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PULMONARY COMPLIANCE AND HISTOLOGY IN RATS EXPOSED TO HYPEROXIC ENVIRONMENTS AT NORMOBARIC AND REDUCED PRESSURES

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Richard B. Pilmer, B.S., M.S.

* * * * * * * *

The Ohio State University 1972

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I express the greatest appreciation to my advisor, Dr. Harold S. Weiss, for patient guidance and advice throughout this study. I am also indebted to Dr. Philip C. Pratt for extensive help in the interpretation of histological changes observed in the course of many experiments.

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I sincerely hope that it will be possible to contribute to science and Air Force technology in a manner that will reflect the work of those mentioned above, and the trust that was placed in me as a candidate for graduate training.
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INTRODUCTION

The breathing of oxygen in high concentrations is followed in all animals thus far tested by a syndrome of undesirable effects known as oxygen poisoning or oxygen toxicity. This problem has been investigated at great length, and older general reviews by Bean (1945), Ohlsson (1947), DuBois (1962), Lambertsen (1966), Pattle (1967), Röth (1967) and Morgan (1968), have been expanded upon by recent reviews more specific to certain aspects of the problem (e.g., Haugaard, 1968, Kafer, 1971, and Clark and Lambertsen, 1971).

Although it has long been known that high levels of oxygen are toxic (Lavoisier, 1783 as cited by Beddoes and Watt, 1796), the subject continues to excite attention. The reasons for this persistent interest are varied, but among them may be cited:

a) philosophical fascination with the fact that a substance vital to life also should be an almost universal poison, b) practical uses for prolonged exposure to increased oxygen in such varied situations as infant incubators, surgical procedures, treatment of diseases, space vehicles, and underwater explorations, and c) unclear mechanisms of action for oxygen as a toxicant, which thus perpetuate a challenge to the investigator.

Excess oxygen depresses respiration in tissues due to mechanisms related to enzyme inhibition (Dickens, 1946, Felig, 1965),
free radical formation (Gerschman, 1962) and peroxidation (Roth, 1967). Morgan (1968) characterized four essential manifestations of the problem: 1) metabolic effects; 2) hematologic and systemic effects; 3) absorption atelectasis and; 4) pulmonary damage. While all tissues and organs may be affected by oxygen toxicity, the lungs remain a prime target for oxygen tensions in the range of one atmosphere (OAP).

Historically, the histological nature of lung injury induced by oxygen toxicity in experimental animals has been interpreted as hemorrhage (Binger, et al., 1927), atelectasis (Rahn, et al., 1963), hyaline membrane formation (Hamman and Rich, 1944; Rosengren, 1967), inflammation and perivascular edema (Bean, 1945), and edema (Hemingway, 1952). Pratt (1958, 1965, 1968) reported however, that histological changes in the alveolar wall, including thickening, capillary congestion and proliferation, appeared to characterize oxygen toxicity in humans. Using these criteria on lungs randomly selected post mortem he was able to identify patients who had been given oxygen therapy during their hospitalization. He also showed that these changes were due neither to the nature of the primary illness nor to post mortem artifacts. Furthermore, the severity of some of the histological changes in the patients seemed out of proportion to the 50% oxygen concentrations normally achieved in oxygen tents, particularly since humans are considered a species relatively resistant to oxygen toxicity (Kafer, 1971).
Our primary reason for adding to the already voluminous literature on the pulmonary aspects of oxygen toxicity centers, then, on these apparent discrepancies between lung changes seen in human patients and those commonly reported in animal experiments. While species characteristics may be involved in these differences, another possible explanation for the human response is that while patients may have started with a low oxygen tension, progressive increases in oxygen level may have been necessary as their illnesses became worse. Since few animal studies have been carried out with progressively increasing oxygen tensions, this was felt to be a worthwhile experimental approach.

Additionally, we felt that including physiological measurements with the histology would contribute to a better understanding of the functional significance and the general mechanism of oxygen damage to the lungs. This we hoped to accomplish by correlating compliance changes with structural changes in the same lung, by examining the effects of the presence or absence of nitrogen in the breathing mixture, and by determining whether aspirated fluid had effects similar to edema formed in situ.

Compliance of excised lungs is a widely used measure of pulmonary function (Pratt, et al., 1961) and has been studied in connection with oxygen toxicity (Beckman and Weiss, 1969). There is no universal agreement, however, as to how compliance is affected—whether it is due to a direct effect of oxygen, or is
secondary to the edema usually found in oxygen poisoned lungs. While it is now widely reported (Kistler, et al., 1967) that the primary oxygen-induced lung lesion is in the endothelial cells of capillaries, leading to increased permeability, exudation, and apparent effects on the surface-active material of the alveolar walls, these changes have not been correlated with functional measurements. Compliance generally has been measured in animals acutely poisoned with oxygen, at concentrations of one atmosphere or higher, in which, because of the rapidity of events, the full spread of histological change may have been obscured. It was hoped that the problem would be clarified by measuring compliance in animals exposed to a wide range of oxygen tensions and durations, by comparing edema induced by aspiration rather than exudation, and by correlating the compliance measurements with histology.

The question of whether toxicity is due to the effects of oxygen per se, or the absence of nitrogen was raised by Penrod (1956), Allen (1963) and others. It is now generally believed that nitrogen plays no role chemically (Wright, et al., 1966) although lack of nitrogen may be physically related to atelectasis (Rahn, 1963; Burger, et al., 1968). While it seems clear that toxicity is proportional to the oxygen concentration and duration of exposure, nevertheless there are reports that the severity of the response is aggravated in the absence of inert gas (Robinson, et al., 1969). We felt that our compliance measurements and
histological procedures could contribute to further clarification of this question.

In summary, this study was designed to determine: 1) the relative pulmonary effects of oxygen given in progressively increasing concentrations versus continuous exposures to a constant concentration, 2) the use of pulmonary compliance to increase understanding of the relationships between function and structural histological changes, 3) the use of light microscopy to relate perivascular edema, capillary congestion, and alveolar thickening to functional values, 4) the effect of the presence or absence of nitrogen on lung function and histology when the animals are exposed to approximately the same oxygen tension, and finally, 5) whether aspirated saline fluid has the same effects as pulmonary edema produced in situ.
HISTORICAL REVIEW

Oxygen Toxicity

Lavoisier discovered oxygen and Priestly was the first to report pathological effects (Bean, 1945). Beddoes and Watt (1796) related dangers and possible therapeutic uses. Early investigation attempted to establish the safe percentage of oxygen administration to provide for normal blood oxygen saturation in diseased patients (Adams, 1912; Barach, 1932; Gambell, 1934). In more recent years the use of oxygen in incubators for premature infants (Ashton, 1953 Manschot, 1954), in surgery, and the prolonged use of oxygen by aquanauts (Behnke, 1940), aircrewmembers and astronauts, has stimulated continued research.

Paul Bert (1878) revealed that oxygen plays a dual role: At low pressures it sustains life— at high pressures, it kills. The lack of tolerance for hyperoxia at one atmosphere (OAP), or at greater pressures (OHP) produces different physiology and pathology in a similar group of animals. Welch (1963) suggested a "time-concentration" response, in which partial pressures of oxygen only slightly elevated over that in air at sea level may be toxic provided exposure time is adequately prolonged.

Roth (1967) reviewed the effects of high oxygen tensions in animals. He divided oxygen toxicity into two classes according
to the areas of involvement: at less than two atmospheres the respiratory tract and eyes are affected; at greater than two atmospheres the central nervous system is the principal area affected (Bean, 1938; Zirkle, et al., 1965). In either case, there is variation between different species as well as between different animals of the same group. As in other areas of investigation, the results of animal experimentation can be only indirectly applied to man.

The literature describes many differences in methods, species, age, and sex of the animals in relation to time of exposure at different oxygen concentrations or pressures, with and without other gases. Other experimental variables include caging, air circulation, carbon dioxide level, humidity, temperature, method of sacrifice, and histological technique.

The Nature of Lung Injury

Smith (1899) was among the first to intensively study the effect of oxygen (O\textsubscript{2}) on the lung. Karsner and Ash (1916) studied exposures in the percentage range of 53–99 percent (O\textsubscript{2}). They were particularly concerned about the degree and rate of lung changes. Rats in 80–96\% oxygen showed congestion, edema, epithelial degeneration, fibrin formation, and pneumonia after 24 to 48 hours. They were among the first to suggest that rats might in some way develop tolerance for oxygen exposure.
By the year 1930, it can be said that there was agreement that oxygen in concentrations greater than 60% caused a rapid deterioration in the mammalian organism (Adams, 1912; Binger, et al., 1927; Cabell, 1927). Concentrations over 70% (OAP) had been found poisonous to dogs, rabbits, rats, guinea pigs, and mice. General symptoms reported from observations of these animals included drowsiness, decreased activity, anorexia, dehydration, weight loss, dyspnea, and cyanosis.

Subsequently, Smith, et al., (1932) exposed Wistar rats to air under pressure to produce an oxygen equivalent of 84%. The pathology found with this increased partial pressure of oxygen (and with nitrogen present) was reported to be similar to that observed by other investigators in animals exposed to increased normobaric concentration of oxygen. In adult rats they found pleural effusion and heavy, dark, edematous lungs. The capillaries of the alveolar walls were engorged and the arteries and veins surrounded by large zones of perivascular edema with varying degrees of cellular filtration. According to Smith:

Young rats, under forty days of age showed an early perivascular edema, and slight cellular infiltration. In rats 100 days old, these changes were more pronounced and in addition there was some desquamation, hyperplasia, and hypertrophy of alveolar wall cells. The apparent resistance of young rats to the pulmonary effects of oxygen other than perivascular edema may be due to an increased cellularity of the alveolar epithelium.

Drinker, et al., (1947) found a progressive increase in cellular hypertrophy and hyperplasia of the alveoli which persisted
for months after return to a room air environment.

Boycott and Oakley (1932) also used rats and came to the conclusion that the cause of death (OAP) was massive pleural effusion. Capillaries were congested, and fluid was found in the alveoli. They concluded that oxygen irritates the endothelium of the pulmonary capillaries so that blood plasma passes through.

Armstrong (1938) reached similar conclusions that environments containing more than 60% oxygen at one atmosphere or equivalent oxygen at reduced pressure, lead to the death of the animal in hours or a few days, and that the cause of death is primarily lung pathology. Armstrong said that pure oxygen was equivalent to the same tension of oxygen in a mixture of gases, and that the presence or lack of nitrogen made no apparent difference. Paine, Lynn, and Keys (1941) reported pulmonary changes in dogs after short exposure (OAP). Distinct lesions were found in animals which had shown no physical symptoms during exposure.

Bean (1945) concluded that the more outstanding pulmonary characteristics of oxygen toxicity are:

Inflammation, congestion, edema, perivascular edema, atelectasis, fibrin formation and consolidation, pneumonia, bronchitis, hypertrophy, hyperplasia, desquamation and degenerative changes of the alveolar cells, and also sclerotic changes with narrowing, thickening, and hyalinization of the pulmonary arterioles.

It should be noted that Bean, Haugaard, (1957, 1968) and
others have pointed out that the pulmonary damage, as well as other changes in the animal, are probably not due to any single mechanism, but rather are the result of many factors acting simultaneously.

The pathological changes observed by Hamman and Rich (1944) in acute diffuse interstitial fibrosis of the lungs have certain characteristics similar to those reported in oxygen toxicity:

The earliest lesion appears to consist in a marked dilatation of the capillaries in the alveolar walls and an exudation of fluid into the walls and alveolar spaces. The walls are, therefore, edematous and swollen. Shreds of fibrin are found within them, and fibrin clots the exudate that escapes into the alveoli to form a thick hyaline membrane.

A recent review of this area (Rosengren, 1967) maintains that hyaline membranes can be similarly induced in rats breathing oxygen.

Ohlsson (1947) believed that the disturbance in the blood carriage of carbon dioxide which occurs in the breathing of oxygen in high concentrations (OAP) was the principal cause of pulmonary damage in oxygen poisoning. Rather than a direct irritant effect from the air side, there was an indirect effect from the blood side. This conclusion was based on histological examination of lung tissue from normal and injured rabbits exposed to 80–90% oxygen. It was also his view that the nitrogen level had no bearing on the symptoms, and that the hypoxemia of injured animals entailed a greater tolerance for the administration of oxygen. Gupta, et al., (1969), in more recent work,
reported that dogs with surgically produced venous admixture (50-55%) were protected against oxygen toxicity; yet all expired eventually after prolonged hyperoxia.

Pratt (1958) reported that a study of pulmonary tissue from persons who had died following prolonged, progressive pulmonary disease, and who had received oxygen therapy for considerable periods, gave rise to the suspicion that oxygen might be adding to the terminal structural abnormality. Oxygen presumably caused not only a form of pulmonary alveolar capillary congestion, but a marked proliferation of the capillaries as well. This was also accompanied by a thickening of alveolar septa, with papillary projections of capillary tufts into the alveolar spaces. The lesion was associated with hemorrhage or slight pulmonary edema. They were present in all lobes, but were often found to be patchy in distribution. A few apparently uninvolved septums were almost invariably found near those showing capillary proliferation. Those patients having the longest oxygen exposure had the heaviest lungs except in instances where greater, pulmonary edema was present because of congestive failure. The changes were most frequently noted when oxygen had been given continuously for ten days or more.

Pratt argued against the concept of inflammation as a result of oxygen irritation because, "oxygen should be just as irritating on the first as on the fifth day. Similarly, it seems logical that simple inflammation should resolve promptly on discontinuation of
oxygen administration."

The mechanism of development of the changes described by Pratt remains to be investigated, although there is evidence that the first step can be explained by capillary dilatation which occurs within minutes after the inception of oxygen exposure, as reported by Comroe (1950).

