Title

Time course and pharmacological intervention in the pulmonary toxicity of nitrogen dioxide, a model of the adult repiratory distress syndrome

Author

Hiatt, Gerald F. S.

Publication Date

1984

Peer reviewed|Thesis/dissertation

TIME COURSE AND PHARMACOLOGICAL INTERVENTION IN THE PULMONARY TOXICITY OF NITROGEN DIOXIDE, A MODEL OF THE ADULT RESPIRATORY DISTRESS SYNDROME

by

GERALD F.S. HIATT

B.A., University of California, San Diego

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

COMPARATIVE PHARMACOLOGY & TOXICOLOGY

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco

Date University Librarian

Degree Conferred: . . . JAN 2 . 1984 . . . . . .

TIME COURSE AND PHARMACOLOGICAL INTERVENTION IN THE PULMONARY TOXICITY OF NITROGEN DIOXIDE, A MODEL OF THE ADULT RESPIRATORY DISTRESS SYNDROME

Gerald F.S. Hiatt

DEDICATION

This work is dedicated to my family, in both the literal and figurative senses. The emotional and moral support given by these people have made fifteen-plus years of educational endeavor an easier and more rewarding undertaking.

I extend my deepest appreciation to Constantine for sharing with me his focus and his inspiration; and to Roger, Dean, Jerry, and Anne for their constant motivation, emotional support and understanding. I extend my appreciation also to Bonnie for allowing me the opportunity, and to Mary for showing me her dedication to research. My dissertation is dedicated to all of you, and to Paul, Ernie and the rest of "the gang" for your support over the last fifteen years.

This work is also dedicated to my parents. Although their patience and understanding have, with reason, been often tried by this long process, Mom and Dad always gave me their support. I only wish Dad could have lived to share this moment, he might even have enjoyed reading this dissertation.

ACKNOWLEDGEMENTS

First and foremost I extend my thanks to Dr. Frederick Meyers for his guidance and for providing me with a "home" at U.C.S.F. Fred taught me much more than pharmacology and toxicology, and has done so with a concern and understanding surpassing that of just an "advisor." My special thanks are given for being there one particular Saturday morning.

Next I thank two gentlemen who provided invaluable assistance throughout my graduate career, Drs. Charles H. Hine and Harold C. Hodge. Dr. Hine constantly attempted to keep matters in perspective and keep me moving in the right direction. I thank him for his concern and efforts, and for serving on my committee. Dr. Hodge has been a great inspiration, a discerning critic and a formidable squash opponent. I thank him for all of these and for being my friend.

My thanks go also to Dr. Robert Phalen, not only for serving on my committee and providing much needed advice, but also for his infectious enthusiasm. I apologize to Bob for turning this into such a weighty matter.

Dr. James Embree provided early inspiration and guidance. In addition, Jim is a good friend who was always ready to help when necessary. I thank him for all of this and more.

CONTENTS

ABSTRACT

INTRODUCTION

ADULT RESPIRATORY DISTRESS SYNDROME....1
HISTORY....1
ASSOCIATED FACTORS....2
CLINICAL....3
PATHOPHYSIOLOGY....4
Structural Considerations....4
Histopathophysiology....6
PULMONARY EDEMA....10
MICROVASCULAR HEMODYNAMICS & FLUID PATHWAYS IN THE LUNG....10
HYDROSTATIC PULMONARY EDEMA....15
PERMEABILITY PULMONARY EDEMA....17
PULMONARY EDEMA OF ARDS....21
MECHANISTIC CONSIDERATIONS....23
Surfactant....23
Histamine....24
Coagulation System....25
Complement/Polymorphonuclear Leukocytes....27
NITROGEN DIOXIDE....31
HEALTH EFFECTS....32
Pulmonary Edema....33
NO_2 TOXICITY AS A MODEL FOR ARDS....37
STATEMENT OF PURPOSE....40

MATERIALS & METHODS

MATERIALS....42
METHODS....44
INHALATION EXPOSURE SYSTEM....44
INHALATION SYSTEM OPERATION....50
DETERMINATION OF NO_2 CONCENTRATION....53
LUNG WATER & BLOOD ANALYSIS....59
Calculations....61
Statistics....64

RESULTS

CONTROL ANIMALS....70
DOSE-RESPONSE EXPERIMENTS....73
TIME COURSE EXPERIMENTS....84
PHARMACOLOGICAL INTERVENTION....94
TERBUTALINE....95
RUTIN....99
INDOMETHACIN....101
DEXAMETHASONE....106
METHYL PREDNISOLONE....109
CLOFIBRATE....110

DISCUSSION

CONTROL ANIMALS....114
HYDROSTATIC/PERMEABILITY PULMONARY EDEMA & WET:DRY RATIOS....117
DOSE-RESPONSE EXPERIEMNTS....120
TIME COURSE EXPERIMENTS....124
0 TO 2 HOURS PI....125
2 TO 6 HOURS PI....127
Early-Hydrostatic Phase....129
6 TO 12 HOURS PI....136
12 TO 18 HOURS PI....139
18 TO 24 HOURS PI....140
Delayed-Permeability Phase....143
RELATION TO ARDS....149
SUMMARY....155
PHARMACOLOGICAL INTERVENTION EXPERIMENTS....156
TERBUTALINE....158
RUTIN....162
INDOMETHACIN....163
DEXAMETHASONE & METHYL PREDNISOLONE....166
CLOFIBRATE....168
Vascular Smooth Muscle....170
Free Fatty Acids....171
Free Radical/Lipid Interactions....173
Coagulation System....181
Thyroxine....184
Leukocytes....186
SUMMARY....189

ABBREVIATIONS....190

REFERENCES....192

ABSTRACT

The development over time of pulmonary injury following inhalation of NO_2, 255 ppm for 120 minutes, was investigated in the guinea pig. This toxic reaction, consisting of pulmonary edema and hyperemic congestion of the lung, models the Adult Respiratory Distress Syndrome (ARDS).

Time course experiments indicated there are two phases to lung injury in this model of ARDS. Accumulations of lung water and blood peak during early, hydrostatically-mediated and again during later, permeability-mediated phases of edemagenesis. The early phase is characterized by accumulation of a "watery" fluid with little accompanying dry weight material. This phase produces few or no overt symptoms and resolves somewhat during the subsequent "latent period." The Delayed-Permeability phase produces accumulation of large amounts of fluid and dry weight material (thought to be plasma proteins and leukocytes), enough to seriously compromise lung function and produce clinical symptoms of respiratory distress. This phase is associated with an intense inflammatory reaction, featuring massive polymorphonuclear leukocyte (PMN) infiltration, within the alveolar zone of the lung. It is this delayed phase that develops both the symptoms and pathology characteristic of the fully developed lesion in ARDS.

Based on data from this and other studies, it is concluded that this two-phase development may be a general feature of ARDS. It is not unexpected that the Early-Hydrostatic phase is not apparent clinically and has therefore been largely ignored. Specific treatment of the Early-Hydrostatic phase may influence the development of the Delayed-

Permeability phase. The results here indicate that early diagnosis of potentially severe ARDS cases by bronchoalveolar lavage may be possible, allowing early initiation of more aggressive therapy in these patients.

Vasodilator (Terbutaline) and anti-inflammatory (Indomethacin and Glucocorticoids) agents did not inhibit the accumulations of lung water and blood in this ARDS model. Terbutaline (+/- $beta_1$ blockade) actually increased both edema and mortality.

Clofibrate demonstrated a striking protective effect, preventing expected mortality and markedly attenuating development of edema and hyperemic congestion. Clofibrate appeared to interfere primarily with the Delayed-Permeability phase of edemagenesis. Based on the data from this and other studies, it is concluded that Clofibrate may exert a pharmacological action significantly interfering with the development of ARDS-type lung injury.

INTRODUCTION

ADULT RESPIRATORY DISTRESS SYNDROME:

Adult Respiratory Distress Syndrome is a term for the clinical presentation of respiratory symptoms related to appearance of diffuse, infiltrative lesions of the gas exchange areas of the lung. The associated pulmonary edema and hypermic congestion of the lung are responsible for the major clincal observation from which the syndrome takes its name, distressed respiration. Although the basic clinical and pathophysiologic features are the same, there are multiple preceeding medical events, not all of which involve the lung, associated with development of this disorder.

HISTORY:

The same basic syndrome has been recongized for years and described under a variety of names: Acute Respiratory/Alveolar Failure, Adult Hyaline Membrane Disease, Capillary Leak Syndrome, Congestive Atelectasis, Da Nang Lung (during the Vietnam War battlefield medical attention was of such high quality that many seriously injured soldiers survived to develop this sequela), Shock Lung and Wet Lung, to mention only a few. The term Adult Respiratory Distress Syndrome was first used by Ashbaugh et al. in 1967 to stress an apparent similarity with the Infant Respiratory Distress Syndrome.

Adult Respiratory Distress Syndrome, or ARDS as it is commonly abbreviated, has become the most popular name for this entire category of respiratory insults, although the possibility of a mechanistic similarity to the syndrome suffered by the newborn is no longer seriously considered. In the neonatal syndrome, respiratory difficulty is the

end result of impaired production of the pulmonary surfactant lining the alveoli (Robertson, 1981). While disruption of surfactant function is a feature of ARDS, it is a result of the alveolar flooding characteristic of the fully developed adult lung lesion (Pattle, 1955 and Said et al., 1965) and not the primary event, as in the infant.

FACTORS ASSOCIATED WITH ARDS:

As alluded to earlier, a wide range of seemingly unrelated medical disorders and other factors can, but do not necessarily, give rise to ARDS. Many of these represent acutely serious conditions in and of themselves. The following table is a compilation, from Hopewell & Murray (1976) and Petty (1981), of some of these medical conditions and other factors sometimes preceeding ARDS.

FACTORS ASSOCIATED WITH ARDS:

SHOCK (any cause)	TOXIC GASES
	Oxygen
INFECTION	Nitrogen Dioxide
Gram-negative sepsis	Ozone
Viral pneumonia	Phosgene
Bacterial pneumonia	
Legionnaire's pneumonia	LIQUID ASPIRATION
	Gastric acid
DISSEMINATED INTRAVASCULAR	Hydrocarbons
COAGULATION	Near drowning
TRAUMA	METABOLIC DISORDERS
Fat emboli	Pancreatitis
Pulmonary contusion	Uremia
Head injury	
Multisystem (any)	MISCELLANEOUS
	Paraquat exposure
DRUG OVERDOSE	Post cardiopulmonary
Heroin	bypass
Methadone	Eclampsia

Although the eventual clinical picture with respect to the lung is the same for all of these diverse medical disorders associated with ARDS, this does not necessarily imply a uniformity of mechanism. One of the disadvantages of grouping such a diversity of medical conditions and other factors into one overall diagnostic category is that important distinctions become blurred. However, many of these conditions do appear to have important features in common and study of these similarities, as well as the differences, may contribute to a more complete understanding of ARDS.

CLINICAL:

Petty and Ashbaugh (1971) delineated the key clinical features defining ARDS. Initially, in spite of other medical conditions, there may be no respiratory symptoms at all. An increase in respiratory frequency (tachypnea), the usual first sign, may only develop several hours (8 to 48) after a preceeding, usually catastrophic, medical event. This latent period permits an intervening treatment, if an effective one can be found.

Tachypnea rapidly develops into labored breathing with shortness of breath (dyspnea). Arterial oxygen pressure (PaO_2) is below normal and carbon dioxide pressure ($PaCO_2$) is increased. Administration of oxygen (O_2) at high concentrations will temporarily correct blood gases, but may ultimately promote development of oxygen toxicity, an ARDS-type syndrome (Clark & Lambertson, 1971). The ability of high concentrations of O_2 to overcome reduced PaO_2 indicates that at this stage poor arterial oxygenation is due to ventilation perfusion mismatching. The chest radiograph may still be fairly clear (Ingram, 1977).

In many cases the disorder inexorably progresses, with the patient becoming cyanotic and increasingly dyspneic. Rales become prominent and the chest radiograph shows bilateral infiltrates in the lung. The lungs become less compliant. Administration of high concentrations of O_2 may no longer improve arterial oxygenation (Petty, 1981), indicating shunting of blood through unventilated areas of the lung. Progressive hypoxemia is the most severe feature of an impending fatal outcome.

In 1972 it was estimated (Lung Program, NHLI) that there were 150,000 cases per year of ARDS in the United States alone. Since that study, the incidence of ARDS appears to be on the rise, although this increase may only represent an increased awareness and reportage of the disorder, not in its occurrance (Hopewell & Murray, 1976). In spite of of the attention and research focused on ARDS in the past 10 or so years, the mortality rate remains high, with estimates ranging from 20% (Douglas & Downs, 1977) to over 80% (Lee et al., 1981).

PATHOPHYSIOLOGY:

Disruption of the alveolar - capillary barrier is the basic lesion in all forms of ARDS. This barrier normally prevents leakage of plasma, and other constituents of blood, out of the pulmonary microvasculature into the interstitium and eventually into the alveoli. The alveolar - capillary barrier is composed of three structures; endothelium, interstium and epithelium (Weibel & Bachofen, 1979).

Structural Considerations:

Pulmonary alveoli are essentially sacs of air confined by a thin epithelium that is supported by connective tissue through which is

entwined an extensive capillary network. The alveolar epithelium is composed mainly of 2 cell types. Although less numerous, the Type I (or Membranous) pneumocytes cover more than 95% of the surface. These are very thin cells, with little metabolic activity, whose main purpose is to establish the membrane across which gas exchange takes place.

The major metabolic capability of the alveolar epithelium lies with the Type II (or Granular) pneumocytes which, although covering less than 3% of the alveolar surface, actually out number the Type I cells. Type II cells are believed to be responsible for synthesis of the surfactant which lines the air-epithelium interface. Surfactant functions to lower the high surface tension within each alveolus. A high surface tension is generated by any fluid-covered surface with a small radius, and creates a force acting to oppose lung expansion as well as drawing fluid into the alveoli. The Type II cells have a high metabolic rate and are resistant to injury. They are thought to be the precursors of Type I cells, proliferating after alveolar injury and differentiating into squamous epithelial cells similar to Type I cells.

