FIBROSING ALVEOLITIS AND ATYPICAL PROLIFERATIVE LESIONS OF THE LUNG

An Experimental Study in Syrian Hamsters

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The term "fibrosing alveolitis" was suggested by Scadding to refer to evolving histopathological processes, both exudative and proliferative, which occur in the alveolar walls rather than in the alveolar spaces. The intraluminal exudate is predominantly of the mononuclear type and the end stage is a diffuse interstitial fibrosis. The fibrosis consequent upon nonresolution of a pneumonia results from organization of the alveolar exudate and is distinct from the progressive fibrosis predominantly in alveolar walls.[1]

Interstitial fibrosis can result from damage to the lungs by a variety of agents, of which only a few are known, such as the chemicals aluminum, beryllium, and nickel carbonyl; the drug hexamethonium, and pneumotropic viruses.[2,3] The category of diseases that cause thickened alveolar walls include: Hamman-Rich syndrome, idiopathic diffuse interstitial fibrosis, "rheumatic" pneumonia, pulmonary manifestations of rheumatoid arthritis, von Recklinghausen's disease, and scleroderma.[4,5]

Entities characterized by extensive proliferation of mononuclear elements within the lung include alveolar proteinosis, primary pulmonary hemosiderosis, desquamative interstitial pneumonia, and eosinophilic granuloma. There is evidence that pulmonary alveolar proteinosis and desquamative interstitial pneumonia can sometimes pursue the same pathway, with interstitial fibrosis and "honeycombing" as the end stage.[6,7] All of these conditions represent puzzling problems in etiology and pathogenesis of proliferation and metabolic activity of the complex cellular population of the lung.

Chemists are often not aware of the cancer-inducing hazards of many chemicals with which they work. N-nitroso-N-methylurethane has been shown in laboratory animals to be a potent carcinogen. Since this compound is widely used in chemistry in the preparation of diazomethane, an alkylating agent, Druckrey and Preussmann have pointed out that N-nitroso-N-methylurethane should be handled with the greatest of care and be replaced in the preparation of diazomethane by the nontoxic N-

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nitroso-N-methyl-*p*-toluene sulfonamide whenever possible.[8] Weisburger and Weisburger have also stressed the hazards associated with the use of N-nitroso-N-methylurethane and related compounds and in addition have advised that chemists should avoid exposure to diazomethane, not only because of its well-known toxic effect but because it has induced lung tumors in mice and rats following inhalation.[9]

The purpose of the present report is to describe the histopathological changes induced in the lungs of Syrian hamsters following subcutaneous administration of N-nitroso-N-methylurethane. The pulmonary lesions induced in the laboratory animals by this chemical closely resemble those that occur in man in association with interstitial fibrosis and atypical epithelial proliferation.

Material and Methods

Experimental Animals. Syrian hamsters, 1 month of age and of either sex were used. The animals were separated by sex, housed in plastic cages in groups of 4, and fed Purina Laboratory Chow daily, supplemented with kale, carrots, and apples 3 times a week.

N-nitroso-N-methylurethane. A 0.2% solution of N-nitroso-N-methylurethane (K & K Laboratories, Inc. Plainview, N. Y.) was made up in 0.5% solution of aqueous ethanol and freshly prepared each week for subcutaneous administration.

Experimental Procedure. A preliminary trial with a 1-mg. dose administered subcutaneously to 5 hamsters resulted in death of all animals within 24 to 48 hr. due to extensive pulmonary hemorrhages and edema. Consequently the test dose used in this experimental study was 0.2 mg. The experimental Group A, and the control Group B, consisted of 12 animals each. Group A received 0.1 ml. (0.2 mg.) of the solution of N-nitroso-N-methylurethane weekly by subcutaneous injection into the interscapular region. The injections were continued for periods ranging from 5 to 6 months and the total dose of N-nitroso-N-methylurethane administered was between 4 and 5 mg. Group B, the controls, received subcutaneous injections of 0.2 ml. of 0.5% solution of aqueous ethanol.