Other reports have maintained that therapeutic oxygen procedures may at times be dangerous unless carefully watched (Moody, 1942; Nash, 1967; Pratt, 1968). While much of the histology described by Pratt has not been widely reported in all species, similar effects have been reported in primates and human subjects. Gable and Townsend (1962) studied needle biopsy samples of lung tissue from high altitude crew personnel who had accumulated over 500 hours of oxygen use in jet aircraft. Capillary alterations were described as variable in different persons, however they explained this possible sequence of events:

"The first change presumably consists of congestion and dilatation of the alveolar septal capillaries. The dilatation becomes progressively more pronounced and the septal walls thicker until the stromal tissue, rather than air spaces, occupies a major portion of each microscopic field. In some instances there is capillary proliferation in the form of papillary tufts projecting into the lumens of alveolar spaces and bronchioles."

This would tend to support Pratt's view (1958) that proliferation is a physiological response of pulmonary vasculature to increased oxygen concentration. The extension from prolonged vasodilatation to capillary proliferation would seem to be a
related occurrence. Pratt, et al., (1961, 1965) also believed that since collapsed lungs are difficult to examine, it might be advantageous to inflate the lungs with air before fixation. This procedure might serve to flatten the capillary tufts against the walls of the alveoli, or at any rate, help to understand more about the development and functional significance of the tufts.

There was also some interest in this procedure because of the report of Durfey (1965) who found mice exposed to 100% oxygen for 48 hours (OAP) were grossly and microscopically similar to air controls if the mice were removed from the high oxygen environment immediately before sacrifice. However, the lungs of mice killed in oxygen, left in oxygen three hours, and then autopsied, were heavy, grossly hemorrhagic, and atelectatic. They resembled closely the typical changes often reported (Gilbert, et al., 1958) as caused by oxygen toxicity.

There is presently general agreement, Pattle (1967) that animals exposed to toxic environments should be removed prior to death and sacrificed for tissue preparation or functional studies.

Holmdahl (Pratt, 1965) stated: "The lungs should be filled with something other than oxygen if oxygen is being given when death occurs. In our intensive care unit, after resuscitation with pure oxygen, when the heart definitely has stopped and we have given up, we ventilate the lungs for 10-15 minutes with air, in order to avoid finding complete atelectasis on the post-mortem table.

Pleural Effusion and Atelectasis

Altschule, et al., (1944) studied the problem of pleural
effusion in humans by comparing the lung volume and respiratory and cardiovascular dynamics before and after thoracentesis. Pleural effusion produced atelectasis, decreased compliance, decreased negativity of intrapleural pressure, and caused shallow respiration. He also noted increased peripheral venous pressure, although no changes of cardiac output or circulation time at rest were found. Pleural effusion impaired respiration and circulation; favored dyspnea and orthopnea; and was most likely to have severe effects in patients with extensive diffuse disease of the lung in addition to the effusion.

Altschule (1944) maintained that the resolution of pulmonary edema, however induced, implied the removal of fluid from the lungs containing considerable amounts of protein (and surfactant material ie., Avery, 1962). The fluid formed was believed to have come from the alveoli. It was felt that if recovery was to occur, this fluid would have to be absorbed chiefly by the pleural lymphatics, and the rate of absorption depended also upon respiratory movement. More recently, Staub, et al., (1967) have related the sequence of events in lung fluid changes.

In the majority of investigations concerned with pulmonary edema associated with oxygen poisoning, the criterion for the evaluation of the degree of edema has been through gross and microscopic examination of the lungs or sections at autopsy. Hemingway, et al., (1952) devised a method of determining the degree of pulmonary edema in small animals after oxygen poisoning. Guinea pigs were killed by guillotine after 48 hours or more exposure in 100% oxygen (OAP).
He determined a lung weight: body weight ratio as an index to the degree of edema (lung weight in grams/body weight in Kg). He found that after 48 hours or exposure, edema begins and increases progressively until death after 4-6 days. The lungs increase in size with the lung weight/body weight ratio more than doubling. Histologically, congestion was not uniform, but localized in regions. According to Hemingway (1952) the lung parenchyma in pulmonary edema is not increased, rather, the lung is expanded by the invasion of fluid.

An interesting observation relative to many histological studies of oxygen poisoned lungs was raised by Shanklin (1964) who stated that formalin fixation failed to preserve the proteinaceous matrix of the fluid of pulmonary edema, an effect which was thought to be compounded during alcohol dehydration or paraffin embedding. Because pulmonary edema is an important part of the lesion complex in hyaline membrane (and oxygen toxicity) disease, he recommended that Helly's fluid be used in the histological study of newborn lungs thought to be involved with hyaline membrane disease.

Penrod (1956), by cannulating one bronchus and occluding the other during the administration of 100% oxygen (OHP) found that in cats he could produce the normally described types of pathology in the open lung but not in the occluded lung. He suggested that his results tended to rule out the possibility of a blood-borne carrier as the causative mechanism for pulmonary pathology; rather, oxygen
directly affected the alveoli. Both lungs in this method were rendered atelectatic, and he concluded that the major cause of pulmonary damage produced by oxygen (OHP) was a reversible atelectasis resulting from a blockade of the smaller air passages.

Others maintained that atelectasis occurred late in the syndrome being more a result than a cause of pulmonary damage—it being primarily associated with compression by massive pleural effusion. Smith et al., (1963) showed that loss of lung compliance occurred late in oxygen exposure. Cedergren, et al., (1959) hinted at functional losses in mice that, after three days in oxygen, had a patchy thickening of the alveolar wall due either to hypertrophy or fluid accumulation in the cells. The splitting of basement membrane and fluid vacuoles between endothelial cells and membrane were also seen.

According to Schaefer, (1964) the absorption of oxygen trapped in non-ventilating alveoli is a physical process to be differentiated from the chemical effects of increased oxygen tension on lung tissue. In breathing 100% oxygen at a reduced pressure, fewer oxygen molecules have to be removed by the perfusing blood than at sea level pressure. Consequently, the collapse time of the alveoli with pure oxygen breathing is shortened to about one sixth of that at normal atmospheric pressure. McHattie and Rahn (1960) observed that mice which died under these conditions sometimes had lung collapse without pulmonary edema.
It would seem that there is general agreement on the response of the alveolar wall when a high concentration of oxygen comes in contact with the alveoli as opposed to a low concentration. With a high concentration, much of the surface epithelium is destroyed (Bowden, 1968) but the capillary is merely injured (Kistler, 1967). The result is a predominantly capillary kind of reaction consisting of exudation. With a low concentration of the irritant, the surface epithelium is perhaps injured but not destroyed, while the injury to the capillary in this case is minimal or absent. Bohm (1966) provided vascular permeability data which tend to support this theory. With lesser amounts of exudation a chronic pneumonitis results with a proliferative response of the alveolar cells. Pratt, (1965) maintains that the latter lesion may resolve and disappear with the passing of time; more recently, Evans et al., (1969), saw processes of cell renewal in the alveoli after oxygen exposure.

While the relationships of pleural effusion, atelectasis, and histological changes remain obscure, there is greater agreement when hyperbaric oxygen results are compared because the pulmonary effect is usually not as pronounced (Roth, 1967). In this case however, it may be that the toxic effect on the nervous system occurs so quickly that the animal goes down hill before there is time for the pulmonary symptoms to develop (Bean, 1945). In the case of various tensions of oxygen at normobaric pressures the situation prevails however, causing pleural effusion, peri-vascular edema, congestion, and
possible organ damage elsewhere.

The Role of Nitrogen

Nitrogen apparently provides a protective effect in our normal physiology, simply as an additional gas in the alveoli which is constant in concentration and pressure. A pneumothorax caused by pure nitrogen injection is sustained longer than one resulting from the injection of air or oxygen (Comroe, 1962). This is the case because the body tissues are saturated with nitrogen, and the blood will take up nitrogen at about one tenth the rate of oxygen absorption.

Since nitrogen is frequently not present in some OAP, OLH, and OHP studies, it may be that nitrogen lack is related to the toxic process of oxygen. Atmospheric nitrogen is generally regarded as a rather inert gas in man which is physically dissolved and constantly present in each of the body tissues and fluids. Helvey (1963) suggests that there is not enough experimental evidence on the absence of nitrogen to support a final conclusion about its importance. That nitrogen is physiologically active even though chemically inert is suggested by diving problems such as nitrogen narcosis and the bends. It may be that only trace amounts of nitrogen are required. The bodily use of trace metals suggests that a difference of 1 or 2 mm Hg nitrogen is conceivably a significant quantity (Helvey, 1963). Interestingly, trace amounts of magnesium and zinc ions are reported to protect rats to some
degree in hypoxia (Radomski, et al., 1970).

While MacHattie and Rahn (1960) raised mice through several generations in the absence of nitrogen in pure oxygen under reduced pressure, without apparent ill-effects, other investigators have shown nitrogen to be important. Volskii (1960), and Allen (1963), indicated departures from normal growth and development of the avian embryo when nitrogen was absent or helium substituted. In 1960 Volskii demonstrated fixation of atmospheric nitrogen during development of the chicken egg, and also showed that in the absence of atmospheric nitrogen it does not develop beyond five days.

Allen stressed the importance of nitrogen, although he did not agree with the Volskii theory of molecular nitrogen incorporation into the substance of the embryo. His results suggested that the vascular system in the absence of gaseous nitrogen fails to develop even though the partial pressure of oxygen is at non-toxic levels. His exposure environment also consisted of the hyperoxic situation with nitrogen replaced by helium or argon. Allen believed that the contribution of nitrogen to the problem of oxygen toxicity was a purely physical one at the molecular level rather than an organic one involving the incorporation of molecular nitrogen into the substance of the egg or developing embryo.

Weiss, et al., (1964) showed that the young chick had a greater tolerance for oxygen (OAP) than mice similarly exposed.
Older birds however, demonstrated a downhill course, although their tolerance was better than mammals. Weiss, Wright and Hiatt (1965) showed hatch of healthy chicks in a helium-oxygen atmosphere, but late embryonic death presumably reduced the hatch rate by as much as 50%. Helium chicks were also smaller, but grew similarly to air controls.

It is possible that the importance of nitrogen will be clarified by additional work in which nitrogen is replaced by another gas such as helium or neon. However, Rhoades (1966) demonstrated metabolic changes in animals exposed to the helium-oxygen atmosphere. When other gases are substituted for nitrogen, additional physical problems of heat transfer from the body surface are introduced.

While there is no evidence showing that lung tissue requires nitrogen directly, nitrogen lack appears to be at least indirectly related to the three fundamental mechanisms which lead to the collapse of an alveolus. Rahn (1963) described these as compression, absorption, and loss of surfactant atelectasis.

**Pulmonary Compliance**

**The Mechanical Properties of Lungs**

The instantaneous pressures applied to the respiratory system or its parts, are equally opposed by pressures developed within the system. These opposing pressures represent the sum of pressures arising from the physical properties of these parts,
and relate to elasticity, flow resistance, and inertia. To consider the mechanical stability of the lung as a functional unit, some of the factors which govern the static properties are considered because ultimately these determine the conditions under which the lung varies its volume with each breath (Radford, 1964). The degree of uniformity of expansion can be observed experimentally only when the space is open—for example, in studies involving excised lungs; in which there is uniformity of pressure on the pleural surface.

Donders was the first to report that lungs collapse on opening the chest because of their own elastic retraction, and that this retractive force increased as the lungs are inflated (as cited by Mead, 1969). Neergaard (1929) provided the first compliance curves for inflation and Radford (1964) summarized the retractive force of the lungs as due to elastic properties, surface tension of air-liquid interface, pulmonary blood volume, and bronchial smooth muscle. Radford stated that the stability of the lung depends upon two factors. One of these involves the surface properties of the lining layers described for extracts of the lung, and the second is due to the arrangement of the elastic elements in the mouths of the alveoli. If either of these is modified, the lung is inhomogeneous mechanically, that is, one area will apparently not be able to ventilate uniformly with another. It is the combination of geometrical hysteresis or nonreversibility of the lung dimensions, with the surface hysteresis of the pulmonary
lining substance which contributes markedly to the mechanical stability of the lung and allows a very large surface area for gas exchange.

The topic of mechanical stability of the lungs is particularly important because it unites lung morphology to lung physiology, and especially to function relating to static mechanical properties of the lungs. Hutchinson furnished the first pressure-volume curves for lungs while Cloetta, also involved with early compliance studies, believed that the lungs were best inflated by lowering the pressure around the lungs rather than by raising the tracheal pressure (as cited by Scarpelli, 1968).

Coryllos and Birnbaum (1932) are frequently cited for their studies of pulmonary gas absorption with bronchial obstruction. They maintained that the integrity of the alveolar endothelium was just as necessary as that of the pulmonary circulation, and that edema of the lung prevented gas absorption. One of the most important contributions of their work was the ability of their method to reliably produce gas free lungs.

Bayliss, et al., (1939) studied the visco-elastic properties of cat lungs. The lungs were ventilated at constant tidal volumes, and using different gases they concluded that gas viscosity could account for only a small part of the total at physiological frequencies. They found that the greater part of the resistance of the lungs to expansion was dependent on the amount of expansion
attained, and not upon the rate at which the expansion was performed. They determined that 80% of the pressure to distend the lungs related to elasticity; 15% to structural viscosity and; 5% was due to air viscosity.

Lawton, et al., (1951) reported that isolated lung-volume curves are non-linear S-shaped curves which tend to be more linear curves in situ. The "critical pressure" was a required pressure point for inflation of the air spaces to take place. They believed that below this, distension of the bronchial tree was the primary elastic response, which tended to explain the linearity of the volume and pressure relationships. A sigmoid-like curve could be approached in situ only with slow volume cycles over a wide range of pressure change.

Setnikar (1955) and Mead, et al., (1969) showed that the reported linearity was due to use of inflation curves over a limited range. To this time both segments of the curves have been recorded and analyzed and it has been noted that the number of alveoli open at a given volume effect the results. Actually, Liebermeister (as cited by Scarpelli, 1968) first noted that the volume change was small as pressure increased from the collapsed state until around 8-10 cm water pressure.

