IN VITRO PULMONARY COMPLIANCE AND PHYSIOPATHOLOGY OF GUINEA PIG LUNGS EXPOSED IN VIVO TO GADOLINIUM OXIDE DUST

by

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A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Physiology

Iowa State University
Of Science and Technology
Ames, Iowa

1965

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INTRODUCTION

Ever since men first realized that the atmosphere in which they lived contained particulate matter which could be inhaled, they have attempted to determine just what effect these particles might have on the functional ability of the lungs. A great deal of research has been done in an attempt to describe the functional processes of the lung, and much information is available concerning the pathological alterations in pulmonary tissue following inhalation of dusts. However, much less has been reported regarding the combination of these two areas. This research is one such endeavor.

In the last few years, scientific interest in the group of elements known as the rare earths has been stimulated. The lanthanons appear in the sixth row of the periodic table, are members of the Group IIIA elements, and include atomic numbers 57 to 71. The lanthanons, together with the element yttrium, which has similar chemical properties, comprise the rare earth elements. The chemical similarities among the lanthanons are well known. These elements are usually divided into light, middle, and heavy weight groups. The element gadolinium belongs to the middle group. Gadolinium, atomic number 64, was discovered in 1886 by Marignac. It has an atomic weight of 156.9, and a density of 7.94. The effects of the oxide of this metal will be studied in the experiments reported in this thesis.

The rare earth metals occur in the earth's crust where

they are as common as some of the better known elements such as lead, tin, zinc, arsenic, mercury, gold, and platinum. The study of the lanthanons from a biologic point of view is essential due to certain properties and uses of these metals. The lanthanons have become widely used in industry and their use will probably increase. They have been used in the manufacture of gas mantles, arc lights, electrodes, in alloys with other metals, in the ceramic and glass industries, and in the electronics industry where they are currently being used as phosphors on color television tubes. In factories where lanthanons are used, the air can contain airborne particles which can be inhaled. The occurence of rare earth compounds among fission products furnishes a further motive for their observation from a biological viewpoint. At least ten radiolanthanons and six radioisotopes of yttrium are formed during atomic fission. The biological dangers of fission products are generally known. Knowledge of behavior of radio-nucleii in the living organism plays an important role in the defense against this potential danger. This problem has aspects in food and industrial hygiene. Following radioactive fallout, radioactive elements appear in the air and can be carried into the body by inhalation. Work has been done to evaluate the toxicity of inhaled stable rare earth oxides. These oxides have been studied chemically and radiologically, and also by histopathologic and hematologic methods. Histologic changes in the

lungs following inhalation of stable rare earths include such changes in the lung parenchyma as alveolar wall thickening and extensive macrophage reaction. To this author's knowledge, no tests have been conducted in order to ascertain the amount of pulmonary functional damage following inhalation of the rare earth dusts. If sufficient alveolar wall thickening occurs, and if impaction of rare earths in the lung continues with increased exposure, then a point may be reached where there is interference with pulmonary function. The objective of this research was to determine if functional deviations did, in fact, occur following an exposure to gadolinium oxide dust by inhalation. Histologic examinations of the lungs were made in an attempt to correlate functional findings with histologic changes in the lung tissue.

REVIEW OF THE LITERATURE

General Considerations

In a research problem which attempts to correlate pulmonary function with pulmonary histopathology, the literature reviewed must necessarily include work accomplished in both areas. In addition, a review of work which characterizes the agent causing pulmonary changes is necessary.

The rare earths make up a fascinating family of elements which, as Gschneidner (1964) indicates, for many years were something of a scientific mystery because their nearly identical chemical properties make them difficult to tell apart, and because the entire group occupies only one position in the Periodic Table. The words "lanthanides" and "lanthanons", which are derived from lanthanum, the first member of this series of fifteen elements, are also commonly used to describe the rare earths. The name "rare earths" is actually a misnomer, for these elements are neither rare nor earths. They are metals, and they are quite abundant. Gschneidner points out that twenty-five per cent of other elements are scarcer than the least abundant naturally occurring lanthanon, thulium.

Pharmacology and Toxicology of Rare Earths

Due to the recent upsurge in the use of rare earth metals in industry and because some of the rare earths are important

fission products, it becomes important to determine their effects when they gain entrance to the body. This has been emphasized by several workers, including Haley et al. (1961) and Kyker (1962). Vickery (1953) and Moeller (1963) have reviewed the chemistry of the lanthanons. Davison (1963) states that since the rare earth cations are not normal constituents of living systems, their action in the body must be interpreted through knowledge of their chemical properties. He further remarks that even though these elements are chemically similar, there are enough differences, when they are introduced into living systems, that few all-inclusive statements may be made for the group. A summary of the whole body of the lanthanons has been given by Kyker (1962). Some of the physical properties of the lanthanons have been enumerated by Magnusson (1963).

The recent increase in work concerning toxicity of the rare earths has produced significant contributions to the literature. Kyker and Cress (1957) studied the acute toxicity of yttrium and lanthanum chlorides. Magnusson (1963) determined the acute toxic effect of injected yttrium, cerium, terbium, holmium, and ytterbium on the rat liver. He observed that an appreciable difference prevails between the toxicities of the light and heavy lanthanons. Graca et al. (1962) have shown that the toxicity of the lanthanons is decreased with a rise in the atomic number. Present knowledge of the internal

behavior of rare earths in living systems has come almost exclusively from the use of radioisotopes of these elements (Kyker, 1962). Recent reviews on the toxicology of the rare earth elements include the work of Davison (1963), Reece (1965), and Haley (1965).

Effects of Rare Earths on the Lungs

Work concerning the effects of the rare earths on the lungs has increased concurrently with the growing use of these elements in industry. In one of the first studies made, Schepers et al. (1955) injected intratracheally into guinea pigs blends of rare earths in which the oxide and fluoride ratios were reversed. They found that the rare earths were progressively retained in the lung tissues, but no gross pathological changes were observed in the experimental animals which would indicate any form of pneumoconiotic fibrosis. Further work by Schepers (1955a), in which rare earth oxide mixtures were intratracheally injected into guinea pigs, revealed prominently thickened alveolar walls containing numerous swollen septal cells. He also found that most of the dust was trapped within focal atelectic areas, and no appreciable chronic cellular reaction of fibrosis occurred in relation to these deposits. Schepers (1955b) also studied the histopathologic changes in the lungs which resulted from prolonged inhalation of rare earth high fluoride dust. The findings

included focal hypertrophic emphysema, regional bronchiolar stricturing, and subacute chemical bronchitis. Pigment was focally retained but provoked no fibrosis or granulamatosis.

Davison (1963) reported on an inhalation study of the pathogenesis of neodymium oxide in mice and guinea pigs. The longest period of exposure was 120 days. Histological changes included a progressive impaction of neodymium in the lungs. It was contained primarily in macrophages and some neodymium was transported to the tracheobronchial lymph nodes where it could be seen in these nodes 100 days after beginning exposure in the mice and after 50 days in the guinea pigs. Lymphocytic hyperplasia was common, but it was not considered to be a specific response to neodymium. Mechanical disruption of the normal architecture of the pulmonary parenchyma was usual after sixty days of exposure. This resulted from the heavy deposition of the foreign material. There were no specific inflammatory reactions to the foreign material during the 120-day period of exposure and observation. The usual granulomatous reaction that is commonly associated with such foreign materials was not present.

Reece (1965) studied the effects of inhalation of yttrium oxide on various parameters in the dog. Histologic changes noted in the lungs of the dogs were alveolar epithelial cell hypertrophy, hyperplasia, and desquamation. He also noted a leukocytic infiltration in the lungs and the presence of dust-laden macrophages in the bronchial lymph nodes of the exposed

dogs. No connective tissue increase was observed.

Talbot et al. (1965) reported on a study in which white mice were exposed to an aerosol of gadolinium oxide at a concentration of 30 mg./meter^3. The longest exposure period was 120 days. The lungs of mice which had been exposed to gadolinium oxide were compared to the lungs of control mice which had been under the same atmospheric and stress conditions, with the exception that they had not been exposed to gadolinium oxide aerosol. A progressive impaction of gadolinium in the lungs was observed as time of exposure increased. The material could not be seen in the tracheobronchial lymph nodes until after 100 days of exposure. There were no acute inflammatory reactions and no fibrosis, but simply thickening of the alveolar mass and an accumulation of dust-laden macrophages. Other organs were normal, and hematological parameters were not significantly different than the control mice values.

Davison et al. (1965) reported on a study made of the organ distribution of a number of rare earth compounds following intrapulmonary injections. Their findings indicated a translocation of the rare earth compounds from the lungs to other organs. They also demonstrated a significant lung burden present 120 days following exposure.

Lung Deposition and Clearance of Particles

Anatomical, physical, and physiological factors which control the fate of inhaled substances have been enumerated by

Morrow (1960) and Hatch and Gross (1964). Dautrebande et al. (1957) state that there is common agreement that larger dust particles settle onto the mucosa of the upper respiratory tract and as the particles become smaller, they penetrate more deeply into the lungs. Morrow suggests that the dominant physical forces involved in the deposition of dust in the respiratory tract are gravitational settling for particles larger than 0.5 micron and Brownian movement impaction for particles smaller than 0.1 micron. Morrow states that the relatively high deposition and retention of small particles (less than one micron) in the lung parenchyma have been repeatedly demonstrated. In guinea pigs and monkeys, efficiency of particle removal in the lung is not significantly different from man; but, because of higher upper respiratory efficiency in these animals, the fraction of inhaled particles which penetrate to and are deposited in the pulmonary air spaces is lower for particles larger than one micron (Hatch and Gross, 1964). Dygert et al. (1949) concluded from data on the toxicity of inhaled uranium dioxide that the intensity of degree of response was a function of particle size, the greater response being associated with smaller particles.

Following deposition of relatively insoluble particulate material in parenchymal regions of the lung, the manner in which the lung clears itself of the material remains an unanswered question despite intensive investigation. Engel (1964)

states that removal of particles may take place in either of two ways: by the capture and expectoration of the particles, or by their absorption into the lymphatic system. Casarett and Milley (1964) review the various concepts which are postulated to be the mechanisms of particle removal. One mechanism presented is the phagocytosis of particles by macrophages normally present. This view is furthered by Karrer (1960), after having experimented with instillation of India ink in mouse lungs, and studying these lungs under the electron microscope. A second mode of particle removal suggested by Casarett and Milley is the direct penetration of the alveolar epithelium by the particle. Gross and Westrick (1954) have supported this view. Casarett and Milley, however, raise several questions concerning this theory. They cite the work of Low (1953) which shows the alveolar epithelium to be continuous, and question the mechanism of differential selectivity of particles. One would have further difficulty explaining why the particle does not remain in the alveolar epithelial cell after having gained entrance. A third theory of particle removal, as stated by Casarett and Milley, is that cells of interstitial or extrapulmonic origin migrate through the alveolar epithelium, engulf particles and subsequently return to an interstitial position. Gross and Westrick feel that this view is physiologically unsound in the light of present knowledge. There is no known consistent way for interstitial cells

to detect and respond to particles in the alveoli.

The final theory of particulate removal advanced by Casarett and Milley (1964) concerns the potential ability of the alveolar epithelial cell. Bertalanffy and LeBlond (1953) suggest that the alveolar tissue is a dynamic tissue where two cell types are renewed at somewhat different rates under physiological conditions. Presumably, the alveolar cells desquamate from the alveolar tissue and later migrate along the walls of the airways. Thus the normal appearance and number of these cells in the alveolar wall are the result of a dynamic equilibrium between their proliferation by mitosis and their loss by desquamation into the air passages. Casarett and Milley (1964) suggest that the alveolar epithelial cells are phagocytic and serve as at least one source of the alveolar macrophage. They also postulate that these cells, having phagocytized material, move either into alveolar spaces or into interstitial tissue depending, in part, on the relation of the cell to adjacent cellular components.

Hatch and Gross (1964) state that, after penetration of the alveolar membrane, lymphatic transport of dust is properly considered a component of alveolar clearance. Karrer (1960) assumes, after having made numerous histopathological observations, that ultimately the free phagocytes enter alveolar septa and that they eventually reach the lymph channels.

Mechanics of Pulmonary Function

Definitions

Elasticity is a property of matter that causes it to return to its resting shape after deformation by some external force. Comroe (1965) describes the elasticity of the lungs by a comparison of lungs with springs which must be stretched by an external force. As more force is applied to the lung, more stretching results and lung volume increases during inspiration. This relation between force and stretch or between pressure and volume can be measured under static conditions. The slope of the line that results from plotting the external force (pressure) against the increase in volume serves as a measure of the stiffness or the distensibility of the lungs and thorax. The closer the slope is to a vertical line, the more distensible are the tissues; and the closer to a horizontal line, the "stiffer" are the tissues. The proper term for this phenomenon is mechanical compliance. It is defined by Mead and Milic-Emili (1964) as the slope of a static volume-pressure curve at a point, or the linear approximation of a nearly straight portion of such a curve, expressed in ml/cm H_2O. Radford (1964) describes another expression of lung elasticity as elastance, or the reciprocal of compliance. This term has the advantage that it increases as the lung becomes less distensible and is thus somewhat easier to visualize than compliance. Compliance measured over a wide range of lung

volumes during slow volume changes is of primary importance when defining the changes in lung elasticity; but a measurement made under conditions of normal breathing, known as functional compliance, is important in describing flow resistance as well as elastic properties. Another term for a functional compliance measurement is dynamic compliance.

The term hysteresis has been used to describe the behavior of mechanical systems in which the result of an applied force lags the force itself. Mead and Whittenberger (1953) apply this term to the respiratory cycle. During a breathing cycle, volume changes of the lungs lag the transpulmonary pressure changes which produce them, and the lungs may be said to manifest hysteresis. A single respiratory cycle forms a closed loop, and the area enclosed, relative to the total volume change, is a measure of the degree of hysteresis. According to Bayliss and Robertson (1939), the pressure needed to support the process of hysteresis should amount to fifteen per cent of the total pressure necessary to inflate the lung during quiet breathing. Cavagna et al. (1962) state that the pressure-volume diagram of the lung in man during breathing is a loop made up of two components, inspiratory and expiratory. The area of the loop is the dynamic work made in both inspiration and expiration. This work is the total to overcome resistance to air flow, pulmonary tissue viscosity, and eventual hysteresis of the lung. If the lung were a perfect elastic body, the last component would be zero.

Historical notes

The study of respiratory mechanics is both old and young. Most of the ideas and techniques in this field were described many years ago. However, at that time, comparatively few measurements concerning mechanics of respiration were made. Most of the measurements have been made within the past fifteen years and were assisted greatly by electrical recording devices and the acceptance of a simple, indirect method for measuring pleural pressure.

Fenn (1964) described the early work of Carson, Donders, and Hutchinson. Carson was the first to study the elasticity of the lungs. In 1820, he attached a water manometer to the trachea and measured the rise in pressure when the thorax of a cat was opened. Donders, thirty-three years later, did the same experiment with some technical and theoretical improvements. He was first to point out that lungs collapsed upon opening the chest wall because of their own elastic retraction, and that this retractive force increases as the lungs are inflated. At approximately the same time, Hutchinson published results obtained on two human lungs immediately post mortem. These values furnished the earliest pressure-volume curves for lungs. Mead (1961) cites later work by Heynsius and Liebermeister. In 1882, Heynsius used Donder's methods to measure retractive pressures at various volumes and got variable results. He noted that if the lungs were allowed to

collapse, re-expansion was not even and tracheal pressure was greater or smaller according to the number of alveoli opened at the same air volume. Liebermeister in 1907 published pressure-volume curves for excised lobes of cat and human lungs inflated from the collapsed state. As pressure was increased, little volume change took place until levels of 8-10 cm. H_2O were reached; whereupon the lungs filled markedly with only small increases in pressure. He attributed this to closure of air passages in collapsed lungs which was reversed, re-establishing continuity between the trachea and the air spaces as the pressure was increased. If Liebermeister had included measurements made during deflation, he would have found smaller pressures during deflation, and the complete volume-pressure cycle from the collapsed state would have been a hysteresis loop.