Necropsies were performed on all animals killed or found dead. Three of the experimental animals were found dead at 8, 17, and 20 months of age. Nine were killed because of marked respiratory difficulties; 1 at 7 months, 3 at 12 months, 3 at 14 months, 1 at 16 months, and 1 at 20 months. Two of the control animals died of acute enteritis at 10 and 12 months respectively. The remainder were killed, 2 at 16 months, 2 at 17 months, and 6 at 20 months of age. Tissues were fixed for histology in 10% buffered formalin and embedded in paraffin. The following staining procedures were carried out: hematoxylin and eosin, Masson's trichrome, periodic acid-Schiff (PAS) routine, PAS following diastase digestion, Wilder's reticulin, Mayer's mucicarmine, alcian blue, Mallory's phosphotungstic acid hematoxylin, Gomori's methenamine silver, and Brown-Brenn.

Results

General

The life span of the experimental animals ranged from 7 to 20 months and the average age at death was 15 months. The average age at death for the control animals was 19 months. The Syrian hamster is very

resistant to pulmonary infection, and microscopic examination of the lungs of the control animals revealed no abnormalities.

All experimental animals showed signs of pulmonary insufficiency. The onset of respiratory distress did not develop at the same time in the animals, but once noted the dyspnea was progressive and increased in severity. Two animals were observed to have rapid respirations after 4 months of treatment with N-nitroso-N-methylurethane. The test substance was administered at weekly intervals, and the first sign of respiratory distress was usually observed at monthly intervals. The ventilatory insufficiency was characterized by rapid, shallow inspirations and prolonged and difficult expirations. The shape of the thorax became altered, with a definite increase in the anteroposterior diameter.

Gross Observations

The significant gross pathology was limited to the lungs. All animals in the experimental group showed similar findings that varied in degree. The lungs were pale, firm, and distended, and they filled the entire thoracic cavity. They had an emphysematous appearance with small blebs and cysts at the periphery. On section the parenchyma revealed increased resistance. The surface was granular, with irregular, patchy, and diffuse distribution of a grayish-white fibrous network. A few lungs had pinpoint areas of hemorrhage and depressed pleural scars. The lungs of 3 animals showed discrete, round, single and multiple, yellow-gray, firm nodules. The air passages often were dilated, and the mucosal surfaces were smooth.

Histological Observations

Many stages of the alveolar reaction, both exudative and proliferative, coexisted in one and the same lung. It seems not unreasonable to suggest that the variation in the pathological picture seen in one and the same lung may represent different stages of the reaction to the stimulus because the N-nitroso-N-methylurethane was administered at weekly intervals. The occurrence of extensive pulmonary hemorrhages and edema, when a preliminary trial with a 1-mg. dose of the chemical was given, suggests that the first stage in the reaction is injury to the capillary wall. The proteinaceous material present in the distal air spaces was homogeneous, sometimes granular and deeply acidophilic (Fig. 1). This intra-alveolar material may represent inspissated serum that extravasated through the injured capillary wall. It was PAS-positive and diastase-resistant. The material was essentially noncellular except for remnants of mononuclear cells. No pathogenic organisms could be identified with Gomori's methenamine silver or Brown-Brenn stains. With phospho-

tungstic acid hematoxylin the alveolar material stained yellow and no fibrin was present. Mayer's mucicarmine and alcian blue stains were negative for mucin. The material was green with orange particles when stained with Masson's trichrome. Frozen tissue was not available for fat stains. The alveolar walls revealed prominent epithelialization, and the lining cells were of the cuboidal type (Fig. 2). This cellular phase is the third stage of the reaction and may be associated with resolution of the process.