Radford, (1957, 1964) improved on the techniques of Bayliss, et al., (1939) to clearly demonstrate the P-V segments of the hysteresis loop.
The elastic recoil of the lungs became an area of great speculation in the interest of explaining hysteresis. Radford, et al., (1964) using excised cat and dog lungs reported that bronchial smooth muscle had little direct effect on lung elastic recoil. Calculation depends on consideration of the changes in free energy of the lungs as they are deflated. During inflation energy is stored by deforming elastic elements, and by the creation of a large air-liquid interface. If the lungs are deflated allowing volume equilibration at successive pressure decrements, the stored energy released during deflation can be measured. More recently, Bachofen et al., (1971) have reported improved methods for analysis of energy and total hysteresis area relationships.

Frank, (1959) studied the effects of acute reversible pulmonary vascular congestion on the elastic behavior of excised cats' lungs. Measurements were made of changes in airway pressure at constantly held lung volumes over a wide range of deflation. To produce vascular congestion, left atrial pressure was raised to 20-30 cm of water pressure. Two effects were noted; one was that the slope of the volume-pressure relations of the lungs was slightly reduced at all levels of deflation; the effect of vascular congestion on the recoiling force of the lungs was a function of the volume of the lungs at which congestion was induced. At large volumes, recoiling force was increased; at intermediate volumes the change was negligible and, at smaller
volumes it was reduced in a manner consistent with the idea that there is a volume of the lungs at which the lungs and blood vessels exert least mechanical stress on each other. This volume was believed to lie close to that in which tidal breathing occurs. According to Frank, (1959):

Vascular congestion leads to an apparent stiffening of the lungs. When edema forms, the effect is exaggerated due to surface phenomena. Vascular congestion by itself had limited effect on the elastic behavior of the lungs.

Pulmonary Compliance in Rats

Rahn, et al., (1963) maintained that an important part of our present knowledge of the elastic properties of lungs has been drawn from experiments on excised lungs. The term static is used in relation to equilibrium conditions which are not possible in the living or dynamic preparation because it is not possible to maintain the lungs at a given state of expansion long enough to allow true equilibrium of all the stresses within them (Radford, 1964). Radford was a proponent of excised lung measurements with the following qualifications:

The principal justification for the application of results of excised lung experiments to living animals is that mechanical characteristics of excised lungs are comparable to those observed in living animals providing that the range of volume change is similar in the two cases.

Pulmonary compliance, defined simply as a volume increase for each unit increase in intra-alveolar pressure has been measured and analyzed in many different ways. Most feel that it is an
accurate expression of the elastic-like properties of the lung if it is measured at points on a pressure-volume curve at which air is not moving into or out of the lung. These points of no air-flow occur near the end of inspiration and again near the end of expiration. Compliance does not include air resistive changes which are the result of friction between the airstream and bronchial wall, turbulence, and other aerodynamic factors. Studies of lung compliance should however yield information about resistance and pulmonary mechanics which, though separate, are influenced by compliance. Values for compliance depend on the initial volume of the lungs, as well as the size of the individual. Comroe (1962) has pointed out that within animals of the same size pulmonary compliance varies with the initial volume of the alveoli which are to be distended by applied transpulmonary pressure.

King (1966) measured pulmonary compliance in the rat under static conditions and reported the following values:

The mean value for compliance was 0.25ml/cm water. Upper airway resistance was estimated to be 53% of the total pulmonary flow resistance. Older non-pathogen-free animals had significantly higher pulmonary resistances than either pathogen-free or younger non-pathogen-free rats.

Stahl (1967) collected data from the literature on respiratory variables and correlated them against body weight on the assumption of an allometric relationship. For the 250g rat he reported
the following respiratory values:

Total lung capacity, 8-11 ml., Functional residual capacity
1-2 ml., tidal volume, 1.5-1.8 ml., Respiration rate, 45-105/minute, and lung compliance 0.30-0.60 ml/cm. water.

The stretching of lungs appears to increase their distensibility and Mead (1970) found that compliance tends to decrease with time, although these reductions could be reversed by single near maximum inflations. The deflation curve from any volume or pressure represents the truly elastic behavior of all those alveoli which have been inflated up to that volume or pressure, although according to Mead, the inflation curves represent the combination of two processes, elastic expansion and recruitment of alveoli (with a change of curvature at the point at which the second process begins).

Mount (1955) was one of the first to record pressure-volume relationships simultaneously:

A respiration pump with inflation pressures of 5-10 cm water was used to produce pressure-volume curves using open chested rats. The output of the manometer circuit fed into the Y-axis of an oscilloscope, while sinusoidal variation in lung volume was recorded directly as a varying voltage from the piston of the sinusoidal pump. Thus, instantaneous values of the pressure-volume coordinates were obtained on the oscilloscope, and the resulting figure was photographed. Lung compliance was indicated by the difference between pressures measured at instants of no gas-flow in the trachea. Compliance values and pressure increased slowly with frequency increase of the sinusoidal pump. The results indicated a large structural viscance in addition to air viscance and elastance components.

**Compliance Studies of Excised Rat Lungs**

Several studies have involved changes in compliance as
measured in excised rat lungs following exposure to various kinds of toxic environments. These are of interest here mainly because of the various methods employed.

Alarie, et al., (1961) studied the influence of microaerosols on P-V curves of excised atelectatic rats' lungs:

One ml of air was injected over a period of 15 seconds with 3 minutes of equilibration time after each injection. A total of 13 ml of air was injected into each set of lungs, with a filling curve plotted by connecting the various pressure readings recorded after the injection of each ml of air. The emptying curve was obtained by releasing the 13 ml of air previously injected into the lungs (1ml/min) and recording the pressure every minute rather than each three minutes.

Their results indicated that substances constricting the respiratory airways, administered in submicronic particles prior to the production of atelectasis, increased the lungs' resistance to the penetration of a given volume of air. Also, dilating substances decreased the resistance to the opening of the lungs while increasing the volume of air required to reach a given intrapulmonary pressure (i.e., bronchiolar dilatation).

Robillard (1964) compared static emptying curves of air-filled and saline-filled lungs to measure both the force due to tissue elasticity and that due to surface tension of the alveolar lining. They found a decrease in surface tension in rats that had breathed aluminum dust similar to Bondurant (1965) who found that the surface tension of extracts of alveolar lining was reduced in animals that had been exposed to cigarette smoke. Robillard (1964) speculated that the decrease in surface tension of the alveolar lining might
serve to protect the lungs against edema. On the other hand, it might also tend to decrease the passive recoil of the lungs, and thus necessitate increased muscular effort during expiration at rest.

Pattle, et al., (1965) found that when isolated rat lungs had been ventilated for a longer period of time during the course of an experiment, both compliance and hysteresis showed a decrease. It was also noted that these changes were more marked with larger tidal volume and lower end-expiratory pressure.

Miller and Bondurant (1962) found cigarette smoke to cause a decrease in surface tension and an increase in surface compressibility. Schoedel (1965) reported similar results with methods which are of interest.

Lung weight and volume were determined before and after recording of the P-V diagrams. These were accomplished at room temperature, and before diagramming the data curves each lung was briefly inflated to remove all observable atelectasis. Inflation was begun at 0 cm water pressure. The lungs were inflated with a syringe to 20 cm water and then emptied at the same speed in a total time of about 20 minutes. The lungs were then inflated rapidly (11 times/min) during intermittent smoke exposure. Average curves were used to show that a disruption of surface forces leads to a decrease in hysteresis, volume lag, and pressure lag with volume change on the deflation side of the loop. He accounted for the fact that the volume of the lungs was greater after taking several P-V diagrams using several methods developed by Clements (1962).

There is a tendency of atmospheric pollutants, in high concentrations to cause pulmonary edema, and in low concentration some may cause chronic pneumonitis. This is of interest because short term toxic environments seem to have a similar effect, and investigators
also considered pathogen-free (King, 1966; Wright, et al., 1966) and age (Saxton, et al., 1946; King, 1966) factors in reporting the mechanical properties of rat lungs. More recently, Somerson, et al., (1971) reported decreased compliance with infection in the rat.

**Oxygen Toxicity and Compliance**

Decreased compliance of from 6 to 50% has been reported in man (Burger, 1967) and this tendency has also been reported in most animals thus far studied including rats (Brooksby, et al., 1966) dogs (Smith, et al., 1963) and rabbits (Rosengren, 1967).

Beckman (1967) and Beckman and Weiss (1969) using air and saline pressure-volume curves on excised rat lungs after 60-66 hours in pure oxygen at 1 atmosphere found a 62% decrease in compliance which correlated with decreased surfactant and an increased tissue rigidity. More recently, Norman, et al., (1971) reported that P-V and Wilhelmy balance studies revealed a reduction in surfactant activity and decreased compliance associated with severe degrees of lung damage in rats exposed to oxygen at 2 atmospheres. Also, addition of nitrogen delayed the onset of pulmonary changes in this study.

While there is general agreement that exposure to one atmosphere of oxygen for prolonged periods leads to edema and death, some differences in surfactant levels recorded are undoubtedly due to different techniques of surfactant extraction (Giammona,
Different effects on surfactant, compliance, edema formation, and general survivability may also be due to various degrees of adaptation in those situations where pure oxygen is not used continuously or where there is an interuption or change in the oxygen environment (Yamamoto, et al., 1970).
METHODS

General Procedures

Compliance determined from air pressure-volume curves and histology by light microscopy were used to study the effects of oxygen toxicity on rat lungs. Although the use of saline P-V curves and lavage for surfactant extraction could have provided additional information on the pulmonary aspects of oxygen toxicity (Beckman and Weiss, 1969), these procedures were not carried out because it was felt that fluid in the alveoli could produce artifacts that would influence the subsequent histological examination. The use of air alone would also make the results on the rat more comparable to the earlier histological observations of Pratt (1965) in humans in which the lungs had been inflated with air after death prior to sectioning.

The environmental conditions outlined below evolved out of efforts to produce lesions similar to those observed in humans in animals at constant and increasing oxygen levels and included:

1. 100% oxygen at one atmosphere of pressure for 48, 60, and 72 hours (acute, normobaric).
2. 40% oxygen at one atmosphere of pressure for four days increasing to 60% oxygen for four days (40-60% stepwise increments).
3. 60% oxygen at one atmosphere of pressure for ten days (chronic, continuous).
4. 100% oxygen at 0.6 atmosphere for ten days (no inert gas).
5. 100% oxygen at 0.8 atmosphere for 12 days (chronic, continuous).

6. 40% oxygen at one atmosphere for four days increasing to 60% and then 80% for four days each for a total of 12 days (40-60-80% stepwise increments).

7. Animals subjected to aspiration of 0.8% saline.

These seven experimental conditions are analyzed either separately or combined in relation to controls or to each other, in experiments labeled I - V of the Results section.

**Animals**

Mature, male, Wistar rats supplied through the Ohio State University animal facilities from the Maxfield Animal Supply Company, Cincinnati, Ohio, were used throughout this study. Rats have been used widely for oxygen toxicity studies because of their availability, susceptibility, and ease of handling. The animals were caged for observation for at least 3-5 days prior to experimental use. Controls and experimental animals were paired by weight as closely as possible, and varied from 250-300 grams at the beginning of exposure to the various environmental conditions.

Younger, lighter rats were used in initial pilot runs, but it was found that there was greater variability in response with some animals showing apparent resistance to oxygen toxicity. Older rats, weighing more than 350 grams usually have shorter life spans in 100% oxygen, possibly because of the presence of other pathological conditions. Therefore, it was decided to use rats of at least 250 grams and no more than 300 grams in weight.

While gnotobiotic type isolators were used in some of the experiments no attempt was made to maintain germ-free conditions.
Wright (1965) showed that germ-free conditions did not increase the ability of rats to survive when exposed to pure oxygen. Nevertheless, diseased lungs (bronchial pneumonia, most commonly) were identified by their spotted or granular appearance and eliminated (Figure 1).

All animals were weighed before and after experimental runs or control confinement. They were individually identified by ear-nicks given at the time of initial weighing. Caging, lighting, watering, and feeding (Ralston Purina micromixed laboratory chow) of experimental and control animals were similar, as were conditions of temperature, relative humidity, and air circulation. The animals were caged in heavy galvanized wire cages suspended above Liter-keep or aluminum foil sprinkled with boric acid powder (to neutralize the urea). Urine and fecal material were removed daily.

**Oxygen and Pressure Environment**

The environmental exposure units consisted of a 22 x 48 x 66 inch rectangular plexi-glass box, similar to that employed by Dines and Hiatt (1964), shown in figures 2 and 3, for conditions of increased oxygen percentage at one atmosphere of pressure. A polyvinyl isolator (Figure 4) was used inside a large low pressure chamber to provide 100% oxygen at less than one atmosphere. The isolator measured 4 x 3 x 2 feet, and was identical to those of Weiss, et al., (1964). Both the rigid box and flexible isolator were maintained at a slight positive pressure by connection via
Figure 1

Grossly Normal Lungs

Diseased Lungs
oxygen mask regulators to cylinders of compressed, dry, 99.5% pure, USP medical oxygen. All leaks were outboard.

In pure oxygen runs at reduced pressure, the animals were placed inside the polyvinyl system, and the low pressure chamber was sealed and taken to 0.6 or 0.8 of an atmosphere (445 or 600 mm Hg.). It was necessary to bring the chamber to ground level pressure once daily for approximately thirty minutes in order to observe and care for the animals. Pressure changes were gradual and never faster than one thousand feet per minute to avoid decompression sickness. Both types of exposure units were fitted with rubber gloves, for easy accessibility to the animals, and contained ports and gas locks so that rats and supplies could be inserted or removed while still maintaining the desired atmosphere.

The large low pressure chamber was monitored by a Chase Brothers A-1 mercurial atmospheric barometer, and maintained at the prescribed pressure by a Barksdale vacuum activator switch which periodically activated a Minneapolis Honeywell magnetic gas dump valve. Vacuum was provided continuously to the large low pressure chamber by a Beach Ross rotary pump (model 6D) which was powered by a 7½ HP Baldor electric motor. TCP oil fumes from the rotary pump were vented out of the building by an overhead sealed exhaust pipe system. During ascent, excess gas from the isolator was vented through a sampling line to the outside of the chamber.