In 1919, Rohrer, as cited by Fenn (1964), obtained pressure-volume curves both in relaxation and with maximum inspiratory and expiratory effort. His experiments differed in that pressures were measured at different lung volumes instead of volumes at different pressures. Rohrer's study was a very sophisticated analysis of all aspects of the mechanics of respiration. In large part, the work in respiratory mechanics since his time may be regarded as filling out the framework he provided. He was, however, far ahead of his time and his excellent contributions attracted very little attention

and never appeared in ordinary textbooks of physiology.

Rahn et al. (1946) repeated the whole pressure-volume problem during World War II. After this work, however, the field developed rapidly and the study was taken up in many laboratories and clinics throughout the world. Perhaps the most important technical advance which contributed to increased in vivo study of respiratory mechanics was the widespread use of esophageal pressures for monitoring intrapleural pressures.

Recent progress

Much of our present knowledge of elastic characteristics of lungs has been obtained from experiments on excised lungs. By the study of the excised lung, the effects of the chest wall and mediastinal structures on inflation may be omitted. Under many experimental conditions, it is not possible to maintain lungs at a given state of expansion long enough to allow true equilibrium of all stresses within them. Radford (1964) applies the term "quasi-static" to this type of study. The principal justification for application of these results to living animals is that the mechanical characteristics of excised lungs are comparable to those observed in living animals provided that the range of volume change is similar in the two cases, and that the time for inflation or deflation of the lungs is also similar. Several methods have been described during in vitro studies of the lungs. Coryllos and Birnbaum (1932) devised a method by which the lungs could be

freed of gas, so that an atelectic lung could be obtained for study. After ventilating the lungs of animals with oxygen for 15-20 minutes, they occluded a cannula which was in place in the trachea, and the gas remaining in the lungs was then absorbed by the pulmonary circulation. This resulted in an essentially gas-free atelectic preparation. Many workers, including Radford (1963), Mead et al. (1957), Pratt and Klugh (1961) and Dautrebande (1962) have used this method in their pulmonary studies.

One method for study of pulmonary function which has gained much favor, especially with those attempting to describe the effects of surface tension in the lungs, is the filling of the lung with saline or Ringer-Locke's solution in the place of air. Radford (1963), Radford and Lefcoe (1955) and Mead et al. (1957) in separate studies utilized this method. The lungs were quickly excised and immersed in a saline or Ringer-Locke bath. The tracheal cannula is connected by a "T"-tube to a water manometer and to a stopcock and syringe which permitted measured volumes to be withdrawn from the bath and injected into the lungs. The tracheal pressure was measured relative to the bath level, and all pressures were recorded as centimeters of H_2O.

Gribetz et al. (1959) devised a unique apparatus for obtaining pressure-volume curves. A fluid-filled burette was connected in series with a bottle partially filled with fluid.

The lung being studied was also situated in the system. Elevation of the burette caused movement of fluid into the bottle with displacement of gas into the lung. The distending pressure of the lung corresponded to the difference in height between the two levels of fluid; it was measured with a water-filled manometer.

Dautrebande (1962) studied the influence of constricting and dilating microaerosols on the pressure-volume curves of isolated atelectatic rat lungs, and noted one change due to technique. He felt that maintenance of the excised lung in air at a temperature of 37°C. was of great importance for reproducibility and quantitative estimation of the results. After excision, the lungs were rapidly placed into a constant temperature (37°C.) plastic chamber. The trachea was tied to a cannula connected to a "T"-tube, one end of which lead to a water manometer. The other end was attached to a syringe graduated in milliliters to which a stopcock had been added in order to control the volume of air injected into the lungs. Pratt and Klugh (1961) and McIlroy and Christie (1952) made use of a respirator box into which the lungs under investigation were placed. The lung was caused to expand by the removal of air from the box, creating a "negative pressure" which simulated the action of the thoracic musculature. Openings in the box were provided for the attachment of a spirometer, manometer, or electronic apparatus. Pratt and Klugh

state that the lung actually inflates only because of the transpleural pressure differential, and thus the use of either positive pressure or "negative pressure" is acceptable as a method of inflation.

Many workers have attempted to separate the forces opposing inflation of the lung, and described each factor individually. Otis et al. (1950) described several forces which the respiratory muscles, in carrying out the breathing movements, would have to overcome. The chest and lungs are elastic in nature and must be stretched during inspiration to accommodate an increased volume. The air, in moving through the respiratory tract, encounters viscous and turbulent resistance and there is probably some additional non-elastic resistance associated with deformation of tissues and with the sliding of organs over one another when they are displaced. Otis et al. (1956) stated that since the lungs are composed of a population of elements or pathways which are not all similar mechanically, they operate asynchronously even when subjected to the same driving force.

The contribution of surface tension to the elasticity of the lungs was first demonstrated in 1929 by Karl von Neergaard (Clements, 1962). He distended lung preparations alternately with air and saline solution and compared the pressures required to do so with each. This experiment, since repeated many times by other workers (Mead et al., 1957; Brown et al.,

1959; Radford, 1963), showed that it takes a higher pressure to distend the lungs with air. Mead et al. (1957) showed that hysteresis is almost absent from the pressure volume diagrams of dog lungs filled with saline. They attributed this phenomenon to the operation of surface tension during inflation. Bayliss and Robertson (1939) divided the pressures required to distend the lungs into 3 parts; the elastic pressure, the viscous pressure, and the airway pressure. They estimated that the pressure required to overcome the elasticity of the lung was 80 per cent of the inspiration pressure; that needed to overcome viscous resistance was 15 per cent, and that needed to overcome airway resistance was 5 per cent. McIlroy et al. (1955) found the tissue viscous resistance to constitute about 30-40 per cent of the total respiratory resistance during quiet breathing in normal adult males. Radford (1964) states that it appears likely that mechanical properties of the alveolar lining cells, connective cell, and macrophages play little part in determining elastic behavior of normal adult lungs. In contrast to adult lungs, fetal lungs may have a significant contribution from tissue cells. Agostoni et al. (1958) have shown that in term fetal guinea pig, cat, and goat lungs, filling with saline solution led to marked static hysteresis of the volume-pressure curves. It seems reasonable to ascribe much of this behavior to the alveolar epithelium, which in fetal animals is columnar or cuboidal,

becoming flattened into a thin stratified layer when the lungs become expanded with air. This finding by Agostoni et al. would lead one to speculate about the possibility of any change in normal tissue cellular elements effecting the pressure-volume curves of the lung.

The force needed to overcome airway resistance, a factor which is minimized with static inflation and deflation of lungs, has been estimated by a number of investigators (Brody, 1954; Mount, 1955; Otis et al., 1956; Campbell, 1959; Goodheart et al., 1959). Amdur and Mead (1958) estimated that the upper airways account for 45 per cent of the total resistance to air flow in the guinea pig. This factor is not present when working with excised lungs. DuBois (1964) has presented a comprehensive review concerning the resistances encountered in breathing.

Research has been done with excised lungs to determine the effects of pulmonary vascular congestion on the compliance of lungs. Frank et al. (1959) studied cat lungs by noting the compliance of the lungs at varied pressures in the pulmonary vessels. They found that only slight changes occurred in elastic behavior of the lungs during deflation when pulmonary vascular pressure was increased from 0 to 16 cm. of H_2O. Frank (1959) further studied the effects of vascular congestion on recoiling force of excised lungs and found that a narrow range of volume exists in which the lungs and blood

vessels exert least mechanical effect on each other. That volume was believed to lie within the range encountered in tidal breathing. Mack et al. (1947) found, while working with isolated preparations of dog lungs, the distensibility of the lungs varied in an inverse manner with the amount of blood in the pulmonary vessels.

Various workers have concerned themselves with pulmonary mechanical differences which may exist among species. Agostoni et al. (1959) made measurements of the pressure-volume relationships of the lung and thorax, lung volumes, dynamic resistance to breathing, breathing frequency, and work of breathing in dogs, cats, rabbits, guinea pigs, and rats. They stated that rabbits and guinea pigs have relatively small lungs so that the volume of the lungs per unit of body weight will differ from other species. Hartroft (1960) compared the size of the pulmonary alveoli in several laboratory animals and man. He found that the alveolar sizes ranged in the following ascending order: mouse, baboon, dog, goat, guinea pig, monkey, rabbit, cat, and man. Aumonier et al. (1958) studied the compliance of the lungs of monkeys, dogs, cats, rabbits, guinea pigs, rats, and mice. They found little difference in the compliance in these different species even though much difference in histological appearance among species exists. Their values for alveolar sizes compared favorably with those of Hartroft (1960). Crosfill and Widdicombe

(1961) determined the compliance of lungs from various species, and agreed that little species variations exist. Frank (1963) made a comparison of the volume-pressure relationship of separate lobes of excised dog lungs using the method of Gribetz et al. (1959). Small differences were found which did not appear to play an important role in causing uneven ventilation seen in vivo in healthy lungs.

Extensive use has been made of compliance and elastance curves in work attempting to describe changes in pulmonary function due to various factors. Dale and Narayana (1935) studied the effects of drugs on the isolated, perfused guinea pig lung. They found that acetylcholine and vagal stimulation both caused bronchiolar constriction as determined by a change in compliance curves. They also attempted to describe the effects of epinephrine, but their results were variable. If the bronchioles were dilated before injection of epinephrine, the epinephrine would produce a bronchiolar constriction. However, if the bronchioles were constricted, injection of epinephrine would cause dilation of the bronchioles. Daly (1938) utilized the isolated perfused lungs of guinea pigs, and found that ventilation of the perfused lungs before perfusion with its own defibrinated blood was smooth and constant. However, the blood was found to contain a "bronchoconstrictor substance" when it was perfused through the pulmonary vessels. Van Liew (1954) studied the pressure-volume characteristics

of the lungs and chest of dogs. When the cervical vagi were blocked, the pressure-volume curves were changed in such a manner that less pressure was required to produce a given volume change. This was attributed to a change in muscular tonus that is normally present during lung distortion due to afferent impulses carried by the vagus nerves. Radford and Lefcoe (1955) evaluated the effects of several drugs on the lungs when surface tension was minimized, and proposed that the effects of bronchiolar constriction was due primarily to interaction with the surface tension effects.

Study of the effects of various aerosols on the lung during inhalation studies has been made by several workers. Dautrebande (1962) has made an extensive study of the effects of dilating and constricting aerosols on guinea pig lungs. He used in vitro methods and evaluated changes in dynamic compliance. Amdur and Mead (1958) have investigated the effects of the industrial hazard of ethylene oxide by inhalation studies in unanesthesized guinea pigs. They found that the irritant effects of ethylene oxide were such that the compliance values were decreased to one-half the pre-exposure values. Balchum et al. (1960) found that there was a decrease in the compliance of the lungs and thorax in dogs which had breathed sulfur dioxide for ten to fifteen minutes, and an immediate and marked increase in the non-elastic resistance to breathing.

Changes in pulmonary function as a result of disease, especially emphysema, has been investigated by Pratt et al. (1961, 1962). In in vitro studies with human lungs obtained post-mortem, they found that emphysematous lungs were excessively compliant when compared to normal lungs. McIlroy and Christie (1954a), using human subjects, found that there is a relative increase in non-elastic resistance in emphysema, and felt that this increase was not wholly due to bronchial obstruction. They speculate that a change in the visco-elastic properties of the lungs accounts for part of the increase in non-elastic resistance.

MATERIALS AND METHODS

Experimental Animals

This experiment utilized 72 guinea pigs. The animals were weighed weekly throughout their treatment period. Mean weights and standard deviations of the male guinea pigs were 817.5±52.4 grams and the females used in this experiment weighed 744.4±51.1 grams. Before exposure to dust was started, the guinea pigs were held in the chambers without dust administration with the same through-put air flow as was used during the experiment. This was continued on a daily basis until the animals became acclimated and exhibited no weight loss. During exposure, the animals were segregated by sex and housed in cages constructed with expanded stainless steel mesh. After removal from the chambers, they were housed in a closed animal room in a standard cage unit containing pine shavings. Feed and water were supplied free choice when the animals were not in the exposure chambers. No food or water was available during the exposure period.

Experimental Design

The experiment was designed as a three way factorial, with treatment, duration of exposure, and sex being the three factors. The guinea pigs were divided into three groups of twenty-four each. Each of these groups contained 12 guinea

pigs (6 males and 6 females) which were exposed to Gd_2O_3 dust, and 12 guinea pigs (6 males and 6 females) which were not exposed to the dust. One group was exposed for 40 days, one group for 80 days, and the other group for 120 days. During the period of exposure the guinea pigs were in the chambers 6 hours per day, 5 days per week.

Method of Exposure

The chambers (Figure 1) were those for which design specifications had been set by Davison (1963) and described by Cook (1961). One chamber contained the guinea pigs being exposed to the Gd_2O_3 dust, and the other chamber was used as a control chamber for the guinea pigs to which exposure to Gd_2O_3 dust was not desired. Air flow through the chambers was 8 cubic feet per minute.

Aerosol Preparation and Generation

The gadolinium oxide used in this experiment was obtained from a commercial source[1] and was guaranteed to be 99.9 per cent purity. Spectrographic analysis revealed the presence of no other rare earth. The only contaminant present was 100 parts per million of calcium. It was received as a finely pulverized powder of unknown particle size. This product was

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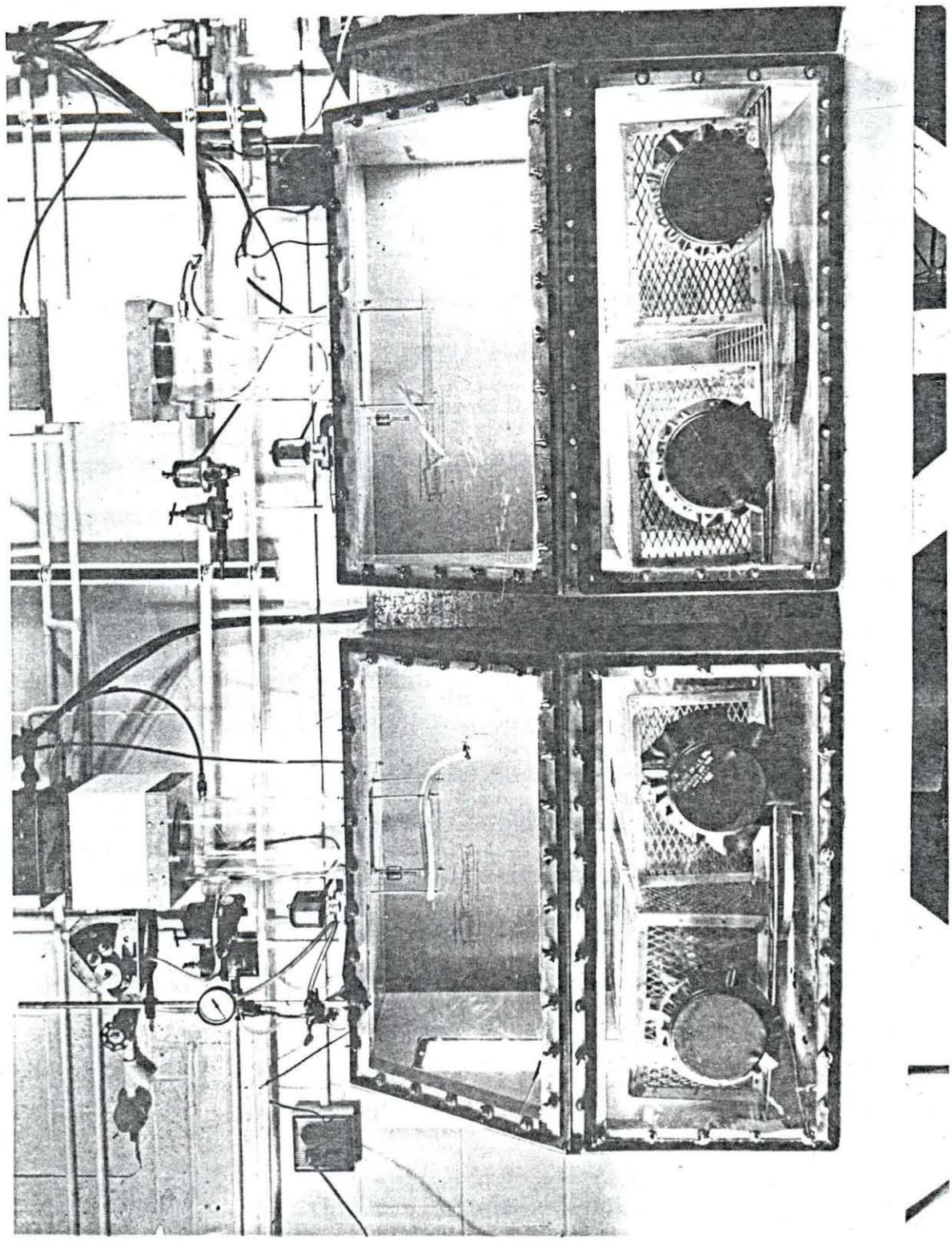
[1]Michigan Chemical Company, St. Louis, Michigan.

