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26. For the experiments on cultured pineal glands, pineals were obtained from animals killed at 12 hours and were maintained for 3 hours in culture (*15*), either in control culture medium or in the presence of various compounds. Pineals were frozen on dry ice and analyzed for protein I content (*16*).
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Lung Fibrosis and Emphysema: Divergent Responses to a Common Injury?

Abstract. *Cadmium chloride, administered intratracheally to golden Syrian hamsters, causes an acute lung injury which evolves into a lesion with functional and morphological features of diffuse fibrosis. With simultaneous feeding of a lathyrogen, β-aminoproprionitrile, this same injury evolves into functional and morphological changes of bullous emphysema. These results suggest that the same lung injury might result in either fibrosis or emphysema, connective tissue synthesis during the healing phase being the critical determinant.*

Interstitial lung fibrosis and emphysema are generally considered to be separate disorders, each having distinguishing clinical, radiological, physiological, and pathological characteristics (*1*). Despite these differences, current concepts suggest common features in pathogenesis. Both disorders are thought to represent a late healing stage after lung injury. Both diseases in humans, as well as in animal-model counterparts, are at some stage associated with inflammation (*2*). It has been proposed that the products of the inflammatory reaction, particularly proteases and oxygen-centered radicals, may play a central role in mediating lung damage that precedes both fibrosis and emphysema (*3*). Even the repair phase after injury may have similarities. Post-injury accumulation of connective tissue proteins is a prominent feature of both emphysema and fibrosis in animal models (*4*).

What determines whether lung damage evolves into fibrosis or emphysema? One factor might be the nature of the initial injury; however, differences in the repair processes might be equally as important. The metabolism of collagen and elastin is of particular interest in the healing phase since these proteins are so important to lung structure and function. We present evidence to support the idea that alterations in the synthesis of these proteins might determine whether emphysema or fibrosis evolves after lung injury. We show that the intratracheal administration of cadmium chloride ($CdCl_2$) to hamsters causes functional and morphological abnormalities that are characteristic of lung fibrosis. With the simultaneous administration of β-aminoproprionitrile fumarate (BAPN), which interferes with normal collagen and elastin synthesis by inhibiting lysyl oxidase (*5*), this same injury results in morphological and functional changes of bullous emphysema.

Female golden Syrian hamsters (LVG outbred strain, Charles River Laboratories) weighing 90 to 120 g were fed either a regular diet (Ralston Purina Rodent Chow) or the same diet to which was added 0.5 percent (by weight) BAPN (Sigma). Seven days later, pentobarbital-anesthetized animals received by intratracheal instillation either 0.5 ml of normal saline containing 0.04 μmole of $CdCl_2$ per 100 g of animal weight or normal saline alone. After 5 weeks, the surviving animals were killed and pressure-volume relationships were measured in the excised lungs. The lungs were then fixed in Formalin at constant pressure (25 cm H_2O), and sections were processed for histopathological examination. To determine the extent of any airspace enlargement, the mean linear distance between alveolar intercepts (L_m) was measured on each lung section (*6*).

After 24 hours, hamsters receiving $CdCl_2$ and either the regular diet or the regular diet with BAPN exhibited signs of respiratory distress, which subsided within a few days. Histopathological examination of the lungs at this stage revealed edema, hemorrhage, and an intense, predominantly neutrophilic, inflammatory reaction.

Nine out of 16 animals receiving $CdCl_2$ and BAPN died between 2 and 5 weeks after instillation of the $CdCl_2$. At autopsy, the lungs from each of these animals contained multiple, large (up to 2 cm in diameter), thin-walled subpleural bullae (Fig. 1a). In some animals a single bulla occupied nearly an entire hemithorax.