Alveolar septal capillaries are bounded by endothelial cells forming a typical non-fenestrated endothelium. This endothelium is more permeable to water than is the alveolar epithelium, probably as a result of numerous pinocytotic vesicles and looser intercellular junctions. Fluid exchange is thought to take place not only across the walls of true capillaries, but also at some arterioles and venules. The site of exchange is therefore referred to as the "microvasculature."

Between the capillary endothelium and the alveolar epithelium is the interstitial space, composed of a ground substance of proteoglycans through which thread connective tissue fibers (both collagenous and

elastic). This septal interstitial space is continuous with that of the junctional or juxta-alveolar regions, the site of intersection of many alveoli. These are the areas which contain the blood vessels. The importance of this structural continuity lies in the fact that the lymphatic capillaries arise within the junctional regions.

Because of the design of this junctional region, lung inflation pulls on fibers distributed radially outward from the septal intersections, the interstitial fluid pressure here is less than in the alveolar septum itself. Hence there is a natural clearance pathway for fluid entering the alveolar wall. It drains along the interstitial fibers toward a region of negative pressure relative to the septum, the junctional interstitium. There it can be cleared by the terminal lymphatics (Weibel & Bachofen, 1979).

Histopathophysiology:

Histopathologic study of patients dying from ARDS reveals a two phase process in the development and progression of the disorder (Nash et al., 1967). Grossly, lungs from patients dying acutely (2 to 7 days) are heavy and consolidated, with a beefy, red appearance and a serosanguineous (containing both serum and blood) exudate. Histologic examination shows capillary congestion, marked interstitial edema, an intraalveolar fibrinous exudate and alveolar hemorrhage. In addition, there are hyaline membranes, thought to be the result of exudation of edema fluid containing high concentrations of plasma proteins (Hopewell & Murray, 1976 and Robin, 1979) lining the gas exchange surfaces of the alveolar ducts and alveoli. These findings are characteristic of a pulmonary edema of high protein content, known as permeability-type

pulmonary edema. These initial findings indicative of permeability edema are considered the acute edematous phase of ARDS.

Patients dying later (usually after 2 weeks) have lungs that are grayish-pink with no exudate, suggesting some fibrinous organization. Microscopically, there is interstitial thickening by a combination of edema fluid, histiocytes, fibroblasts and increased numbers of collagen fibers. The normal flat alveolar epithelium has been replaced by large rounded or cuboidal cells (hyperplasia of type II pneumocytes). This is the phase of early interstitial fibrosis (Nash et al., 1967).

Evidence from patients dying during intermediate periods, who manifest a combination of the two patterns, indicates a progressive nature for ARDS. Initial alveolar injury results acutely in an edematous, congestive reaction which, if survived, may progress to fully developed pulmonary fibrosis or emphysema (Hopewell & Murray, 1976). These two chronic developments have recently been postulated to be divergent responses to similar lung injury (Niewoehner & Hoidal, 1982).

The clinical features of ARDS are easily understood in the light of these observations. Pulmonary edema, regardless of cause, produces arterial hypoxemia and decreased lung compliance. These in turn lead to the tachypnea, cyanosis and dyspnea noted clinically.

It has been shown that fluid accumulation in the lung proceeds sequentially, the extra-alveolar interstitium (areas around large blood vessels and airways) filling first, followed by the alveolar interstitium and then the alveoli themselves (Staub et al., 1967). There are increasing possibilities for interference with O_2 uptake, leading to hypoxemia, as this process proceeds.

Poor oxygenation of arterial blood can be evident even in the absence of alveolar edema. Small peripheral airways and blood vessels are exposed to interstitial hydrostatic pressure. Interstitial fluid accumulation increases the hydrostatic pressure within this region and therefore decreases the pressure gradient acting to keep these airways and blood vessels open, as a result their diameter is decreased (Guyton, 1965 & Iliff, 1971). Small airway constriction or closing leads to poorly ventilated areas of lung and therefore to ventilation/ perfusion mismatching prior to flooding of the alveoli (Laver et al., 1970). When relatively small areas of ventilation/perfusion mismatching exist, administration of high concentrations of O_2 can re-establish lowered PaO_2 by dissolving enough O_2 in the blood from well ventilated areas to load hemoglobin from those areas more poorly ventilated.

As fluid accumulation progresses through the interstitium the alveolar wall itself becomes thickened. This thickening can theoretically produce a diffusion limitation for O_2 across the alveolar-capillary barrier, especially in lungs already compromised by ventilation/perfusion mismatching (King et al., 1974).

With the onset of alveolar flooding, ventilation/perfusion mismatching reaches the point of frank shunting wherein blood is flowing through completely non-ventilated, fluid-filled, regions of the lung. At this point, even high concentrations of O_2 are not able to correct PaO_2 (Laver et al., 1970) and cyanosis becomes increasingly severe. When the situation becomes extreme, even larger airways fill with fluid. Terminally, pulmonary edema froth appears even in the bronchi and trachea.

The decreased compliance characteristic of ARDS is also due to the accumulation of excess water in the periphery of the lung. As the interstitium fills with water it becomes stiffer and therefore more force is required to expand the lung, leading to dyspnea. In addition to this loss of elasticity, the decreases in lung volume upon narrowing and obliteration of airspaces contribute to the decreased compliance, the lung becoming less compliant at smaller volumes (Sharp et al., 1961).

As the alveoli fill with fluid, there is disruption of surfactant and inactivation, by proteins present normally in the plasma and now entering the alveoli, of its ability to lower surface tension (Taylor & Abrams, 1966). This loss of the ability of surfactant to lower surface tension, which opposes expansion of the lung, contributes to the decreased compliance of the lung.

The tachypnea and dyspnea of ARDS are a result of the patient breathing with less compliant lungs. Smaller degrees of lung expansion require less effort and hence rapid shallow breathing (tachypnea) is preferred. Expansion, to any degree, of stiff lungs is a labored and somewhat painful experience (dyspnea) and may be made more unpleasant by stimulation of irritant receptors, termed J receptors, in the interstitial wall (Paintal, 1970).

Thus it can be appreciated that all of the clinical and pathophysiologic features of ARDS are traceable to increased permeability of the alveolar-capillary barrier against leakage of plasma out of the microvasculature.

PULMONARY EDEMA:

All of the clinical and pathophysiologic features of the Adult Respiratory Distress Syndrome are a direct consequence of excess fluid accumulating in the parenchyma of the lung. This condition is known as pulmonary edema.

There are two distinct types of pulmonary edema, which can be distinguished experimentally and clinically. These are hydrostatic pulmonary edema and permeability pulmonary edema. In both types, fluid enters the lung parenchyma at a rate faster than it can be removed by the lymphatic channels draining the lung. Differentiation between these types is made on the basis of the defect in fluid balance leading to this increased rate of fluid accumulation.

MICROVASCULAR HEMODYNAMICS & FLUID PATHWAYS IN THE LUNG:

The movement of fluid between the microvasculature and the parenchyma of the lung (or any organ) is governed by the balance of hydrostatic fluid pressure, between the microvascular lumen and the surrounding interstitum, and the oncotic pressure developed between these same spaces. Hydrostatic forces are determined by hemodynamics and oncotic forces by concentrations of proteins and other osmotically active (molecular weight > 60,000) species. Modifying the effect of these pressure gradients are the characteristics of the membrane(s) across which fluid must diffuse.

In the normal lung there is always some cycling of water out of the microvasculature through the interstitium and into the lymphatics, by which it is eventually returned to the circulation. There is, there-

fore, a continual excess of water transudation compared to resorption across the endothelial wall. It is only when the rate of entry into the interstitium becomes excessive that problems develop.

The classical description of microvascular fluid balance is that of Starling (1896), and takes the basic form:

$$\text{Flow} = \text{Permeability} \times \text{Pressure}.$$

In Starling's equation the permeability of the intervening membrane is multiplied by a summation of the influences of the hydrostatic and oncotic pressures:

$$Q_f = K_f \left[(P_{mv} - P_{pmv}) - R\ (II_{mv} - II_{pmv})\right]$$

where Q_f is the net transvascular fluid flow, P_{mv} is the hydrostatic fluid pressure within the microvascular lumen, P_{pmv} is the hydrostatic fluid pressure in the perimicrovascular interstitium, II_{mv} is the oncotic pressure within the microvascular lumen and II_{pmv} is the oncotic pressure in the perimicrovascular interstitium.

Values for most of the variables in the Starling equation are not known with certainty for the lung, II_{mv} is the only variable for which a value is agreed upon, it is the oncotic pressure of normal plasma. The value of P_{mv} has traditionally been estimated by the equation:

$$P_{mv} = P_{la} + 0.4\ (P_{pa} - P_{la})$$

where P_{la} equals left atrial and P_{pa} pulmonary artery pressures. Recent support for this equation has been found (Holloway et al., 1983). Measurements of lung lymph protein concentration by Staub's group (Staub, 1975) and the results of Nicolayson et al. (1975) indicate that II_{pmv} is approximately 70% of II_{mv}. Microcapsular implantation studies have indicated that P_{pmv} may range as low as -5.0 to -15.0 mmHg (Snashall & Hughes, 1981). The conclusion drawn from consideration of

all of these estimates is that both the hydrostatic and oncotic pressure gradients across the microvascular endothelium are within the same range. More important than the magnitude of the forces however, is how they change with different physiologic states and the effects they produce upon alteration of the membrane across which they act.

K_f in the Starling equation is the microvascular membrane filtration coefficient, which is a measure of the transmembrane fluid flow per unit driving pressure. It is the product of the membrane's hydraulic conductivity per unit area and the surface area available for exchange. This is the permeability term in the general flow equation. Because of the difficulty in achieving the necessary experimental conditions, all other variables in the Starling equation must be either fixed or measured, K_f has only been estimated and the values do not inspire much confidence (Erdmann et al., 1975).

The last term, R, is the microvascular reflection coefficient for plasma proteins, reflecting the membrane's permeablility to proteins relative to water. This term takes into account the fact that the microvascular membrane is not a perfect semipermeable membrane for plasma proteins, there is some continual leakage. Therefore full osmotic pressure, due to the concentration gradient for plasma proteins across this membrane, is not developed. A membrane which is completely impermeable to any given species has a reflection coefficient (R) equal to 1.0 for that species. Conversely, a value of zero for the reflection coefficient denotes a membrane offering no resistance at all to passage of that species. In the normal lung, R = 0.9 (McNamee et al., 1979).

Analysis of the Starling equation predicts two types of situations leading to increases in fluid filtration into the lung. Increases in

the driving pressure, either hydrostatic ($P_{mv} - P_{pmv}$) or oncotic (II_{mv} II_{pmv}), could lead to increased fluid alone. An alteration of the membrane's permeability (as reflected by K_f) could lead to increases in both fluid and protein flow. The former is hydrostatic pulmonary edema and the latter is permeability pulmonary edema.

In either case, the pathways traversed by this excess fluid leaving the microcirculation are the same. The interstitium of the lung functions to keep the gas exchange surface relatively dry, even in the event of "leaks" in the vessels closely apposed to that surface.

A great portion of the alveolar interstitium prevents the accumulation of water by simply not existing. The septum separating adjacent alveoli can be characterized as having a thick and a thin regions, with capillaries in between. In the thick regions, interstitial fibers and ground substance form a true interstial space running between the capillary endothelium and the alveolar epithelium. In the thin regions however, there is no interstitium, the basement membranes of the endothelium and epithelium are fused into one unit. It is believed the majority of gas exchange takes place across thin regions, indeed it is towards this region that the interstitial fibers displace the capillary upon stretching of the septum during inspiration (Weibel & Bachofen, 1979). Kapanci et al. (1974) observed "contractile cells" attached to both endothelium and epithelium, thus spanning the width of the alveolar interstitium in the thick regions. These may counter fluid accumulation there by contracting to decrease compliance of the compartment. Although the alveolar septum is the site of fluid leakage during an imbalance in the Starling equation forces, fluid is rapidly drained.

Just as the alveolar interstitium is designed to stay dry, the extra-alveolar, or junctional, interstitium is designed to draw in fluid. This area is composed of loose connective tissue surrounding the pulmonary blood vessels and conducting airways. These areas are defined by the junction line of 3 or more alveolar septa. They are therefore supported by fibers projecting radially outward. With expansion of the lung, these fibers are stretched thus exerting tension on the loose connective tissue and lowering hydrostatic pressure within. This suction effect contributes to drainage of excess fluid out of the alveolar septum into the extra-alveolar/junctional interstitium. The lymphatic capillaries of the lung arise within this compartment and fluid here gains entrance to the lymphatic system (Weibel & Bachofen, 1979).

The gradient of hydrostatic pressure, established by the structure of the interstitium and decreasing from the alveolar septum to extra-alveolar/junctional interstitium, is likely responsible for the sequence of fluid accumulation in edematous lungs. Excess fluid preferentially accumulates in the looser connective tissue of the extra-alveolar/junctional interstitium. There it forms perivascular or peribronchiolar "cuffs" of fluid. The alveolar septum becomes distended with fluid only later in the process (Staub et al., 1967).

The pathway taken by fluid, excess or otherwise, extravasated from the alveolar microvasculature is therefore described. Traversing the endothelial wall into the alveolar septum, the fluid is rapidly suctioned through the septum and into the junctional spaces. Here it is taken up by the terminal lymphatics, moves out of the lung and is eventually returned to the circulation.

HYDROSTATIC PULMONARY EDEMA:

Hydrostatic pulmonary edema is the result of an overall increase in the hydrostatic pressure driving fluid out of the microvasculature. In this type of pulmonary edema the endothelial walls of the microvasculature retain their usual ability to seive macromolecules. As a result, plasma proteins are prohibited from leaving the microvasculature. The fluid entering the interstitium, and eventually the alveoli, has a low (relative to plasma) protein concentration.

Theoretically, there are two conditions potentially leading to development of this type of pulmonary edema. The hydrostatic pressure gradient ($P_{mv} - P_{pmv}$) across the endothelial wall could increase or the oncotic pressure ($\Pi_{mv} - \Pi_{pmv}$) gradient could decrease.