Figure 1. Control and exposure chambers used in this experiment. The Wright dust feed generator is shown in place above the exposure chamber



reduced to a moderately uniform particle diameter by two stages of grinding. In the first stage the oxide was pulverized in a Pica blender mill[1] using stainless steel vials and balls for eight minutes. The second stage of grinding consisted of manually regrinding the powder suspended in absolute ethanol in a mortar with a ceramic pestle. The alcohol was removed from the mixture by burning, and a satisfactory dry dust remained. This gadolinium oxide was then stored in an evacuated dessicator until it was used. The suspension of the aerosol in the air of the inhalation chamber was accomplished by the use of the Wright dust feed mechanism[2] (Figure 3). It operated by scraping dust from a cylinder tightly packed with gadolinium oxide, then removing the dust with a stream of air. This dust laden air from the Wright dust feed mechanism was injected into the main stream of chamber air in the throat of the chamber just below an upstream filter. This assured that the aerosol was the only foreign material in the chamber atmosphere. The dust was introduced into the top of the chamber in such a manner that incoming air gave an even distribution in all parts of the chamber as demonstrated by Cook (1961). The concentration of aerosol in the chamber was dependent on the gear ratio of the

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[1]Pica Blender Mill, Model 3800, Pitchford Scientific Instruments Corporation, Pittsburgh, Pennsylvania.

[2]Purchased from L. Adams, Ltd., London, England.

Wright mechanism, the air pressure used to remove the dust from the scraper, and the replacement rate of the air in the chamber. Dehumidification of the air in the Wright dust feed mechanism was necessary to prevent clogging of the scraper plate. This was accomplished with a water trap at the air compressor unit and by an air filter and dehumidifying unit in the line before the air passed the scraper blade.

Aerosol Concentration

In order to determine the amount of dust available for breathing by the guinea pigs, it was necessary to sample the chamber air daily. Sampling was accomplished by drawing 16.8 $ft.^3/hr.$ of chamber air across a cellulose acetate filter[1] for 15 minutes. The filter was held in a stainless steel open end filter holder which was affixed to the end of a stainless steel tube. After sampling, the filter was collected for chemical determination of the gadolinium oxide which had impinged on its surface.

The method described by Fritz et al. (1958) was used to determine the amount of gadolinium oxide on each filter. This method uses arsenazo 3-(2- arsenophenylazo) - 4,5 - dihydroxy -2,7 naphalene disulfonic acid for the quantitative colorimetric determination of gadolinium oxide and other rare

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[1]Millipore DA-650m filter, Millipore Filter Corporation, Bedford, Massachusetts.

earths. The method is applicable to the determination of very low concentrations of rare earth and is not highly pH dependent.

In this experiment the gadolinium oxide was eluted from the filter with 50 per cent acid. This was diluted to a total volume of 50 ml. with ion free water. A 1 ml. aliquot of this dilution was used for the determination. The sample was buffered with a triethanolamine buffer, purified arsenazo reagent added, and the pH adjusted to 8.2 with ammonium hydroxide. The resulting color was measured against a reagent blank, prepared with an unexposed filter, at 570 mμ in a spectrophotometer.[1] The results were reported as mg./M^3 of air when compared to a calibration curve for Gd_2O_3.

Mean concentrations, standard deviations, and 99 per cent confidence intervals for the mean for each exposure period are listed in Table 1. The concentration of dust at each exposure period was approximately 20 mg./M^3.

Table 1. Chamber atmosphere concentration means, standard deviations, and 99 per cent confidence intervals

Exposure time	Mean and Standard Deviation (mg. Gd_2O_3/M^3)	Confidence Intervals
40 day	20.64 ± 3.37	$19.41 < \mu < 21.87$
80 day	20.82 ± 6.98	$18.76 < \mu < 22.88$
120 day	20.77 ± 6.18	$19.30 < \mu < 22.24$

[1]Coleman Jr. Spectrophotometer. Coleman Instruments, Inc., Maywood, Illinois.

Mass Distribution and Particle Size Determination

Since the size of the particle which is inhaled primarily determines what portion of an inhaled dust is retained in a lung, and also affects the depth to which it penetrates before being deposited, it was deemed necessary to approximate the size of particles in the chamber atmosphere and also determine the per cent of the total mass generated which consisted of particles less than 1 micron. A Casella cascade impactor[1] (Figure 2) was utilized for determining the mass distribution of the generated aerosol. This instrument consists of a system of four air jets impinging in series on glass discs. The jets are progressively finer, so that the speed and, therefore, the efficiency of impaction of particles on the discs increase from jet to jet when air is drawn through at a constant rate. Between the fourth stage and the line to the vacuum pump is a filter unit. A glass coverslip was coated with stop-cock grease and affixed to each of the stages. The stop-cock grease allowed for retention of the impacted particles. The same type of filter used for sampling the chamber concentration was placed in the filter unit. The largest particles were removed at the first stage, and the smallest particles were removed on the filter. A size

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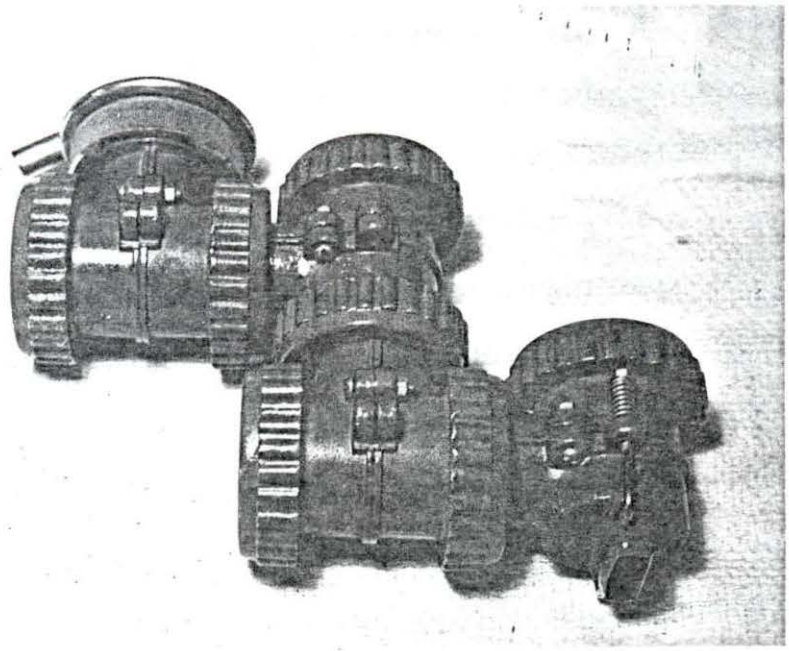
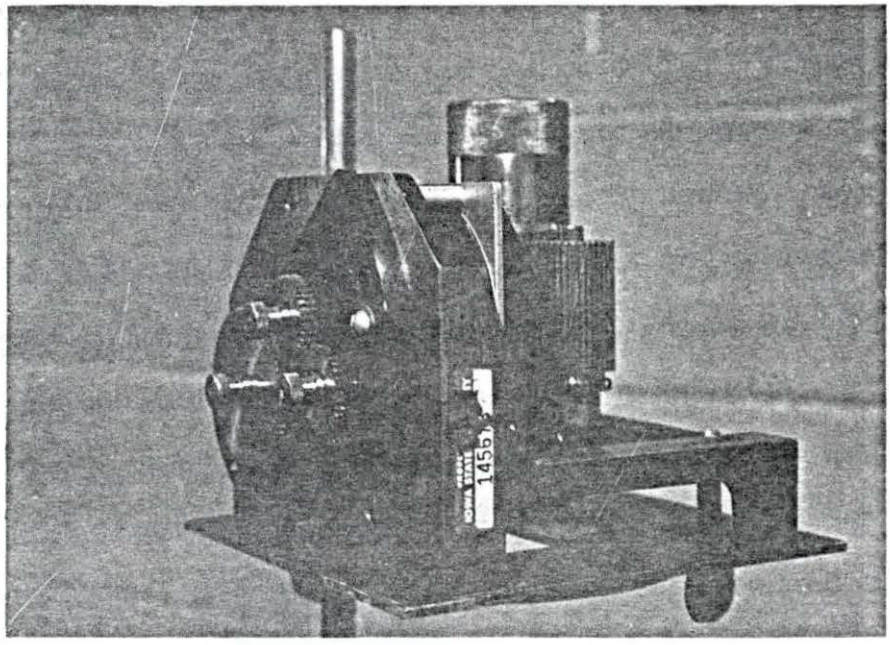
[1]Purchased from Mine Safety Appliance, Pittsburgh, Pennsylvania.

Figure 2. Casella cascade impactor

Figure 3. Wright dust feed generator

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gradient thereby resulted which assisted in the assessment of the samples. A time of 30 seconds was used for collection of samples so that impacted particles would not tend to pile up and cause deflection of subsequent particles. The method described for determining chamber aerosol concentration was used for the quantitative determination of gadolinium oxide impacted at each stage of the filter. Hot, dilute nitric acid (1:1) melted the stop-cock grease and thus released the particles so that they could go into solution. It was then determined that 92 per cent of the mass was collected at stage 3, stage 4 and the filter.

It was also necessary to know the size of the particles collected at the various stages. For this determination, electron microscope grids were mounted in the line of deposition at each stage and particles were impinged directly on these grids for counting. Air flow through the cascade impactor was started prior to placing it in the chamber atmosphere. Sampling for three seconds from the chamber atmosphere was found to be optimal for impacting large numbers of particles with a minimum of piling. The method of particle size analysis was the same as that used for samples collected on the electrostatic precipitator. Mean diameters and standard deviations for stages 3 and 4 were found to be 0.563 ± 0.531 microns and 0.382 ± 0.331 microns, respectively.

In order to determine the mean particle diameter of the

generated aerosol, the chamber atmosphere was sampled with a point-to-plane electrostatic precipitator.[1] Precipitation was accomplished by drawing chamber air through the instrument at a flow rate of 2.5 ft.^{3}/hr. This process removed a random sample of particles from the chamber air and deposited them on an electron microscope grid overlaid with a carbon film, where they could be photographed with the electron microscope. The maximum diameter horizontal with the top or bottom edge of the electron photomicrograph (Figure 4) was determined with a particle size analyzer.[2] Further analysis of data obtained from the analyzer gave a mean particle diameter and standard deviation of 0.22 ± 0.21. These figures were based on 1000 particles photographed at random from the grid and represented that portion of the population that could be seen and measured at a magnification of 4,125 times.

In Vitro Compliance Measurement

The method by which compliance measurements were achieved in this experiment utilized principles as described in experiments of Dale and Narayana (1935), Mack *et al.* (1947), Lawton and Joslin (1951), Radford (1955), Mead *et al.* (1957), Frank

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[1]Precipitator constructed by Iowa State University Instrument Shop, Ames, Iowa. Power source for precipitator was designed and constructed by Mr. A. O. Stattleman, Ames, Iowa.

[2]Particle size analyzer, Model TGZ-3, Carl Zeiss, Oberkochen/Wuertt., West Germany.

Figure 4. Electron photomicrograph of a typical field of particles precipitated with the electrostatic precipitator. Original picture was taken at 4125 magnifications

(1959), Gribetz et al. (1959), Pratt and Klugh (1961), and Dautrebande (1962). After each group of guinea pigs had undergone treatment as prescribed by the experimental design, they were anesthetized with 6 per cent pentobarbital sodium and placed on positive pressure respiration with a respirator.[1] The trachea was isolated through a ventral midcervical incision, and a thread was passed under it, to be used later for occluding the trachea. A midline incision was made from xiphoid cartilage to umbilicus, and the skin was dissected laterally. The abdominal cavity was then opened, the diaphragm incised, and the lungs were observed. At the moment when full inspiration was observed, the thread around the trachea was tightened, and the trachea occluded. This procedure was followed so that the lungs would not collapse, thus preventing internal surfaces to become apposed. The expanded lung was then removed from the thoracic cavity, and taken to the inflation chamber where compliance determinations were carried out within the following five minutes.

The inflation chamber (Figure 5) was a 500 cc. bottle which was situated in a constant temperature water bath. A layer of water was placed in the chamber below the point to which the lung would expand, in order to achieve a moist environment which would closely simulate the conditions in the

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[1] Physiograph respirator Model V100KG, E and M Instrument Company, Houston, Texas.

thoracic cavity. The temperature of the water bath was set at a point which would hold the air temperature in the inflation chamber at $37^{o}\pm2^{o}$ C.

A 50 cc. syringe mounted on a constant rate injector[1] (Figure 7) was utilized for injection of air into the lung. The setting on the injector provided 7.62 cc. of air per minute to the lung. This value was chosen in order to provide time for equilibration of the air within the alveoli, and at the same time inject air rapidly enough so that some relationship to *in vivo* inspiration might exist.

