function in the lungs devised by Setnikar\(^2\) and elaborated by Mead\(^3\) which hypothesized collagen fiber bundles acting independently of elastin fiber bundles and limiting distensibility of the lung. The increase in TLC\(_{25}\) in the elastase-treated animals suggests that elastin and collagen fibers acted in series or that elastase treatment and the subsequent repair process secondarily altered the collagen network.

The present work was, therefore, undertaken in an attempt to further evaluate the relationships between altered lung connective tissue and mechanical behavior in a system reflecting only tissue properties and carried out in vitro in order to eliminate changes during the repair process in the original in vivo experiments.

Degassed lungs were incubated with either an elastase, collagenase, or buffer solution immediately after excision. Saline filled, quasi-static, volume pressure curves were obtained on normal and treated excised hamster lungs.

Deflation curves of mean data are shown for the three groups of lungs. Zero volume (V\(_L\)) is taken to be tissue volume or that volume of the lung prior to incubation. The ordinate is volume expressed as a percentage of total fluid within the lung. Care was taken to ensure that total fluid volume was similar in the normal and treated lungs. The abscissa is transpulmonary pressure in cm H\(_2\)O (P\(_L\)).

The mean curve of elastase-treated lungs has lower recoil pressures and increased C\(_r\) in the low- to mid-V\(_L\) controls. Yet, elastase treated lungs sustain normal recoil pressure and C\(_r\) at high V\(_L\). In contrast, the mean curve of collagenase-treated lungs has normal recoil pressure and C\(_L\) at low to mid V\(_L\), but lower recoil pressure and significantly increased C\(_r\) at high V\(_L\). Comparable volumes were attained at the end of inflation in the three groups. P\(_L\) at full inflation was not significantly different in the elastase-treated and control groups, but was significantly lower in the collagenase-treated lungs.

These results indicate that specific connective tissue protein digestion results in specific and independent derangements in the deflation volume-pressure curve of hamster lungs. Hence, they support the concept of functional autonomy of elastic fiber bundles and collagen fiber bundles. The inconsistency of our previous results with this concept, in particular the increased TLC\(_{25}\) after elastase treatment, may have occurred as a result of the following. It is possible that the collagen network was altered by the increased stress to which it was submitted during breathing after the disruption of the parallel elastic fiber network. Alternatively, collagen fibers or a linkage with elastin fibers in the living animal may have been altered by enzymatic injury related to the cellular response in the lung which was initiated by the original elastase insult. These considerations merit further investigation.

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**Session 7: Experimental Lung Injuries**

**Pulmonary Effects of Paraquat Poisoning**

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Paraquat (1,1'-dimethyl-4,4'-dipyridylum chloride) is a powerful herbicide and it is thought to act by turning into a stable free radical, methyl viologen, in the presence of powerful reducing agents. In plants the reduction of paraquat to the radical ion is likely brought about by photosystem 1, followed by a spontaneous reaction with oxygen to form O\(_2^+\) radical ion and regenerated paraquat. O\(_2^+\) reacts either spontaneously or enzymatically to produce oxygen and hydrogen peroxide and the latter is considered to be the damaging agent in plants. A similar reaction is postulated in animals (but with a differing reducing agent) with the superoxide damaging cell membranes. It is possible that oxygen, ozone and radiation may damage the lung also through superoxide and free radical formation.

We have been able to examine the lungs of three patients who died nine to 22 days after suicidal ingestion of paraquat. We have also investigated the acute pulmo-
FIGURE 1. A paper-mounted, whole-lung section of the patient dying 17 days after ingestion of paraquat shows marked microcyst formation, most obvious in the anterior half of the lung. Note the formation of large cystic spaces in the tip of the lingula (× 0.45).

nary effects of paraquat in rats to determine the effectiveness of the morphologic lesions as an assay system for paraquat poisoning.

Human Cases

The three patients were 16, 31 and 35 years old and died 9, 17 and 22 days following ingestion of paraquat respectively. Severe ulcerative and necrotic lesions of the oropharynx, transient hepatocellular dysfunction and renal failure occurred in the patients. Respiratory failure developed rapidly in the patient who died on the ninth day post-ingestion. In the other patients, respiratory

FIGURE 2. A close-up of the paper-mounted whole-lung section shows the microcyst formation more clearly and this is in sharp contrast to the relatively normal lung tissue which presents a relatively solid appearance (× 1.7).

failure developed more slowly and an evolving radiologic pattern was observed in the lungs, with an early phase of intra-alveolar exudation being succeeded by the appearance of linear shadows and cystic spaces. One lung was received from the coroner in two of the cases; a complete autopsy was done on the third.