Air circulation within the animal environment units was maintained by Muffin fans (14 watt, 50-60 cps). Carbon dioxide
was absorbed from these relatively sealed units by pumping inside gases continuously through an externally located cylinder of soda lime and then back into the isolator, or by absorption on trays of soda lime placed inside the units. Either method kept CO₂ below 0.5%, according to periodic CO₂ analysis accomplished by sampling the environment with a Spinco (Model LB-1) CO₂ medical gas analyzer. A Beckman (Model E-2) oxygen analyzer in series with the CO₂ analyzer provided the oxygen percentage. All environmental variables were read and recorded at least three times daily.

In experiments where the percentage of oxygen was varied rather than the pressure, the desired partial pressure of oxygen was automatically regulated with a Space/Defense Corporation (ESS-DEE) two gas atmospheric controller, powered by a Trygon (T 50-750; 0-50 volt) DC power supply unit.

Excess moisture was removed by passing environmental gases through a copper tube condenser unit cooled by a refrigerated anti-freeze solution. Movement of gases through the condenser was accomplished by an American Standard blower powered by a General Electric explosion proof, ozone free, induction motor. Relative humidity and temperature were determined by Abbeon and Bacharach instruments which were similarly calibrated for the plexi-glass box and polyvinyl isolator. Temperature and humidity fluctuations within the laboratory were moderate, ranging from 18-26 °C and 40-65 percent respectively throughout the experiments. There was
a smaller range within individual runs.

**Preparation of Rat Lungs**

Pressure-volume curves were run on the excised lungs of all rats exposed to the various environments and on corresponding controls of approximately the same weight prior to preparation of the tissues for histological examination. The rats were sacrificed by an overdose of Sodium Pentabarbital ("Diabtal," 60mg) 1 ml IP after their withdrawal from the environment. The throat was opened before breathing movements ceased, and the trachea exposed. A string was placed loosely around the trachea until breathing stopped and then tightened after opening the chest. The lungs were thus collapsed to atmospheric pressure but not atelectatic. Two cuts were then made laterally in the rib cage, resulting in a large Y-shaped opening through which the lungs could be removed with less danger of accidental puncture by cut ribs or instruments. Lung and trachea removal was a delicate process requiring considerable care to avoid punctures (if this did occur, leaking lungs were discarded when so identified by their abnormal P-V curves).

After the lung and trachea were removed, they were weighed in air and their buoyancy in saline was determined. The heart and pulmonary vessels were left attached and intact to lessen the chance of accidental puncture or fluid entry, similar to the methods of Lawton (1951). Heart tissue was removed later and weighed to
provide a correction for true lung weight. The string used to tie the trachea (as far superior as possible) also prevented fluid entry into the lungs and served as a tie point for saline buoyancy measurement. In the approximate 15 minutes of procedure just described, the lungs were considered to be in equilibrium with room temperature.

After weighing, the tracheal ligature was removed, and a small teflon tipped cannula slipped into the trachea. The trachea was tied securely to the cannula with two ligatures to insure a good seal. The tip of the cannula was well above any bifurcation to insure even inflation of all lobes of the lung simultaneously.

**Continuously Recorded Compliance Measurements**

The lungs with tracheal cannula in place were suspended in a 150ml rubber-stoppered bottle. A connection was made through the stopper from the cannula to a volume recorder (Med-Science, Model 160) which measured the volume of air into or out of the lungs in response to a reduction or increase of pressure around the lungs produced by an automatic infusion syringe (Colson Constant Flow System, Model 1055). Thus, when the automatic syringe removed air from the jar, causing the lungs to inflate, air was drawn in from the volume recorder. The bottle had approximately 1/2 inch of saline in the bottom to maintain humidification and thus prevent drying of the tissues.

Pressure was detected from a T connection in the line between
the syringe and the jar by both a water manometer and a Statham Strain Gauge pressure transducer (Model P23B). As air moved from around the lungs, a negative pressure was produced, but by changing the polarization this was registered on the records as relative positive pressure within the lungs. Intra-lung pressure however, remained essentially atmospheric, in accordance with the design of the volume meter. A cartesian diver in the volume meter sensed a pressure change of less than 1mm H₂O and activated a piston and cylinder servo mechanism to either deliver or remove air from the lungs until the pressure returned to ambient.

A linear transducer was adapted to the sliding arm of the piston, and the output of the transducer was transmitted to the Y axis of a Mosely Autograph X-Y recorder (Model 3S). Pressure output from the Statham Strain gauge transducer was connected to the X axis through a Grass (Model 5B) amplifier. Thus, pressure-volume curves (i.e., hysteresis loops or compliance curves) were plotted automatically as the lungs were inflated and deflated. A Grass ink-writing oscillograph was included to provide a separate time-pressure record, while the pen on the sliding arm of the volume recorder also provided separate volume record for cross-check and calibration purposes. A block diagram of this apparatus is presented in Figure 5, and a picture of the complete system in Figure 6.

Before each group of lungs (usually about four were run each day) were tested for compliance, the X-Y recorder, volume meter, and Grass
Block Diagram of Apparatus for Automatic Registration of Pressure—Volume Curves from Excised Rat Lungs

Figure 5
polygraph were calibrated by withdrawal and injection of air by calibrated syringe from or into the lung bottle. Pressure was calibrated by the water manometer and volume by the syringe displacement.

After calibration, the lungs were pre-inflated to 15 cm H$_2$O transpulmonary pressure and returned to ambient pressure before compliance curves were recorded, similar to the methods of Pratt (1961) in his studies of human lung compliance. This procedure served to provide a check for uniform inflation, detection of leakers, and avoidance of atelectasis. The lungs were then inflated to 20 cm water and returned to ambient pressure in a period of approximately 2$\frac{1}{2}$ minutes. This cycle was followed by two faster inflation-deflation cycles of approximately 1$\frac{1}{2}$ minutes duration each. The two inflations at the same rate of pressure change were accomplished to determine the reproducibility of the pressure-volume curves under the conditions and methods employed. The temperature around the system was controlled within a 3°F range, and averaged 80°F. The time required for pre-inflation and production of three compliance curves was approximately 60 minutes from sacrifice of the animal.

It was found that each successive inflation tended to trap a small amount of air in the lungs; to compensate for this, the X-Y recorder was brought back to zero before each inflation. To determine the relationship between initial air trapped and final air trapped, and the distensibility of the lungs, the lungs were again
Weighed in saline after completion of the compliance curves. After removal of the lungs from the weighing in saline, they were blotted gently to remove excess moisture, and then weighed in air utilizing an Ainsworth "Right-A-Weigh" single pan balance.

In an earlier series of approximately thirty-five separate experiments, it was determined that with our apparatus the traditional stepwise lung inflation-deflation method of compliance measurement with an equilibration period of 15–60 seconds at each step produced an irregular compliance loop that was consistently similar to the continuous curve. When a line was drawn between points of equilibration recorded at each end-time interval, the slope was not significantly different than a continuously recorded line. It was felt therefore, that a continuous P-V curve was satisfactory for determining "quasi static" compliance.

**Residual Air in the Lungs**

Lung density data were obtained for use in analysis with various aspects of the compliance curve. Presumably, lungs which are less distensible after oxygen exposure due to loss of surfactant, congestion, or edema, will attain a lesser volume at maximum pressure (20 cm H$_2$O), and also trap a smaller amount of air after the inflation-deflation cycles. Trapped air should show itself in two ways: The point at which the retraction curve returns to the Y axis upon reaching ambient pressure; and the difference in buoyancy of the lungs weighed before and after inflation.
The process for determining trapped air was as follows: A beam balance sensitive to 0.01 grams (Dial-O-Gram, Ohaus) was slightly modified by removing the pan and attaching in its place a wire loop. On the end of the wire a weight (60g) was attached, centered below a hook used to attach the lungs; the end of the wire loop with the weight and the lungs was immersed in a beaker of saline (Baxter "Tis-U-Sol"). This gave a tare value. Thus, with the lungs attached they tended to push upward if they were buoyant and hence decrease the weight of the system. The change in weight of the system was recorded as a measure of air in the lungs at the time of measurement. Frank (1963) used a procedure similar to this; he estimated the lung tissue specific gravity to be 1.065. Although the specific gravity of the immersant we used was less (about 1.01) it was felt that this introduced a negligible error in the measurements and residual air volume was calculated on the basis of one ml per gram weight decrease.

Rate of Filling; Maximum Pressures; Volumes

In order to establish guidelines and a standardization among all P-V curves so that the results could be later analyzed and compared statistically, maximum pressure and volume limits were established. Other investigators have filled gas-free rat lungs to as high as 30 cm water pressure (Saxton, et al., 1946; Lawton, et al., 1951) and reported on only those lungs which did not leak. Our early trial and error efforts showed that when lungs were
filled with air beyond approximately 20 to 25 cm water pressure, they tended to develop leaks. Although many lungs remained intact, it was felt that this selective process could influence the results.

It was thus decided that a maximum pressure of 20 cm water was adequate to give a large range of expansion and still avoid leaks due to over-inflation. When this pressure was reached, the automatic syringe was reversed so that the pressure within the lung immediately began to fall. The maximum volume reached depended on the compliance (volume change per unit of pressure change) for the individual lung.

The rate of filling was set relatively low in comparison to the normal respiration rate of the rat in order to avoid airway resistance and tissue inertia forces. The normal rate of respiration in the rat is between 50-60 breaths per minute (Rhoades, 1966). The time required to complete one average air pressure-volume curve was approximately 120-180 seconds or at an estimated rate of 1/2 or 1/3 breaths per minute respectively. The rate of syringe volume change was 2.7 or 5.4 cc per minute, but it should be noted that while syringe movement was constant, filling time for an individual set of lungs is variable being dependent on the size and the compliance.

Typical laboratory recordings of compliance curves for a normal control rat are shown in Figures 7 and 8. In order to later determine the sequence of curves drawn, a color code was
Curves 1 and 2

- Normal Rat - Control: 291 gm.
- Identification: D 14
- Lung-Heart Weight Air: 3.606 - 1.678 = 2.12g
- Buoyancy in Saline: Frame Wt: 60.03 (Before) Lung + Frame Wt: 59.18
  (After) Lung + Frame Wt: 59.22
- Heart + Ext. Tissue Wt: 1.928 - 1.478 = 0.450g.

Date: 9/27/65
Temp: 80°F

Figure 7
used changing the X-Y recorder pen twice on each set of lungs with curves 1 (green) and 2 (red) drawn on one side of the graph paper and curves 3 (red) and 4 (green) on a second sheet.

Using this system, compliance from beginning to end inflation (0-20 cm water pressure) in normal animals ranged from 0.26 to 0.36 ml/cm water, similar to the mean value for the range of compliance in rats reported by King (1966) and Stahl (1967).

Analysis of the results of preliminary studies with this P-V apparatus involving approximately 100 experiments with rats weighing from 98-385g led to the conclusion that the continuous method of compliance measurement was satisfactory for the following reasons:

1. The classical hysteresis loop was produced by normal lungs, just excised, without plotting pressure and volume information separately.

2. These curves were reproducible at various speeds and produced slopes not significantly different whether done stepwise or continuously. Four curves could be produced by the same set of lungs without causing overstretching or leaks.

3. Averaged curves 3 and 4 were nearly identical, and found to be not significantly different from each other. Since all lungs were given the same sequence of events, and because the fourth inflation-deflation loop was recorded just before the last buoyancy measurement, only this last drawn mean loop was analyzed in the Results section.

Rats Subjected to Saline Aspiration

One group of unanesthetized animals of similar age and weight were sacrificed by asphyxiation accomplished by total immersion in a 0.85% saline solution at room temperature as in
methods reported by De Boer, et al., (1970). A glass chamber of approximately 35 x 60 cm with water inlet at the bottom and gas outlet at the top was filled with saline in about 60 seconds. The animals were not restrained but rapid flooding minimized movement prior to death. About 5-10 breaths under water were observed prior to cessation of respiratory efforts. The animal was removed after about two minutes and the lungs excised with subsequent procedure similar to that of the other experimental groups.

Preparation of Tissues for Histology

After compliance measurements and weighing, the lungs were fixed in 10% formalin solution for later histological preparation. One side was additionally infused with fixative: the right main bronchus was tied off close to the trachea, sealing off the anterior, median, posterior, and post caval lobes (or collectively the right side). Fixative was then injected via the trachea until the left lobe, which is a single large lobe in the rat, reached moderate distension no greater than its estimated size at maximal inspiration in vivo. Before the injecting syringe was removed, a ligature was tied around the trachea so that fixative would remain entrapped during submersion of the complete lungs in formalin. A fixative soaked gauze pad was then placed over the lungs to insure that all parts were in contact with the solution. Heart and associated extraneous tissues were removed just prior to fixation, weighed and discarded.
Lung tissue was later prepared for histological examination by light microscopy under the supervision and guidance of Dr. Philip C. Pratt, Department of Pathology, of the Ohio Tuberculosis Hospital. (Dr. Pratt is presently with the Duke University Medical Center, Durham, North Carolina). After the usual dehydration and embedding procedures, paraffin sections were cut at six microns and stained with hemotoxylin and eosin.

During histological examination, observations of one section through the left lobe of the lung tissue, were scored on a 0-3 point system for each of four characteristics: 1. Perivascular edema; 2. Capillary congestion; 3. Alveolar septal widening (with proliferation if noted as only + or -) and 4. Infection. If pneumonia were detected, the slide for that animal was removed. An average of the subjectively estimated numerical ratings was accomplished to provide an overall numerical index for the degree of pathological findings for the group of animals from a particular environment for later comparison to functional values.

In Figure 9 the photomicroscopy illustration represents the scoring for perivascular edema based on an overall inspection of the microscopic field at 100X and a closer observation of certain areas at 400X. The photomicroscopy of Figure 10 is shown at 400X and represents scoring of capillary congestion and alveolar septal thickening.
This illustration (100X) represents scoring for perivascular edema of approximately 2.0 seen in animals exposed to 100% oxygen at 1 atmosphere for 60 hours. The average score for the group was 1.70 ± 0.15 and was intermediate between 1.63 ± 0.17 seen at 48 hours and 1.81 ± 0.18 seen at 72 hours; (see Table 1, Results).
This photo illustrates capillary congestion and alveolar thickening as seen in the 40-60-80% increasing oxygen (Experiment IV). The congested capillaries are best seen in the septa along the lower margin of the field, but are present in varying degrees throughout. The darkest ovoid bodies are cell nuclei; the lighter gray ones are erythrocytes, all of which are situated within capillary lumens. This field itself would rate 3+ congestion and 2+ alveolar thickening, but the extent of the change, or the relative amount of lung tissue involved would be included in the overall estimate of the degree recorded for the whole animal.