An electronic recorder[2] (Figure 6) was used to acquire a simultaneous record of the intrapulmonary pressure and the volume of air which was injected at any given pressure. Since the air was injected at a constant rate, the time parameter paper speed during inflation enabled the investigator to determine the volume of air displaced from the syringe at any time period. The measurement of pressure in the system was accomplished by the use of a pressure transducer[3] which was placed in the system between the injector and the lung. The pressure transducer was calibrated with a mercury manometer. Immediately

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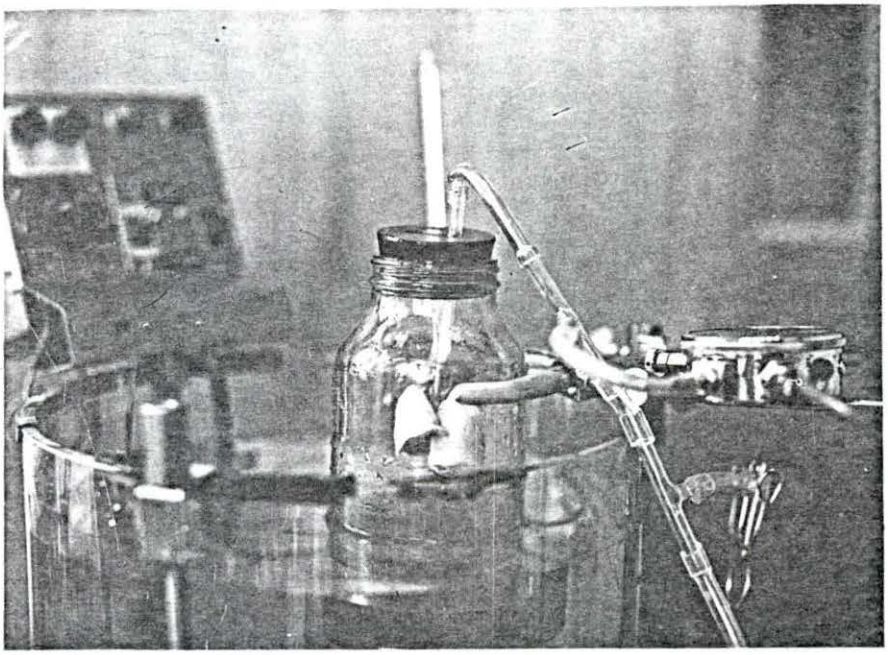
[1]Harvard Apparatus Company.

[2]Physiograph Recorder, E and M Instrument Company, Houston, Texas.

[3]Physiograph Pressure Transducer, E and M Instrument Company, Houston, Texas.

Figure 5. Inflation chamber in water bath with lung in position for inflation

Figure 6. Electronic recorder on which pressure-volume tracings were made



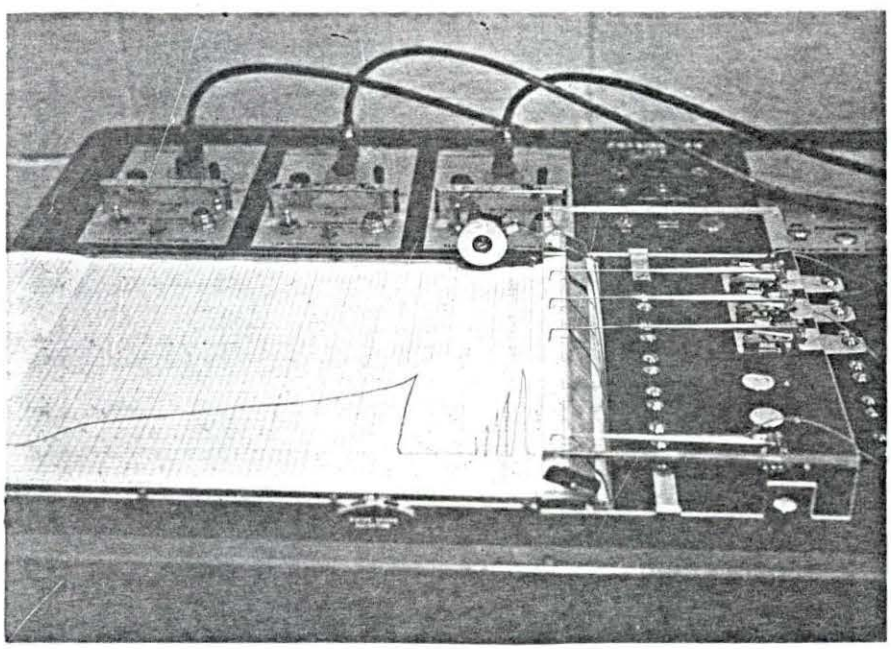
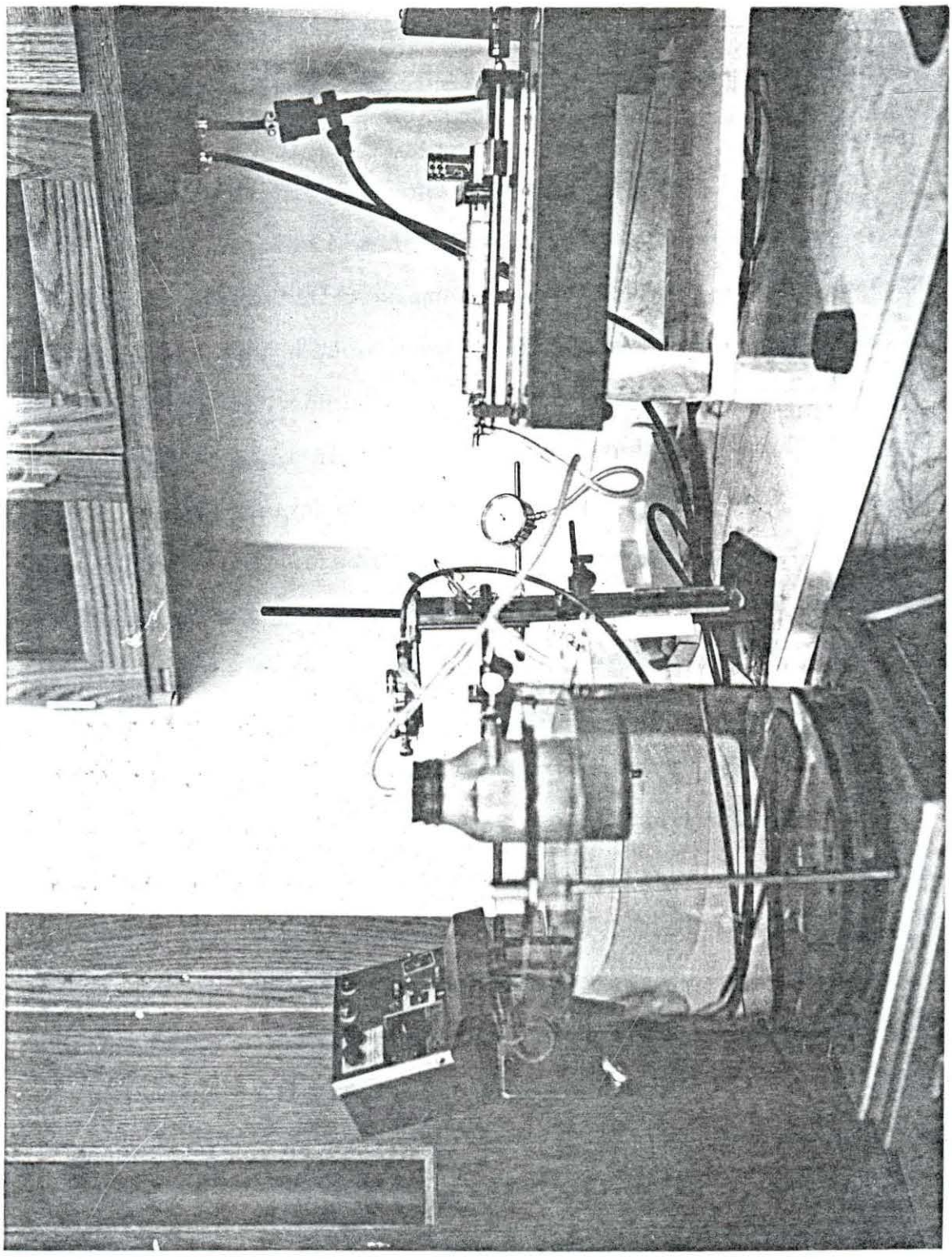


Figure 7. Constant rate injector, pressure transducer, and inflation chamber in place in water bath



following the inflation of each lung, the recorder was calibrated for pressure, so that measurement of the height of the tracing at any point would determine the exact pressure at that point.

Immediately before placing the lung in the inflation chamber, the trachea was severed below the ligation thread and the lung was allowed to collapse. During preliminary investigations, it was found that the lungs would more readily open when inflation was begun if they were not allowed to remain collapsed for more than a short period of time. The lung was then secured by the trachea to a glass cannula which had been inserted through a stopper in the chamber. Another opening in the stopper was provided so that pressure in the inflation chamber would not oppose the inflation of the lungs. The cannula was connected by a series of glass and plastic tubings to the 50 cc. syringe mounted on the constant rate injector.

When the lung had been secured to the cannula, a preliminary inflation was made. Previous writers, especially McIlroy (1952) and McIlroy and Christie (1952) have shown that in order to obtain consistent and comparable data for the compliance of excised lungs, it is necessary to conduct the measurements after the lung has previously been inflated under standard conditions. This principle was followed in this experiment, so that the lungs were treated the same way and would thereby begin the recorded inflation under relatively

similar conditions. The lung was then allowed to collapse by opening a "T" in the system. The "T" was closed, and the recording to be used for analysis was made. Most of the lungs treated in this manner opened easily, inflated smoothly, and exhibited compliance curves which were similar and from which inferences could be made.

Histological Examination

Following inflation and recording, the lungs were removed from the chamber and sections were harvested for histological evaluation. These tissues were immediately placed in 10 per cent formalin, sectioned and then stained with hematoxylin and eosin, and examined microscopically.

RESULTS AND DISCUSSION

Determination of *In Vitro* Compliance

The functional test used in this experiment was devised as a method for making comparisons between lungs subjected to different environments. Although not intended to advance the basic knowledge of pulmonary physics, it did serve as a method for correlating functional and histological studies. Several methods were examined for possible use before arriving at the procedure of choice.

Problems which arose during preliminary investigation of pulmonary function tests should be examined. The method for freeing lungs of gas, as described by Coryllos and Birnbaum (1932) was attempted on several guinea pig lungs. After removing completely atelectic lungs from the thoracic cavity, inflation was attempted using a 20 cc. hypodermic syringe for the quantitation of volume, and a water manometer in the system for determination of pressure changes. It was found that the intra-tracheal pressure required before inflation of the lungs began, varied quite markedly from lung to lung. Some of the atelectic lungs would open and begin inflation at 5 cm. water pressure, and some would not open until 40 cm. water pressure was reached. The extreme pressures required to open some of the atelectic lungs can probably be attributed to the fact that many surfaces within the lung were apposed, and the

surface tension between these surfaces was sufficient to require very high opening pressures. However, the fact that this was not a constant finding, tends to decrease the strength of this reasoning.

Radford (1963) has extensively applied the method of saline filling of lungs for pulmonary compliance studies. Since the effects of surface tension in the lungs are minimized by this method, it was thought that this method might prove superior to others. Filling of the lungs began at more uniform pressures, but in many instances after the lung had been partly filled, leaks developed and the lung had to be discarded. Gribetz et al. (1959) when inflating lungs with saline, also found that lungs treated in this manner had an increased tendency to leak. Also, with saline injection very little fluid was passively forced outward from the lungs after injection, and most attempts to withdraw fluid failed. Since a preliminary expansion of each lung was desired, failure to regain injected fluid precluded the use of this method in this experiment. Whether this phenomenon was due to bronchiolar muscle constriction which trapped fluid in the lungs, or due to elastic bundle action or some other factor remains an unanswered question.

In this experiment, as described earlier, the lungs were taken from the thoracic cavity in an inflated state. After the lung had collapsed, pressure was immediately applied, and the

preliminary inflation was accomplished. Recording of the following inspiration produced compliance curves which were comparable. The differences in opening points were taken into account by plotting the curves as elastance and omitting the portion of the curve which was due to this opening pressure variation when determining the slope of the curve. The linear approximation of a nearly straight portion of the curve was made, (Figures 8, 9, 10, 11), and the values of these slopes, expressed as mm. Hg./ml. air, were the measurements used for statistical analysis. Mead et al. (1957) describe irregular expansion of air filled lungs. This phenomenon was observed in the early stage of inflation in this experiment, but with further inflation a stage was reached when all regions appeared to expand uniformly. It was desired that most of the slope of the elastance curves would be composed of this stage of uniform expansion. By doing so, the effects of surface tension would be minimized.

Thirteen lungs were discarded because of failure to open at very high pressures or because the lung developed a leak during inflation. When a very high pressure was reached, and the lung suddenly opened, the intratracheal pressure built up was sufficient to cause rupture of the portion of the lung which opened first. Leaks of unknown origin in a few of the lungs were attributed to either fragility of the lung tissue or puncture at the time of removal from the thoracic cavity.