Although alteration of the oncotic pressure gradient could conceptually result in increased transendothelial fluid flow, because the pulmonary microvascular membrane is not impermeable to proteins this situation is not observed. The oncotic pressure gradient across the microvascular membrane develops passively and quickly, relative to the development of pulmonary edema. Therefore, any changes in protein concentration on one side of the endothelial membrane are rapidly compensated for by changes on the other side (Vaughn et al., 1974).

Alterations in hydrostatic pressure, however, are very common. Elevations of microvascular hydrostatic pressure (P_{mv}) are produced mainly as a result of abnormalities in cardiac function. With left ventricular failure, the heart does not empty fully at the end of each systole (increased afterload). Blood backs up through the left atrium and into the pulmonary circulation, from the venous side to the micro-

vasculature. High pressures develop in the blood therein and this increased hydrostatic pressure forces fluid into the parenchyma of the lung. This is the type of mechanism producing the edema (pulmonary and otherwise) classically associated with congestive heart failure.

The Starling equation predicts a linear increase in fluid filtration with increased microvascular hydrostatic pressure. Although this occurs, frank pulmonary edema does not immediately result. The protein content of the interstitial tissue provides a protective mechanism against this event. As fluid flows through the interstitium and leaves via the lymphatic system, protein is washed out of the perimicrovascular regions. Normally, there is a balance between the amount of protein leaving via this pathway and that entering from the microvasculature. However, with increased amounts of fluid flowing through the interstitium, proteins are washed out at a faster rate and the protein concentration therein falls. This decrease in protein concentration produces an increase in the oncotic pressure gradient across the endothelial wall. The result is a decrease in the net force driving fluid out of the microvasculature, countering somewhat the effects of the increase in hydrostatic pressure. It has been estimated that up to 50% of the effect of increased hydrostatic pressure can be countered via this dilution of interstitial protein (Erdmann et al., 1975). It is only when this "protein wash out" safety mechanism is overwhelmed that pulmonary edema develops.

In a classical set of experiments on hydrostatic mechanisms of pulmonary edema, Guyton & Lindsey (1959) determined in dogs that an increase in left atrial pressure to over 25 mmHg is necessary to pro-

duce accumulation of interstitial fluid in the normal lung, as measured by increased Lung Wet/Dry and Wet Lung/Body weight ratios. However, if the protein content (and hence oncotic pressure) of blood is reduced by 50%, an elevation to only 11 mmHg produces these increases. In a much more sophisticated experiment, in which the caudal mediastinal lymph nodes of sheep were chronically catheterized to obtain samples of essentially pure lung lymph, Erdmann et al. (1975) measured lymph flow and its protein concentration. Lung lymph collected in this manner is believed to accurately represent interstitial fluid in both protein concentration and rate of accumulation (Staub et al., 1975 and Nicolayson et al., 1975). These investigators found a normal lung lymph flow equal to approximatley 6 ml/hour with a protein concentration 0.69 that of plasma (lymph/plasma or L/P protein ratio). Elevations of pulmonary microvascular pressure produced approximately linear increases in lymph flow and corresponding decreases in lymph protein concentration, verifying the predictions of the Starling equation for conditions modelling hydrostatic pulmonary edema. These experiments also verified the protective effect of the wash out of interstitial protein. The difference in plasma to lymph osmotic pressure increased by only half of the induced increase in microvascular hydrostatic pressure. In addition, extravascular lung water did not increase until microvascular hydrostatic pressure had more than doubled.

PERMEABILITY PULMONARY EDEMA:

Alteration of the microvascular endothelial wall so that its ability to filter protein and other macromolecules is greatly decreased or

lost results in Permeability pulmonary edema. It is this type of pulmonary edema that is the highly destructive feature of ARDS.

In this type, the fluid entering the parenchyma has a relatively high protein concentration. In terms of the Starling equation, K_f, the membrane's conductivity, is increased thus allowing increased transendothelial flow of fluid. Although R, the reflection coefficient for protein, theoretically does not have to decrease along with the increase in K_f, this is almost invariably the case (Staub, 1978). The decrease in the membrane's ability to inhibit protein flux results in a diminution of the oncotic pressure gradient as a factor controlling fluid flux and the s (II_{mv} - II_{pmv}) term in the Starling equation loses significance. Since II_{pmv} rapidly equalizes with II_{mv}, interstitial protein wash out cannot function as a protective mechanism against fluid accumulation. Transendothelial fluid flow is then determined almost exlcusively by the product of K_f x (P_{mv} - P_{pmv}). It can therefore be expected that in permeability pulmonary edema small increases in hydrostatic pressure can have dramatic effects on fluid accumulation. In fact one of the key features of pemeability pulmonary edema is that excess lung water accumulates in spite of pulmonary wedge pressures that are normal or even lower than usual (Fein et al., 1979).

Experiments similar to those of Erdmann et al. (1975) have been performed on sheep whose microvascular permeability is increased by administration of E. coli endotoxin (simulating gram - negative septic ARDS). These experiments (Brigham et al., 1979) resulted in an increase in lung lymph flow with increased protein concentration. In the steady state, lymph flow was 5 times control and lymph/plasma protein ratio was elevated (to 1.15 times control), in contrast to the results with

increased hydrostatic pressure. These results are clearly indicative of a situation of increased permeability at the level of the exchange vessels. Analysis of concentration ratios for proteins with different molecular radii showed that the endothelial wall retained some ability to seive macromolecules. Increases in permeability were greatest for proteins of smaller radius, although permeability was increased dramatically for all proteins studied (radius 35 to 100 angstroms). It was earlier shown in a similar model (Pseudomonas aeruginosa infusion in the same animal preparation), by Brigham et al. (1974), that no pulmonary edema, detected as EVLW/dP (extravascular lung water/dry parenchymal tissue) weight ratio, was present in sheep surviving the period of increased microvascular permeability. There were increases in lung lymph flow, with elevated lymph protein clearance, (similar to the endotoxin experiment) in all of these animals. Two sheep dying during the steady state period did have increased EVLW/dP ratios, indicative of serious pulmonary edema.

The previous experiment on endotoxin-mediated increases in microvascular permeability can be compared with experiments on hydrostatically-mediated increases in lymph flow (Bowers et al., 1977) to determine the relationship between increases in hydrostatic pressure and lung lymph flow in both types of fluid leakage. Small increases in microvascular hydrostatic pressure (10 cm H_2O raised to less than 15 cm H_2O) produced large (4 fold) increases in lung lymph flow in the permeability experiments. Larger (10 cm H_2O raised to 25 cm H_2O) increases in pressure produced smaller (2 fold) lung lymph flow increases in the hydrostatic experiments.

In considering ARDS it is important to make clear the distinction between hydrostatic and permeability forms of pulmonary edema.

The hydrostatic form can be characterized as high pressure, low protein pulmonary edema. The high microvascular hydrostatic pressures in this form produce accumulation of a relatively protein-poor fluid since microvascular wall retains its ability to filter protein. The "defect" here is usually at a site (the ventricular wall for example) well removed from the exchanging vessels of the lung. In this type of pulmonary edema, decreasing hydrostatic pressure within the pulmonary circulation should have beneficial effects.

By way of contrast, the permeability form can be characterized as low pressure, high protein pulmonary edema. In this form, loss of ability of the endothelium to filter large molecular weight substances results in accumulation within the lung of fluid with a protein concentration close to that of plasma, with little or no elevation in driving pressure. It is clear the "defect" in this type of pulmonary edema is at the level of the microvasculature itself. Any manipulation decreasing hydrostatic pressure should produce dramatic attenuation of the rate of edema formation.

The contrasts between these 2 types of mechanisms leading to pulmonary edema were investigated by Vaughan et al. (1974) with sheep chronically catheterized for lung lymph collection. They examined the rate of clearance in lung lymph of labelled albumin administered IV. When a left atrial balloon was inflated to increase microvascular pressure 2 fold over normal, lymph flow also doubled but albumin clearance from the lung increased to a lesser extent. Infusion of Pseudomonas bacteria produced no detected increase in microvascular pressure, but

lymph flow rose to 8 fold normal and albumin clearance from the lung increased 5 fold.

It is therefore apparent that 2 very different mechanisms lead to the production of pulmonary edema. The characteristics of each are important not only with respect to treatment, but also with regard to achieving a mechanistic understanding of the events operative in such clinical situations as congestive heart failure and ARDS.

PULMONARY EDEMA OF ARDS:

The physiological and clinical features of ARDS do not allow distinction between the 2 types of pulmonary edema. It is the histological and pathological findings, along with newer sophisticated clinical laboratory measurements, that assign ARDS to the category of permeability pulmonary edema.

Ultrastructural studies are lacking on the main types of permeability pulmonary edema (models of sepsis with Pseudomonas or endotoxin) discussed thus far. Brigham et al. (1974) did publish and comment on one micrograph showing widening of interlobular septa, perivascular cuffing with fluid and some alveolar edema.

Strong parallels to experiemntal permeability pulmonary edema have been made by clinical studies on the pulmonary hemodynamics and protein handling in ARDS patients. Fein et al. (1979) analyzed 24 patients with severe pulmonary edema. These were split into 2 groups on the basis of cardiovascular status and their pulmonary edema fluid analyzed for protein content, along with their plasma. Eighteen of the patients showed no evidence of heart disease and had normal (0 to 15 mmHg) or slightly elevated (< 20 mmHg) pulmonary wedge pressures. In this group

the edema fluid/plasma protein concentration ratio always exceeded 0.6. In the patients with diagnosed cardiogenic edema, with pulmonary wedge pressures ranging 23 to 37 mmHg, the edema fluid/plasma protein concentration ratio averaged 0.46. The high protein, low pressure group included a number of cases with medical conditions typically leading to ARDS: shock, trauma, infection and drug overdose. Thus the classical distinction between hydrostatic and permeability pulmonary edema, made on the basis of protein concentration and hydrostatic pressure, in the experimental setting holds also for clinical forms. Pathological findings in the ARDS group (hyaline membranes, blood cell aggregates in pulmonary vessels, fibrosis in addition to alveolar edema and hemorrhage) were also distinct from the hydrostatic group (alveolar edema, hemorrhage only). By definition, the ARDS patients in this study had permeability pulmonary edema and the cardiogenic patients had hydrostatic pulmonary edema.

In another study on permeability in ARDS, Anderson et al. (1979) identified 2 patient groups with pulmonary edema: those with sepsis and those with heart failure (HF) following myocardial infarction. Clearance of labelled albumin from the blood into a tracheal tube was then measured to estimate pulmonary capillary permeability. Septic patients cleared the albumin almost 10 times faster than the HF patients. All patients had comparable degrees of pulmonary edema and the septic patients had normal pulmonary wedge pressure and serum oncotic pressure. The increased protein clearance in the septic patients could therefore be attributed to increased pulmonary microvascular permeability. A similar finding was reported by Robin et al. (1972). Two patients with

septic shock were shown to have protein concentrations in their pulmonary edema fluid very close to that of plasma.

These clinical studies support the correlation between ARDS and permeability pulmonary edema. Patients in these studies exhibited the hallmarks of a permeability-mediated increase in transvascular fluid movement: low/normal hydrostatic pressures and "edema" fluid with high protein concentration.

MECHANISTIC CONSIDERATIONS:

Over the past 10 years has come a complete re-evaluation of our understanding of permeability pulmonary edema and the pathogenesis of ARDS. There has been a dramatic increase in the number of studies on experimental and clinical forms of permeability pulmonary edema and whole new theories have evolved about the underlying mechanism(s).

Surfactant:

Early theories regarding the mechanism operative in permeability pulmonary edema were concerned with disruption of surfactant function (Pattle, 1955). Because of the potentially high surface tension at the air-liquid interface of the alveolar epithelium, it was reasoned that loss of surfactant function would create a strong negative pressure drawing fluid into the alveolus. This concept was reinforced by the clinical and histological similarities of both permeability pulmonary edema and ARDS with Respiratory Distress Syndrome of the Newborn (Petty & Ashbaugh, 1971). The latter syndrome has been attributed to deficient surfactant production in the fetus (Avery & Mead, 1959 and Adams et al., 1970). However, it is now clear that disruption of surfactant

promotes alveolar collapse and atelectasis, but in and of itself does not lead to pulmonary edema (Staub et al., 1975).

The confusion and controversy surrounding the possible mechanistic involvement of surfactant illustrates a recurring problem in research on the causes of pulmonary edema; separation of cause and effect. No one disputes the findings of alterations of surfactant, leading to diminished ability to lower surface tension, in cases of pulmonary edema or ARDS (Said et al., 1965 and Petty et al., 1977). One of the logical conclusions from these observations is that disruption of surfactant could be the cause of the corresponding pulmonary edema, especially in light of the ready availability of a reasonable explanation, provided by the surface forces operative within the alveolus. The alternative conclusion, that the disruption of surfactant structure and function is a result of pulmonary edema and not the cause, is less attractive, no matter how true.

Histamine:

Another early theory, possibly the first to postulate involvement of components of inflammation, concerned the increased microvascular permeability produced by histamine. Pietra et al. (1971) found that histamine was capable of producing an edematous reaction in the lungs. This edema was produced in the peribronchiolar interstitial tissue and was mimiced by compound 48/80, a low molecular weight polymer of p-methoxy-N-methylphenethylamine which degranulates mast cells thus releasing histamine. It was therefore postulated that histamine release might be involved in producing certain types of pulmonary edema.

Observations within the same experiment cast doubt on this interpretation however. Tracer studies showed that the site of leakage was confined to the post-capillary venules in the bronchial circulation, with no effect on the pulmonary circulation. In addition, the duration of leakage in these experiments was extremely limited, lasting only 10 to 15 minutes. In a later experiment, Brigham & Owen (1975) infused histamine into sheep chronically catheterized for lung lymph collection. They found a sustained increase in lymph flow, at high protein concentration with prolonged infusions of histamine and claimed increased pulmonary microvascular permeability. Persson et al. (1978) also produced an edematous reaction in the lung by exposing guinea pigs to aerosols of histamine, an effect countered by administration of beta-receptor agonists.