In other areas there was a hypercellularity of the alveolar walls with a tendency toward papillary formation. The hyperplastic alveolar cells were embedded in a thickened interstitial tissue. Disintegration of the alveolar material led to the formation of prominent acicular clefts (Fig. 3 and 4). Numerous desquamative alveolar cells were present in the alveolar lumina and the cytoplasm of some contained PAS-positive granular material and the nuclei were often pyknotic. The presence of PAS-positive material within the histiocytes of the alveoli represents phagocytosis and this may be a principal mechanism for clearing of the alveolar material together with the process of absorption. The alveolar cells showed varying degrees of swelling and vacuolization. The septa in some instances were thickened by lymphocytic and mononuclear infiltrates (Fig. 5 and 6).

Single or sometimes multiple spherical, sharply outlined concretions composed of irregular concentric rings or laminations were found free in alveolar lumina. Occasionally these concretions were associated with desquamated alveolar lining cells forming a syncytium (Fig. 7). Low cuboidal epithelial cells lined the honeycombed pulmonary parenchyma, and the interstitial thickening of the distal air spaces was often marked. The fibrosis was both reticular and collagenous and of loose or compact structure. The lumina were filled with masses of desquamated alveolar cells; the cytoplasm was granular and gave a positive PAS reaction. A few cells contained iron pigment (Fig. 8). The desquamation of the alveolar cells and the marked fibroblastic thickening of the walls were the late lesions and may represent impairment of the alveolar clearing process. The lungs of all the experimental animals showed the lesions that have been described. However, there was variation in degree and extent of involvement.

Atypical Acinar Proliferation. The small discrete nodules noted grossly in 3 of the animals revealed a characteristic histological pattern of pulmonary adenomatosis. The nodules were not encapsulated, and the closely packed columns of cuboidal or columnar cells showed no striking variation in size or shape. The tumor cells were arranged in an acinar pattern and were supported by sparse stroma of fibrous tissue (Fig. 9).

Small foci of adenomatous hyperplasia was observed in 5 of the animals only on microscopic examination.

There was variation in the degree and type of atypical acinar proliferation encountered. All of the animals showed foci of epithelial atypism associated with epithelialization of the distal air spaces. The cells showed variation in nuclear size, shape, and hyperchromatism. In 4 cases the distal air spaces were lined by a mucus-secreting bronchiolar type of epithelium (Fig. 10). An example of an extreme and striking degree of atypical hyperplasia was noted on microscopic examination of a centrally depressed wedge-shaped pleural scar. The periphery of the pleural scar was composed of dense, compact, collagenous tissue. The alveolar tissue adjacent to the scar was composed of irregular acinar structures lined by extremely anaplastic cells that bulged irregularly into the lumen. There was marked variation in nuclear size and shape, bizarre forms, mitotic figures, hyperchromatism, and prominent nucleoli (Fig. 11 and 12). The distinction between atypical epithelial proliferation and neoplasia is difficult. Although this lesion histologically resembled a primary anaplastic adenocarcinoma of the lung, no metastases were noted, and thus the diagnosis of carcinoma was not made.

Discussion

It is well recognized that the alveolar membrane may be the target tissue for inhaled irritants or toxic substances. That the mode of action of drugs, chemicals, or toxins on the alveolar membrane may be a remote one rather than a local one has not received the attention it deserves. Appreciation of the role of the alveolar lining membrane has provided evidence that the lung is not a passive organ merely for exchanging oxygen and carbon dioxide but that it is a metabolically active one.[10]

The present study has shown that, in Syrian hamsters, N-nitroso-N-methylurethane has a selective and remote action on the alveolar membrane of the lung. The lesions induced resemble histologically those seen in certain obscure pulmonary diseases in man that are characterized by interstitial fibrosis and atypical epithelial proliferation. The primary change in Montana progressive pneumonia of sheep, and jagziekte and Kimberley horse disease is in the interalveolar tissue, and interstitial fibrosis may be greatly marked in the advanced stages of these diseases.[11,12] Theiler in 1918 demonstrated, by feeding experiments, that a toxic principle in the plant *Crotalaria dura* was the etiological agent of jagziekte in horses, thus showing for the first time the affinity of a plant toxin for the epithelial cells of the alveoli.[13] Recent studies by Gardiner, Royce, and Bokor on Kimberley horse disease also incriminate a plant, *Crotalaria crispata,* as the etiological agent. They produced pulmonary

lesions in rabbits by whole-plant feeding or by administration of alkaloidal mixtures containing fulvine. The essential nature of the lesion was proliferation of the epithelial elements of the alveolar walls and interstitial fibrosis.[12]