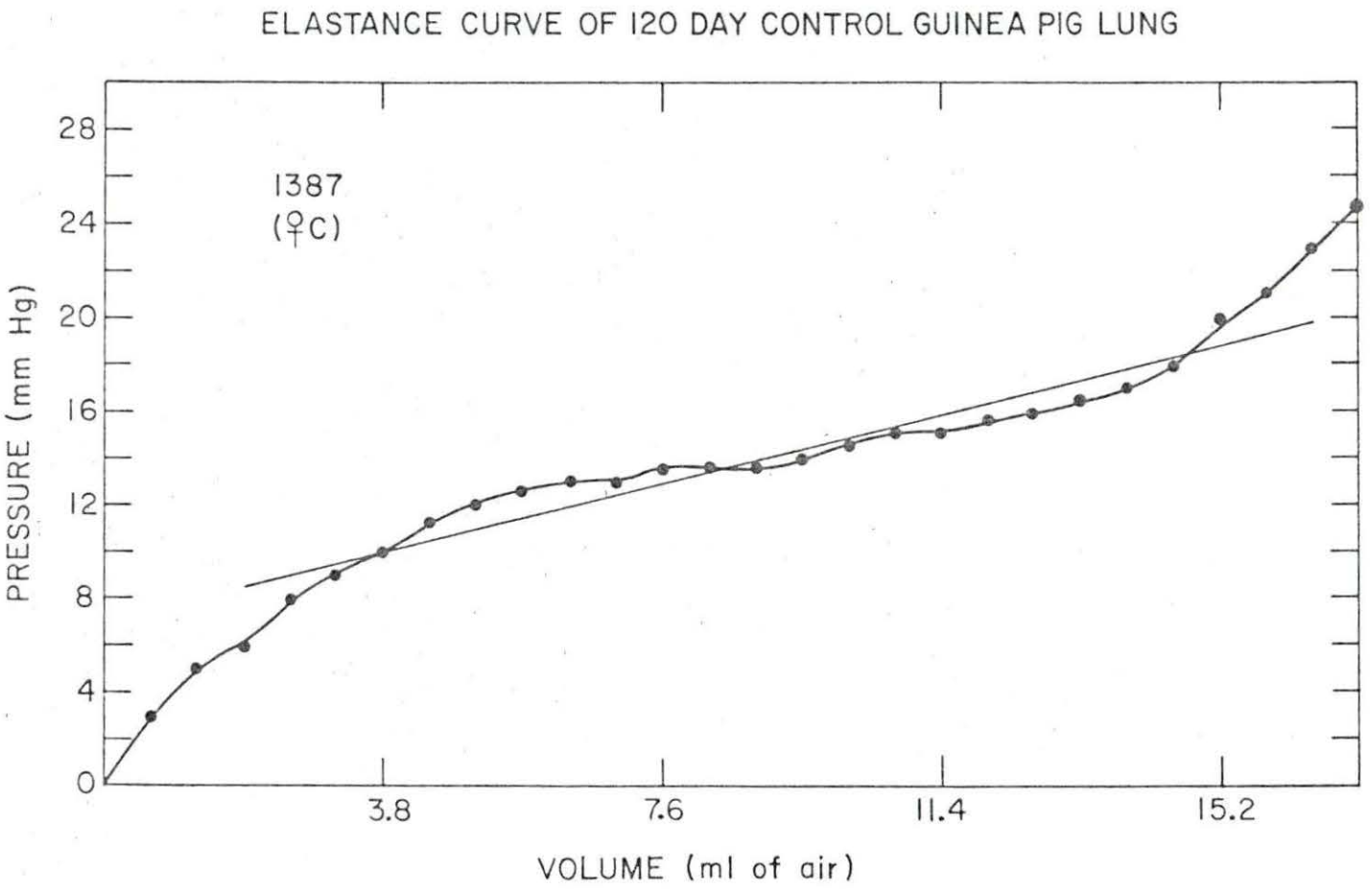


Figure 8. Elastance curve of 120 day control guinea pig lung

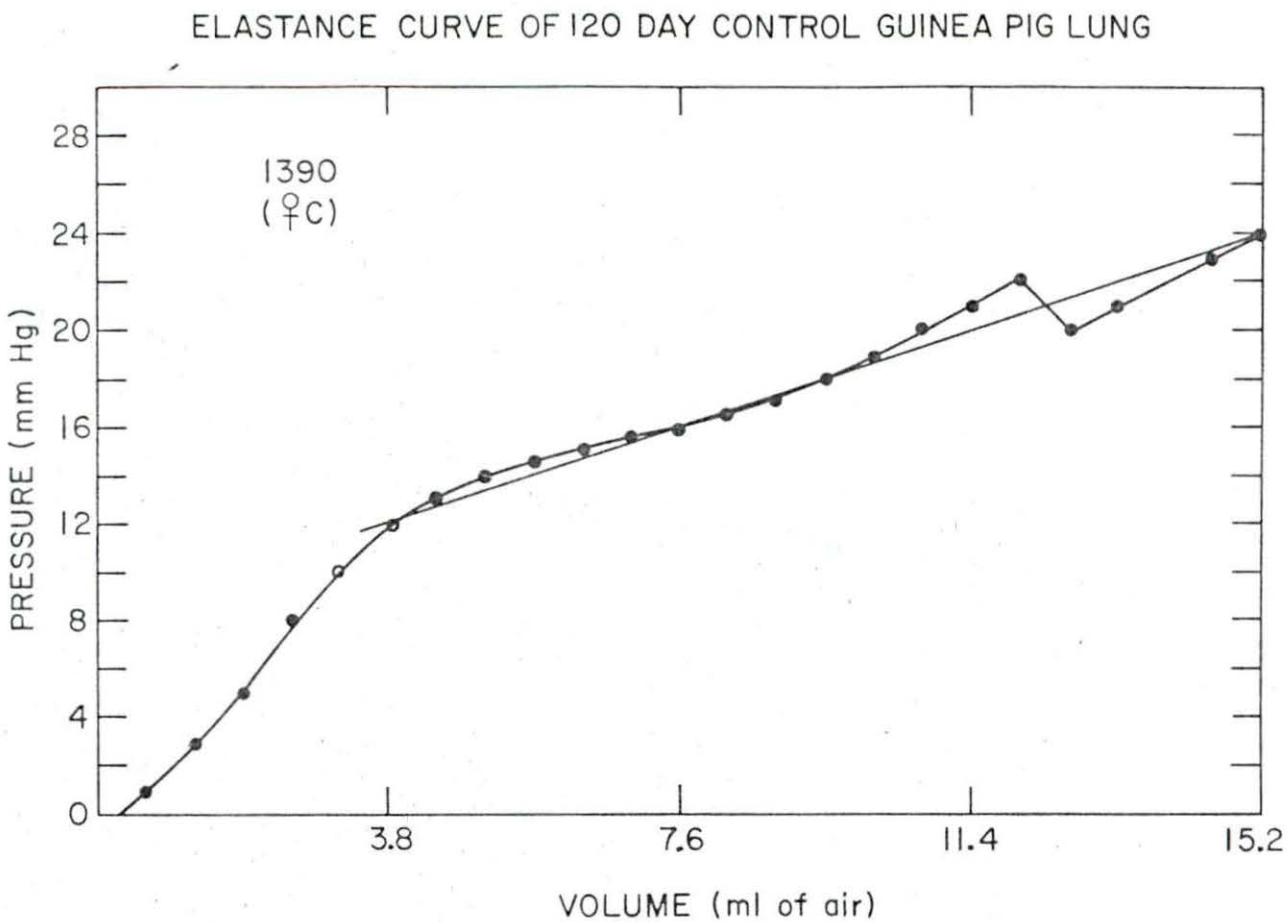


Figure 9. Elastance curve of 120 day control guinea pig lung

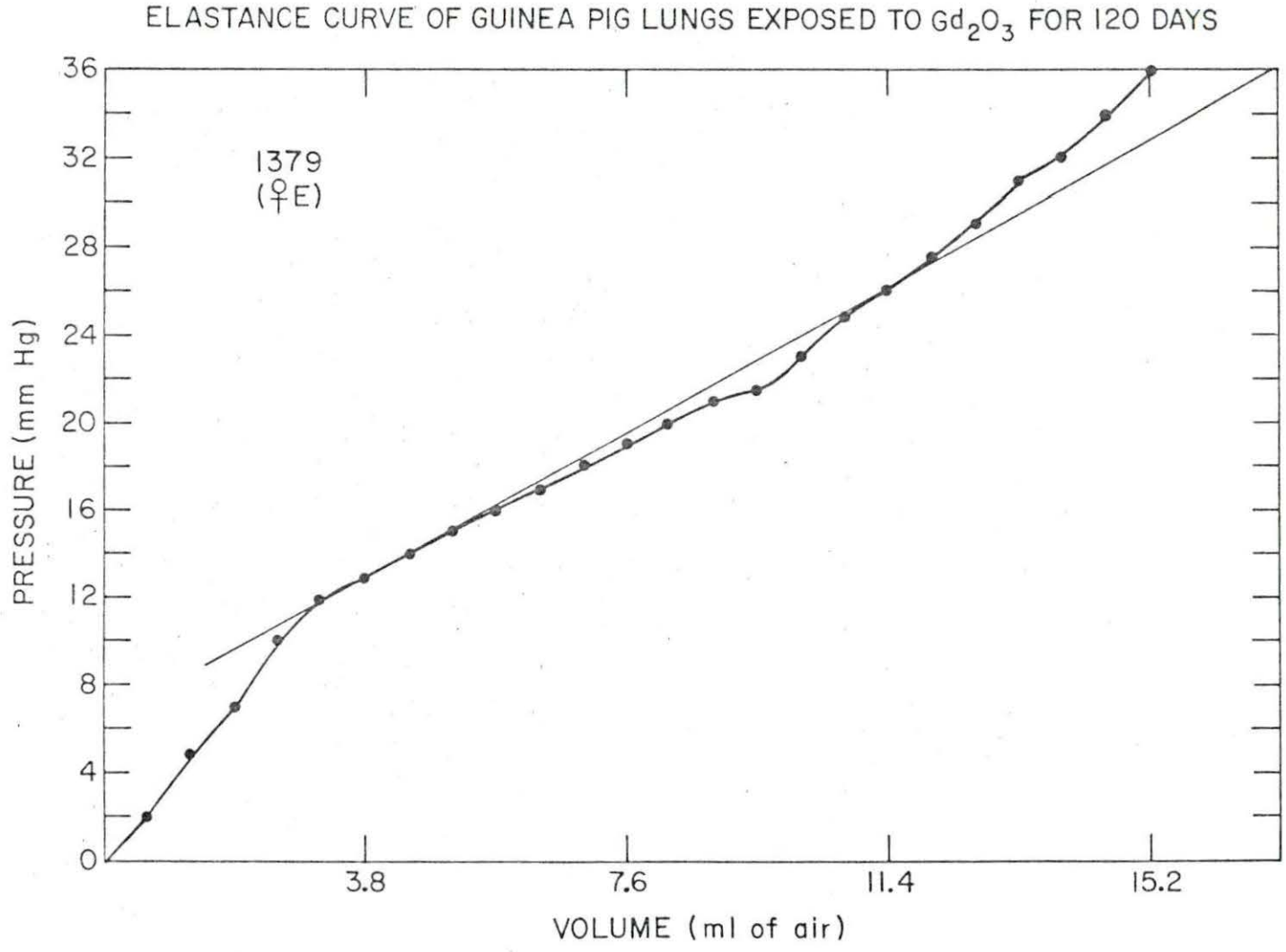


Figure 10. Elastance curve of guinea pig lungs exposed to Gd_2O_3 for 120 days

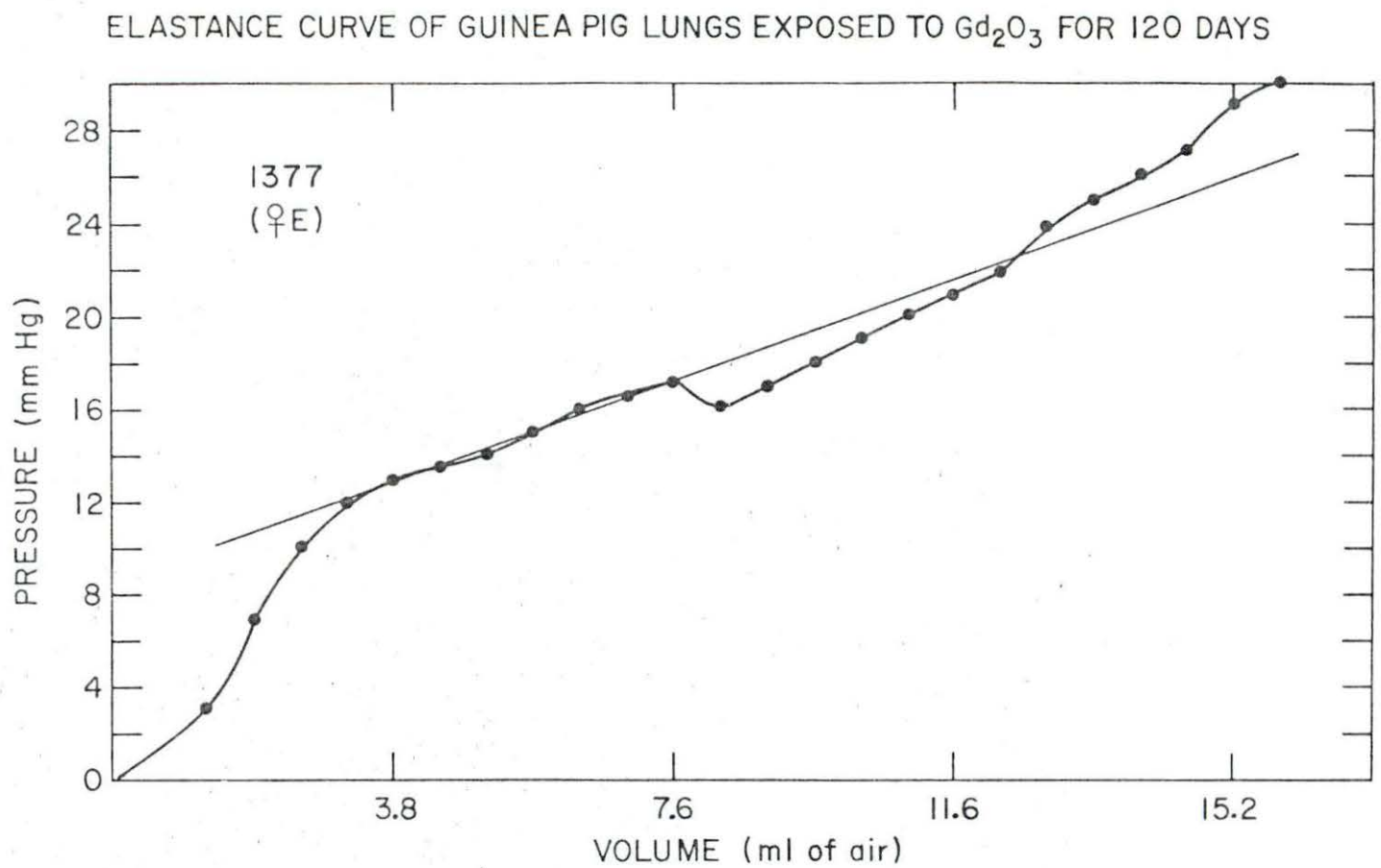


Figure 11. Elastance curve of guinea pig lungs exposed to Gd_2O_3 for 120 days

Statistical Analysis

This experiment was originally designed with six observations or guinea pigs per subclass, but since some of the lungs either failed to open or developed a leak during inflation, the subclasses when analysed contained unequal numbers of observations. Therefore, the analysis made on the results was a 3-way factorial with unequal numbers of observations per subclass. Yates (1934) reported that the use of an unweighted means analysis was justified when the number of observations in each subclass are not too different. When the results were investigated, it was found that eight subclasses contained five observations, two subclasses contained six observations, one subclass contained three observations, and one contained four observations. Since these numbers do not represent a wide variation in observations per subclass, the unweighted means analysis was the analysis of choice. The mean of each subclass was calculated, Table 2, and the 3-way factorial analysis was begun.

Table 2. Table of means*
(Unweighted means analysis)

Exposure (days)		40	80	120
Controls	M	.4060	.4780	.2875
	F	.4240	.3880	.3500
Treated	M	.3450	.4300	.5620
	F	.3600	.4240	.4467

*mm. Hg./ml. air

The first procedure in the analysis was to determine if the variances within the subclasses were homogeneous. This was accomplished by the use of Bartlett's test, as described by Winer (1962). It was found that the Chi square at eleven degrees of freedom was 14.047, which was non-significant at the 0.20 level. Therefore, homogeneity of variance was assumed and the analysis was continued.

A preliminary analysis of variance was calculated, and it was found that there was a significance at the 0.10 level, which would indicate the possibility of significant effects and of interactions in the data. The final analysis of variance on the means, Table 3, was made, and the only significant factor was the treatment vs. duration interaction, which was significant at the 0.01 level. This would indicate that the control group, over the periods of forty, eighty, and one hundred twenty days of exposure reacted in a different manner than did the exposed group over the same periods of time.

Table 3. Analysis of variance of subclass means

Source	d.f.	SS	MS	F
Sex	1	.001118	.001118	< 1
Duration	2	.004337	.002168	< 1
Trt vs. control	1	.004571	.004571	1.60
Sex vs. trt	1	.000781	.000781	< 1
Sex vs. duration	2	.002155	.001078	< 1
Trt vs. duration	2	.033818	.016909	5.91*
Trt vs. sex vs. dur.	2	.008889	.004444	1.55
Error	47	.134496	.002862	
Total	58			

*P < 0.01

Since the analysis of variance showed no significance due to sex, the sex factor was omitted, and a two-way table was obtained. A linear and quadratic test for trend, Table 4, was made on the control group and exposed group. In the control group there is no evidence of any kind of trend over duration. In the exposed group, however, there is strong evidence of a linear trend and no evidence of a quadratic trend. This would indicate that within the exposed group, increase in duration of exposure produces a significant linear increase in the slope of the elastance curve; i.e., the compliance of the lungs exposed to gadolinium oxide progressively decreased, and the lungs were less elastic than those of the control guinea pigs. Since all factors during the lives of the two groups of guinea pigs were relatively equal, except for the exposure to gadolinium oxide dust, the assumption can be made that the extensive macrophage reaction and lymphoid hyperplasia seen in histological sections resulted in impaired function of the lungs of those guinea pigs exposed to the dust.

Table 4. Test for linear or quadratic effect

Contrast	d.f.	MS	F
Control - linear	1	.009264	3.24
Control - quadratic	1	.005830	2.04
Exposed - linear	1	.023058	8.06*
Exposed - quadratic	1	.000002	.00095
Error	47	.002862	

*P < 0.01

Histological Evaluation

The inflation procedures to which each lung was subjected enhanced the value of histological sections. Since the lungs had been distended more than would normally occur in tidal breathing, and because they remained somewhat distended after undergoing inflation, the histologic sections gave a good perspective when being viewed for the purpose of determining alveolar wall thickening. Reece (1965) infused 10 per cent formalin in the lungs of dogs which had undergone inhalation of yttrium oxide, but felt that the infusion washed out many macrophages which were in the alveoli. This infusion was not necessary in this experiment, and the resulting sections are deemed a true representation of the effects of gadolinium oxide aerosol.