It was subsequently shown, by tracer studies (Pietra et al., 1979), that even prolonged histamine infusion produces leakage only in the bronchial circulation with no interstitial or alveolar edema. Thus, while histamine may play a role in the initial inflammatory reaction to lung injury and has the potential to produce increases in microvascular permeability, it most likely has little effect in the sustained reaction leading to pulmonary edema.

Coagulation System:

A number of the components of the coagulation system have been been implicated in permeability pulmonary edema and ARDS. Most of the attention has centered on the potential role of platelets, fibrin and the microemboli formed by their interaction.

Platelets contain a number of substances capable of increasing microvascular permeability including histamine, serotonin and bradykinin. In addition, they contain or can synthesize other permeability-enhancing agents and a number of mediators of inflammatory action by other cells. Platelets have been shown to be sequestered in the lungs of patients with ARDS (Schneider et al., 1980). Intratracheal instillation of native or synthetic platelet activating factor produces an acute inflammation of the lung similar to that of ARDS (Camussi et al., 1983). This condition was characterized by edematous thickening of the alveolar septa with infiltration of polymorphonuclear leukocytes and macrophages into the septa and the alveoli. With progression of the reaction there was alveolar edema and eventually the development of pulmonary fibrosis.

Evidence regarding their role in the increased pulmonary vascular permeability in ARDS-type models has been conflicting however. Vaage (1976) reported increased lung microvascular permeability after platelet embolization, although the effect was transient. Binder et al. (1980) also observed a transient (1 hour) period of increased microvascular permeability after platelet embolization within the lung. Platelets remained depleted in the circulation long after this period although microvascular permeability had returned to normal. It therefore appears that any independent direct action platlets have in inducing permeability pulmonary edema is short lived.

The situation with regard to fibrin, and its degradation products, is similar. Haynes et al. (1980) examined patients with ARDS and compared them to patients with similar preceeding medical conditions who did not develop ARDS. The ARDS patients had increased amounts of D-

antigen (a fibrin/fibrinogen degradation product) in their serum. It was observed that D-antigen may either be a "marker" for ARDS or may be involved in its development. In experimental animals the D-antigen has been shown to produce effects mimicing ARDS (Manwaring et al., 1978) including rapid respiration, hypoxemia and increased pulmonary microvascular permeability. Again, however, there are conflicting reports. Complete depletion of circulating fibrinogen was found (Binder et al., 1979) not to prevent the increased microvascular permeability of the pulmonary circulation following glass bead microembolization.

Microembolization is believed to mimic the condition wherein platelet-fibrin thrombi impact in the pulmonary circulation. Microemboli composed of platelets and fibrin are often found in the lungs of patients dying from ARDS (Saldeen, 1976) and in vivo microembolization with thrombin has produced increases in microvascular permeability in the pulmonary circulation (Malik & van der Zee, 1977). Again, however, the results of Binder et al. (1979) are not in agreement. These coagulation factors certainly have the potential for contributing to a mechanism involved in the production of permeability pulmonary edema and the pathophysiologic changes of ARDS. The extent, if any, of that contribution is currently unclear.

Complement/Polymorphonuclear Leukocytes:

Perhaps the most interesting and promising mechanistic indications concerning permeability pulmonary edema and ARDS have been with respect to the possible involvement of complement and polymorphonuclear leukocyte (PMN) activation. The PMN, when activated, can generate active oxygen species such as the hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide

(H_2O_2) and the superoxide anion (O_2^-), any of which has the potential for damaging the endothelium (Badway & Karnovsky, 1980). In fact, activated PMNs have been shown to damage endothelial cells in culture (Sacks et al., 1978) via the action of oxygen radicals.

In addition to oxygen radicals, PMNs also contain proteases, acid and neutral, which are capable of inflicting severe tissue damage. The PMN neutral proteases can degrade elastin, collagen, proteoglycan and basement membrane (Janoff et al., 1979). Not only can the PMN proteases destroy tissue, they can also amplify an inflammatory response by cleaving fibrinogen, the Hageman factor and complement. Another effect of oxygen radicals can be inactivation of the antiproteases that normally protect the lung from such damage (Carp & Janoff, 1980), thus allowing a cycle of PMN-mediated damage that can be self-reinforcing.

At autopsey, PMNs have been found in great numbers in the lungs of patients with ARDS (Pietra et al., 1981). Bronchoalveolar lavage (BAL) fluid from patients with ARDS contained 10 to 100 times as many cells as that from normals. Many of the cells were PMNs as compared to no PMNs from normals (Fowler et al., 1981). Analysis of BAL fluid has also revealed active PMN elastase in 17 of 24 ARDS patients studied (McGuire et al., 1982). Those patients without active elastase were found to have an elastase inactived by $alpha_1$-proteinase in their BAL fluid. This elastase was found to cleave and activate a number of inflammatory proteins, including complement proteins. None of the normals studied had measurable elastase activity in their BAL fluid. Perhaps the most impressive clinical study with regard to possible PMN involvement is that of Hammerschmidt et al. (1980). They analyzed samples of plasma from random patients with a medical condition often

preceeding ARDS and found a striking correlation between the presence of "C5a-like" PMN aggregating activity and the later development of ARDS. In those cases that did not progress to ARDS there was no correlation with presence of this aggregating activity.

Experimental work further supports the possible involvement of complement and polymorphonuclear leukocytes in the development of permeability types of pulmonary edema. Injection of rabbits with complement -activated plasma leads to a reaction resembling ARDS, with tachypnea and hypoxemia (Hohn et al., 1980). Vascular plugging by PMNs was observed, as was the development of endothelial damage with interstitial edema. These changes did not develop in animals depleted of their PMNs. Phorbol myristate acetate (PMA), which activates PMNs in vivo, produces a permeability-type of increased lung lymph flow in chronically catheterized sheep (Loyd et al., 1983). Subsequent to an initial pulmonary hypertension, lung lymph flow was increased while maintaining a normal protein concentration, the hallmark of a permeability type of increased flow. In isolated/perfused lungs this effect has been shown due to the generation of oxygen radicals by the PMA-stimulated PMNs (Shasby et al., 1982). It has also been shown that prior depletion of PMNs attenuates development of increased lung lymph flow or pulmonary edema in a number of experimental models of ARDS. These include microembolization in the sheep, normally producing increased lung lymph flow with a high protein concentration (Flick et al., 1981), and pulmonary edema due to inhalation of high concentrations of oxygen, normally producing increased lung wet weights and extravascular lung water (Shasby et al., 1982a).

There is, therefore, a strong body of evidence supporting the possible involvement of activation of complement and polymorphonuclear luekocytes in the production of permeability pulmonary edema and ARDS. Complement activation is certainly a feature of many of the medical conditions preceeding ARDS (sepsis, trauma, etc.) and PMNs are definately capable of producing many of the pathophysiologic changes characteristic of these lung injuries.

A note of caution should perhaps be added. Much of the evidence supporting the possible involvement of components of the coagulation and complement systems, as well as PMNs, comes from the observation of these proteins and cells within the alveoli or other areas accessable by BAL. It should be kept in mind that one of the criteria for permeability pulmonary edema is breakdown of the barrier(s) to leakage of just these types of substances from the plasma. It is very possible that these materials are present in the alveoli not because they are the cause of the edematous reaction, but that the loss of the barrier to leakage allows them access to this area. As with surfactant, it is important to establish the distinction between cause and effect.

NITROGEN DIOXIDE:

Nitrogen dioxide (molecular formula NO_2) is a reddish-brown gas existing in equilibrium with its dimer, dinitrogen tetroxide (N_2O_4), a chocolate-brown liquid. The ratio of NO_2 to N_2O_4 at body temperature (37°C) is approximately 30:70. The molecular weight of NO_2 is 46.01, with the boiling point of the NO_2-N_2O_4 mixture being 21°C, close to room temperature. NO_2 has a strong odor and high oxidation rate, making it extremely corrosive.

Nitrogen dioxide concentrations in air are usually expressed as either parts-per-million (ppm) or micrograms per cubic meter (ug/m^3) with the conversion factor (at 25°C and 760 torr) being:

$$ppm = (ug/m^3)/1880$$

(Natl Air Quality Crit Advisory Comm, 1971).

Present in environmental, indoor and industrial settings, nitrogen dioxide is a seemingly ubiquitous potential health hazard. Low concentrations are present in the ambient air of most urban areas (1968 hourly averages in Pheonix = 0.036 ppm and Los Angeles = 0.070 ppm, Committee on Medical and Biological Effects of Air Pollutants, 1977). These levels are thought to contribute to the increased incidence of respiratory illness in urban areas (Shy & Love, 1980). Exposure to moderate concentrations (10 to 30 ppm) for extended periods is used in the laboratory to produce a model of emphysema (Freeman et al., 1968). High concentrations (>50 ppm) can be generated in industrial situations such as metal cleaning processes, electric arc welding, dynamite blasting, diesel engine operation or missile refueling and have been involved in a number of fatal accidents (NIOSH Crit Doc, 1976 and Yockey et al., 1980).

HEALTH EFFECTS:

Evidence from a multitude of studies in biologic systems indicates that the major toxic effects of NO_2 are produced in the deep lung, in the area of the terminal airways and gas exchange surfaces.

At very low concentrations, NO_2 is an irritant to the respiratory tract, producing increased airway resistance in humans. Beil & Ulmer (1976) exposed volunteers to graded low levels of NO_2 (1.0, 2.5, 5.0 and 7.0 ppm) for 2 hours. They noted increased airway resistance at 2.5+ ppm NO_2. In a longer exposure (5.0 ppm for 14 hours), the initial increased airway resistance, which occurred during the first half hour, resolved somewhat by 2 hours. There was then another increase in airway resistance between 6 and 14 hours of exposure. In addition to increases in airway resistance, von Nieding et al. (1973) observed a significant decrease in CO diffusing capacity in humans breathing 5.0 ppm NO_2 for 15 minutes.

Of necessity, studies with higher concentrations or longer exposure periods are carried out only on experimental animals. A number of these studies have investigated the effects of chronic exposure to relatively low levels of NO_2.

Mice exposed to 0.5 ppm NO_2 for 3 to 12 months developed changes in alveolar structure (Blair et al., 1969). Alveoli were seen to be expanded in all exposed animals. Permanent alteration of pulmonary function has been observed in rats exposed to 0.8 ppm NO_2 for their natural lifetime. While these animals exhibited sustained tachypnea, there was no microscopic evidence of obstructive disease (Freeman et al., 1966).

Exposure to 2.0 ppm NO_2 continuously for life resulted in early evidence, cytoplasmic blebbing and loss of cilia, of respiratory injury in rats (Freeman et al., 1968). Acute exposure at similar levels (1.5 to 3.5 ppm) produced increased susceptibility, up to 123% above control, to infection in mice (Ehrlich, 1966).

At concentrations an order of magnitude higher, emphysematous changes develop in the deep lung. Rats continuously exposed to 10 to 18 ppm NO_2 exhibited enlarged air spaces with alveoli showing evidence of having lost septa (Freeman et al., 1968 and 1968a). Riddick et al. (1969) exposed dogs to 25 ppm NO_2 for 6 months and noted fully developed bullous emphysema in one animal and small bullous lesions in the lungs of the 5 other exposed dogs. They also observed increased amounts of collagen in bronchiolar and alveolar areas.

At these higher levels, evidence of more severe injury occurs acutely. Ciliary destruction in the rat was noted at exposure to 15 to 25 ppm NO_2 for 4 hours (Stephens et al., 1972). There is also the appearance of acute injury in the alveoli. Alveolar macrophages were observed ingesting fibrin after a 24 hour exposure of rats to 17 ppm NO_2 (Evans et al., 1971).

Pulmonary Edema:

Overt development of pulmonary edema has been reported for both long exposures at relatively low concentrations and short exposures at high concentrations. Wagner et al. (1965) observed frank pulmonary edema in dogs after 3 to 6 months exposure to 1 to 5 ppm NO_2. In the same study, 50% of guinea pigs exposed to 65 ppm NO_2 for 8 hours succumbed to lung edema.

Hine, Meyers & Wright (1970) carried out the most complete study to date on the acute toxicity, with respect to pulmonary changes, of NO_2. They exposed 5 species (mice, rats, guinea pigs, rabbits and dogs) to various concentrations of NO_2 and reported on mortality and histologic changes in the lungs occurring both acutely and during the following year. Deaths occurring shortly (prior to 96 hours) after exposure were due to pulmonary edema, the lungs becoming filled with fluid so that they sunk when placed in water. Fluid oozed from the cut surface of the lungs and was present in the bronchial tree. Histologically, there was proteinaceous fluid in the alveoli and the interstitium along with hyperemic congestion of the alveolar walls. This pulmonary edema developed in a dose - dependent manner in all 5 species. Exposures to concentrations below 50 ppm, for periods up to 24 hours, produced no mortality due to pulmonary edema, the only deaths being 2 guinea pigs succumbing from asphyxia. Fractional mortalities in most species resulted from exposures to 50 ppm for times in excess of one hour. At 100 ppm NO_2, mortalities in all species occurred for exposures from 0.5 to 2 hours. Above 200 ppm NO_2 all species had poor survival rates in all exposures. Long term effects were noted in animals surviving the exposures. About one third of the survivors showed signs of pulmonary fibrosis at 30 days post-exposure, these tended toward resolution by 3 to 6 months. In three cases there was development of chronic inflammation in the form of bronchiolitis fibrosis obliterans. Many of these findings on acute development of pulmonary edema are substantiated by the observations of others (Kleinerman & Cowdrey, 1968, La Towsky et al., 1941, Carson et al., 1962 and Fenters et al., 1971).