New disease entities of the lung in man that have been described in recent years are pulmonary alveolar proteinosis and desquamative interstitial pneumonia (DIP).[14,15] The fact that many theories have been proposed for the etiology and pathogenesis of these diseases is ample proof that very little is known regarding them.[14,16–19] The possibility that pulmonary alveolar proteinosis is not a new disease has been suggested by Larson and Gordinier. They offered as the most plausible explanation for the apparent appearance of the disease, the more recent use of lung biopsy as a diagnostic technique.[20]

Liebow, Steer, and Billingsly have reported similarities between alveolar proteinosis and DIP, particularly in the littoral proliferation and desquamation of the large alveolar cells. These authors have also observed that the alveolar reaction in Montana progressive pneumonia in sheep and in jagziekte bears a particularly striking resemblance to DIP.[15]

Experimental models in laboratory animals may well reflect the probable stages in the development of similar lesions that are seen in man. In the present study, there is support for the idea that following absorption of N-nitroso-N-methylurethane into the main circulation, it reaches the lung and causes capillary injury with increased permeability. Hemorrhage into the alveolar spaces was prominent when a high dose of the chemical was administered. That N-nitroso-N-methylurethane has an affinity for the pulmonary epithelium is evidenced by the proliferation of the alveolar cells. Knowledge in regard to the metabolic fate of N-nitroso-N-methylurethane in the body is not known, but certain chemicals may be fixed by tissues and thus exert a cumulative effect. The experimental work by Gardiner, Royce, and Bokor of feeding *Crotalaria crispata* to rabbits suggests that the alkaloid has such an effect. The alveolar cell proliferation was progressive and continued to develop indefinitely after the feeding or parenteral administration of the plant had been discontinued.[12] The atypical acinar proliferations observed in this study suggest that regenerative hyperplasia, epithelial atypism, and neoplasia may represent a final common pathway. Squamous carcinoma and alveolar-cell carcinoma of the lung have been induced in rats following intravenous administration of N-nitroso-N-methylurethane.[8]

The prevalent concept is that the most likely etiological agent for primary lung cancer and obscure pulmonary disease (e.g., alveolar proteinosis) must be cigarette smoke, a toxic inhalant, or an unidentified

virus.[14,18] Many diverse agents may act on the lung and reach there by routes other than inhalation. The findings in the present study suggest that a search should be made for environmental agents, chemicals, drugs, and other substances that are ingested, absorbed from the skin or other sites, or administered parenterally, which reach the lung by the main circulation and have a selective action on pulmonary epithelium. Studies relating to the metabolism of the alveolar lining membrane may be relevant to the problem of lung cancer in man and will further our understanding of other disease processes in which the alveolar membrane is altered.

Summary

The effect of subcutaneously administered N-nitroso-N-methylurethane was studied in Syrian hamsters. The wide spectrum of pathological changes observed in the lungs of the experimental animals include: PAS-positive proteinaceous material in the alveolar spaces, acicular clefts, prominent epithelialization of the alveolar walls, masses of desquamated cells in the lumina, pulmonary corpora amylacae, cystic spaces, and intense interstitial fibrosis. The atypical acinar proliferation was characterized by air spaces lined with mucus-secreting bronchiolar type of epithelium, pulmonary adenomatosis, and irregular shaped cystic spaces lined by anaplastic cells in peripheral areas of the lung associated with dense scarring.