Lungs of guinea pigs exposed to gadolinium oxide progressed through a changing sequence as the exposure time was increased. Sections from control and exposed lungs are shown in Figures 14 through 21 in an attempt to demonstrate the degree of deviation from normal lung histology. An indication of the sequential changes which took place can be easily demonstrated by a comparison of Figures 12, 15 and 19. The histologic sections in this manuscript are typical of the 72 lungs examined.

The most apparent histologic change as a result of 40 days of exposure (240 exposure hours) to gadolinium oxide was a

mild swelling and proliferation of septal cells (Figure 14). Occasional macrophages were seen in viewing the entire group of 40-day exposed lung slides, but the numbers were not sufficient to consider them a deviation from normal. A comparison count was not made of the lymphocytic nodular hyperplasia present in the exposed and control lungs, but upon macroscopic viewing of the two groups of stained slides, an increase was definitely apparent in the lung sections of guinea pigs exposed to gadolinium oxide.

At 80 days of exposure (480 exposure hours), a more extensive parenchymal cell reaction is evident (Figures 15 and 17). The predominant finding at 80 days was the presence of numerous septal and alveolar macrophages. This was expected in view of the findings of Schepers (1955), Davison (1963), Reece (1965) and Talbot et al. (1965). According to Hatch and Gross (1964), these alveolar macrophages or "dust cells" are desquamated alveolar cells. The similarity between the alveolar macrophages and attached, rounded alveolar cells as described by Hatch and Gross was also noted. Alveolar epithelial cells become alveolar macrophages by a process of hypertrophy and hyperplasia of intact cells. This process in itself would contribute to a thickened septal wall and could possibly interfere with normal pulmonary function. The extreme hypertrophy of alveolar epithelial cells prior to desquamation is shown in Figure 17. There is an apparent

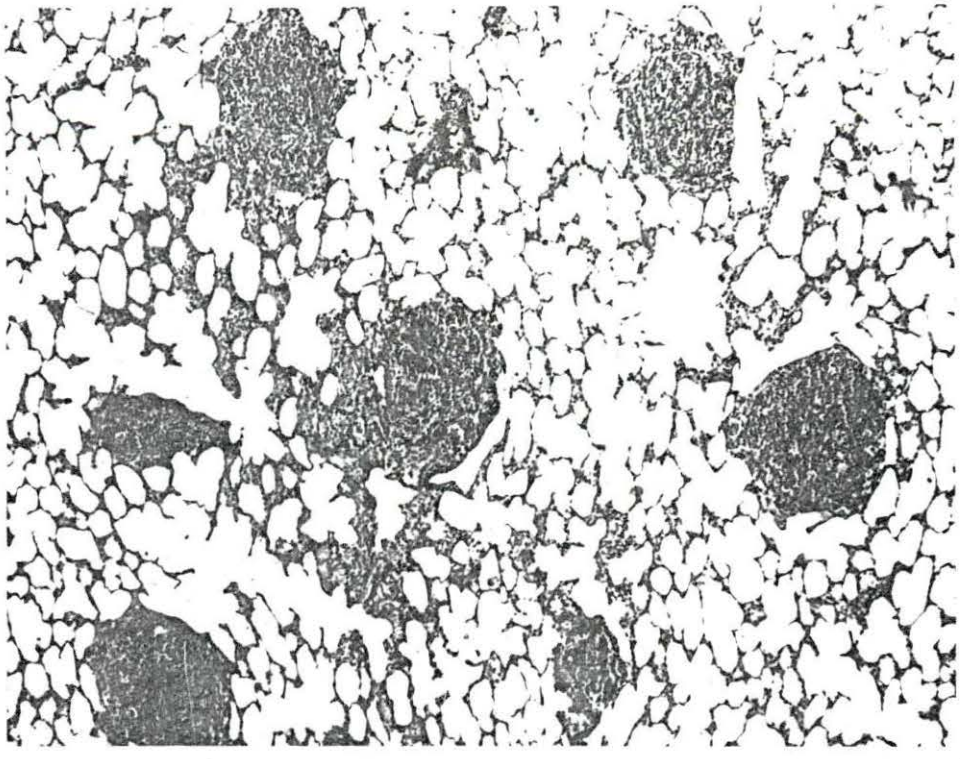
general increase in lymphocytic nodular hyperplasia when a comparison is made between 40 and 80-day exposed lungs.

Histologic changes after 120 days of exposure to gadolinium oxide dust include thickening of the alveolar walls (Figures 19 and 21). Certain portions of the septa are as many as 3 and 4 cells thick. Formation of macrophages in the septa and the presence of dust-laden macrophages in the alveoli are especially noticeable in Figure 21. All 120-day exposed lungs exhibited heavy infiltration of lymphocytic nodules, and it is speculated that this increase was great enough to physically hinder the normal expansion of the lung. When comparing the pulmonary tissue changes with the results of the functional determinations and statistical inferences, it seems that the condition of the pulmonary parenchyma could be determined by the method presented in this thesis. However, the limitations of statistical findings as well as the sensitivity of the method should be kept in mind. It would seem that the method should be able to detect significant changes at 120 exposure days before being considered reliable. The fact that it was possible to detect a linear trend, or sequential change, in the lung tissue is a significant finding.

One factor other than thickened alveolar walls and lymphoid infiltration should be mentioned as a possible cause of an increased elastance slope. Miller (1947) states that guinea pigs have far more airway smooth muscle than other

Figure 12. Histological section of guinea pig lung exposed to gadolinium oxide for 40 days. x 55. H. and E. stain

Figure 13. Higher magnification of guinea pig lung shown in Figure 12. x 136. H. and E. stain



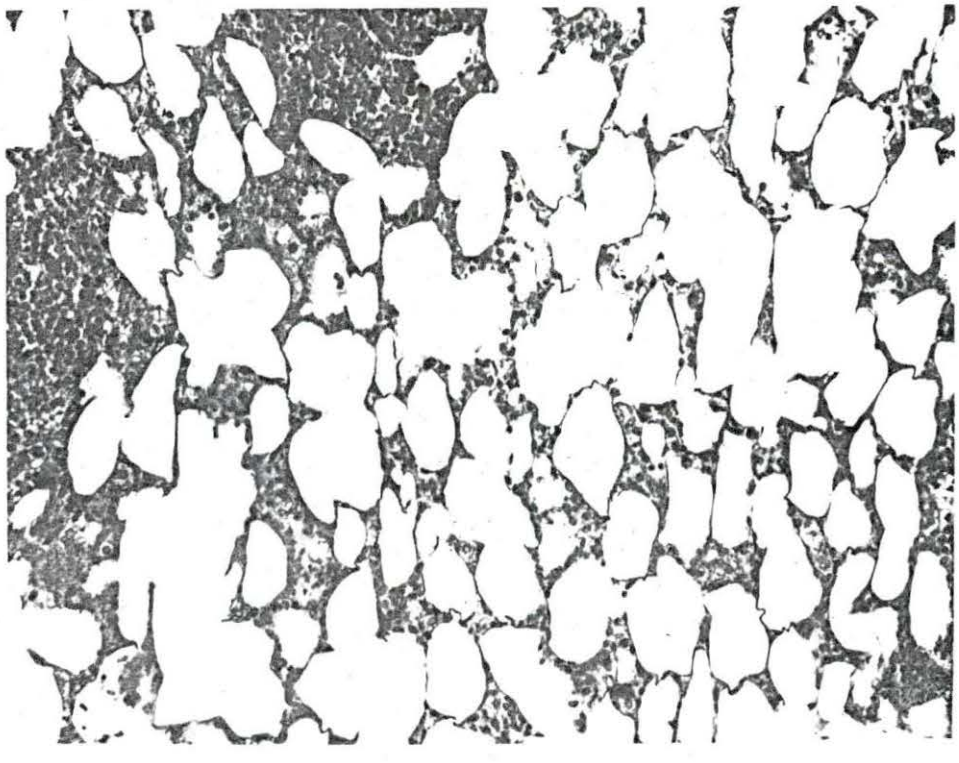
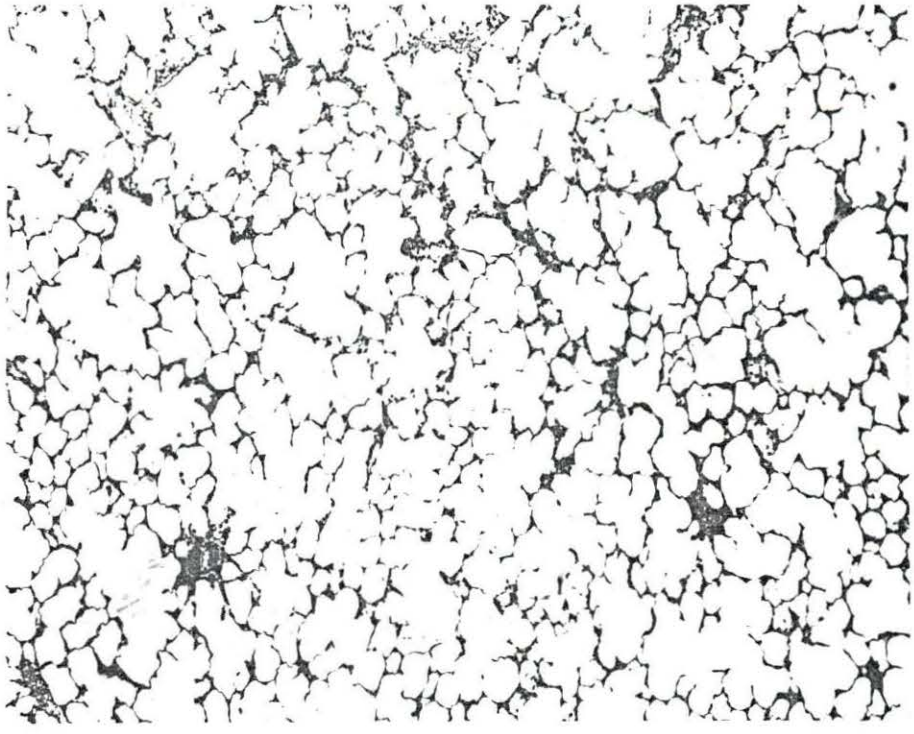


Figure 14. Lung from a control guinea pig held in an inhalation chamber through 80-day treatment period. x 55. H. and E. stain

Figure 15. Lung from a guinea pig exposed to an aerosol of gadolinium oxide for 80 days. Thickened septa are evident. Moderate lymphoid hyperplasia is not uncommon in most guinea pig lungs. x 55. H. and E. stain



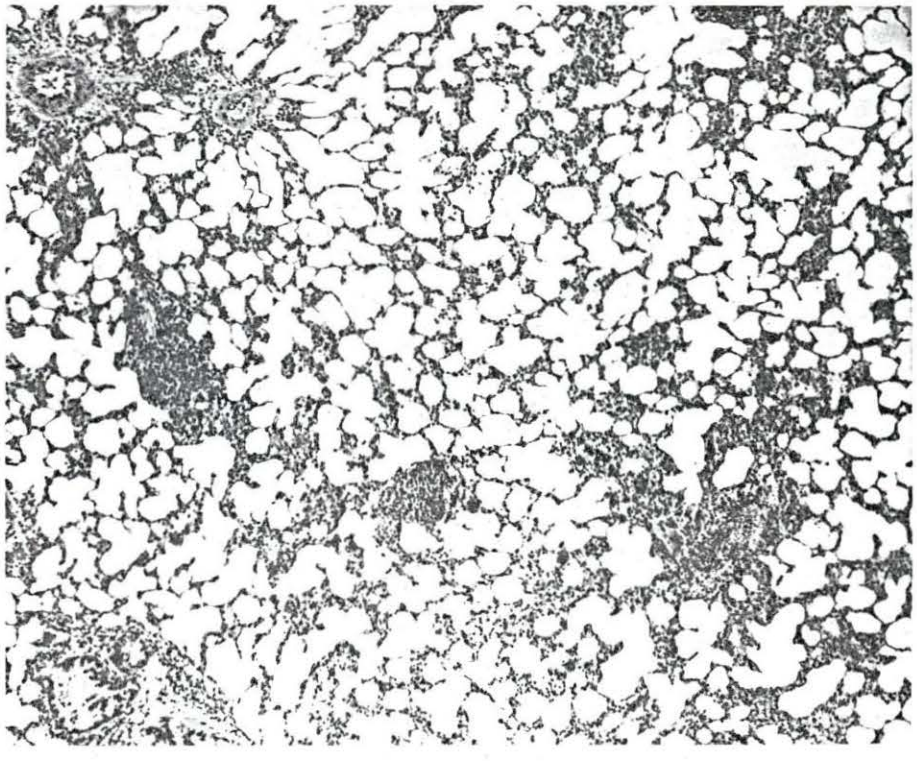
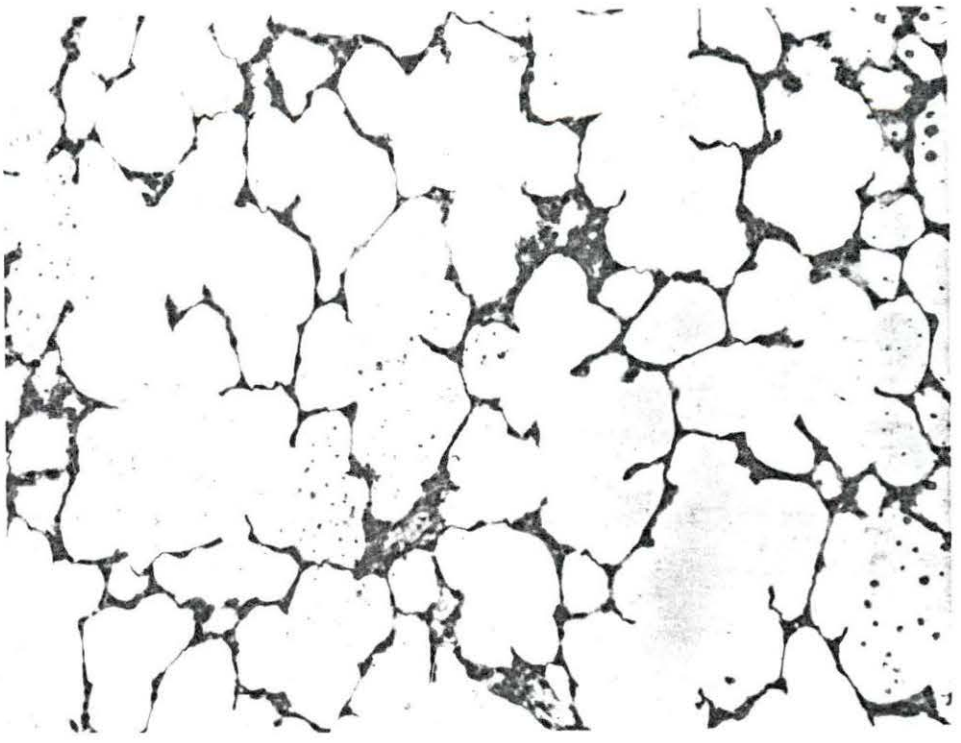


Figure 16. Lung section from control guinea pig held in an inhalation chamber through 80-day treatment period. x 136. H. and E. stain

Figure 17. Histological section from lung of guinea pig exposed to gadolinium oxide dust for 80 days. Thickened septa and lymphoid hyperplasia are noticeable. Some dust-filled macrophages are evident. x 136. H. and E. stain