Pulmonary edema secondary to inhalation of high concentrations of NO_2 belongs in the permeability-type classification. One of the hallmarks of this type of pulmonary edema is extravasation of proteinaceous fluid from the microvasculature and its eventual appearance in the alveoli. Many of the reports on NO_2-induced pulmonary edema document leakage of fluid with a high protein content. Alveolar fluid observed by Hine et al. (1970) was described as "proteinaceous material within the alveolar spaces" and fibrin was occasionally noted. After exposure of rats to 17 ppm NO_2 for 24 hours, Stephans et al. (1972) noted alveolar accumulation of fibrinous debris and small alveolar hemorrhages. Analysis of BAL fluid from hamsters exposed to 17 or 22 ppm NO_2 for 48 hours revealed increases in total soluble protein (DeNicola et al., 1981). Lungs from these hamsters were edematous as evidenced by increased Lung Wet/Dry weight ratios and the presense of frothy fluid in their tracheas. The most definative observation with respect to NO_2-induced leakage of protein from the pulmonary microvasculature was made by Langloss et al. (1977). In their publication, a transmission electron micrograph, from a cat exposed to 80 ppm NO_2 for 3 hours, clearly shows fibrin strands emanating from a capillary in an alveolar septum. Intra-alveolar fibrin was noted as a consistent finding in their animals.

Even low level exposure results in significant loss of protein from the pulmonary circulation, an indication of the trend taken in more severe injury. Sherwin & Carlson (1973) collected BAL fluid from guinea pigs exposed to less than 1 ppm NO_2 for 1 week. Protein concentrations in the BAL fluid from exposed animals averaged 50% higher than in BAL fluid from controls. In a previous study, Sherwin & Richters

(1971) reported increased levels of tritiated serum in the lavage fluid from mice exposed to 30 ppm NO_2 for 24 hours.

Unfortunately, the other hallmark of permeability pulmonary edema, low or relatively normal microvascular hydrostatic pressure, is not documented in the literature. There are no reports on pulmonary wedge (approximating microvascular) pressure in experiments in which definate pulmonary injury was produced. One group, Abraham et al. (1979), measured cardiopulmonary function in sheep at the end of an exposure to 15 ppm NO_2 for 4 hours. Although they observed no increase in pulmonary wedge pressure, it is clear from the rest of their data that no detectable pulmonary injury whatsoever resulted from the exposure. Measurements were made on PaO_2, $PaCO_2$ and pulmonary resistance, compliance and hemodynamics. The only change observed was an increase in airway resistance at one time point, evidence of only a minor response to NO_2. Another study (Dowell et al., 1971) reported a slight decrease in pulmonary arterial pressure during a 1 hour exposure of dogs to 16 ppm NO_2. Without concurrent left atrial pressures, however, one cannot even estimate microvascular pressure during the exposure.

Although not a consistent finding in all types of permeability pulmonary edema, the presence of coagulation proteins and PMNs in the alveolar interstitium and the alveoli are characteristics of ARDS-type pulmonary edema. A number of investigators have reported both of these ARDS markers in NO_2-induced pulmonary edema. Many of the reports on the presence of fibrin in the alveoli have already been discussed with regard to leakage of proteinaceous fluid subsequent to NO_2 exposure. Margination of PMNs and platelets within alveolar wall vessels was observed by Dowell et al. (1971) in beagles exposed to 16 ppm NO_2

for 1 hour. Kleinerman & Cowdrey (1968) reported PMN inflitration of alveoli from hamsters exposed to 50 ppm NO_2 for 2 to 3 days. Similar alveolar inflitration by PMNs was noted in the cats exposed by Langloss et al. (1977) to 80 ppm NO_2 for 3 hours. More definative studies were carried out by DeNicola et al. (1981) and Gardner et al. (1969). DeNicola's group measured a number of cytological and biochemical parameters in hamsters exposed to 12, 17 or 22 ppm NO_2 for 48 hours. They concluded that the most sensitive indicator of pulmonary injury was increased numbers of PMNs recoverable in lavage fluid. Ten-fold increases were obtained at even the lowest NO_2 concentration in the study. Gardner's group analyzed cells recovered by bronchoalveolar lavage from rabbits exposed to 40 ppm NO_2 for 3 hours. An increase in excess of 10-fold was noted in PMNs acessable to the BAL washing.

The response to inhalation of high concentrations of NO_2 can therefore be characterized as production of a permeability type of pulmonary edema. This edematous reaction is severe enough to cause death in a large proportion of exposed animals. Analysis of histological observations and the edema fluid as collected by BAL indicates a high protein concentration and the participation of coagulation proteins and PMNs.

NO_2 TOXICITY AS A MODEL FOR ARDS:

This permeability pulmonary edema, produced in response to inhalation of high concentrations of NO_2, has all of the characteristics of being a good model for ARDS. The main features of ARDS, as discussed earlier, are the production of a permeability type pulmonary edema with

a well defined histologic appearance, consisting of deposition of proteinaceous fluid and characteristics of an intense inflammatory reaction in and around the alveoli. In addition, there is often a characteristic latent period between the predisposing event and the manifestation of respiratory symptoms secondary to the accumulation of edema fluid in the lung. Many cases of ARDS which survive the acute edematous phase progress to pulmonary fibrosis or emphysema.

Consideration of the pulmonary edema produced in response to NO_2 reveals that this toxic effect shares all of these above characteristics with ARDS. The permeability nature of NO_2-induced pulmonary edema has just been discussed. As reported by Hine et al. (1970) and La Towsky et al. (1941) the gross and histologic appearances of NO_2 injured lungs are similar to those of lungs from patients dying acutely from ARDS. Lungs from NO_2 exposed animals dying acutely showed hyperemic consolidation with a mottled surface over which solid, dark-red areas alternated with relatively normal or emphysematous areas. These lungs were heavy and a serous, bloody fluid oozed from the cut surface. Histologic signs included capillary congestion or engorgement, interstitial/alveolar edema, and extravasation of fibrin into alveoli. All of these observations are consistent with those of the acute phase of ARDS.

A latent period between exposure and development of respiratory symptoms is characteristic of NO_2 toxicity. This latent period is most often noted in reports on cases of human poisoning from NO_2 (Milne, 1969 & Yockey et al., 1980) and ranges from 4 to 36 hours in duration, just as with ARDS.

If the acute edematous phase is survived, as with prolonged exposures to low concentrations of NO_2, a fibrotic or emphysematous

reaction often develops (Hine et al., 1970 & Freeman et al., 1968). Again this feature of NO_2 toxicity closely parallels the clinical situation with respect to ARDS.

The applicability of NO_2-induced pulmonary edema as a model of ARDS has been noted by Hopewell & Murray (1976) in their discussion of the clinical syndrome.

STATEMENT OF PURPOSE:

There are two general features of both Adult Respiratory Distress Syndrome and the toxic reactions that model it that differ qualitatively from other exogenously precipitated pathologic conditions. These two features are the existence of the characteristic latent period, between the precipitaing event and the onset of respiratory symptoms, and the organ specificty, regardless of etiologic agent or route of exposure, of the resulting damage.

This study was designed to investigate a specific question with regard to the latent period: Is the delay in onset of respiratory symptoms in ARDS due to a delay in onset of pulmonary injury? Alternatively, it was considered that there could be immediate pulmonary injury but that signs and symptoms of distressed respiration became manifest only sometime later, when some "threshold" in accumulated injury had been exceeded. It was felt that an answer to this question would contribute information towards the answer to a more basic question with regard to ARDS: Is the pulmonary injury in ARDS a direct effect of the precipitating event/agent or an indirect response of the body to a more non specific initiating event?

For the case of the NO_2 toxicity model of ARDS the question adopted a more refined form: Are the delayed signs and symptoms of distressed respiration due to a delay in onset of the edemagenic process? The alternative being an immediate onset in edema formation with signs and symptoms occurring only later when enough excess lung water had accumulated to exceed the lung's ability to function more or less normally.

Analysis of the time course of accumulation of excess water and blood in the lung was anticipated to provide an answer to the question regarding onset of pulmonary injury in NO_2 toxicity. Experiments were therefore planned in which animals would be exposed to NO_2 and sacrificed, for analysis of lung water and blood content, sequentially over 24 hours.

It was also anticipated that an understanding of the time course of development of pulmonary edema would allow prediction of pharmacologic agents that could conceivably interfere with the edemagenic process. Once the time course became clear it was possible to identify agents with the potential for influencing, more specifically than the agents currently in use clinically, the devlopment of pulmonary edema.

The more immediate goal of the experiments on pharmacological intervention in NO_2 toxicity was to provide some mechanistic insight to the events preceeding ARDS. It was felt that an analysis of the effect, whether ameliorative or exacerbative, of pharmacologic agents with well defined actions on pulmonary edema development would contribute to an understanding of those events leading to ARDS. A secondary goal was to begin to explore a more effective therapy than is currently available for the 150,000 patients who develop ARDS every year in the U.S.

MATERIALS & METHODS

MATERIALS:

Animals:

Male guinea pigs of the Hartley strain, weighing 250 to 550 gm, were used in all experiments. The guinea pigs had free access to food and water at all times except when in the inhalation exposure chamber. Between the end of exposure and the time of sacrifice the guinea pigs were housed 2 per cage in the laboratory for observation, otherwise they were housed in the U.C.S.F. Animal Facility. Individual animals showing signs of respiratory infection or other health problems were not entered into the experiments. None were deleted for these reasons after exposure to NO_2.

Chemicals:

Except where noted, all chemicals and reagents used were obtained from Sigma (St. Louis, MO). Phosphoric acid (85%) was obtained from Fisher Scientific (Santa Clara, Calif.) and copper sulfate from Aldrich (Milwaukee, Wisc.). Drugs used for anesthesia and the series of experiments on pharmacological intervention were obtained either from Sigma or, when available commercially in unadulterated injectable form, from the Inpatient Pharmacy at U.C.S.F.

Inhalation Exposure System:

Most of the materials for construction of the inhalation exposure system were obtained from Fisher Scientific, Thomas Scientific (San Francisco, Calif.) or Cole-Parmer (Chicago, Ill.). The vacuum pump was purchased from Graingers (South San Francisco) and the PVC pipe

from a local plumbing supply store. The Chemisorb for cleaning dilution air and the Neoprene closed cell sponge used to seal the chamber doors were kindly supplied by Dr. Robert Phalen (U.C. Irvine Air Pollution Health Effects Laboratory).

Calibration of Flowmeters:

Flowmeters used in these experiments were calibrated against both a wet test meter (Singer/American Meter AL-18), using methods described by Nelson (1971), and against a standardized dual-flowmeter system in the Maintenance Lab of the Respiratory Care Facility at U.C.S.F., under the supervision of Al Lopez.

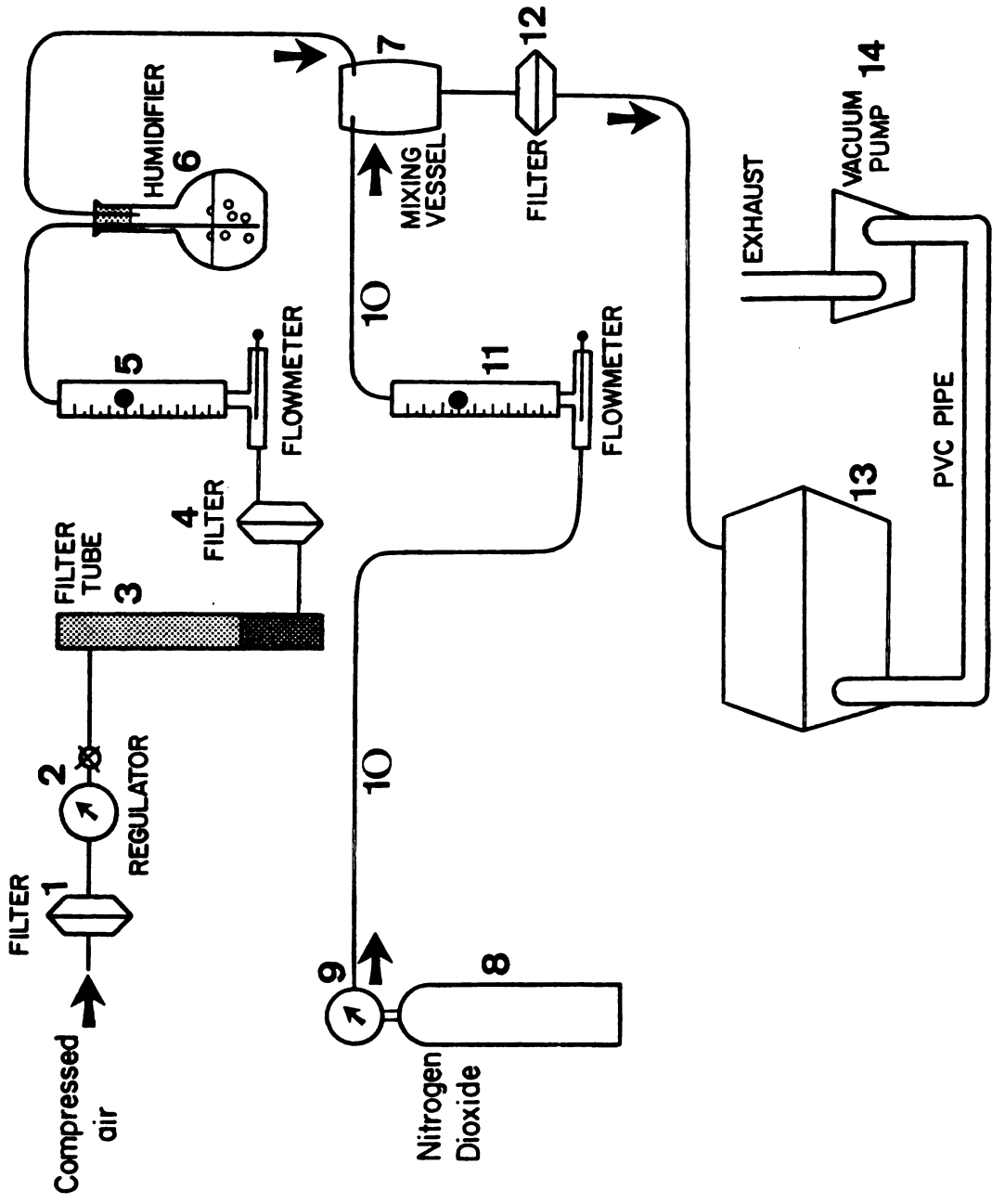
Advice:

Advice and guidance concerning materials and shop techniques used in the manufacture of the inhalation system were kindly provided by Mr. Charles Lee (U.C.S.F. Department of Pharmacology).