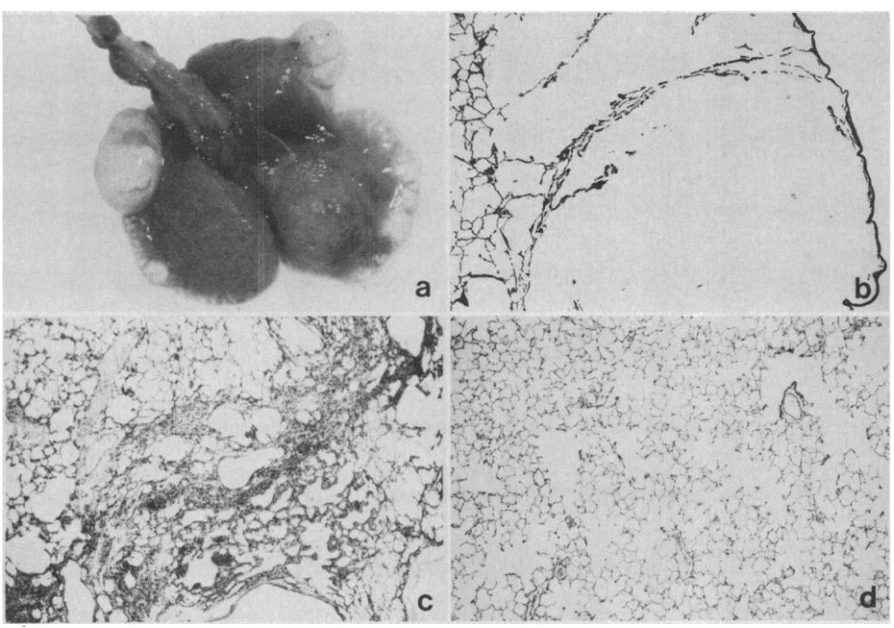

Fig. 1. (a) Air-filled lung from a hamster that had received $CdCl_2$ 5 weeks before and was also fed BAPN. The multiple subpleural bullae occurred only in this treatment group. (b) Histologic section showing a bulla and adjacent emphysematous changes in the same lung. (c) Diffuse lung fibrosis in an animal that received $CdCl_2$ but no BAPN. (d) Normal hamster lung. (All photomicrographs are ×22.)

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The immediate cause of death in these animals appeared to be spontaneous pneumothorax, as the lungs were partially collapsed prior to the opening of the thoracic cavity and transpleural air leaks were demonstrated in excised lungs. Five of the seven surviving animals also had multiple bullae. Pressure-volume relationships in these seven lungs showed overinflation and increased compliance characteristic of emphysema (Fig. 2). The outer walls of the bullae consisted of only a thin band of fibrous tissue (Fig. 1b), and in many places the cavity communicated freely with adjoining alveolar spaces. Many adjacent regions also exhibited lesser degrees of airspace enlargement which resembled panlobular emphysema. Most lungs in this group also had fibrotic areas similar to that shown in Fig. 1c. (A normal lung is shown in Fig. 1d.) Because most bullae did not remain expanded during tissue processing, they were specifically excluded from L_m measurements. Even with this systematic underestimate, the L_m in this group (83 ± 5 μm, mean ± 1 standard error of the mean) was significantly increased over that of controls (59 ± 2 μm, $P < .05$).

All animals receiving $CdCl_2$ and the regular diet survived to the end of the 5-week period. Macroscopic bullae were present in none of the lungs from this group. When inflated, the pleural surface over many lung regions appeared puckered and retracted. Pressure-volume measurements showed small lung volumes with decreased compliance (Fig. 2). There were broad bands of condensed parenchyma and thickened alveolar walls which stained positively for collagen (Fig. 1c). In other areas, alveolar architecture was distorted and airspaces appeared dilated but in no instance were there bullous emphysema lesions like those shown in Fig. 1b. The average L_m in these animals (74 ± 4 μm, $P < .05$) was significantly increased over that of controls, confirming the impression of airspace enlargement (*7*). There were no discernible functional or morphological changes in animals that received BAPN but no $CdCl_2$ (L_m = 62 ± 2 μm).