A probable sequence of lesions could be observed in the lung. In all patients, there was considerable topographic variation in the severity and nature of the lesions (Fig 1 and 2). In the patient dying nine days after ingestion of paraquat there was marked intra-alveolar edema in much of the lung. In addition, there was active fibroblastic proliferation of the lung interstitium. Hyaline membranes were present lining some of the air spaces and were becoming incorporated into the wall, and early "honeycombing" with disorganization of the alveolar pattern of the lung was apparent. This process was much more obvious in the other two cases (Fig 2) and both showed distinctive areas where the lung structure was entirely disorganized and replaced by many small (0.5-2.0 mm) cysts lined by fibrous tissue (Fig 3). A second type of fibrosis was observed as well. This was characterized by preservation of the framework of the lung

FIGURE 3. Lung structure is completely disorganized resulting in the formation of small cysts separated from each other by fibrous tissue. Compare Figure 4 (× 53).

FIGURE 4. Pre-existing air spaces are filled by loose fibrous tissue. Walls of an alveolar duct (center) and alveoli can be made out (× 53).
room temperature for one hour and then processed saline-injected control. At the appropriate time, the ani-

lead citrate for electron microscopic examination. Two to fixative for two hours and were then

of the lesions as given in the literature. The tissue from the lungs removed and fixed by intratracheal instillation. Four rats were examined to Epon. One

tions of the animals when the tissue was examined. The observer was not aware of the experimental condi-

paraquat dichloride, in a concentration of 10 mg/ml in sterile normal saline solution, was given intravenously in a dose of 25 mg paraquat dichloride per kg body weight to 13 rats; six rats served as controls and received 2.5 ml normal saline/kg body weight. Four rats were examined with prior knowledge of their experimental conditions—one control and three animals sacrificed at 24, 48 and 72 hours after paraquat administration respectively. These animals were used to gain experience of the lesions of paraquat poisoning and reinforce available descriptions of the lesions as given in the literature. The tissue from the remaining animals was randomized and coded so that the observer was not aware of the experimental conditions of the animals when the tissue was examined. Three animals were sacrificed at 6 hours, 14.5 hours, 24 hours, 48 and 72 hours after intravenous injection, and at each time there were two paraquat-injected animals and one saline-injected control. At the appropriate time, the ani-

did some necrotic debris in the air spaces. At 48 hours, the epithelial thickening was more obvious (Fig 7) and occasionally there appeared to be focal gross thickening with many mitochondria as described by Smith and Heath.1 Frank epithelial necrosis now became apparent and in some areas the epithelium had stripped off the basement membrane. At 72 hours, the changes were more obvious and at this stage numerous perivascular and peribronchial “prefibroblasts”2 were apparent.

In all animals, the changes were spotty and normal alveoli could be seen close to abnormal ones and occasional sections were entirely normal, whereas abnormalities might be obvious on other blocks from the same animal. Endothelial lesions were not observed and consistent airway epithelial lesions were not apparent although flattening of epithelium and bars in non-ciliated cells were seen in some of the paraquat animals, but not in the controls. Some of the cells contained both mucus granules and granules typical of Clara cells (Fig 8).

Animal Studies

Nineteen specific pathogen free (*** on Medical Research Council scale) female Sprague-Dawley rats delivered in filtered boxes, kept in normal cages, weighing 150 to 200 gm were used. Analytical standard paraquat dichloride, in a concentration of 10 mg/ml in sterile normal saline solution, was given intravenously in a dose of 25 mg paraquat dichloride per kg body weight. Four rats were examined with prior knowledge of their experimental conditions— one control and three animals sacrificed at 24, 48 and 72 hours after paraquat administration respectively. These animals were used to gain experience of the lesions of paraquat poisoning and reinforce available descriptions of the lesions as given in the literature. The tissue from the remaining animals was randomized and coded so that the observer was not aware of the experimental conditions of the animals when the tissue was examined. Three animals were sacrificed at 6 hours, 14.5 hours, 24 hours, 48 and 72 hours after intravenous injection, and at each time there were two paraquat-injected animals and one saline-injected control. At the appropriate time, the ani-

some of the controls. A variety of changes were seen electron microscopically. Six hours after paraquat, the experimental animals could not be distinguished from the controls. At 14.5 hours after paraquat, the mitochondria of type II cells were pale and swollen and the osmiophilic bodies in these cells were abnormal and densely and concentrically laminated (Fig 6). These changes were absent in control animals. In addition, there appeared to be an excess quantity of osmiophilic material with much tubular myelin in the air spaces of the paraquat animals. There were more foamy macrophages in the air spaces of the experimental animals. Some of the cells contained both mucus granules and granules typical of Clara cells (Fig 8).