METHODS: INHALATION EXPOSURE SYSTEM

An inhalation system, for exposure of small to moderately sized laboratory animals to gases, was designed and built as a part of this project. The basic concept was provided by Dr. Francis Weir, a former graduate of this laboratory, of the University of Texas Health Science Center at Houston. Refinements to this design and suggestions on practical operation were provided by Dr. Robert Phalen of the U.C. Irvine Air Pollution Health Effects Laboratory. This dynamic inhalation system operates on the principle of gas-stream mixing (Nelson, 1971) whereby a single dilution, with clean air, is made to a moving stream of the toxic gas at high concentration. This moving stream, diluted to the appropriate test concentration, is then directed through the chamber housing the animals. The major advantages of such a dynamic system are maintenance of a constant supply of the toxic gas at the desired concentration and constant removal of certain metabolic and waste products (water, carbon dioxide, ammonia and heat) from the animals' environment.

In the present system, air from a compressor is first passed through a filter [# 1 in the following diagram] (AMF Micro-Klean) to remove any dust and oil mist. Pressure is then regulated [2] (Matheson 40-L) to 14 psi prior to the stream entering a large filter tube [3] filled with a dessicant (Drierite) and a reactive material (Chemisorb) to remove oxidant and organic contaminants. A 0.45 um filter [4] (Motor Guard D-10) then removes any particulate matter prior to the stream entering a rotameter-type flowmeter [5] (Matheson 701-PBX with # 75 tube) with a needle valve. Here the dilution air is controlled to the proper flow for mixing with the toxic gas under study. Just before mixing, the air



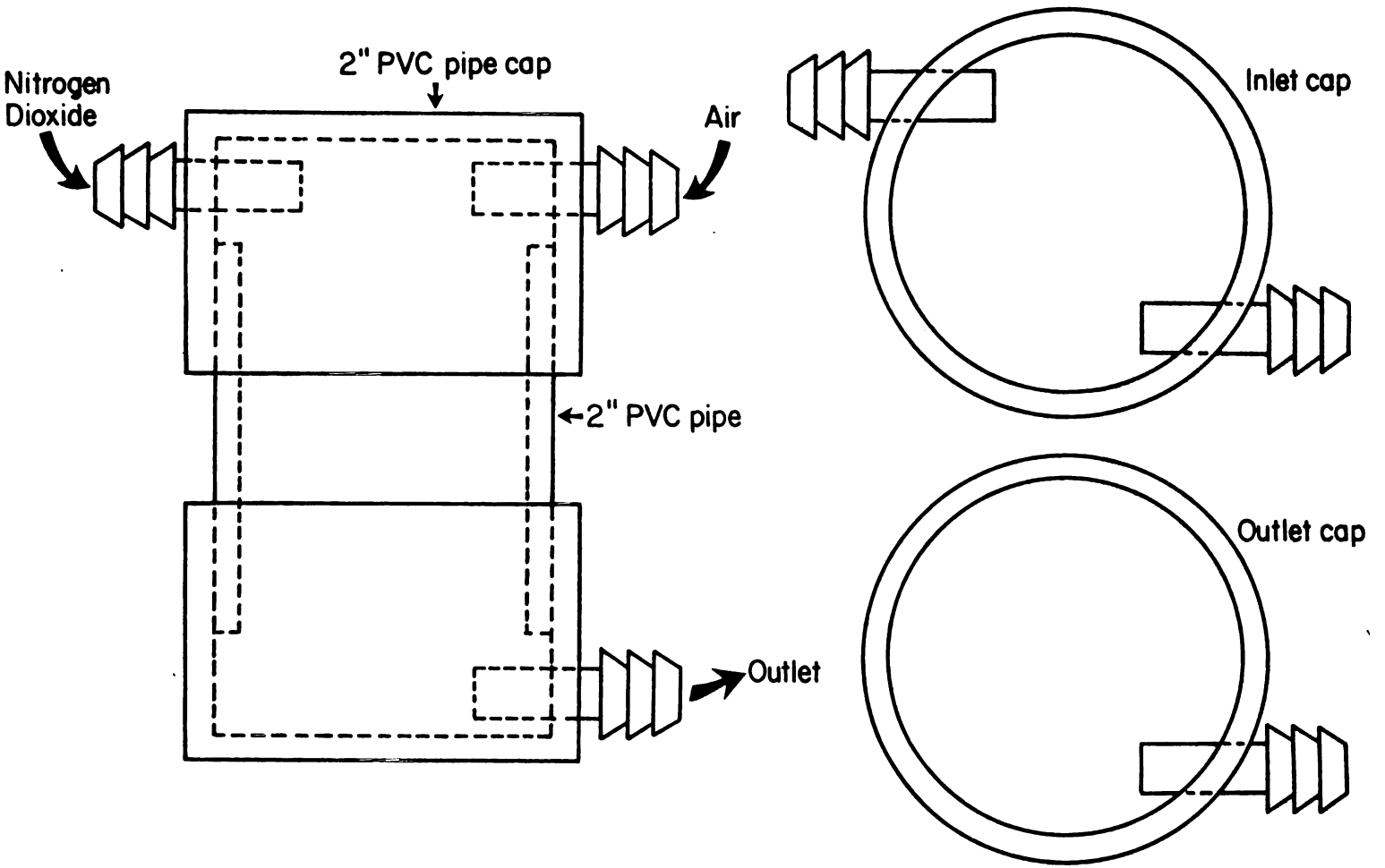
FLOWCHART OF INHALATION EXPOSURE SYSTEM

is bubbled through a humidifier [6] where the relative humidity of the final chamber atmosphere can be controlled by altering water depth. In the present series of experiments, chamber humidity was kept at 40 to 60%. From the humidifier, air enters a vessel [7] for mixing with the nitrogen dioxide.

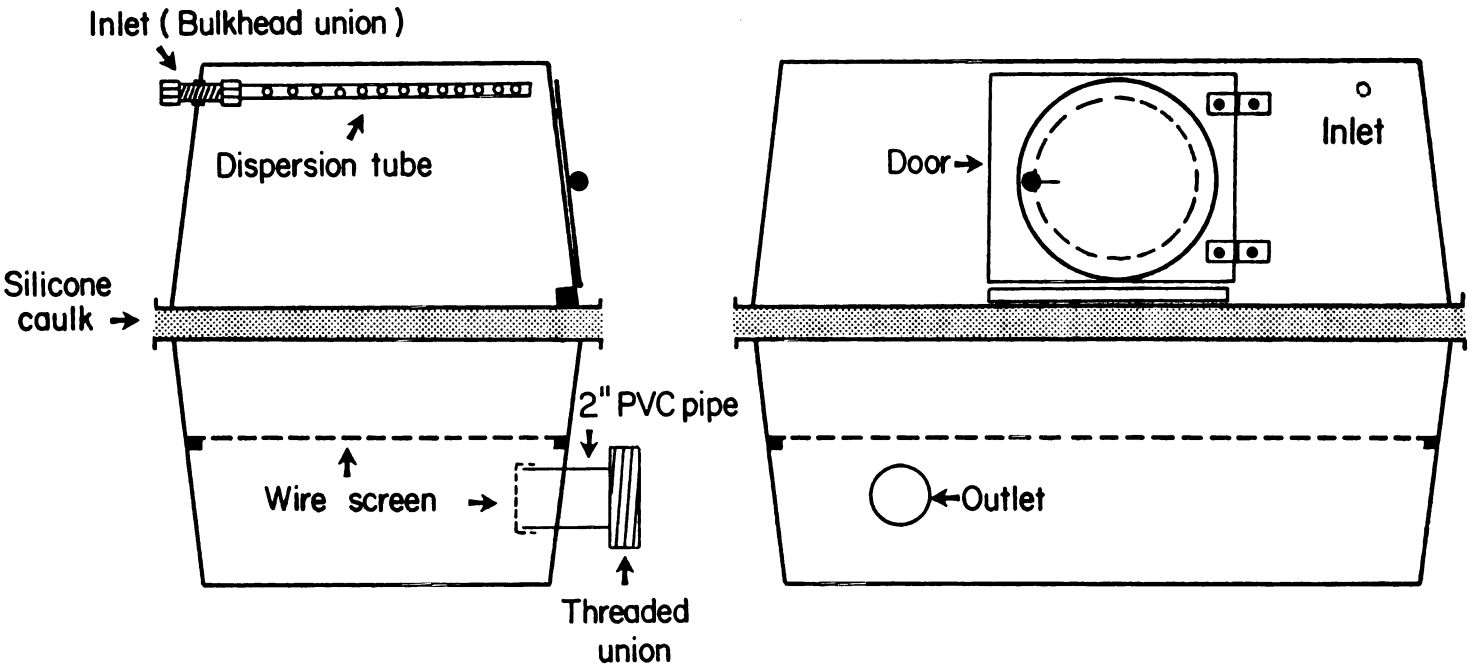
Nitrogen dioxide was purchased commercially (Matheson or Liquid Carbonic) in cylinders of 0.5% concentration. Gas from the cylinder [8] was regulated [9] (Matheson B-15-660) to 14 psi and conducted through Teflon tubing [10] to a rotameter-type flowmeter [11] (Gilmont #12 with capillary valve) constructed entirely of glass and Teflon. Here the flow was adjusted to achieve the desired chamber concentrations of NO_2. Teflon tubing [10] then conducted the 0.5% NO_2, at the metered rate, to the mixing vessel [7].

Mixing of the dilution air and nitrogen dioxide was achieved in a vessel (diagram on following page) constructed from polyvinylchloride (PVC) pipe expressly for this purpose. The vessel is an approximately 6" piece of PVC pipe capped and sealed at both ends. At the top of the vessel are two gas inlets placed 180° apart and oriented tangentially. The dilution air and toxic gas enter from opposite sides and mix while swirling down the vessel. The gas mixture exits the vessel through an outlet placed tangentially at the bottom. This diluted test mixture is filtered once more [12] (Gilman A/E, 0.3 um filter) before entering the exposure chamber [13].

The exposure chamber (diagram on 2nd following page) was constructed by inverting one large (51 x 41 x 22 cm) polycarbonate animal cage (Fisher Scientific) over another and "glueing" them together with a silicone adhesive. A large hole was cut in one side, and covered with



VESSEL CONSTRUCTED FOR MIXING NO_2 WITH DILUTION AIR



CHAMBER FOR INHALATION EXPOSURE OF SMALL LABORATORY ANIMALS

a plastic (Plexiglass) door which was sealed with a Neoprene closed cell sponge material, for moving animals into and out of the chamber. While in the chamber, animals are supported on a wire screen situated above the outlet tube and the floor. During operation, the floor of the chamber is covered with NaCl to absorb water and dessicate any urine and feces produced during the experiment and to retard bacterial growth and ammonia formation.

The incoming test atmosphere is distributed throughout the chamber by a dispersion tube running across the top of one side. Gas exits this dispersion tube through holes drilled approximately every 2 cm along its length. The gas outlet is situated in the opposite bottom corner of the chamber. This outlet and all subsequent plumbing were constructed with PVC pipe 2" in diameter. Over the face of the outlet is a polypropylene screen to filter out animal hair and dander.

Gas exiting the chamber is pulled through the system by a vacuum pump [14] (Ametek 115937) controlled by a rheostat. The output of this vacuum pump is directed into a fume hood, exhausting the waste gas from the laboratory.

This system of gas flow control wherein the moving gas stream is both "pushed" (by the relatively high pressures of both the diluent air and toxic gas) and "pulled" (by a variable vacuum source downstream from the chamber) allows fine control over both flow through and pressure within the chamber.

METHODS: INHALATION SYSTEM OPERATION:

This inhalation system was designed to provide controllable concentrations of NO_2 at flow rates high enough for rapid equilibration of the chamber and removal of metabolic by-products, which have the potential to interfere with the experiment.

Chamber volume was calculated from the interior dimensions to be 73.0 liters. Therefore 4 animals (each weighing 500 gm maximum and therefore comprising a maximum volume of 0.53 liters), the usual number in each experiment, represent a little over 2 liters, less than 5% of the chamber volume. This volume promotes the best uniformity of exposure by not interfering with even atmosphere distribution and providing minimal surface area for adsorption of the test atmosphere (Public Health Monograph #57).

An inhalation chamber flow rate greater than 0.5 liter per rat (corresponding on a mass:mass basis, to greater than 1.0 liter per guinea pig) has been found to keep water, heat and carbon dioxide buildup inside the chamber within acceptable limits (Gage, 1970). The present system operates normally at flow rates of 22 to 26 liters/minute, far in excess of this minimal value necessary to counter metabolic by-product accumulation. At these flow rates there was no tendency for the chamber to cool excessively and temperatures during exposures remained within 2°C of room temperature. Acceptable flow rates are defined in terms of flow (F in liters/minute) in relation to total chamber volume (V in liters) and have been found to be in the range:

$$F/\text{minute} = (0.1 - 1.0)\ V \qquad \text{(MacFarland, 1976).}$$

The flows in these experiments ranged 0.30 to 0.36 times chamber volume.

An advantage of a high flow rate is that chamber equilibration occurs rapidly. The equation relating chamber volume and flow rate to the time required to reach 99% equilibration is:

$$t_{99} = 4.605 \times \frac{\text{chamber volume (V)}}{\text{flow rate (F)}}$$

(MacFarland, 1976). For the present system, with a chamber volume of 73.0 liters and a minimal flow rate of 22 liters/minute, equilibration (defined as a less than 1% concentration difference between incoming gas and chamber atmosphere) occurred in less than 16 minutes.

TYPICAL EXPOSURE:

For a typical exposure the compressed gases, dilution air and NO_2, were turned on in concert with the vacuum pump. Flowmeters were adjusted to the appropriate settings, based on experience, and rechecked often during the first 10 to 15 minutes. The vacuum pump was also adjusted via the rheostat to keep chamber atmospheric pressure close to -0.5 inches of water relative to ambient. Keeping chamber pressure negative relative to the laboratory insured that any leaks present would be inward and not risk exposure of laboratory personnel to NO_2.