N-nitroso-N-methylurethane, a known potent carcinogen, is widely used in chemistry for the preparation of diazomethane, an alkylating agent. In the present study there is support for the concept that environmental agents that reach the lungs by routes other than inhalation may be relevant to the problem of lung cancer in man. The search for chemicals, drugs or other substances that have a selective affinity for pulmonary epithelium may further our understanding of lung cancer and other diseases in which the alveolar membrane is altered.

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LEGENDS FOR FIGURES

FIG. 1. Proteinaceous material in alveolar lumina. Minimal thickening of walls and epithelialization. Hematoxylin and eosin stain. × 85.

FIG. 2. The exudate in alveolar lumina PAS-positive, diastase-resistant. Periodic acid-Schiff stain. × 135.

FIG. 3. Hyperplasia of alveolar cells, interstitial fibrosis, and acicular clefts. Masson's trichrome stain. × 135.

FIG. 4. Note prominence of acicular spaces and remnants of cells in alveolar lumina. Hematoxylin and eosin stain. × 135.

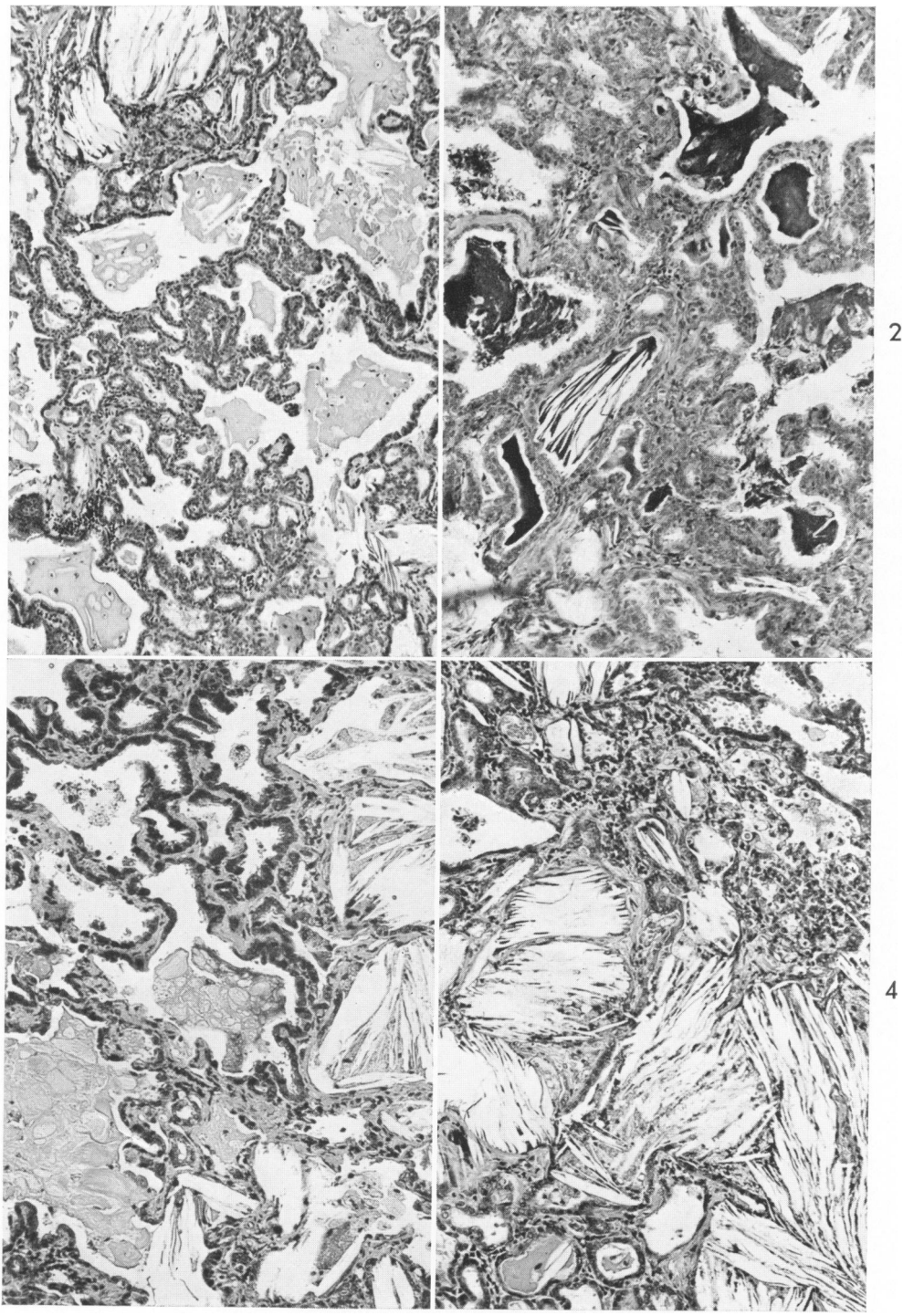
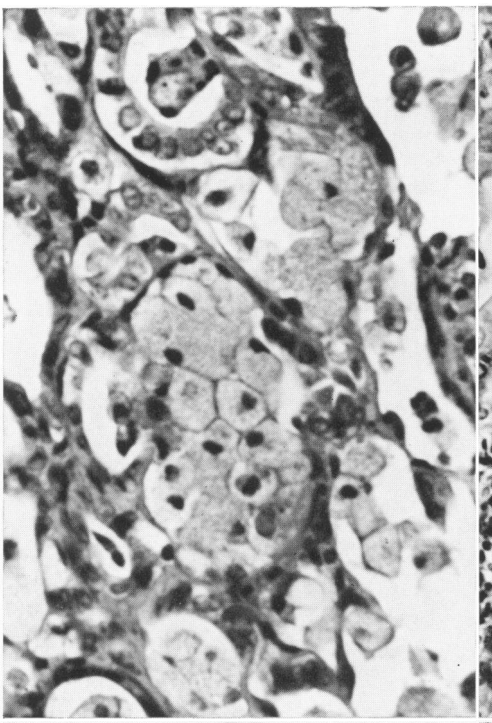