14 blocks were examined from each animal. Blocks were selected to include both parenchyma and peripheral airways.

In general, cellular changes as seen by the light microscope examination were not helpful in distinguishing experimental animals from controls up to 48 hours after injection of paraquat. However, marked congestion was observed in about half of the paraquat animals, but not in any of the controls. A variety of changes were seen electron microscopically. Six hours after paraquat, the experimental animals could not be distinguished from the controls. At 14.5 hours after paraquat, the mitochondria of type II cells were pale and swollen and the osmiophilic bodies in these cells were abnormal and densely and concentrically laminated (Fig 6). These changes were absent in control animals. In addition, there appeared to be an excess quantity of osmiophilic material with much tubular myelin in the air spaces of the paraquat animals. There were more foamy macrophages in the air spaces of the experimental animals. These changes were also present at 24 hours after administration of paraquat, and in addition at this time, focal swelling of the type I epithelium became apparent as did some necrotic debris in the air spaces. At 48 hours, the epithelial thickening was more obvious (Fig 7) and occasionally there appeared to be focal gross thickening with many mitochondria as described by Smith and Heath.1 Frank epithelial necrosis now became apparent and in some areas the epithelium had stripped off the basement membrane. At 72 hours, the changes were more obvious and at this stage numerous perivascular and peribronchial “prefibroblasts”2 were apparent.

In all animals, the changes were spotty and normal alveoli could be seen close to abnormal ones and occasional sections were entirely normal, whereas abnormalities might be obvious on other blocks from the same animal. Endothelial lesions were not observed and consistent airway epithelial lesions were not apparent although flattening of epithelium and bars in non-ciliated cells were seen in some of the paraquat animals, but not in the controls. Some of the cells contained both mucus granules and granules typical of Clara cells (Fig 8).
Forty-eight hours after administration of paraquat, there is swelling of the epithelium of type I cells. Note that the endothelium is normal ($\times 16,000$).

Dark homogeneous blobs (DHB) were seen in type I alveolar epithelial cells in the animals 48-72 hours after paraquat (Fig 9). Endothelial blebbing, focal interstitial edema and apparently increased pinocytosis were observed frequently in controls so that they could not serve as useful distinguishing features between paraquat and controls.

**DISCUSSION**

Our experimental work indicates that paraquat-treated animals can be distinguished regularly from experimental animals 24 hours after administration of paraquat, and probably even at 14.5 hours. Before this time, lesions are not consistently found in paraquat-treated animals, but marked pulmonary congestion was found in some experimental animals and not in controls. Our experience is thus similar to Smith and Heath who used a higher dose of paraquat (40 mg/kg body weight). Our results also suggest that the early phase of paraquat poisoning is different from early oxygen poisoning (and ozone and radiation) where damage to endothelial cells plays so prominent a part. However, oxygen poisoning shares with paraquat the special sensitivity of type I epithelial cells to damage. Our experimental studies also suggest the pathogenesis of the subacute lesions noted in human lungs. Two distinct forms of fibrosis exist. One is characterized by the formation of microcysts and interstitial fibrosis and it seems likely that this is brought about by alveolar wall destruction. This is likely a consequence of epithelial necrosis, basement membrane dissolution and alveolar collapse. The destroyed alveolar walls come together and fibroblastic proliferation results in fibrosis and formation of a fine honeycomb pattern. This lesion is quite similar to fibrosing alveolitis in the human for which it is a useful model. The other process is less clear in its pathogenesis and is characterized by the formation of abundant loose fibrous tissue within alveolar spaces. It may be that this represents the organization of protein-rich edema fluid which pours into alveoli at an early stage of paraquat poisoning. As a hypothesis, it is suggested that it occurs when the epithelial cells are damaged but not destroyed and overall alveolar architecture is preserved. The presence of this remarkable type of fibrosis has been observed by others in paraquat poisoning. Perhaps the marked edema noted in the patient who died soonest after ingestion represented this lesion at an early phase. It should be noted that at this stage patients often have renal failure (as did the patient referred to here) so that the edema may in part be due to fluid overload. The fibrotic and proliferative lesions as well as marked edema are very similar to the lesions described in oxygen poisoning. Since our patients were treated with oxygen, the possibility must be considered that the lesions were caused or potentiated by oxygen. The lesions are clearly not caused by therapeutically administered oxygen since clinical pulmonary involvement occurs at a stage before oxygen is administered and also occurs in animals to which oxygen has not been administered. Also, the fibrotic lesions are clearly of several days’ duration, whereas the oxygen was administered only terminally. However, some features of acute oxygen poisoning, notably severe edema, may have been an added feature to the underlying paraquat lesion.
The experimental data also suggest that lung lesions may be present long before they are clinically apparent. Classically, pulmonary complications become apparent only about a week after paraquat ingestion, but lesions are regularly apparent in animals long before this.