Initial equilibration was considered to have occurred when there was no change in flowmeter readings for longer than 20 minutes. At this time the first sample of the chamber atmosphere was taken for NO_2 analysis, a process requiring another 20 to 25 minutes. If the chamber NO_2 concentration was at the desired level, the animals were placed in the chamber and the starting time noted. If not, the flowmeters were re-adjusted and another 20 minutes allowed for re-equilibration prior to repeat sampling and analysis of the atmosphere.

The animals were left in the chamber for 120 minutes, the standard length of exposure for these experiments. This exposure length was chosen as long enough to even out the disruptive effects of opening the chamber for animal introduction, but short enough that food and water could be reasonably witheld. During the exposure both the flowmeters and the pressure gauge on the chamber were continuously monitored and any necessary corrections made in gas flows or vacuum motor speed. At approximately the halfway point (60 minutes into exposure) and again near the end of the exposure additional samples of the chamber gas were taken and analyzed. The final NO_2 concentration for the exposure was calculated as the average of all the samples taken just prior to and during the exposure.

For exposures with measured NO_2 concentrations differing more than 5 ppm NO_2 from the desired level, minor adjustments were made in the length of exposure to match the actual CT product (concentration in ppm NO_2 times the length of exposure in minutes) to that of the desired exposure. For example, an exposure planned for 120 minutes at 255 ppm NO_2 would have a CT product = 30,600. If the average NO_2 concentration during the exposure was found to be 260 ppm, the exposure was shortened to 118 minutes (CT product = 30,600). If this required an adjustment of greater than 5 minutes, the experiment was aborted.

At the end of the exposure period, the NO_2 gas cylinder was shut off and the animals removed from the chamber and returned to cages in the laboratory. There they had free access to food and water and were available for observation during the time until sacrifice for analysis of lung water and blood content. The entire system was flushed with clean air for at least 30 minutes before being shut down.

METHODS: DETERMINATION OF NO_2 CONCENTRATION:

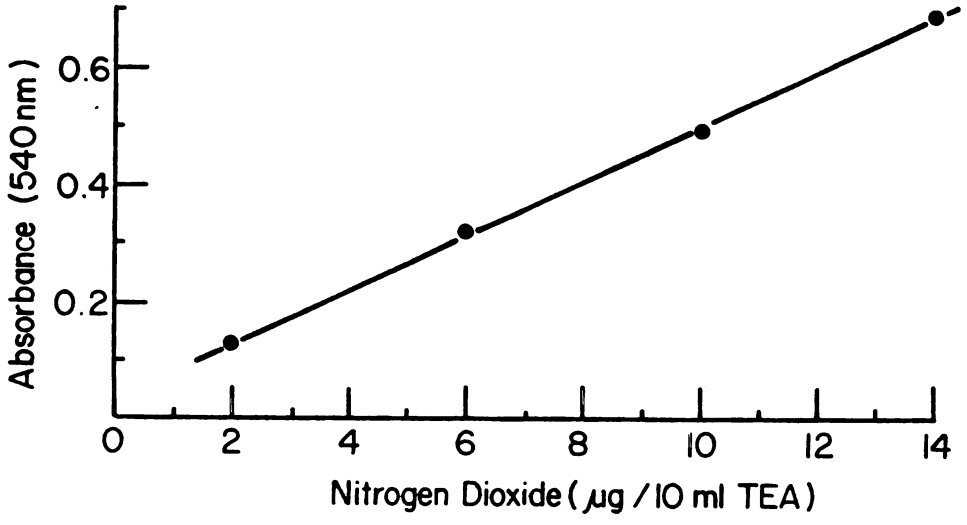
Chamber NO_2 concentration was determined colorimetrically by the method of Blacker (1973), a modification of the popular Saltzman (1949) method. In the Blacker version of the method the separate solutions for absorbing NO_2 and developing the colored product are mixed sequentially after the gas is sampled and absorbed into solution. In the Saltzman version, all of the reagents are pre-mixed before sampling. The Blacker assay was found to more reliable and reproducable in this investigator's hands.

At the start of each exposure day, a standard curve for the Blacker assay was generated. To a series of 25 ml Erlenmeyer beakers were added 1.0, 3.0, 5.0 and 7.0 ml of a $NaNO_2$ stock solution. This solution was prepared with a NO_2^- concentration equal to 2.0 ug/ml. An absorbing solution, triethanolamine (TEA) [15.0 mg/ml], was added to each beaker to bring the total volume to 10.0 ml. Therefore, the final concentrations of the standard solutions were 2.0, 6.0, 10.0 and 14.0 ug NO_2^-/10 ml TEA, respectively. Next, 1.0 ml of 0.02% hydrogen peroxide solution was added to the reaction, followed by 10.0 ml of Blacker sulfanilamide solution (prepared by dissolving 10.0 gm sulfanilamide in 400 ml distilled H_2O, adding 50.0 ml of 85% phosphoric acid and diluting to 500 ml with H_2O). Color development was then begun by adding 1.0 ml of a N-1-naphthylethylenediamine dihydrochloride (NEDA) solution (prepared by mixing 0.5 gm NEDA with 500.0 ml distilled H_2O) to each of the beakers, with mixing. After allowing 15 minutes for full color development, the absorbance of each of the solutions was read spectrophotometrically (Beckman DU) at 540 nm against a blank prepared in the same

manner, but without NO_2^- added initially to the TEA solution. The standard curve was plotted as [NO_2^-] (ug/10 ml TEA) against Absorbance (540 nm) and normally yielded a straight line (see following page). Preparation of a standard curve for each experiment was not absolutely necessary, but allowed an easy check on spectrophotometer function as well as the condition of solutions used in the assay. Exhaustion of the TEA, sulfanilamide or NEDA solutions could be quickly determined by an abnormal standard curve.

For sampling of the chamber gas, 2 syringes were prepared. Into a disposable 35 ml syringe were pipetted 10.0 ml of the TEA absorbing solution and the syringe was capped with a 3-way stopcock. A gas-tight 50 ml syringe made entirely of Teflon and glass (Hamilton) was also fitted with a 3-way stopcock. This gas-sampling syringe was then attached via its own 3-way stopcock to another 3-way stopcock in the wall of the chamber near the breathing zone of the animals. With the stopcocks open, the syringe was flushed 3 times with chamber gas. The syringe was then filled to the 20 ml mark and the stopcocks closed. Mixing of the gas and the TEA solution was achieved by attaching the 2 syringes via their stopcocks and bubbling the gas into the TEA. The gas-TEA mixture was further mixed by flushing back-and-forth between the connected syringes 10 times. The gas was then left in contact with the TEA, in one of the syringes, for 5 minutes to allow for complete absorption. After this 5 minute period, the TEA was transferred to a 25 ml Erlenmeyer beaker and the hydrogen peroxide, sulfanilamide and NEDA solutions added as with preparation of the standard curve. Absorbance of the colored product was read spectrophotometrically at 540 nm.

BLACKER NO_2 ASSAY - STANDARD CURVE



$[NO_2]$	Abs (540nm)
2.0	0.126 (0.002)
6.0	0.302 (0.002)
10.0	0.494 (0.003)
14.0	0.686 (0.004)

Standard curve for the determination of $[NO_2]$ by the method of Blacker (1973). Each point represents the average of 10 determinations. Concentrations represent NO_2^-, in ug per 10 ml of the TEA absorbing solution-the standard amount used in sampling chamber atmosphere, and must be multiplied by a correction factor to obtain the corresponding gas concentrations. Absorbance values are expressed as mean (sem).

Absorption of NO_2 from the gas phase into TEA, as the NO_2^- ion, does not occur in a one-to-one stoichiometry. For the present assay the correct stoichiometry has been found to be:

$$1 \text{ ug } [NO_2]_{gas} = 0.63 \text{ ug } [NO_2]_{ion} \text{ in TEA}$$

(Blacker, 1973), a relationship that was verified by this investigator. The value obtained from the standard curve must therefore be multiplied by the reciprocal of 0.63 (1.587 = "correction factor") to yield the correct NO_2 gas concentration. This value, in turn, was divided by 1.886×10^{-3} to convert the gas concentration from ug/ml to ppm. All of these values and calculations were recorded on a separate data sheet, kept with the data sheet on exposure conditions, for each experiment. On the following pages are reproduced the data sheets for recording exposure conditions (next page) and recording data and performing the calculations involved in the NO_2 analysis (second page).

EXPOSURE REPORT:

Expt #_____ Date:_____

Title:_____

$[NO_2]$:_____ ppm Duration:_____ min

CT value: _____

AIR:

Flowmeter (Matheson SS):_____ = Flow:_____ l/min

Pressure:_____ psi Humidifier:_____ cm H_2O

Vacuum motor:_____ (rheostat setting)

NO_2:

Flowmeter (Gilmont # 12):_____ = Flow:_____ l/min

Pressure:_____ psi

Total Use = (_____ min on) x (_____ l/min) = _____ l

- divided by 28.32 = _____ cf

TIMES:

Gas On:_____ AM/PM

Equilib time:_____ min

Start Expos.:_____ AM/PM

End Expos.:_____ AM/PM

Total On:_____ min

Gas Off:_____ AM/PM

CHAMBER: Pressure:_____ inches H_2O

ANIMALS:_____

NOTES:_____

_____

_____

NO_2 ASSAY: Date: _____

Expt #: _____

STANDARD CURVE:
- standard procedure
- 1.0 ml NEDA added at t = 0 to start color development
- absorbance (540 nm) read at t = 15 min

NO_2^- sln	TEA (ml)	NO_2 (ug/10ml)	H_2O_2	Sulfanil	Trans/Abs (540)
1.0 ml	9.0	2.0	1.0 ml	10.0 ml	_____/_____
3.0 "	7.0	6.0	"	"	_____/_____
5.0 "	5.0	10.0	"	"	_____/_____
7.0 "	3.0	14.0	"	"	_____/_____

- standard line calculated as NO_2^- (ug/10 ml TEA) vs Absorbance

slope = _____ intercpt = _____

CHAMBER NO_2: Measured $[NO_2]$ = _____ ppm
- standard procedure with gas sample

Sample vol: _____ cc

#	Trans/Abs (540)	NO_2(ug)/sample*	NO_2/cc**	x 1/CF	NO_2 ppm†
1	_____/_____	_____	_____	_____	_____
2	_____/_____	_____	_____	_____	_____
3	_____/_____	_____	_____	_____	_____

* from standard curve to yeild NO_2^- (ug/10 ml TEA)

** divided by volume of sample to yeild NO_2^- (ug/cc)

† 1 ppm = 1.886×10^{-3} ug/cc

NOTES: _____

METHODS: LUNG WATER AND BLOOD ANALYSIS

Lung water and blood contents were analyzed by a modification of the method (Selinger et al., 1975) developed in Norman Staub's laboratory from the conservation of mass principle as applied to lung water (Pearce et al., 1965).

The animals were sedated with pentobarbital (35 mg/kg, IP) and surgical anesthesia was induced with methoxyflurane administered via face mask. A blood sample was drawn by cardiac puncture, mixed with heparin and set aside for later analysis of hematocrit, hemglobin concentration and specific gravity. The skin and superficial musculature over the neck and thorax were opened to expose the sternohyoid muscles in the neck and the sternum in the thorax. The sternohyoid muscle was split and the trachea was clamped, a procedure followed immediately by severence of the right and left common carotid arteries to produce death by exsanguination, a procedure which minimizes postmortem water accumulation in the lung (Boyd & Knight, 1963). After death of the animal, the sterum was split to expose the lungs and heart. The trachea was cut above the clamp and dissected free of mediastinal tissue. The lungs, with the heart attached, were dissected free of the thorax and the block of tissue gently washed in 0.9% saline.

Right and left sets of lobes were then dissected free of the heart, the surfaces gently blotted dry of excess saline and weighed immediately in separate pans (Lung Wet weight, Q_{lb}, being equal to the sum). One set of lobes was then homogenized (Brinkmann Polytron) in an equal mass of distilled water. This set of lobes was dried to constant weight in an oven at 85°C and the dry weight (Lobe Dry weight) recorded.

The other set of lobes was homogenized in a like manner, distilled water being added to lyse red blood cells to release their hemoglobin, and then centrifuged at 30,000 x gravity for one hour (Sorvall RC2B or Beckman L5-40 ultracentrifuge). These centrifugation conditions yield a supernatant with no residual turbidity from incompletely sedimented tissue (Selinger et al., 1975). The resulting supernatant was collected and its hemoglobin concentration ($[Hb]_s$) determined by colorimetric measurement, at 540 nm (Beckman DU), of the reaction between hemoglobin and Drabkin's reagent producing cyanmethemoglobin (Drabkin & Austin, 1935). This same method was used to measure hemoglobin concentration ($[Hb]_b$) in the peripheral blood sample. Specific gravity measurements were made on both the lung supernatant (SG_s) and blood (SG_b) samples using the method of Phillips et al. (1950). Aliquots of the fluid to be measured were dropped into a series of copper sulfate solutions of graded density and their behavior (whether rising or falling) was noted. Drops remain suspended in solutions of density equal to their specific gravity.

An aliquot of the blood sample was weighed (Blood Wet weight) immediately upon opening the collection tube and then dryed in an oven to constant weight (Blood Dry weight). Capillary tubes were filled and centrifuged at 6000 x gravity for 5 minutes (Clay-Adams Readacrit) to separate red blood cells and plasma for hematocrit determination. Hematocrit (%) was calculated as:

(volume of red blood cells/volume of whole blood) x 100.