It is impossible to completely illustrate all of the details in this method of estimating the intensity of reactions. Changes are usually not uniform throughout the tissues, for example, some alveolar septa here are thickened considerably less (400X).
Histology values for the three criteria (indication of infection was used to eliminate the animal from consideration) and composite scores were compared using non-parametric statistical methods. For each individual criterion i.e., perivascular edema, capillary congestion, or alveolar thickening, the scores ranged from 0-3, and for the composite score the possible range was 0-9. Where statistical differences in histology were indicated, the results were based on comparison to controls by non-parametric methods of Fisher's Exact Test for Probability using Bradley's (1968) tables of distribution free statistical tests (as cited by Snedecor, 1967).

**Data Analysis**

Three P-V curves from each set of lungs were analyzed initially by measuring the volume to 1/10 of an inch every 0.5 inch along the X (pressure) axis. (Metric measurement was not used initially because the metric graph paper available at the time was of a softer texture and difficult to work with). Volume values in inches were measured for the ascending (inflation curve to the right, and descending (deflation) curve to the left (note arrows on Figure 7) and values transferred to punch cards for computation and analysis on the IBM 7094 system including the 1620 computer and attached 1627 plotter.

Calibration was such that on the Y axis (ordinate) 6 inches provided for a range of 0-12 ml of air volume, or 1 ml volume for
each \(\frac{1}{2}\) inch of verticle displacement, while on the X axis (abscissa) 7 inches provided for a range of 0-20cm of water pressure, or 1 cm water pressure for each \(\frac{7}{20}\) inch of horizontal displacement. The plotter system produced a hysteresis loop or compliance curves based on the average results of eight animals and related controls for each experimental condition. All curves were so plotted, but only the last drawn (or curve 4) was analyzed in detail as mentioned before.

Figures presented in the results section have one additional modification to reduce the error due to edema, atelectasis, or varying degrees of trapped air. Similar to the methods of Beckman and Weiss (1969) and Somerson et al. (1971), the X-axis was calibrated in relation to total lung volume instead of the filling volume. The total lung volume was obtained as follows: To each volume of change due to filling or emptying there was added a volume representing the corrected lung weight (assuming a density of one) and a volume representing retained gas (measured as buoyancy in 0.9 percent saline immediately after the last inflation-deflation cycle).

Data taken from individual graphs and records were run on a Wright-Patterson AFB computer for determination of mean, standard deviation, and standard error, with respect to the following: Body weight before (at the beginning of experimental confinement) and after (at the time of sacrifice); Lung weight;
Lung Weight ratio; Lung buoyancy; Static compliance (for points of theoretical momentary non-flow) between 0 and 20cm water; Slope at 10cm water Inflation and Deflation (to provide compliance at the mid point of inflation and deflation) Volume % 10/20 (the volume at 10 cm water pressure deflation/the volume at 20cm water pressure inflation) to provide information about the contractive characteristics and stabilities of the lungs; and Area of the P-V or hysteresis loops.

Snedecor (1967) was used as a statistical reference for the analysis of variance, including F ratio and D values (used to indicate whether one mean differed more than by chance from the average of control or another group means for the .05 and .01 levels; eg., Tukey’s D contrast or comparison) for significant differences, correlations, and regressions. When data from the same animal taken at different times were compared (eg., Body weight before and after exposure), the paired difference method, using an Olivetti Underwood computer program, was employed.
RESULTS

I. Acute Normobaric Oxygen Toxicity: Comparison of Normal and Experimental Rats Exposed to 100% Oxygen at 1 Atmosphere of Pressure for 48, 60, and 72 hours.

The animals showed typical dyspnea upon removal from the chambers; two from the 72 hour group expired upon withdrawal before sacrifice and were discarded. As indicated in Table 1, mean animal weight for the four groups was not different at the beginning of the experiment. (In Table 1, "start" and "end" differentiate body weight at the beginning of exposure from that taken just after sacrifice. For controls, "end" represents the weight after 72 hours in room air). It can be seen that loss in weight had begun at 48 hours and was progressively more apparent at 60 and 72 hours. Nevertheless these weight losses are relatively moderate, reaching a maximum of 7.4% at 72 hours.

Lung weight increased progressively with exposure, reaching a two-fold increase by 72 hours. The lung weight/body weight ratio also shows significantly marked and progressing elevation at each extension of exposure, reflecting primarily the lung weight increase but also the simultaneous loss of body weight. Lung buoyancy for the 48 hour animals was slightly greater than controls, but was less than controls at 60 and 72 hours. While analysis of variance (ANOVA) indicated that buoyancy differences

60
TABLE 1

COMPARISON OF CONTROL AND EXPERIMENTAL RATS EXPOSED TO 100% OXYGEN AT ONE ATMOSPHERE OF PRESSURE FOR 48, 60, AND 72 HRS

<table>
<thead>
<tr>
<th>Variable Measured</th>
<th>Control</th>
<th>48 hrs.</th>
<th>60 hrs.</th>
<th>72 hrs.</th>
<th>P Value from ANOVA</th>
<th>Tukey's D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td>272±8.6</td>
<td>279±8.5</td>
<td>273±6.0</td>
<td>283±5.1</td>
<td>NS</td>
<td>0.56/0.66</td>
</tr>
<tr>
<td>Lung Weight (g)</td>
<td>270±7.5</td>
<td>274±6.1</td>
<td>265±5.1</td>
<td>265±4.6</td>
<td>NS</td>
<td>1.7/1.6</td>
</tr>
<tr>
<td>Lung wt (g/kg)</td>
<td>1.5±0.072</td>
<td>2.1±0.023</td>
<td>2.6±0.033</td>
<td>3.2±0.109</td>
<td>.01</td>
<td>0.56/0.66</td>
</tr>
<tr>
<td>Body wt (g/kg)</td>
<td>5.2±0.22</td>
<td>7.2±0.219</td>
<td>9.6±0.311</td>
<td>12.0±0.189</td>
<td>.01</td>
<td>1.7/1.6</td>
</tr>
<tr>
<td>Lung (ml)</td>
<td>279±9.5</td>
<td>287±6.1</td>
<td>263±5.1</td>
<td>261±6.6</td>
<td>NS</td>
<td>1.7/1.6</td>
</tr>
<tr>
<td>Buoyancy</td>
<td>1.3±0.25</td>
<td>1.4±0.21</td>
<td>0.9±0.24</td>
<td>0.9±0.11</td>
<td>NS</td>
<td>1.7/1.6</td>
</tr>
<tr>
<td>Total Lung vol (ml)</td>
<td>2.8±0.16</td>
<td>3.6±0.219</td>
<td>3.5±0.239</td>
<td>4.1±0.269</td>
<td>.01</td>
<td>0.54/0.63</td>
</tr>
</tbody>
</table>

*Significantly Different from Controls at the .05 and .01 levels, Respectively.
were not statistically significant, buoyancy of 48 hour lungs was greater than for 60 or 72 hours.

Average P–V curves for the four groups are shown in Figure 11 and the compliances derived from them are listed in Table 1. To reduce error due to edema or atelectasis, the Y-axis was calibrated in terms of total lung volume instead of filling volume. The total lung volume at the start of inflation was obtained by adding the retained air volume (measured as end buoyancy in 0.9% saline) to the corrected lung weight in air (assuming a density of one) taken just prior to fixation of the tissues for histology. All subsequent volumes were obtained by adding to the starting value the volume of air infused.

It can be seen in Figure 11 that all the P–V curves fall within the total lung volume range of the controls and therefore that the compliance values are not likely to be distorted by stretch or over-distension of lung tissue. It can also be seen that while the 48 hour curve is elevated above the control, the two follow fairly parallel courses over most of inflation and deflation. As expected, then, compliance values differ only slightly between controls and the 48 hour animals (Table 1, under Compliance 0–20). By contrast, 60 and 72 hour lungs could not be inflated to the levels of the control lungs, and their P–V curves are considerably flattened. Compliance for the 60 and 72 hour lungs is as expected, significantly reduced on the order of 50%, not only for the 0–20cm range, but also for the 10cm points of
Figure 11

Pressure - Volume Curves of Control and Experimental Rats Exposed to 100% Oxygen at 1 ATM of Pressure for 48, 60, and 72 Hours
both inflation and deflation. The reduction of compliance in the 0–10cm range during deflation is particularly marked.

In contrast to the decreased compliance, lung stability, estimated as % of maximum volume retained at 10cm water deflation, suggests improvement with 60 and 72 hours of oxygen exposure. Similar conflicting results between compliance and stability index have been found before (Gruenwald, 1965; Beckman and Weiss, 1969) and have brought forth suggestions that lung stability estimates based on % retained volume are unreliable in rats. Throughout these experiments it was found to be contradictory and consequently was eliminated from subsequent analyses.

Area of the P-V hysteresis loop is increased in the 48 hour animals relative to controls and decreased in the 60 and 72 hour lungs.

In contrast to slightly better compliance at 48 hours, histology indicates at this time a marked increase in perivascular edema and a lesser increase in alveolar thickening, but little change in capillary congestion (Table 1; Figures 12-14). Apparently the early edematous tissue changes are not great enough to effect a decreased compliance at 48 hours. At 60 hours (Table 1 and Figure 9) there are further increases in perivascular edema and alveolar thickening and a slight increase in capillary congestion, although these last mentioned histological changes appear relatively small compared to the 50% or greater decrease in
Control rat. No exposure to oxygen. The field includes a small bronchus and an associated pulmonary artery as well as alveolar ducts and alveoli. All structures are within normal limits for comparison with other illustrations (100X).
Figure 13

PHOTOMICROSCOPY 48 HOURS EXPOSURE TO 100% OXYGEN AT 1 ATM

Rat, killed after 48 hours exposure to 100% oxygen at 1 atm. The field includes the same structures as in the control. The only apparent reaction is widening of the adventitia of the artery by edema fluid (100X).
Rat, killed after 72 hours of exposure to 100% oxygen at 1 atm. Perivascular edema is still more marked. The cells in the adventitial space include polymorphs and fibroblasts (100X).
compliance which occurred over the same interval. Between 60 and 72 hours, the most marked histological effects are a 70% further increase in both capillary congestion and alveolar thickening. These changes are associated with only rather small further decreases in compliance over that at 60 hours.

Some of the discrepancies between observed tissue changes and measured changes in compliance (Table 1) are exemplified by the composite histology scores: by 48 hours the composite score has about doubled whereas overall compliance (0-20) is, if anything, slightly improved. Between 48 and 60 hours, the composite score only increased by 10%, but overall compliance is halved. Between 60 and 72 hours however, the two variables change more similarly: composite histology has increased a further 31% and overall compliance decreased an additional 33%. On the whole, lung weights changed in the same manner as did histological scoring.

Because the rat is subject to respiratory infections, this was also scored in the histology for each individual animal throughout the study. Where traces of infection were noted, this was never greater for the experimental group than for the controls. The mean score for infection, considering all animals, was 0.3±0.2 which shows that there was a trace of infection (mild, chronic murine pneumonia) in less than half of the animals. While scoring for infection was maintained, there was little
deviation between groups, and it was subsequently eliminated from the tables. In those few individual instances where there were indications of marked infection or disease other than those normally attributed to oxygen toxicity, the animals were eliminated from consideration.

II. 40%-60% Oxygen Stepwise Increments:
Comparison of Normal and Experimental Rats Exposed to 40% Oxygen at 1 atmosphere of Pressure for 4 Days Increasing to 60% Oxygen for 4 Days.

Forty percent oxygen for four days followed by sixty percent oxygen for the same time interval had little effect on body weight and lung weight, although lung buoyancy appeared to increase as shown in Table 2. The P-V curves were similar (Figure 15) and compliance was little affected (Table 2). Nevertheless, there was a ten-fold increase in the score for perivascular edema, and a seven-fold increase in the score for capillary congestion although only a small increase in alveolar thickening was recorded. The composite histology score was almost three-fold greater than in controls. While the final composite score was not as high as that for 72 hours of 100% oxygen (Table 1), the relative changes over their respective controls were similar in the two experiments. The photomicroscopy was characterized by a more congestive appearance (Figure 16). The small compliance changes, however, were more like those seen after 48 hours of oxygen. The failure of these histological changes to be reflected by an increase in lung
**TABLE 2**

<table>
<thead>
<tr>
<th>Variable Measured</th>
<th>Control</th>
<th>60-70% Inc</th>
<th>P ANOVA</th>
<th>Tukey’s D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body (g) Start Weight End</td>
<td>267±2.6</td>
<td>276±10.3</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Lung (g) Weight</td>
<td>1.72±0.11</td>
<td>1.64±0.06</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Lung wt (g/kg) Body wt</td>
<td>5.92±0.30</td>
<td>5.64±0.25</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Lung (ml)</td>
<td>0.70±0.15</td>
<td>0.34±0.21</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Total Lung vol (ml) 0 cm H2O</td>
<td>2.4±0.12</td>
<td>2.6±0.13</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Compliance (ml/cm H2O) Overall</td>
<td>0.36±0.13</td>
<td>0.37±0.13</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Inflation 10 cm</td>
<td>0.37±0.06</td>
<td>0.35±0.04</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Deflation 10 cm</td>
<td>0.34±0.04</td>
<td>0.33±0.03</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Area, total Hysteresis Loop (cm²)</td>
<td>7.4±1.62</td>
<td>6.4±0.88</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Perivascular Edema</td>
<td>0.13±0.08</td>
<td>1.25±0.16**/</td>
<td>.01</td>
<td>0.61/0.72</td>
</tr>
<tr>
<td>Capillary Congestion</td>
<td>0.13±0.08</td>
<td>0.88±0.17*</td>
<td>.05</td>
<td>0.49/0.58</td>
</tr>
<tr>
<td>Alveolar Thickening</td>
<td>0.62±0.06</td>
<td>0.81±0.23</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Composite Histology</td>
<td>0.84±0.12</td>
<td>2.94±0.10**</td>
<td>.01</td>
<td>0.54/0.63</td>
</tr>
</tbody>
</table>

* Difference from Control Statistically Significant
Figure 15

PRESSURE - VOLUME CURVES OF CONTROL AND EXPERIMENTAL RATS EXPOSED TO 40% OXYGEN AT 1 ATM (4 DAYS) INCREASING TO 60% OXYGEN (4 DAYS) FOR A TOTAL OF (8 DAYS)
This illustration shows a field generally comparable to that in the 40-60-80% increasing group (Experiment IV). Perivascular edema is moderate and can be seen in the lower right corner. Alveolar septa are thickened in this case, as compared with the unexposed control, but are not as thickened as in the 40-60-80% specimen. This particular view would represent approximately 1+ congestion, 1+ thickening, and 2 perivascular edema. Capillary congestion and thickening were scored under increased magnification (100X).
weight, as they were in 100% oxygen, also seems noteworthy.