FIG. 5. Masses of desquamated cells fill alveolar spaces. Lining cells are predominantly cuboidal type. Hematoxylin and eosin stain. × 400.

FIG. 6. Septa thickened by lymphocytic and mononuclear cell infiltrate. Variation in size and shape of alveolar lining cells. Hematoxylin and eosin stain. × 260.

FIG. 7. Pulmonary corpora amylacae. Desquamated alveolar cells forming syncytium. Hematoxylin and eosin stain. × 690.

FIG. 8. Intense interstitial fibrosis with prominent thickened walls. Irregular-shaped cystic spaces lined by flattened cuboidal cell. Masses of desquamated alveolar cells in lumina. Hematoxylin and eosin stain. × 135.



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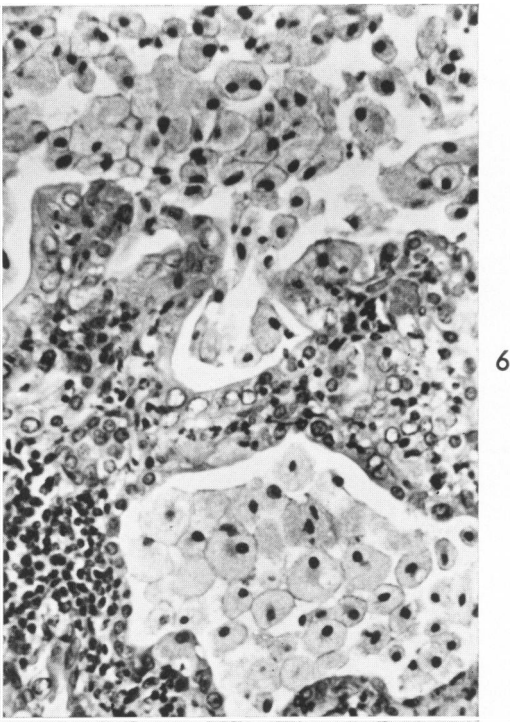