The results of this study demonstrate that $CdCl_2$-induced lung injury in hamsters can be modified by BAPN to produce a lesion that is predominantly emphysematous rather than fibrotic. It is not known whether this dimorphic response is unique to $CdCl_2$. We used $CdCl_2$ because it affords a convenient method for inducing lung injury and because both short-term and long-term exposures have been reported to cause "emphysema-like" lesions (*8*). We also observed airspace enlargement in animals receiving $CdCl_2$ alone, but whether this represents alveolar wall destruction, an essential feature of emphysema (*9*), or merely distortion and overdistention of alveoli adjacent to fibrotic zones is difficult to establish. In any case, the resultant pattern of organ dysfunction is typical of fibrosis and not of emphysema. However, the giant bullae observed in animals given both $CdCl_2$ and BAPN represent unequivocal evidence of alveolar wall disruption, and the functional changes are typical of emphysema.

We propose the following mechanisms to explain our observations. We suggest that $CdCl_2$ with or without BAPN does induce alveolar wall destruction, possibly through the action of endogenous proteases. The synthesis of connective tissue after the acute injury strengthens damaged but intact alveolar walls and prevents further airspace enlargement but may contribute to the restrictive pattern of lung dysfunction. In contrast, interference with postinjury collagen and elastin synthesis in BAPN-treated animals permits further alveolar wall destruction, either from continued proteolysis or from stress-induced separation of previously damaged connective tissue fibers. Bulla formation eventually results.

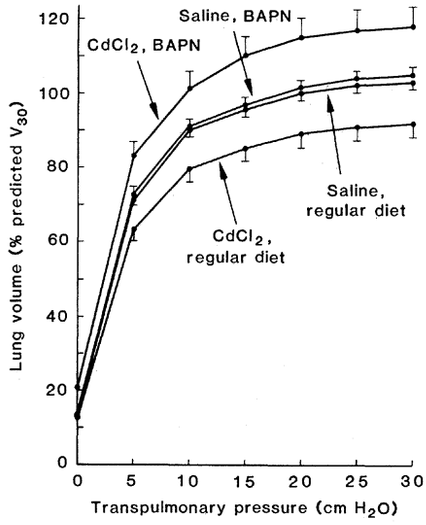

Fig. 2. Lung volume as a function of transpulmonary pressure (P_{TP}) for each of the four treatment groups (N = 6 to 13). Freshly excised lungs were mounted in a heated (37°C), humidified chamber. With a constant-volume history, lungs were deflated from a P_{TP} of +30 cm H_2O (V_{30}) to 0 cm H_2O at a constant rate (10 ml/min) while P_{TP} and volume change were measured continuously. Minimum air volume at a P_{TP} of 0 cm H_2O was determined by volume displacement. Predicted lung volumes at V_{30}, based upon body weight, had earlier been established for normal female animals. At each P_{TP}, lung volumes are significantly increased ($P < .05$) over controls in the group receiving $CdCl_2$ and BAPN but are significantly decreased in the $CdCl_2$-treated animals on a regular diet.

In view of the fact that BAPN has biological effects other than its inhibitory effect upon lysyl oxidase, the mechanisms proposed are tentative (*10*). We cannot exclude the possibility that BAPN subtly modifies the initial injury. However, in other experiments bullae formed even though BAPN feeding was begun a week after $CdCl_2$ instillation. Since the acute inflammatory reaction is subsiding at that time, this would clearly implicate an effect of BAPN upon repair as the mechanism of bulla formation.

Our results, as well as earlier work, suggest that collagen and elastin formation may play a central role in determining not only the severity but also the nature of organ dysfunction after lung injury (*11*). Current research into the pathogenesis of fibrosis and emphysema has focused largely upon mechanisms of injury. We suggest that common lung toxins, such as cigarette smoke, might have equally important effects upon the healing phase after injury.

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