III. Chronic, Continuous Oxygen (no inert gas)
Comparison of Normal and Experimental Rats Exposed to 60% Oxygen at 1 Atmosphere of Pressure and 100% Oxygen at 0.6 Atm for 10 Days.

Sixty percent oxygen at 1 atmosphere of pressure or 100% oxygen at 0.6 atmosphere for 10 days had little effect on body weight (Table 3). Lung weights and the lung weight/body weight ratio increased slightly in both experimental groups, but the effect missed statistical significance. Both treatments tend to decrease buoyancy, but also not significantly. Some similarities in gross physical parameters between these ten day exposures and 48 hours at 100% oxygen (Table 1) or the 40-60% increasing experiment (Table 2) are suggested.

Average P-V curves for the three groups fall in the same total volume range (Figure 17). The curve for the 60% oxygen at 1 atmosphere group reaches higher volumes than either the control or 100% oxygen at 0.6 atmosphere group, and while overall compliance is elevated in this group, it does not attain statistical significance (Table 3). Area of the hysteresis loop is, however, elevated significantly for the rats in 60% oxygen at 1 atmosphere. The similarities between ten days at the 60% oxygen level and 48 hours of 100% oxygen, or 40-60% for eight days are again evident. While the effect is certainly not marked, the absence of nitrogen may aggravate the effect of oxygen toxicity slightly.
CONPARISON OF CONTROL AND EXPERIMENTAL RATS EXPOSED TO 60% OXYGEN AT 1 ATM AND 100% OXYGEN AT 0.6 ATM (10 DAYS)

<table>
<thead>
<tr>
<th>Variable Measured</th>
<th>Average ± SE (6 Animals per group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Body (g) Start Weight</td>
<td>270±9.5</td>
</tr>
<tr>
<td></td>
<td>End</td>
</tr>
<tr>
<td>Lung (g) Weights</td>
<td>1.9±0.15</td>
</tr>
<tr>
<td>Lung wt (g/kg)</td>
<td>6.6±0.39</td>
</tr>
<tr>
<td>Lung (ml) Buoyancy</td>
<td>0.6±0.25</td>
</tr>
<tr>
<td>Total Lung vol (ml)</td>
<td>2.5±0.16</td>
</tr>
<tr>
<td></td>
<td>0 cm H₂O</td>
</tr>
<tr>
<td></td>
<td>Overall (cm)</td>
</tr>
<tr>
<td></td>
<td>Inflation 10 cm</td>
</tr>
<tr>
<td></td>
<td>Deflation 10 cm</td>
</tr>
<tr>
<td></td>
<td>Area, total Hysteresis Loop (cm²)</td>
</tr>
<tr>
<td></td>
<td>Peri-vascular Edema</td>
</tr>
<tr>
<td></td>
<td>Capillary Congestion</td>
</tr>
<tr>
<td></td>
<td>Alveolar Thickening</td>
</tr>
<tr>
<td></td>
<td>Composite Histology</td>
</tr>
</tbody>
</table>

* Difference from Control Statistically Significant
Figure 17

PRESSURE - VOLUME CURVES OF CONTROL AND EXPERIMENTAL RATS EXPOSED TO 60% OXYGEN AT 1 ATM AND 100% OXYGEN AT 0.6 ATM (10 DAYS)

LEGEND

CONTROL
60% O₂ AT 1 ATM.
100% O₂ AT 0.6 ATM.
Figure 18

PHOTOMICROSCOPY 60% OXYGEN AT 1 ATM (10 DAYS) CONTINUOUS

Rat killed after 10 days' exposure to 60% oxygen at 1 atmosphere. The field includes a small pulmonary artery, alveolar ducts and alveoli. There is some perivascular edema but congestion and alveolar thickening are not uniformly seen (100X).
Rat, killed after 10 days' exposure to 100% oxygen at 0.6 of an atmosphere. The appearance is exactly comparable to 60% oxygen results (Figure 18).
Histological data (Table 3) indicate that either oxygen exposure produced about a five-fold increase in perivascular edema, a three-fold increase in capillary congestion, and about a 70% increase in alveolar thickening. While there is little apparent difference between 60% oxygen at 1 atmosphere and 100% oxygen at 0.6 atmosphere (Figures 18 and 19) the nitrogen free group tends to have higher scores, somewhat in agreement with their slightly higher lung weight/body weight ratio and slightly lower compliance. The composite scores suggest further that ten days of exposure to the equivalent of 60% oxygen was certainly no worse, and perhaps slightly better (compared to own controls) than four days at 40% increasing to 60% for four days (Table 2).

IV. Chronic, Continuous Oxygen Versus 40%-60%-80% Stepwise Increments:
Comparison of Control and Experimental Rats to 100% Oxygen at 0.8 Atmosphere (12 Days) and 40-60-80% Oxygen at 1 Atm for 4 Days at Each Level for a Total of 12 Days.

(Note: 0.8 atm of oxygen was used instead of 80% oxygen because it offered a more feasible experimental situation at the time, and our previous results at the 60% oxygen level (Experiment III) suggested little effect from the presence or absence of nitrogen).

It can be seen from the results (Table 4) that 100% oxygen at 0.8 atm for 12 days caused a significant 8.5% loss in body weight, slight increase in lung weight, significant increase in the lung weight/body weight ratio and a decrease in buoyancy. By contrast, the 40-60-80% group gained slightly in body weight, but showed a nearly 50% increase in lung weight. The lung weight/body
<table>
<thead>
<tr>
<th>Temperature</th>
<th>Calorific Value</th>
<th>Heat of Combustion</th>
<th>Fuel Analysis</th>
<th>Final Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>190/250°C</td>
<td>15.0/16.5</td>
<td>14.5</td>
<td>13.5/14.0</td>
<td>12.5/13.0</td>
</tr>
<tr>
<td>900/1060°C</td>
<td>20.0/25.0</td>
<td>23.0</td>
<td>22.0/25.5</td>
<td>21.0/22.0</td>
</tr>
<tr>
<td>210/280°C</td>
<td>18.0/20.0</td>
<td>17.5</td>
<td>15.0/16.5</td>
<td>13.0/14.0</td>
</tr>
<tr>
<td>230/240°C</td>
<td>20.0/21.0</td>
<td>21.0</td>
<td>20.0/20.5</td>
<td>18.0/19.0</td>
</tr>
</tbody>
</table>

*Note: Values are approximate and may vary.*
Figure 20

PRESSURE - VOLUME CURVES OF CONTROL AND EXPERIMENTAL RATS EXPOSED TO 100% OXYGEN AT 0.8 ATM (12 DAYS) AND 40-60-80% OXYGEN AT 1 ATM FOR 4 DAYS AT EACH LEVEL FOR A TOTAL OF (12 DAYS).

LEGEND
CONTROL
100% O₂ @ 0.8 ATM.
40-60-80% INCR.
weight ratio was also significantly elevated and buoyancy decreased, similar to but more pronounced than the continuous oxygen group.

The P-V curves fall in the same volume range (Figure 20), although obviously flattened for both experimental groups. Compliance, as might be expected, is therefore decreased in both, but more so in the 40-60-80% group (Table 4), where the overall 0-20cm value falls to about half that of controls. This greater compliance decrease in the 40-60-80% oxygen group is also reflected in the significantly decreased area of hysteresis loop.

All histological scores are increased by the oxygen treatments but considerably more so by the 40-60-80% sequence. In particular, the score for perivascular edema is doubled and the score for alveolar thickening tripped over that of the continuous oxygen group. These differences are contrasted in Figures 21 and 22. The composite histology score of 4.99 for the 40-60-80% series is the highest of any experimental group tested, including those animals close to death after 72 hours of pure oxygen at 1 atmosphere (Table 1).

V. Comparison of Control and Experimental Rats Subjected to Saline (0.85%) Aspiration.

The lungs of animals subjected to saline aspiration were about two times heavier than controls with the lung weight/body weight
Figure 21

PHOTOMICROSCOPY 100% OXYGEN AT 0.8 ATM (12 DAYS) CONTINUOUS

Rat, killed after 12 days' exposure to 100% oxygen at 0.8 of an atmosphere. There is moderate perivascular edema but other changes are not apparent (100X).
Rat, killed after exposure to 40–60–80% oxygen at 1 atm for 4 days at each increment. There is perivascular edema and also a generalized thickening and increased cellularity of the septa of the alveolar ducts and alveoli. These changes resemble those seen in human lungs after oxygen therapy following their terminal illness (100X). This tissue is also shown at 400X in Figure 10.
ratio also significantly increased about two fold (Table 5). With regard to these criteria, the results were similar to animals exposed to pure oxygen for 72 hours (Table 1). By contrast, lung buoyancy was significantly increased (almost doubled) in the drowned rats compared to controls, whereas in oxygen exposure the tendency is for buoyancy to decrease.

Average P-V curves are shown in Figure 23 and to aid in comparisons the 72 hour 100% oxygen curve from Figure 11 has been added. The most obvious difference lies in the flat, narrow P-V loop for the oxygen rats compared to either controls or saline animals. Also, the inflation segment for the saline rats differs from the controls first in the nearly horizontal segment to a pressure of around 14cm water, then followed by a sharp rise to almost the same total volume as controls at 20cm. Taking the tangent of the angle for the ascending inflation segment of the saline animals between 16-20cm water it was found that the mean of 40.0± 3.17 was not only significantly greater than the oxygen exposed rats (19.4± 2.83) but also greater than controls (30.2± 3.11).

Another difference from hyperoxia was that the deflation or retraction line of the saline curve had a unique "dip" between 10 and 6cm water pressure (Figure 23). This characteristic was not seen in any other family of curves.

The area of the hysteresis loop is about the same for saline as controls, but is significantly less than either in oxygen
### TABLE 5

**COMPARISON OF CONTROL AND EXPERIMENTAL RATS EXPOSED TO SALINE ASPIRATION**

<table>
<thead>
<tr>
<th>Variable Measured</th>
<th>Average ± SE (8 Animals per group)</th>
<th>Control</th>
<th>0.5% Saline</th>
<th>P ANOVA</th>
<th>Tukey's D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body (g) Start Weight</td>
<td></td>
<td>283±4.9</td>
<td>302±7.3</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Lung (g) Weight</td>
<td></td>
<td>1.6±0.07</td>
<td>3.4±0.18**</td>
<td>.01</td>
<td>0.56/0.66</td>
</tr>
<tr>
<td>Lung wt (g/kg) Body wt</td>
<td></td>
<td>5.7±0.23</td>
<td>11.3±0.60**</td>
<td>.01</td>
<td>1.3/1.6</td>
</tr>
<tr>
<td>Lung (ml) Buoyancy</td>
<td></td>
<td>0.80±0.18</td>
<td>1.5±0.25</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Total Lung vol (ml) O cm H2O</td>
<td></td>
<td>2.1±0.14</td>
<td>4.9±0.09**</td>
<td>.01</td>
<td>0.54/0.69</td>
</tr>
<tr>
<td>Overall 0-20 cm</td>
<td></td>
<td>0.36±0.06</td>
<td>&quot;34±0.09&quot;</td>
<td>.05</td>
<td>0.13/0.16</td>
</tr>
<tr>
<td>Inflata 10 cm</td>
<td></td>
<td>0.36±0.09</td>
<td>0.05±0.01**</td>
<td>.01</td>
<td>0.12/0.19</td>
</tr>
<tr>
<td>Deflata 10 cm</td>
<td></td>
<td>0.30±0.04</td>
<td>0.36±0.05</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Area, total Hysteresis Loop (cm²)</td>
<td></td>
<td>7.28±0.76</td>
<td>7.64±0.86*</td>
<td>.03</td>
<td>2.7/3.2</td>
</tr>
<tr>
<td>Peri-vascular Edema</td>
<td></td>
<td>0.3±0.15</td>
<td>0.3±0.12</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Capillary Congestion</td>
<td></td>
<td>0.6±0.25</td>
<td>0.2±0.15</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Alveolar Thickening</td>
<td></td>
<td>0.3±0.15</td>
<td>0.1±0.12</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Composite Histology</td>
<td></td>
<td>1.2±0.13</td>
<td>0.7±0.15</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

* Difference from Control Statistically Significant
Figure 23

PRESSURE - VOLUME CURVES OF CONTROL AND EXPERIMENTAL RATS SUBJECTED TO SALINE ASPIRATION

LEGEND
- CONTROL
- 72 HOUR OXYGEN
- SALINE ASPIRATION
poisoning (Table 1). Histologically (Table 5), the saline animals were found to be normal by the light microscopy methods used, and not significantly different from controls with regard to any of the criteria.

**Combined Data**

To show more clearly the interrelationships among the various treatments, several of the measured variables (i.e., lung weight, lung weight/body weight ratio, buoyancy, overall compliance, hysteresis area, and composite histology score) were re-evaluated as a fractional change from their respective controls (Table 6). It was felt that this procedure would reduce most of the initial differences that may have existed among the various batches of animals with regard to such factors as size, intercurrent infection, season, etc., and thus facilitate intergroup comparisons.