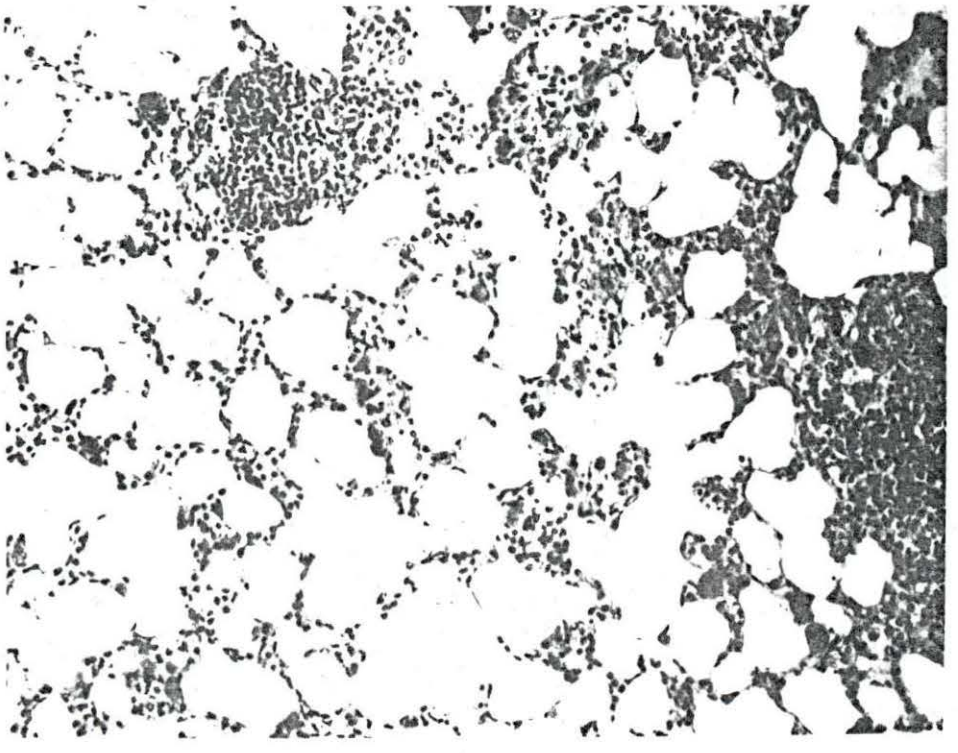
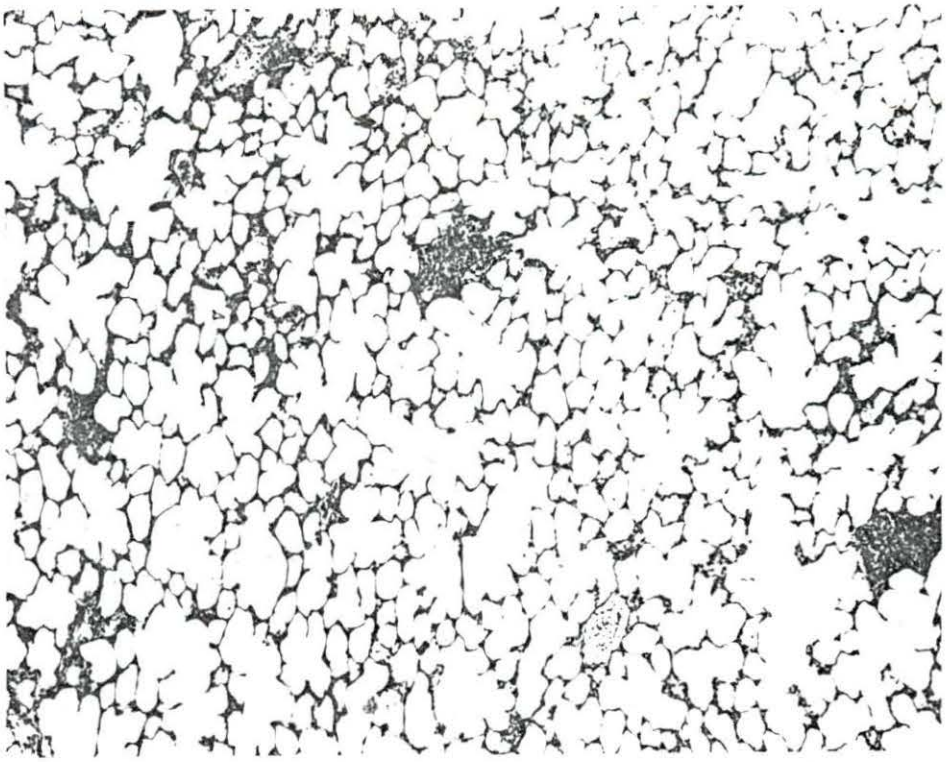


Figure 18. Histological section from lung of control guinea pig held in inhalation chamber through 120-day treatment period. Some septal thickening is evident. x 55. H. and E. stain

Figure 19. Histological section from lung of guinea pig exposed to gadolinium oxide dust for 120-day treatment period. Extreme septal thickening is present, and lymphoid response is greater than normal lung. x 55. H. and E. stain



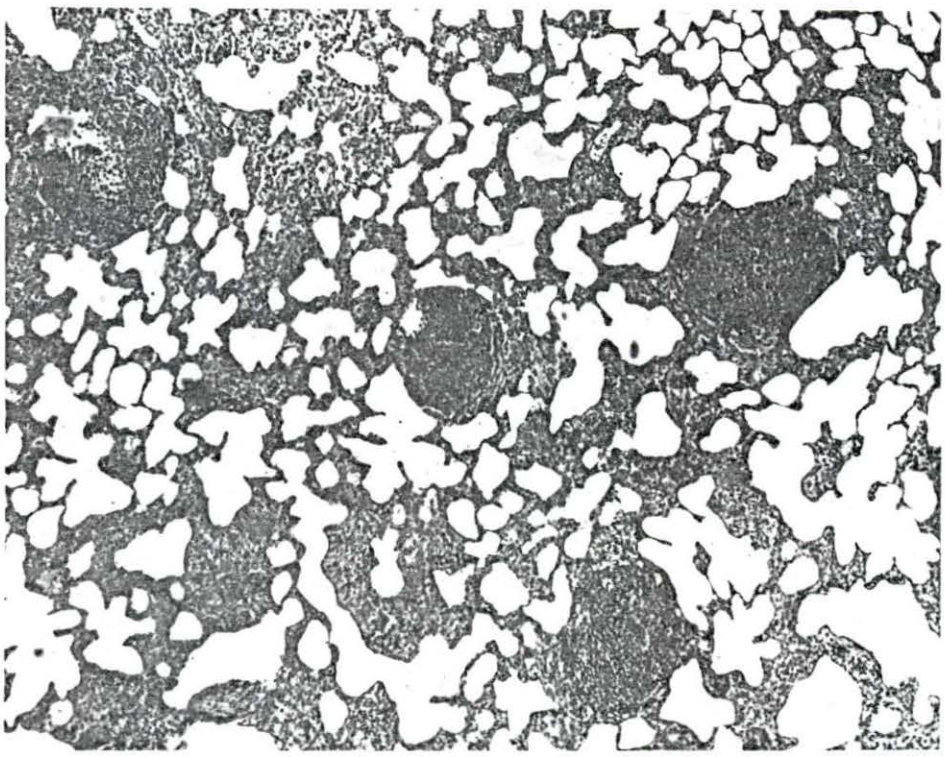
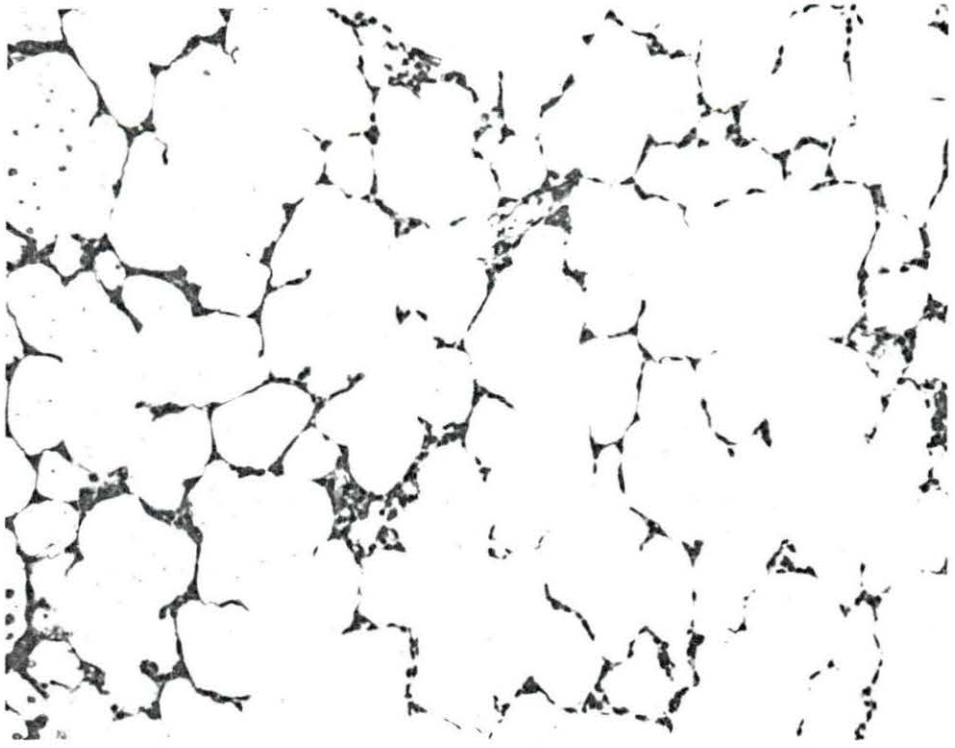
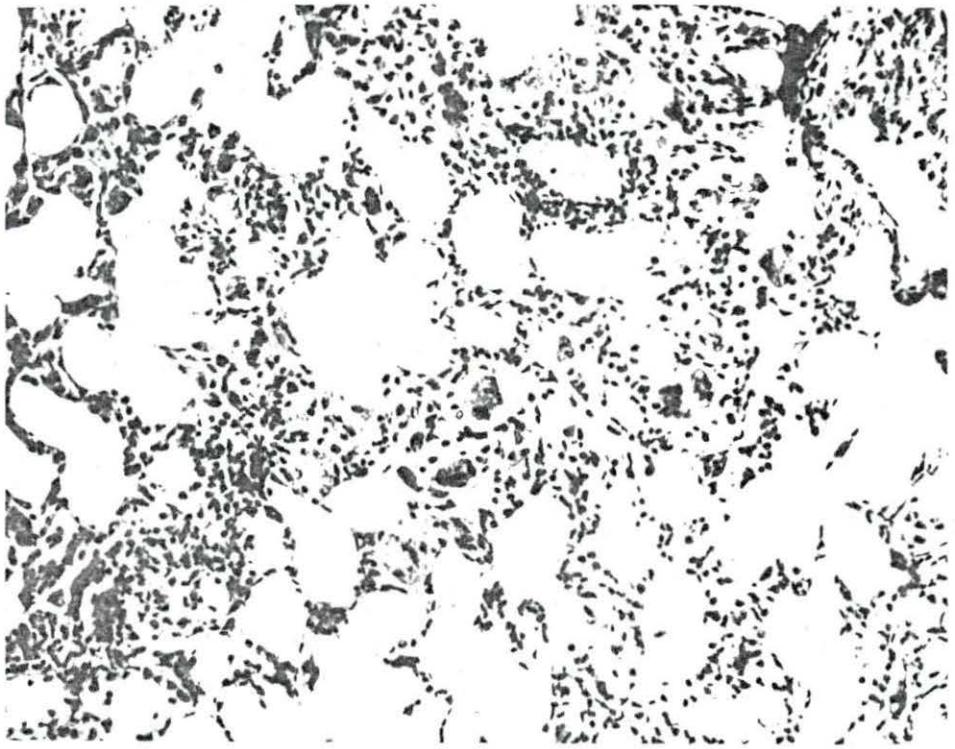


Figure 20. Lung section from control guinea pig lung held in inhalation chamber through 120-day treatment period. x 136. H. and E. stain

Figure 21. Lung section from a guinea pig exposed to gadolinium oxide dust for 120-day treatment period. Thickened septa are evident and large macrophages laden with dust are prominent. x 136. H. and E. stain





species. Radford and Lefcoe (1955) state that with bronchoconstriction some of the small bronchioles may close off entirely and when closed, larger pressures are required to open them. Widdicombe et al. (1962) found that the inhalation of charcoal dust by cats caused an immediate change in the compliance curve. This, however, was an acute experiment, and this reflex bronchoconstriction due to stimulation of irritant receptors in the airways may not apply to a more chronic type experiment. It might be suggested that other experimentation be undertaken, possibly utilizing another experimental animal with less tendency for lymphoid response to irritation, and smaller amounts of bronchial muscle in the lungs. Another, more sensitive technique might also be devised.

The conspicuous absence of pulmonary fibrosis throughout the sections of exposed lung tissue would seem to warrant some comment. Throughout the reports of Schepers et al. (1955), Davison (1963), Reece (1965), and Talbot et al. (1965) the absence of pulmonary fibrosis is apparent. Some of Schepers' experiments with rare earth exposure in the guinea pigs ran as long as 2 years. He states that the injurious action of any dust may also express itself by way of its effects on the bronchi and alveolar membranes even when no fibrotic reaction is initiated by it. Davison states that this absence of any fibrous response might lead to the assumption that the rare earth dusts were nontoxic, but mention is made of the fact

that some agents which are not harmful to the animal lung are toxic to the human lung and that harmful effects are not always seen with short exposure periods. He also states that the absence of fibrous reaction would make detection of antemortem exposure difficult. In view of the findings in this short-term experiment, and with limitations of the sensitivity of the method considered, it is suggested that regular compliance determinations of workers exposed to rare earth dust by inhalation may have some merit in the antemortem diagnosis of pulmonary functional damage.

SUMMARY

The purpose of this experiment was to determine the effects of inhaled gadolinium oxide on the functional ability of the pulmonary system. Changes which had been described by previous experimenters after histopathologic examination of lungs exposed to rare earths motivated the synthesis of this research. It was supposed that if these alterations were capable of interfering with the function of the lung, they could be detected.

Seventy-two guinea pigs were divided into three groups of twenty-four each. Each group contained twelve control animals and twelve exposed animals. The groups were exposed for six hours per day, five days a week, for periods of 40, 80 and 120 exposure days. The gadolinium oxide aerosol generated into the exposure chamber atmosphere was a dust in which 92 per cent of the mass was made up of particles less than one micron diameter.

After treatments of the prescribed periods of time, the guinea pigs were anesthetized, the lungs removed, and compliance measurements were made. These measurements were plotted as elastance curves, the slope of each curve was determined, and a statistical comparison was made in an attempt to describe any differences which existed in the control and exposed lungs.

Analysis of the data was accomplished by subjecting the

results to a 3-way factorial analysis. Treatment, duration of exposure, and sex were examined for effects. The analysis of variance revealed a significant interaction between the treatment and duration of exposure factors, and further analysis for trend disclosed a highly significant linear trend in the exposed groups.

Histopathologic changes in the exposed groups and the amount of deviation from normal lung tissue presuppose a difference in functional ability. These changes included alveolar cell hypertrophy, septal cell thickening, lymphoid hyperplasia, and macrophage formation.

From results obtained from this relatively short-term experiment, it is hypothesized that a compliance measurement may have some merit in the detection of pulmonary functional damage where usual methods of detection of alterations due to dust inhalation are unsatisfactory.