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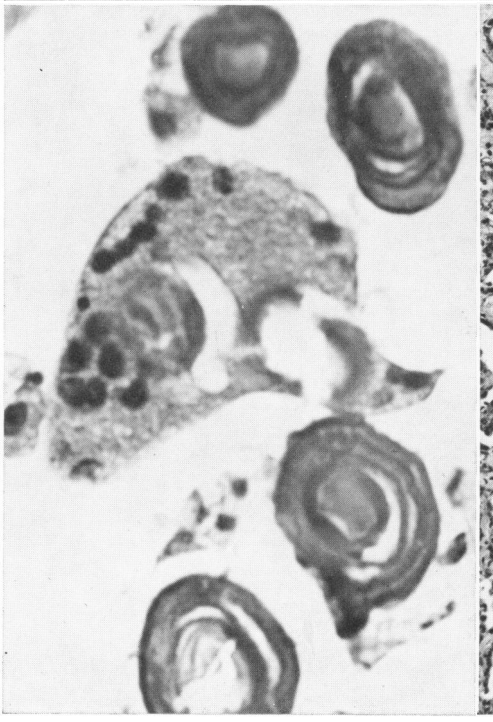

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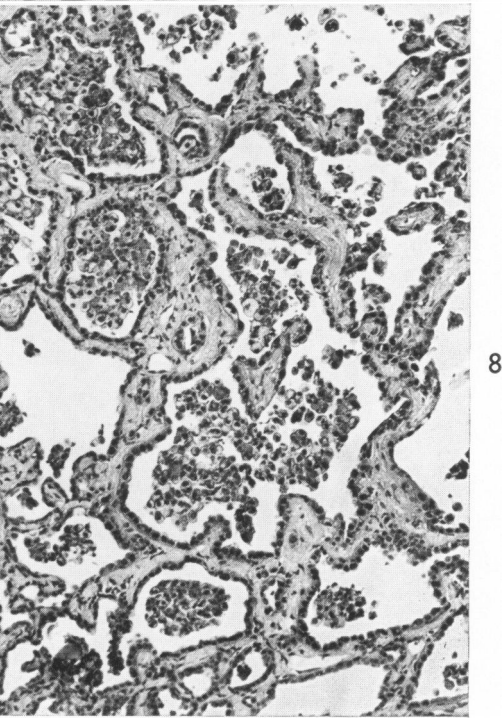

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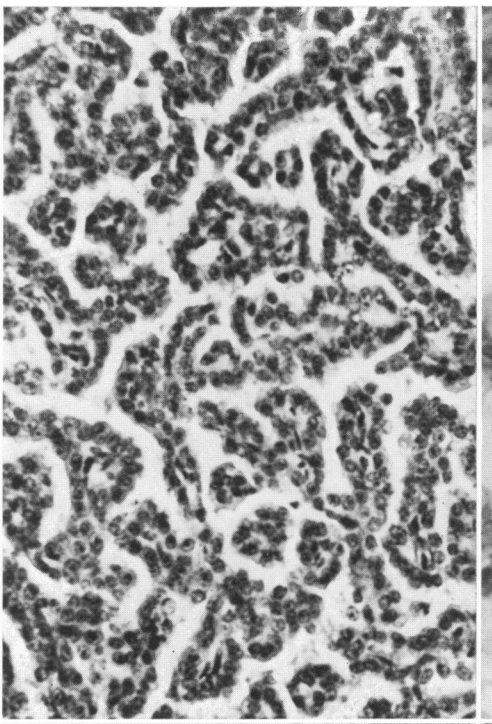
FIG. 9. Pulmonary adenomatosis. Note uniform structure of cells supported by delicate stroma. Hematoxylin and eosin stain. × 290.

FIG. 10. Atypical acinar proliferation. Air spaces lined by mucus-secreting bronchiolar type of epithelium. Hematoxylin and eosin stain. × 610.