The evidence suggests that histology is more severely effected by stepwise increase in oxygen tension than by continuous exposure to oxygen at less than 100% oxygen at 1 atmosphere. Forty percent oxygen for four days followed by sixty percent for four days (Experiment II) produces a greater relative increase in composite score than sixty percent oxygen for ten days (Experiment III) and almost equal to the severely toxic 72 hour 100% oxygen rats of (Experiment I). Even more dramatic is the increase in relative histology score of the 40-60-80% group with four days at each level (Experiment IV), which is twice as great as the relative increase
# Table 6

Combined data from all experiments, with variable treated as a change (ratio) from their respective control groups.

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Plotting Symbol</th>
<th>Oxygen Treat.</th>
<th>Lung Wt/Body Wt Ratio</th>
<th>Lung Wt</th>
<th>Buoyancy</th>
<th>Overall Compliance</th>
<th>Area</th>
<th>Comp. Histol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>HRS</td>
<td>100% Oxygen @1 Atm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>48 Hrs.</td>
<td>1.52</td>
<td>1.40</td>
<td>1.31</td>
<td>1.07</td>
<td>1.26</td>
<td>2.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 Hrs.</td>
<td>1.85</td>
<td>1.73</td>
<td>0.69</td>
<td>0.54</td>
<td>0.76</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72 Hrs.</td>
<td>2.31</td>
<td>2.13</td>
<td>0.69</td>
<td>0.43</td>
<td>0.61</td>
<td>3.50</td>
</tr>
<tr>
<td>II.</td>
<td>□</td>
<td>4 Da.Ea.</td>
<td>0.95</td>
<td>0.94</td>
<td>1.71</td>
<td>1.03</td>
<td>1.14</td>
<td>3.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40–60% Increas.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 Days Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III.</td>
<td>△</td>
<td>Inert N₂</td>
<td>1.03</td>
<td>1.05</td>
<td>0.67</td>
<td>1.19</td>
<td>1.43</td>
<td>2.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60% O₂ @1 Atm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100% O₂ @0.6 Atm</td>
<td></td>
<td>1.04</td>
<td>1.05</td>
<td>0.50</td>
<td>0.97</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV.</td>
<td>▿</td>
<td>4 Da.Ea.</td>
<td>1.71</td>
<td>1.76</td>
<td>0.30</td>
<td>0.53</td>
<td>0.57</td>
<td>6.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40–60–80% Increas.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 Days Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100% O₂ @0.8 Atm</td>
<td></td>
<td>1.36</td>
<td>1.18</td>
<td>0.46</td>
<td>0.74</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 Days Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V.</td>
<td>☯</td>
<td>Aspiration</td>
<td>1.98</td>
<td>2.13</td>
<td>1.88</td>
<td>0.68</td>
<td>1.05</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.85 Saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
either of 12 days of 0.8 atm of 100% oxygen or of any other exposure. However, a lesser differential effect is seen between stepwise and continuous oxygen on functional measurements, i.e., the 40-60% treatment (Experiment II) had only slightly greater effect on lung weight and lung compliance than 60% oxygen for 8 days, and the 40-60-80% treatment (Experiment IV) is only moderately worse than continuous 0.8 atm of 100% oxygen.

Further inspection of the data by correlation analysis of the ratios (Table 7) suggests that change in compliance is more dependent on change in lung weight \( r = -0.81 \) or lung weight/body weight ratio \( r = -0.85 \), than it is to change in histological score \( r = -0.30 \). The greatest correlation of all, however, was between compliance and hysteresis loop total area \( r = 0.94 \) whereas compliance was not significantly related to buoyancy \( r = 0.27 \).

While none of the changes in functional measurements are significantly correlated with changes in histology, two approached significance: the \( r \) of \(-0.64\) with buoyancy and the \( r \) of \(-0.55\) with hysteresis loop area. Apparently the histological measurements did not reflect edema at all since the correlations with lung weight and lung weight/body weight ratio were very low: i.e., \( r = 0.07 \) and \(-0.08\).
### TABLE 7

CROSS-CORRELATION AMONG THE COMBINED DATA OF TABLE 6: (r Values)

<table>
<thead>
<tr>
<th></th>
<th>Lung Wt/Lung Buoyancy</th>
<th>Overall Compliance</th>
<th>Hyster. Area</th>
<th>Composite Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Wt/</td>
<td>+0.96**</td>
<td>-0.85**</td>
<td>-0.32</td>
<td>-0.08</td>
</tr>
<tr>
<td>Body Wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung Wt</td>
<td></td>
<td>-0.10</td>
<td>-0.81**</td>
<td>-0.56</td>
</tr>
<tr>
<td>Buoyancy</td>
<td>+0.01</td>
<td></td>
<td>+0.27</td>
<td>+0.46</td>
</tr>
<tr>
<td>Overall Compliance</td>
<td>-0.85**</td>
<td>-0.81**</td>
<td>+0.27</td>
<td></td>
</tr>
<tr>
<td>Hysteresis Area</td>
<td>-0.32</td>
<td>-0.56</td>
<td>+0.46</td>
<td>+0.94**</td>
</tr>
<tr>
<td>Composite Histology</td>
<td>-0.08</td>
<td>+0.07</td>
<td>-0.64</td>
<td>-0.30</td>
</tr>
</tbody>
</table>

*, ** For n=9 (7df), r must= 0.67 for P=0.05, and r must= 0.80 for P= 0.01.
DISCUSSION

Among several observations made in this study which lend themselves to further analysis and discussion are the following:

1. Continuous versus stepwise increases in oxygen exposure.
2. Histological versus functional measurements.
3. Oxygen toxicity in the presence or absence of inert gas.
4. Time course of the toxic process.
5. Relationships among functional measurements.
6. Effects of oxygen versus saline aspiration on P-V curves.

Continuous Versus Stepwise Increases in Oxygen Exposure

Results with the acute two and three day exposures of rats to 100% oxygen indicated functional and histological changes (Table 1) similar to those widely reported in the literature (see, e.g., Bean, 1945; Kistler, et al., 1967; Haugaard, 1968; Beckman et al., 1969) but differed somewhat from Pratt's histological observations in humans (1965) in that congestion and septal widening seemed far less severe. When we exposed normal rats to a stepwise increase in oxygen, from 40% at 1 atmosphere to 60%, four days at each level (Table 2), the only apparent significant effect was in the histology, as shown by perivascular edema and capillary congestion. There seemed to be no effect on lung weight or compliance. For this reason, in a subsequent trial the 40–60% stepwise exposure
was lengthened by four additional days at 80% oxygen. These rats developed alveolar septal thickening and other changes (capillary proliferation) comparable to those seen in terminal human patients using identical histopathological techniques (Pratt, 1968). Also, the pattern of increased lung weight and decreased compliance was significant (Table 4). Continuous exposure to a 60% level with or without nitrogen, and 80% oxygen without nitrogen for equivalent periods of time produced apparently less severe histological and functional effects. Thus, the evidence from this study suggests that stepwise increase in oxygen tension is more detrimental to normal animals than continuous exposure to any of the oxygen levels used, particularly with regard to histology. That the histopathology is a form of early adaptive change is not completely ruled out however.

In Figure 24, bar graphs have been constructed to illustrate two series of trials (40-60% and 40-60-30%) where the stepwise versus continuous exposures were compared (i.e., Experiments II, III, and IV; Tables 2, 3, and 4). Only two variables are examined here, overall compliance and composite histology, using the ratio to control in each case (from Table 6) to permit the experiments to be put into the same frame of reference. In both series of trials, compliance is relatively reduced in the stepwise exposures compared to the continuous exposures (13% and 26% lower). Also in both series, histological changes are relatively more severe in the stepwise exposures than in comparable continuous exposure i.e.,
Figure 24

**Relative to Controls**

- **Continuous Exposure in Oxygen**
  - 40-60% Oxygen 4 Days Each (Exp II) 1.03
  - 60% Oxygen, 10 Days (Exp III) 1.19
  - 40-60-80% 4 Days Each (Exp IV) 0.53
  - 100% Oxygen, 0.8 ATM, 12 Days (Exp IV) 0.74

- **Stepwise Increase in Oxygen**
  - 40-60% Oxygen 4 Days Each (Exp II) 3.34
  - 60% Oxygen, 10 Days (Exp III) 2.69
  - 40-60-80% 4 Days Each (Exp IV) 4.57
  - 100% Oxygen, 0.8 ATM, 12 Days (Exp IV) 3.56

**Legend**
- Continuous
- Stepwise

**Overall Compliance**

**Composite Histology**
(24% and 96% higher in the 40-60% and 40-60-80% experiments).
In both series it should be noted that the continuous trial was at
the level of the highest of the stepwise increments, and therefore
should be considered the more severe exposure.

These results may explain the apparently different and
more severe pathology observed by Pratt (1968) in the lungs of
human patients treated with oxygen than that commonly associated
with oxygen exposure in the laboratory animals. Terminal patients
may be exposed to progressively increasing concentrations of
oxygen particularly if they fail to make progress with their
diseases. According to Pratt:

Humans were given oxygen therapeutically only when
they were seriously ill from a variety of causes, and
usually at an oxygen concentration level well below
100%. In the course of treatment, the oxygen level is
often increased although oxygen by catheter, mask, or
tent rarely if ever reaches 100% concentration. Also,
patients often have increasing oxygen flow and concen-
tration over a period of several days.

While oxygen therapy may contribute to the final structural
abnormalities in humans the role of changes in lung mechanics
in the demise of patients as discussed by Pratt (1965, 1968)
remains uncertain. This is suggested by the observation that
the effect of stepwise increments in oxygen may be greater on
histological appearance than on impairment of function as
measured by compliance (Figure 20). Patients receiving oxygen
therapy may well have died sooner from their disease if not
given supplemental oxygen, but further experiments might provide
data for safer schedules of oxygen administration to minimize lung damage.

This difference between constant and increasing oxygen appears to have been missed before, possibly because previous work with hundreds of different combinations of oxygen concentration and pressure generally have been at one prescribed level from outset to termination. We know of only one other experiment (Barach, et al., 1944) in which the oxygen level has been increased stepwise during the course of the run.

**Histological Versus Functional Measurements**

The analysis of the stepwise versus continuous oxygen exposures in the previous section pointed up some of the discrepancies between histological score and compliance noted in other phases of this study. With the correlations between histology and compliance, lung/body weight ratio, etc., not shown to be significant (Table 7) it is evident that there is an inconsistent relationship between microscopic appearance and those measures used in this study to represent function of the rat lung. Nevertheless, correlations approached significance in some cases, (eg., $r = -0.64$ with buoyancy and $-0.55$ with hysteresis loop area). It also should be noted that these measures of function are limited to only a portion of those which could be applied to the excised lung. It remains conceivable that in vivo measurements relating to respiratory rate, tidal volume, minute volume and perhaps diffusion could
show different relationships with histology.

Part of the discrepancy between histology and compliance seems to be due to time factors, with histological changes apparently occurring sooner than functional changes in oxygen exposure. For example, in Experiment I (e.g., Table 6) at 48 hours compliance is within a normal range whereas composite histology score has increased by 229%. By 60 hours, however, the two measures of lung damage are more comparable; compliance having been halved and histology increased by 2\(\frac{1}{2}\) fold. Also, treatments like saline aspiration depressed compliance somewhat (Table 5) but had virtually no effect on histology (at least under standard light microscopy).

The process of congestion and perhaps proliferation is apparently associated with decreased compliance only when there is simultaneous edema as shown by increased lung weight (Tables 2-4). Mild congestion (Table 3) may even be associated with an increased compliance in the absence of increased lung weight. On the other hand, Beckman, et al., (1971) found decreased compliance in monkeys without significant alteration of the lung/body weight ratio or gross histological appearance following head trauma. That this result was due to another process such as bronchiolar constriction seems possible however. Additional work is needed to isolate and clarify the relationships between alveolar edema, airway edema, and bronchiolar constriction in excised lung studies.
That congestion occurs later than edema in sub-human primates was shown by Robinson, et al., (1969). Pure oxygen at 1 atm produced the exudative response which later resolved and was followed by a proliferative phase (14 days). It was their belief that:

It may be possible that if an animal lives through the early acute toxic phases the proliferative type lesions appear, characterized by hyperplasia of the granular pneumocytes that make up a major portion of the alveolar epithelium.

It would seem possible that a stepwise increase in oxygen (Table 4) may produce a more moderate form of edema with increased or decreased compliance. Frank (1959) showed that congestion by itself did not greatly affect compliance in excised lungs, and believed that this congestive response might be protective or adaptive. Schaffner (1967) using electron microscopy rationalized a "protective" thickening of alveolar walls to increase the diffusion distance:

There is widening of septa with capillary protrusion into the alveoli so that surface area for diffusion is decreased; there is also an increasing number of capillaries in the septa to expose less blood for diffusion.

While it is not possible to directly compare rats, monkeys, and humans, both the acute exudative and the chronic proliferative lesions have been seen in dogs, rats, and mice (Robinson, et al., 1969; Kafer, 1971) and terminal humans (Pratt, 1965).

**Oxygen Toxicity in the Presence or Absence of Inert Gas**

In only one experiment (Experiment III) was there a direct
comparison made between the toxic effects of oxygen in the presence or absence of nitrogen. Sixty percent oxygen at one atmosphere produced a slightly less severe response, both histologically and functionally than 100% oxygen at 0.6 atmospheres (Tables 3 and 6). This would indicate perhaps some role for absorption or compression atelectasis in increasing the severity of the oxygen toxic response (Rahn, et al., 1963). However, with only one trial available and with the changes found quite small, caution should be exercised in extending these findings. Additionally, one must consider carefully how closely the two trials compared in oxygen partial pressure at the alveolar level where the effective toxic action presumably takes place. At 0.6 atmosphere in 100% oxygen we can estimate the alveolar PO₂ to be 448 torr (P_B) - 47 torr (PH₂O) - 40 torr (PCO₂), or 448 - 87 = 361 torr. In 60% oxygen at 1 atmosphere the alveolar PO₂ may seem to be higher [(747 - 87)(0.6) = 396 torr]. However, it is probably some 30-40 torr lower than the calculated 396 torr for the same diffusion-utilization reasons that in normal air alveolar PO₂ is about 100 torr whereas one would expect it to be closer to 140 if it were just diluted by CO₂ and H₂O. Thus the alveolar oxygen is probably similar in the two systems.