At present, it is still not clear from our data whether the airway epithelium is involved or not and we are studying the matter further. As noted, rather nonspecific alterations were seen in some paraquat animals, but not in all of them, and it is clear that airway epithelium shows lesions which, if real, are far less obvious than those seen in alveolar epithelium. Absence of lesions in airway epithelium would be of considerable interest. It is not clear why certain organs and cells are particularly sensitive to paraquat. The similarity to oxygen poisoning and the additive effect of oxygen to paraquat poisoning5 has suggested that paraquat may particularly affect the lung because of its high ambient oxygen. Were this the case, then airway epithelium should be especially involved but this is not the case. Alternatively, cells may be particularly sensitive because paraquat goes selectively to them. This is a hard question to solve in the lung interstitium where the epithelium appears sensitive and the endothelium relatively resistant. Paraquat is highly soluble in water and thus ordinary autoradiographic techniques cannot be used and, in addition, high resolution is necessary to separate endothelium from epithelium. However, the presence or absence of radioactive paraquat in airway epithelium should be relatively easy to determine and we are at present examining this problem.

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Pathophysiology of Experimental Canine Interstitial Lung Disease*

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Patients with diffuse interstitial lung disease, characterized by restriction of lung volumes, impaired diffusion and decreased compliance, frequently manifest ventilatory disturbances such as dyspnea, tachypnea and alveolar hyperventilation. In the milder stages of the diseases, these abnormalities of ventilation may be present only during exercise. The physiologic mechanism underlying such excessive ventilatory drive has not been identified, but could involve neural stimuli arising in bronchopulmonary sensory receptors whose afferent pathways are in the vagus nerves. Previous attempts to investigate this possibility have generally involved studies on anesthetized animals in whom acute bronchopulmonary disturbances have been produced, such as the imposition of an external respiratory elastic load applied to the airway. There are both practical limitations and theoretical objections to this approach. Accordingly, we have examined the respiratory physiologic disturbances that occur in conscious dogs during the course of interstitial lung disease induced by the intravenous administration of complete Freund’s adjuvant.1

MATERIAL AND METHODS

Our studies were performed on four dogs trained to stand quietly and to run on a treadmill. Once trained they underwent surgical preparation that consisted of creation of an exteriorized cervical vagal loop on each side of the neck.2 The carotid arteries were placed in a subcutaneous position to make them readily accessible for future cannulations prior to each study. Finally, a permanent tracheostomy was created to allow the later insertion of an endotracheal tube during the studies. Following surgery, at least one month was allowed for healing. All studies were performed with the dogs conscious. Initially, the dogs were studied repeatedly in the healthy state over a three-month period to establish normal values of each of the variables for each dog, since each dog served as his own control for the disease studies.

RESULTS

Following the control studies, interstitial lung disease was induced in the dogs by the intravenous administration of complete Freund’s adjuvant, 0.3 ml/kg on each of two days. The physiologic measurements were repeated at frequent intervals throughout the course of the disease, a period of six to eight weeks, following which there was complete recovery. Histologic studies were performed on eight other dogs whose lungs were removed at varying intervals following injection of Freund’s adjuvant. The pulmonary reaction to the adjuvant consisted of two temporally distinct phases. One week following injection there was a diffuse interstitial pneumonitis characterized by edema and increased cellularity of alveolar walls. The cells consisted predominantly of histiocytes, but many lymphocytes, plasma cells and polymorphonuclear leukocytes were also present. The histiocytes stained heavily for lipid which was presumably derived from the paraffin oil of the Freund’s adjuvant. However, no lipid-staining material could be detected in the pulmonary blood vessels. By two weeks following injection, the histologic picture had changed considerably. The pulmonary abnormality now was a proliferative granulomatosis, resembling sarcoido-