FIG. 11. Atypical acinar proliferation associated with wedge-shaped pleural scar. Large anaplastic cells line the spaces. Hematoxylin and eosin stain. × 135.

FIG. 12. Higher magnification of Fig. 11. Irregular masses of anaplastic cells with prominent nucleoli line alveolar space. Hematoxylin and eosin stain. × 690.



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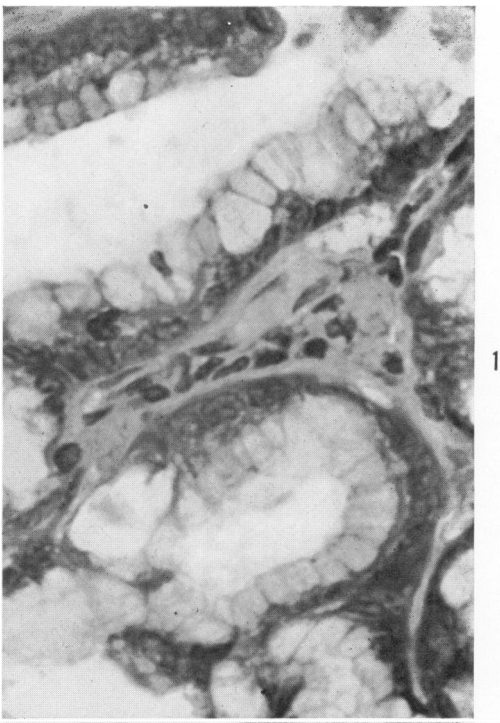

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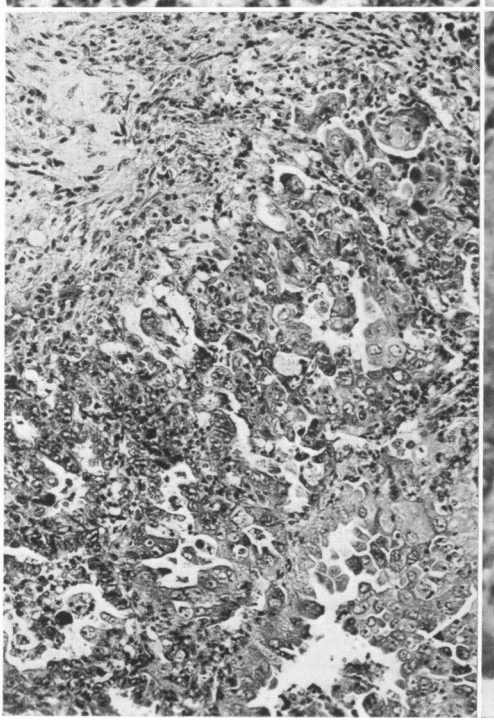

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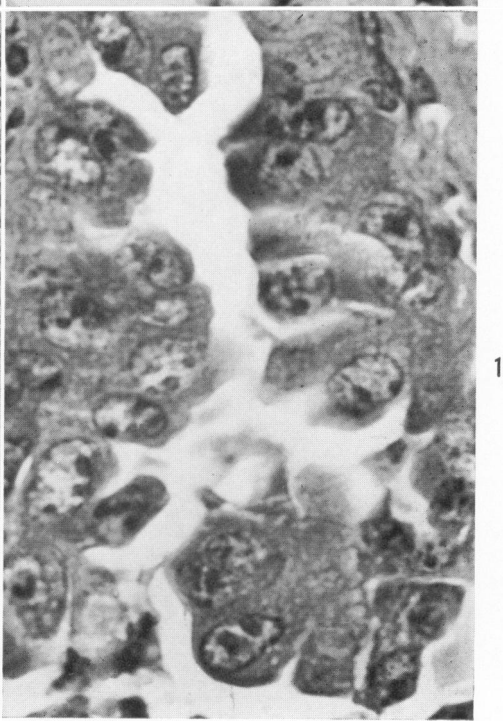

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