The small effect of nitrogen in oxygen toxicity observed here would agree with the findings of Wright, et al., (1966), in which mice survived equally when their exposure to 100% oxygen was interrupted either by air or lowered PO₂ without nitrogen.
The issue remains alive however, and there are still reports that nitrogen delays the toxic process: Leon, et al., (1970) showed differences in rates of hemolysis with and without nitrogen, and Normán, et al., (1971) reported that lung damage was delayed by the presence of nitrogen.

**Time Course of the Toxic Process**

Experiment I in which measurements were made at 48, 60, and 72 hours in 100% oxygen at 1 atmosphere provided an opportunity to follow the time course of changes in compliance and histology. In Figure 25 the relative changes of histology, lung/body weight ratio, and compliance (from Table 6) have been graphed for comparison. It can be seen that the progressively more severe histology and higher weight ratios are associated with a general decrease in compliance. However at 48 hours, compliance is essentially unaffected whereas marked histopathological changes and increased lung weight have already occurred.

These results are in general agreement with those found in the literature. Hamman and Rich (1944) associated the earliest lesion of oxygen toxicity with dilatation of the capillaries and exudation of fluid into the alveoli. Kistler (1967) using the electron microscope and morphometric methods stated that histological changes did not occur before 24 hours, but were great enough after 48 hours to cause functional changes. Hemingway (1952) showed progressive increase in lung weight and lung/body
Figure 25

RELATIVE CHANGES* IN HISTOLOGY, LUNG WEIGHT/BODY WEIGHT RATIO AND COMPLIANCE IN 48-72 HOURS OF 100% OXYGEN AT 1 ATM.

LEGEND: ▲ HISTOLOGY
× LUNG WT/BODY WT
⊙ COMPLIANCE

*Ratio of Experimental Group Compared to Respective Control Group
weight ratio in guinea pigs exposed to pure oxygen for 48 to 72 hours. Smith (1963) provided evidence to show that the loss of lung compliance occurred late in oxygen exposure of dogs. Beckman and Weiss (1969) found an approximate 50% decrease in lung compliance after 60-66 hours of 100% oxygen at 1 atmosphere, similar to what we would expect on the basis of our 60 and 72 hour animals (Table 1).

It is interesting that the early (48 hour) effects of acute oxygen exposure and the chronic exposures (40-60% increasing and 60% continuous oxygen) were characterized by a slight increase in compliance over controls whereas histopathology was already marked. This appears contrary to some reports in the literature (Hughes, et al., 1970). The reasons for these differences remain obscure.

Relationships Among Functional Measurements

Table 7 shows that changes in total lung compliance, to which considerable functional importance is attached, is strongly correlated with changes in lung weight, lung/body weight ratio, and hysteresis area but poorly correlated to changes in buoyancy. The high negative correlation and significant regression of compliance on lung/body weight ratio (Figure 26) was as might be expected, assuming that the increase in lung weight is due at least in part to intra-alveolar edema which is known to disrupt the surfactant layer and thus decrease compliance by increasing surface tension (Scarpelli, 1968). It is less likely that tissue
CHANGE IN COMPLIANCE AS A FUNCTION OF CHANGE IN THE LUNG WEIGHT/BODY WEIGHT RATIO, (ALL EXPERIMENTS)

(Changes calculated as a ratio to respective controls.)

\[ y = 1.54 - 0.49x \]

\[ r = -0.85 \pm 0.10 \]

Figure 26
elasticity decreased, in view of past work which showed that fluid retention following vascular perfusion may actually increase the compliance measured by saline filling (Beckman, 1967).

The highly significant positive correlation \( r=0.94 \) between the total area within the P-V loops and overall compliance supports the idea that hysteresis is due to surface forces. This conclusion follows from other studies (Beckman, et al., 1969) suggesting that compliance changes were due primarily to the disruption of surfactant. However when area was compared to lung/body weight ratio the regression was not significant \( r=-0.56 \) suggesting that factors other than simple fluid accumulation affect the hysteresis loop.

While there did seem to be a direct relationship between buoyancy and the other functional measurements, it is difficult to explain why these did not reach statistical validity. Theoretically, any increased surface tension would tend to decrease the amount of trapped gas; yet the correlation between compliance and buoyancy is only 0.27. Other factors apparently enter into the resultant retained volume; possibly the correlation would have been better if initial buoyancy were used, since this value increased by an average of 32% after the last loop was produced compared to just before the first inflation. Still another factor which may have biased the correlation was the unusually large increase in buoyancy in saline aspiration which was associated with a decrease in compliance. It should also be pointed out
that buoyancy had the best correlation with histological score 
\((r = 0.64)\) of any functional measurement.

It may be of importance to note again that despite the obviously low compliance of the oxygen poisoned lungs, the percent of maximum volume retained on deflation to 10cm was higher than controls and indicative of highly stable lungs. These results would lend support to the findings of Gruenwald, (1963), Beckman and Weiss (1969) and others who reported that in the rat stability measurements based on the deflation limb may be misleading.

**Effects of Oxygen Versus Saline Aspiration on P-V Curves**

The low correlation \((r = -0.30)\) between composite histology score and compliance indicates that the functional effects of edema, estimated by histopathology, are not as great as the effects of edema determined by lung weight \((r = -0.81)\). The crux of the edema problem appears to be in its distribution: Whether within airways, perivascular, intra-alveolar, or interstitial. Standard histological techniques apparently will not quantify fluid within air spaces and thus register primarily perivascular and interstitial changes. The correlation of 0.07 between composite score and lung weight (Table 7) suggests that the perivascular and interstitial changes contribute little to heavier lungs. The varying effects of the different distribution of edema on compliance would presumably be through factors listed
by Bachofen, et al., (1970) as: airway closure, disruption of surfactant, alterations in alveolar shape, or alveolar collapse.

Saline aspiration was attempted in the interest of producing equivalently edematous lungs by a process other than oxygen toxicity. Earlier work had shown that drowned rats had lungs of almost identically increased weight as those exposed to pure oxygen for 72 hours. If the rats were anesthetized prior to aspiration of 0.85% saline, the lungs produced were heavier than 72 hour oxygen animals, therefore unanesthetized animals were used similar to the procedures of DeBoer (1970).

Lung weights were similarly elevated by 72 hours of oxygen exposure and saline aspiration (Table 6), indicating comparable degrees of edema. However, overall compliance was decreased on the order of 60% in the 72 hour oxygen animals but only about 30% in the saline aspiration group. Furthermore, the shape of the P-V curves was markedly different (Figure 23). The oxygen rats show uniformly low compliance over the full 20cm water pressure range, whereas the P-V curve for the saline animals starts out flat until about 14cm water, whereupon it rises steeply to near normal lung volume. Additionally, the deflation or retraction curve was unique in having a dip at around 8cm water pressure.

The almost non-existent compliance during the beginning of air filling in the saline rats is compatible with the concept of widespread closure of airways which open suddenly as a pressure
of around 14 cm water is reached. The increased buoyancy of the lungs from the saline rats (Table 5) argues against alveolar collapse. This finding is in agreement with Hughes, et al., (1970) who stated that peribronchial and intra-airway edema may cause airways to close on deflation at higher distending pressures resulting in the trapping of larger volumes of gas. This apparent closure may also be responsible for the dip in the deflation curve at around 8 cm water.

Perhaps even the small decrease in overall compliance which did occur in saline aspiration may be misleading, being due to the short interval in which alveolar filling occurred after the airway obstruction was overcome, since the slope of the inflation curve was similar to controls during this period. It is unlikely that decreased compliance was due to any change in the airways themselves, for although Mead (1969) states that airways are compliant structures, their expansion only contributes 2-3% of the total. The absence of histopathology would suggest that little entrance of saline into the alveoli themselves takes place, although there are reports of electron microscopy histopathology and loss of surfactant in drowning (Whayne, et al., 1968; Butt, et al., 1970).

In contrast to the "diphasic" P-V curve and increased buoyancy of saline aspiration, there is the uniformly low-compliant curve and decreased buoyancy in oxygen sick animals. Similar flat curves are seen in normal animals in which saline has been forced across the alveolar membrane from the pulmonary
vasculature in an effort to wash out surfactant (Beckman and Weiss, 1969). The flat air P-V curves could not be explained in terms of tissue recoil (as estimated by fluid filling and emptying), indicating that increased surface tension was the primary mechanism. Direct measurement of the phospholipid washed out of these lungs supported the idea that oxygen toxicity had decreased the quantity of pulmonary surfactant. However, widespread blockage of airways could not be completely ruled out, particularly if the closure was due to increased surface tension in the airways. The present study showing a markedly different air P-V and lung buoyancy pattern in a situation where airway closure seems to be the predominant process tends to support loss of alveolar surfactant as a key mechanism in oxygen toxicity.

The results of this study should be extended to problems of drowning with caution, since the effects of aspirated isotonic saline as used here may differ considerably from that produced by the more typical situations involving fresh water or seawater.
SUMMARY

The cause and functional significance of the wide range of pulmonary phenomena which have been associated with oxygen toxicity remain obscure. Edema, atelectasis, and decreased compliance have been reported in acute hyperoxia (100% oxygen at 1 or more atmospheres of pressure) while congestion, septal thickening and capillary proliferation have been more frequently attributed to chronic hyperoxia (less than 100% oxygen or less than 1 atmosphere of pressure).

Hospitalized patients requiring high oxygen levels appear to be afflicted with more varied and perhaps more severe lung damage than experimental animals subjected to oxygen toxicity. Although species differences may be involved, another possibility is that patients may be exposed chronically to progressively increasing oxygen concentration during therapy, compared to the more acute constant oxygen level usually used in the laboratory.

In this study some of the questions relative to oxygen toxicity were examined by measuring and comparing histological and functional changes in the lungs of rats under a variety of simulated clinical and laboratory situations. These included acute versus chronic oxygen exposures; continuous, uniform versus stepwise increments of increased oxygen level; hyperoxia in the presence and absence of nitrogen; and pulmonary edema produced by oxygen in contrast to edema.
following aspiration of 0.85% saline.

Some 150 mature, male Wistar rats varying in size from 250 to 300 grams were used in nine trials. Oxygen exposure took place in sealed, recycling plastic chambers, either rigid or flexible, with the oxygen level controlled automatically. The CO₂ was absorbed by soda lime and excess moisture removed by refrigerated condensers. Exposures under one atmosphere were accomplished by placing the flexible plastic chambers inside a hypobaric chamber and reducing the pressure to the desired level.

Compliance was the prime variable used to assess function. The pressure-volume (P-V) curves from which compliance (change in volume/change in pressure) was taken were produced by continuous inflation and deflation of excised lungs with air at about 5½ ml/min to a maximum pressure of 20 cm water pressure. Four P-V curves were registered in sequence on an X-Y recorder with the last used in analysis. The third and fourth curves were generally superimposable, indicating that the results were reproducible and reliable. Retained or trapped gas was evaluated from buoyancy measurements on the lung after the last curve. Lung weight, by itself or as a ratio to body weight, was used to estimate edema.

The basic histological categories measured were perivascular edema, capillary congestion, and alveolar thickening. Following the P-V curves, lungs were stored in 10% formalin (one lobe was first filled with fixative), then processed for standard light microscopy.
(6 μ sections, H and E stain). One slide from the formalin filled lobe was arbitrarily selected for scoring on a scale of 0-3 for each category. The three scores were also summed for a composite score. In order to bring both the histological and functional data from all the experiments into a common reference frame, the percent deviation from controls (relative change) was calculated within each experiment.

Two series of experiments showed that stepwise increase in oxygen tension was apparently more detrimental than uniform continuous exposure. For example, relative histology score was 24% higher and relative compliance 14% lower in 40% oxygen for four days followed by 60% oxygen for four days than after eight days of continuous 60% oxygen at one atmosphere. Even greater differences were noted when four days each at 40, 60, and then 80% oxygen, consecutively, was compared to 12 days of 100% oxygen at 0.8 atm (i.e., histology 96% higher; compliance 28% lower).

Histology and function were poorly correlated within individual experiments. Histological changes appeared to precede changes in lung weight and compliance as well as being relatively more severe after similar exposures. When change in composite histology score was compared to change in compliance over the 9 separate experiments, the correlation coefficient (r) was -0.30 (0.67 needed for \(P=0.05\)); for change in lung weight, \(r\) was +0.07. The best correlation was with buoyancy (\(r=-0.64\)). Within the functional measurements,
compliance was significantly correlated with lung weight ($r = -0.81$).

Considering all groups, the greatest loss of compliance was, from most to least, seen in 72 hour 100% oxygen, 60 hour 100% oxygen, 40-60-80% increasing oxygen, saline aspiration, and 100% oxygen at 0.8 atm for 12 days continuously.

Only slight evidence was gathered on the role of inert gas (i.e., the presence or absence of nitrogen) in oxygen toxicity. In one trial, 0.6 atm of 100% oxygen appeared to cause slightly more severe symptoms than 60% oxygen at 1 atm. The difference however was neither statistically nor practically significant.

The effect of edema on function appears to be dependent upon where the edema occurs. In oxygen toxicity, perivascular edema, as seen histologically, was poorly correlated with compliance, in contrast to high correlations between compliance and edema evaluated by lung weight increase. Edema induced by saline aspiration (drowning) caused the same lung weight increase as acute oxygen toxicity and yet had far less effect on compliance. Furthermore, the P-V curve in saline aspiration had a markedly different shape than in oxygen toxicity. It was concluded that saline aspiration primarily blocked airways but had minimal effects on the alveoli. This provides further evidence that depression of compliance in hyperoxia is mediated through effects on pulmonary surfactant rather than through bronchial constriction or obstruction.
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