BIBLIOGRAPHY

Agostoni, E., A. Taglietti, F. Agostoni, and I. Setnikar
1958 Mechanical aspects of the first breath. Journal of Applied Physiology 13:344-348.

Agostoni, E., F. F. Thimm, and W. O. Fenn
1959 Comparative features of the mechanics of breathing. Journal of Applied Physiology 14:679-683.

Amdur, M. O. and J. Mead
1956 The respiratory response of guinea pigs to inhalation of ethylene oxide. Archives of Industrial Health I4:553-559.

Amdur, M. O. and J. Mead
1958 Mechanics of respiration in unanesthetized guinea pigs. American Journal of Physiology 192:364-368.

Aumonier, F. J., M. L. Crosfill and J. G. Widdicombe
1958 The relationship between compliance and histology in different species. Journal of Physiology 141: 20F.

Balchum, O. J., Jerzy Dybicki, and George R. Meneely
1960 Pulmonary resistance and compliance with concurrent radioactive sulfur distribution in dogs breathing $S^{35}O_2$. Journal of Applied Physiology 15:62-66.

Bayliss, L. E. and G. W. Robertson
1939 The visco-elastic properties of the lungs. Quarterly Journal of Experimental Physiology 29:27-47.

Bernstein, L.
1957 The elastic pressure-volume curves of the lungs and thorax of the living rabbit. Journal of Physiology 138:473-487.

Bertalanffy, F. D. and C. P. LeBlond
1953 The continuous renewal of the two types of alveolar cells in the lung of the rat. Anatomical Record 115:515-536.

Brody, A. W.
1954 Mechanical compliance and resistance of the lung-thorax calculated from the flow recorded during passive expiration. American Journal of Physiology 178:189-196.

Brown, E. S., R. P. Johnson, and John A. Clements
1959 Pulmonary surface tension. Journal of Applied Physiology 14:717-720.

Campbell, E. J. M.
1959 Volume pressure diagram of the lungs and transmural pressure of the airways. Journal of Applied Physiology 14:153-154.

Casarett, L. J. and P. F. Milley
1964 Alveolar reactivity following inhalation of particles. Health Physics 10:1003-1011.

Cavagna, G., G. Brandi, F. Saibene, and G. Torelli
1962 Pulmonary hysteresis. Journal of Applied Physiology 17:51-53.

Clements, J. A.
1962 Surface tension in the lungs. Scientific American 207(6):120-130.

Comroe, J. H.
1965 Physiology of Respiration. Yearbook Medical Publishers, Inc. Chicago, Ill.

Cook, R. M.
1961 A system for administering air-borne contaminants by inhalation. Unpublished M.S. thesis. Library, Iowa State University of Science and Technology, Ames, Iowa.

Coryllos, P. N. and G. L. Birnbaum
1932 A theory of absorption in atelectasis. American Journal of Medical Science 183:347-350.

Crosfill, M. L. and J. G. Widdicombe
1961 Physical characteristics of the chest and lungs and the work of breathing in different mammalian species. Journal of Physiology 158:1-14.

Dale, A. S. and B. Narayana
1935 Observations on the perfused lungs of the guinea pig. Quarterly Journal of Experimental Physiology 25:85-97.

Daly, I. de B.
1938 Observations on the blood-perfused lungs of the dog, guinea pig and Macacus rhesus, with special reference to "spontaneous" lung movements. Quarterly Journal of Experimental Physiology 28:357-403.

Dautrebande, L.
1962 Microaerosols. Academic Press, Inc. New York, N.Y.

Dautrebande, L., H. Beckman, and W. Walkenhorst
1957 Lung deposition of fine dust particles. Archives of Industrial Health 16:179-187.

Davison, F. C.
1963 Critical controlled inhalation study of pathogenesis of neodymium oxide in mice and guinea pigs. Unpublished Ph.D. thesis. Library, Iowa State University of Science and Technology, Ames, Iowa.

Davison, F. C., W. O. Reece, and R. B. Talbot
1965 Organ distribution following intrapulmonary injection of rare earth compounds. United States Atomic Energy Commission Report COO-1170-3.

DuBois, A. B.
1964 Resistance to breathing. In Fenn, W. O. and H. Rahn, eds. Handbook of Physiology: Section 3. Respiration. Vol. 1. pp. 451-462. American Physiological Society, Washington, D.C.

Dygert, H. P., C. W. LaBelle, S. Laskin, V. C. Pozzani, E. Roberts, J. J. Rothermel, A. Rothstein, C. J. Spiegl, C. F. Spague, Jr., and H. E. Stokinger
1949 Toxicity following inhalation. In Voegtlin, C. and H. Hodge, eds. Pharmacology and Toxicology of Uranium Compounds. Vol. 1:423 through Vol. 2:700. McGraw-Hill Book Company, Inc., New York, N.Y.

Engel, S.
1964 Comparative anatomy and pulmonary air-cleansing mechanisms in man and certain experimental animals. Health Physics 10:967-971.

Fenn, W. O.
1964 Introduction to the mechanics of breathing. In Fenn, W. O. and H. Rahn, eds. Handbook of Physiology: Section 3. Respiration. Vol. 1. pp. 357-362. American Physiological Society, Washington, D.C.

Frank, N. R.
1959 Influence of acute pulmonary vascular congestion on recoiling force of excised cats' lungs. Journal of Applied Physiology 14:905-908.

Frank, N. R.
1963 A comparison of static volume-pressure relations of excised pulmonary lobes of dogs. Journal of Applied Physiology 18:274-278.

Frank, N. R., E. P. Radford, Jr., and James L. Whittenberger
1959 Static volume-pressure interrelationships of the lungs and pulmonary blood vessels in excised cats' lungs. Journal of Applied Physiology 14:167-173.

Fritz, J. S., M. J. Richard, and W. J. Lane
1958 Spectrophotometric determination of rare earths. Analytical Chemistry 30:1776-1779.

Goodheart, C. R., F. J. Haddy, F. G. Grodins, and R. V. Ebert
1959 Elastance and resistance of lungs-chest of dogs. American Journal of Physiology 196:525-528.

Graca, J. G., F. C. Davison, and J. B. Feavel
1962 Comparative toxicity of stable rare earth compounds. II. Effect of citrate and edetate complexing on acute toxicity in mice and guinea pigs. Archives of Environmental Health 5:437-444.

Gribetz, I., N. R. Frank, and M. E. Avery
1959 Static volume-pressure relations of excised lungs of infants with hyaline membrane disease, newborn and stillborn infants. Journal of Clinical Investigation 38:2168-2175.

Gross, P. and M. L. Westrick
1954 The permeability of lung parenchyma to particulate matter. American Journal of Pathology 30:195-207.

Gschneidner, K. A., Jr.
1964 Rare earths: the fraternal fifteen. Division of Technical Information Extension, U. S. Atomic Energy Commission, Oak Ridge, Tennessee.

Haley, T. J., K. Raymond, N. Komesu, and H. C. Upham
1961 Toxicological and pharmacological effects of gadolinium and samarium chlorides. British Journal of Pharmacology 17:526-532.

Haley, T. J.
1965 A review of the pharmacology and toxicology of the rare earth elements. Unpublished report presented at Atomic Energy Commission Rare Earth Conference, Ames, Iowa, April, 1965. Mimeographed. Laboratory of Nuclear Medicine and Radiation Biology of the Department of Biophysics and Nuclear Medicine, School of Medicine, University of California at Los Angeles, Los Angeles, California.

Hartroft, W. S.
1960 The size of pulmonic alveoli of common laboratory animals and man. American Review of Respiratory Diseases 81:744.

Hatch, T. F. and P. Gross
1964 Pulmonary deposition and retention of inhaled aerosols. Academic Press, Inc., New York, N.Y.

Karrer, H. E.
1960 Electron microscopic study of the phagocytosis process in lung. Journal of Biophysical and Biochemical Cytology 7:357-366.

Kyker, G. C.
1962 Rare earths. In C. L. Comar and F. Bronner, eds. Mineral Metabolism. Vol. 2, Part B. Academic Press, Inc., New York, N.Y.

Kyker, G. C. and E. A. Cress
1957 Acute toxicity of yttrium, lanthanum, and other rare earths. Archives of Industrial Health 16: 475-479.

Lawton, R. W. and D. Joslin
1951 Measurements on the elasticity of the isolated rat lung. American Journal of Physiology 167:111-118.

Low, F. N.
1953 The pulmonary alveolar epithelium of laboratory mammals and man. Anatomical Record 177:241-264.

Mack, I., M. Grossman, and L. N. Katz
1947 The effect of pulmonary vascular congestion on the distensibility of the lungs. American Journal of Physiology 150:654-660.

Magnusson, G.
1963 The behavior of certain lanthanons in rats. Acta Pharmacologica et Toxicologica 20, Supplement 3: 1-95.

McIlroy, M. B. and R. V. Christie
1952 A post-mortem study of the visco-elastic properties of normal lungs. Thorax 7:291-294.

McIlroy, M. B. and R. V. Christie
1954a The work of breathing in normal subjects. Clinical Science 13:127-136.

McIlroy, M. B. and R. V. Christie
1954b The work of breathing in emphysema. Clinical Science 13:147-154.

McIlroy, M. B., J. Mead, N. J. Selverstone, and E. P. Radford, Jr.
1955 Measurement of lung tissue viscous resistance using gas of equal kinematic viscosity. Journal of Applied Physiology 7:485-490.

Mead, J.
1961 Mechanical properties of lungs. Physiological Reviews 41:281-330.

Mead, J. and J. Milic-Emili
1964 Theory and methodology in respiratory mechanics with glossary of symbols. In Fenn, W. O. and H. Rahn, eds. Handbook of Physiology: Section 3. Respiration. Vol. 1. pp. 363-376. American Physiological Society, Washington, D.C.

Mead, J. and J. L. Whittenberger
1953 Physical properties of human lungs measured during spontaneous respiration. Journal of Applied Physiology 5:779-796.

Mead, J., J. L. Wittenberger, and E. P. Radford, Jr.
1957 Surface tension as a factor in pulmonary volume-pressure hysteresis. Journal of Applied Physiology 10:191-196.

Miller, W. S.
1947 The lung. 2nd ed. Charles C Thomas, Springfield, Illinois.

Moeller, T.
1963 The chemistry of the lanthanides. Reinhold Publishing Corporation, New York, N.Y.

Morrow, P. E.
1960 Some physical and physiological factors controlling the fate of inhaled substances. Health Physics 2:366-378.

Mount, L. E.
1955 The ventilation flow resistance and compliance of rat lungs. Journal of Physiology 127:157-167.

Nisell, O. I. and L. S. G. Ehrner
1956 A simple apparatus for measurement of pressure volume relationships in respiration. Journal of Applied Physiology 8:565-567.

Otis, A. B.
1964 The work of breathing. In Fenn, W. O. and H. Rahn, eds. Handbook of Physiology: Section 3. Respiration. Vol. 1. pp. 463-476. American Physiological Society, Washington, D. C.

Otis, A. B., W. O. Fenn, and Hermann Rahn
1950 Mechanics of breathing in man. Journal of Applied Physiology 2:592-607.

Otis, A. B., C. B. McKerrow, R. A. Bartlett, Jere Mead, M. B. McIlroy, N. J. Selverstone, and E. P. Radford, Jr.
1956 Mechanical factors in distribution of pulmonary ventilation. Journal of Applied Physiology 8:427-443.

Otis, A. B., H. Rahn, and W. O. Fenn
1946 Venous pressure changes associated with positive intrapulmonary pressures; their relationship to the distensibility of the lung. American Journal of Physiology 146:307-317.

Pratt, P. C., A. Haque, and G. A. Klugh
1961 Correlation of postmortem function and structure in normal and emphysematous lungs. American Review of Respiratory Diseases 83:856-865.

Pratt, P. C., M. A. Haque, and G. A. Klugh
1962 Correlation of postmortem function and structure in panlobular pulmonary emphysema. Laboratory Investigation 11:177-187.

Pratt, P. C. and G. A. Klugh
1961 A technique for the study of ventilatory capacity, compliance, and residual volume of excised lungs and for fixation, drying, and serial sectioning in the inflated state. American Review of Respiratory Diseases 83:690-696.

Radford, E. P., Jr.
1963 Mechanical stability of the lung. Archives of Environmental Health 6:128-133.

Radford, E. R., Jr.
1964 Static mechanical properties of mammalian lungs. In Fenn, W. O. and H. Rahn, eds. Handbook of Physiology: Section 3. Respiration. Vol. 1. pp. 429-450. American Physiological Society, Washington, D.C.

Radford, E. P. and N. M. Lefcoe
1955 Effects of bronchoconstriction on elastic properties of excised lungs and trachea. American Journal of Physiology 180:479-484.

Rahn, H., A. B. Otis, L. E. Chadwick, and W. O. Fenn
1946 The pressure-volume diagram of the thorax and lung. American Journal of Physiology 146:161-178.

Reece, W. O.
1965 Effects of exercise and inhaled yttrium oxide on blood lactic acid, erythrocyte volume, plasma volume and lung histology in dogs. Unpublished Ph.D. thesis. Library, Iowa State University of Science and Technology, Ames, Iowa.

Schepers, G. W. H.
1955a The biological action of rare earths. I. The experimental pulmonary histopathology produced by a blend having a relatively high oxide content. Archives of Industrial Health 12:301-305.

Schepers, G. W. H.
1955b The biological action of rare earths. II. The experimental pulmonary histopathology produced by a blend having a relatively high fluoride content. Archives of Industrial Health 12:306-316.

Schepers, G. W. H., A. B. Delahant, and A. J. Redlin
1955 An experimental study of the effects of rare earths on animal lungs. Archives of Industrial Health 12:297-300.

Snedecor, G. W.
1956 Statistical methods. 5th ed. Iowa State College Press, Ames, Iowa.

Talbot, R. B., F. C. Davison, and W. O. Reece
1965 Inhalation toxicity of gadolinium oxide in mice. Unpublished manuscript. Veterinary Medical Research Institute, Ames, Iowa.

Van Liew, H. D.
1954 Contribution of vagus nerves to pressure-volume characteristics of chest and lungs in dogs. American Journal of Physiology 177:161-163.

Vickery, R. C.
1953 Chemistry of the lanthanons. Butterworth's Scientific Publications, London, England.

Widdicombe, J. G., D. C. Kent, and J. A. Nadel
1962 The mechanics of bronchoconstriction during inhalation of dust. Journal of Applied Physiology 17:613-616.

Winer, B. J.
1962 Statistical principles in experimental design. McGraw-Hill Book Company, Inc., New York, N.Y.

Yates, F.
1934 Analysis of multiple classifications with unequal numbers in the different classes. Journal of the American Statistical Association 29:51-66.

ACKNOWLEDGEMENTS

The writer wishes to express his appreciation to his major professor, Dr. Richard B. Talbot, for his counsel, suggestions, and encouragement through the course of this study.

Special thanks is expressed to Mrs. Donna Ruhl, Department of Statistics, for statistical consultation and assistance in interpretation of results.

The author would also like to express appreciation to Mr. Clifford Annis, B.S., for supervision of the experimental animals and for photographic assistance; to Mrs. Genevieve Swenson for preparation of excellent histologic sections; and to Mrs. Ruth Cooper and Mrs. Julie Van Hovel for aid in typing of this manuscript.

Acknowledgement is also given to the Department of Physiology and Pharmacology for financial assistance through Physiology Training Grant, GM 1043-03S1, Research Training Grants Branch of the National Institute of General Medical Sciences, National Institutes of Health, Bethesda, Maryland. Financial support for this research by the United States Atomic Energy Commission (contract AT-11-1 (1170)) is gratefully acknowledged.

Finally the writer would like to thank his wife, Peggy, for her support and understanding during this period of study and experimentation.