Calculations:

Residual blood content of the lungs is proportional to the hemoglobin concentration of the lung supernatant. Assuming that red blood cells in the lung have the same hemoglobin content as those in the periphery, the supernatant/blood hemoglobin ratio multiplied by the wet weight of the lung yeilds the residual blood mass. This value must be corrected for differences in density and in fractional water content between the two fluids (Pearce et al., 1965). There is disagreement in the literature concerning the exact form of the equation relating supernatant hemoglobin concentration to lung blood content. Pearce et al. (1965) cited Rapaport et al. (1956) and multiplied venous hematocrit by 1.055 to estimate pulmonary hematocrit. Staub's group (Snashall et al., 1979) cited a study by Studer & Potchen (1971) concluding that pulmonary hematocrit is 0.9 times that of venous blood. The most reasonable compromise between these two positions is to calculate residual lung blood content with no correction made to peripheral hematocrit. Therefore the calculation used in this study was:

Q_b: residual lung blood mass

$$= Q_h \times \frac{[Hb]_s}{[Hb]_b} \times \frac{Fwh}{Fws} \times \frac{SG_b}{SG_s}$$

where Q_h was the sum of Lung Wet weight and weight of distilled water added during homogenization. $[Hb]_s$ & $[Hb]_b$ were the hemoglobin concentrations, and SG_s & SG_b the specific gravities, of the supernatant and blood respectively. Fwh & Fws, the fractional water contents of the homogenate and supernatant respectively, were calculated as:

$$\frac{\text{Tissue Wet weight} - \text{Tissue Dry weight}}{\text{Tissue Wet weight}}$$

The volume of the residual blood in the lung was then obtained:

V_b: lung blood volume

$= {}^{Q_b}/_{SG_b}$

The blood-free lung mass was calculated by subtracting residual lung blood mass from Lung Wet weight:

Q_l: blood - free lung mass

$= Q_{lb} - Q_b$

Extravascular lung water was then derived by the equation:

EVLW: extravascular lung water

$= (Q_h \times Fwh) - (Q_b \times Fwb) - Q_w+$

where Fwb is the fractional water content of the blood, calculated in the same manner as for homogenate and supernatant, and Q_w+ is the mass of distilled water added during homogenization.

The final variable necessary for analysis of lung water and blood content is lung tissue dry weight. This value represents the mass of pulmonary tissue minus blood (both wet and dry components) and water contents:

dP: lung tissue dry weight

$= Q_{lb} - Q_b - EVLW$

The data were further refined according to standard convention. The Lung Wet/Dry weight ratio is a wet:dry measure which includes the contribution of residual blood in the lung. The amount of increase in this ratio is thus a measure of the overall response to a toxic insult, such as inhalation of NO_2. The EVLW/dP ratio is a wet:dry measure taking into account parenchymal tissue and neglecting both wet and dry components of the residual blood. An increase in this ratio is there-

fore a more direct indicator of pulmonary edema as separate from hyperemic congestion. The percentage of Lung Wet weight that is accounted for by extravascular lung water (% EVLW in Wet) provides a sensitive indicator of "pure" water increases with respect to the overall weight increases caused by influx of water, protein and other blood components.

In the case of permeability types of edema, where there is increased leakage of plasma proteins in addition to water into the lungs, these wet:dry ratios may not change as dramatically as expected due to increases in both the numerators (wet measures) and denominators (dry measures). A more accurate indication of the increases in extravascular lung water and dry components of the lung is obtained by examination of both wet and dry measures separately. These are expressed as the ratio (gm/kg) to body weight (BW) in order to eliminate differences based on the size of the animal. Lung Wet/BW & Lung Dry/BW weight ratios correspond to those wet:dry measures incorporating residual blood while EVLW/BW & dP/BW correspond to those excluding residual blood.

Residual blood content (as mass or volume) is an indication of the severity of hyperemic congestion produced in response to a toxic insult. In these experiments, residual blood in the lung was expressed in relation to both dry parenchymal tissue mass (dP) and body weight (BW) as the Blood/dP and Blood/BW measures.

Representative data collection and summary sheets are included here (following pages) to illustrate the raw data obtained in the experiments and the calculations used in analysis of pulmonary edema and hyperemic congestion. Each animal was assigned its own data sheets and those of any one experiment were collected and analyzed together.

STATISTICS:

Group means were compared by the Student's t test and mortality ratios were compared by the z test of proportions (Zuwaylif, 1974). A p value less than 0.05 was considered significant for all comparisons.

ANIMAL REPORT: Date: _____

Animal # _____ Expt # _____

Species: _____

Sex: _____

Weight: _____ gm

EXPERIMENT:

Title: _____

$[NO_2]$: _____ ppm Duration: _____ min

Pretreat: _____ Dose: _____ mg/kg, Interval: _____ hours

Time/Date of sacrifice: _____ Latent period: _____ hours

Anesthesia: Pentobarbital (35 mg/ml): _____ ml + methoxyflurane

PE/HC DATA SUMMARY:

Lung Wet Weight (Q_{lb}): _____ gm EVLW: _____ gm

Lobe Wet Weight: _____ gm Tissue Dry Weight (dP): _____ gm

Lobe Dry Weight: _____ gm Wet - blood (Q_l): _____ gm

Lung Blood Vol (V_b): _____ ml Lung Blood Mass (Q_b): _____ gm

Lung $^{Wet}/_{Dry}$ Weight Ratio: _____ $^{EVLW}/_{dP}$ ratio: _____

Dry ($^{Wet}/_{Lung\ W/D}$): _____ gm $^{EVLW}/_{BW}$: _____ gm/kg

$^{Wet}/_{BW}$: _____ gm/kg $^{dP}/_{BW}$: _____ gm/kg

$^{Dry}/_{BW}$: _____ gm/kg % EVLW in Wet: _____ %

$^{Q_b}/_{dP}$ ratio: _____ $^{Q_b}/_{BW}$: _____ gm/kg

NOTES: _____

_____

_____

PERIPHERAL BLOOD:

Animal # _____ Expt # _____

HCT: _____ % _____ % Average: _____ %

Hemoglobin:
- 20 ul sample, 5.0 ml Drabkin's sln

$$[Hb] = \frac{Abs. - .00 \ *}{.026}$$

Trans/Abs (540): _____/_____ Hemoglobin: _____ gm/100 ml

_____/_____ _____ "

$[Hb]_b$: _____ gm/100 ml

Whole Blood Weights:
Sample: _____ ml

Wet weight:			Dry weight:		
	gm	(blood+pan)		gm	(blood+pan)
-_____	gm	(B pan)	-_____	gm	(B pan)
	gm	(blood)		gm	(blood)

Whole Blood Specific Gravity:
-by Copper Sulfate falling drop method

SG_b: _____

*Value for hemoglobin equation determined from standard curve for each batch of Drabkin's solution.

NOTES: _____

_____

_____

LUNGS:

Animal # _____ Expt # _____

Wet Weights:

gm (Rt lobes+pan) gm (Lf lobes+pan)

-_____ gm (R pan) -_____ gm (L pan)

gm (Rt lobes) gm (Lf lobes)

Homogenization (Brinkmann Polytron):

Added dH_2O: _____ ml (Rt lobes) Tube: _____

Added dH_2O: _____ ml (Lf lobes) Tube: _____

Polytron setting: _____ Time: _____ sec.

Dry Weight: (Rt or Lf) lobes

gm (lobes+pan)

-_____ gm (R/L pan)

gm (lobes)

Centrifugation:

Model: _____ Rotor: _____

Speed: _____ rpm Time: _____ min

Supernatant: (Rt or Lf) lobes Tube #: _____

Hemoglobin: sample vol: _____ ml. Drabkin's vol: _____ ml

Trans/Abs (540): _____/_____ Hb equivalent: _____ gm/100 ml

_____/_____ _____ "

$[Hb]_E$: _____ gm/100 ml

Supnt Weights: Wet Vol: _____ ml Dry

gm (super+pan) gm (super+pan)

-_____ gm (S pan) -_____ gm (S pan)

gm (super) gm (super)

Specific Gravity: SG_s: _____

EXTRAVASCULAR LUNG WATER: Expt # _____

Animal # _____

Q_{lb}: wet weight of lung

= _____ (Rt lobes) + _____ (Lf lobes) Q_{lb} = _____ gm

Q_w+: weight of added water

= _____ (Rt lobes) + _____ (Lf lobes) Q_w+ = _____ gm

Q_h: weight of lung homogenate
= lung wet weight + weight of added water

= _____ (Q_{lb}) + _____ (Q_w+) Q_h = _____ gm

Fwh: fractional water-homogenate R or L lobes

$$= \frac{\text{(lobe wet wt + added water) - (lobe dry wt)}}{\text{(lobe wet wt + added water)}}$$

Fwh = _____

$$= \frac{(\quad \text{gm} + \quad \text{gm}) - (\quad \text{gm})}{(\quad \text{gm} + \quad \text{gm})}$$

Fws: fractional water-supernatant

$$= \frac{\text{(super wet weight) - (super dry weight)}}{\text{(super wet weight)}}$$

Fws = _____

$$= \frac{(\quad \text{gm}) - (\quad \text{gm})}{(\quad \text{gm})}$$

rFw: ratio $^{Fwh}/_{Fws}$ rFw = _____

Fwb: fractional water-blood

$$= \frac{\text{(blood wet weight) - (blood dry weight)}}{\text{(blood wet weight)}}$$

Fwb = _____

$$= \frac{(\quad \text{gm}) - (\quad \text{gm})}{(\quad \text{gm})}$$

HCT_p: pulmonary hematocrit

HCT_p = _____

= _____ % (HCT)

$[Hb]_s$: supernatant hemoglobin conc (includes Q_w+)

$[Hb]_s$ = _____ gm/dl

$$= \text{\_\_\_\_\_}\ [Hb]_E \times \frac{\text{20 ul}}{\text{\_\_\_\_\_ ul (vol)}} \times \frac{\text{5.0 +} \quad \text{ml}}{\text{5.02 ml}}$$

EXTRAVASCULAR LUNG WATER (p 2)

Expt # _____

Animal # _____

$[Hb]_b$: blood hemoglobin concentration

$$= \frac{\text{Abs (540)} - 0.00 *}{0.026}$$

$[Hb]_b$ = _____ gm/dl

rHb: ratio $[Hb]_s/[Hb]_b$

rHb = _____

SG_b: specific gravity-blood

SG_b = _____

SG_s: specific gravity-supernatant

SG_s = _____

rSG: ratio SG_b/SG_s

rSG = _____

Q_b: lung blood mass

Q_b = _____ gm

$$= Q_h \times \frac{[Hb]s}{[Hb]b} \times \frac{Fwh}{Fws} \times \frac{SGb}{SGs} \quad = Q_h \times rHb \times rFw \times rSG$$

V_b: lung blood volume

$$= Q_b/SG_b$$

V_b = _____ ml

Q_l: blood-free lung mass

$$= Q_{lb} - Q_b$$

Q_l = _____ gm

EVLW: extravascular lung water

$$= (Q_h \times Fwh) - (Q_b \times Fwb) - Q_w +$$

EVLW = _____ gm

dP: lung tissue dry weight

$$= Q_{lb} - Q_b - EVLW$$

dP = _____ gm

*Value for hemoglobin equation determined from standard curve for each batch of Drabkin's solution.

RESULTS

RESULTS: CONTROL ANIMALS

Measurements of lung water and blood content were made in 22 normal guinea pigs ranging in weight from 250 to 550 gm. This wide weight range allowed detection of any correlations between the parameters of lung water analysis and body weight (BW). The control animals all spent time in the exposure chamber under conditions simulating those of an exposure to NO_2, with exception of NO_2 being added to the gas stream. As with the experimental animals, these were observed for 24 hours after "exposure" and then sacrificed for lung water and blood analysis. Most of the control experiments were performed early in the project, while the techniques involved in NO_2 exposure and concentration assay were being perfected, and prior to the series on NO_2 exposure. The toxic response to NO_2, as studied in these experiments, is of sufficient severity that prior, rather than concurrent, controls do not introduce any confounding factors.

In these animals, the Lung Wet/Dry weight ratio averaged 5.21 and the EVLW/dP weight ratio 4.18. Both of these ratios showed some negative correlation (r values approximately -0.70) with body weight. The component parameters (Lung Wet & Lung Dry and EVLW & dP weights) were all directly correlated (r values approximately 0.90) with BW.

Because of these correlations, and the opposite trends between ratios and individual parameters, expression of each parameter as a ratio to body weight was found to provide the best means for comparing data. Therefore the data are expressed as Lung Wet/BW & Lung Dry/BW and EVLW/BW & dP/BW, all of which show no correlation with BW. The percentage of EVLW in total wet weight of the lung averaged 70.3 % and also showed no correlation with BW.

Residual blood content in the lungs from control animals varied widely, possibly due to some lack of standardization of technique during early experiments in addition to the expected individual variation, but averaged 0.318 gm. This parameter also showed high correlation (r value 0.72) with BW. Blood content is therefore also expressed as the ratio to BW (Blood/BW) for comparative purposes. Blood/BW averaged 0.83 in the controls.

CONTROL DATA

LUNG WATER AND BLOOD DATA IN GUINEA PIGS

	Mean	(sem)	r *
Body Weight (gm)	385.7	(17.5)	---
Lung Wet/Dry weight ratio (gm/gm)	5.21	(.04)	- 0.69
Lung Wet weight (gm)	2.4716	(.1040)	0.89
Lung Dry weight (gm)	0.477	(.024)	0.89
Wet/BW (gm/kg)	6.46	(.13)	- 0.42
Dry/BW (gm/kg)	1.24	(.03)	- 0.11
EVLW/dP weight ratio (gm/gm)	4.18	(.04)	- 0.68
EVLW weight (gm)	1.735	(.071)	0.88
dP weight (gm)	0.419	(.021)	0.88
EVLW/BW (gm/kg)	4.54	(.10)	- 0.44
dP/BW (gm/kg)	1.09	(.03)	- 0.08
% EVLW in Lung Wet weight	70.3	(.41)	- 0.23
Lung Blood weight (gm)	0.318	(.017)	0.72
Blood/dP ratio (gm/gm)	0.77	(.14)	- 0.18
Blood/BW (gm/kg)	0.83	(.03)	- 0.23

These values represent the results of lung water and blood analysis of the control animals (n = 22). Animals were exposed in the chambers to filtered room air under conditions similar to those of an exposure to NO_2.

*Correlation coefficient of parameter vs. body weight.

EVLW = extravascular lung water weight
dP = dry lung parenchymal tissue weight
BW = body weight

RESULTS: DOSE-RESPONSE EXPERIMENTS

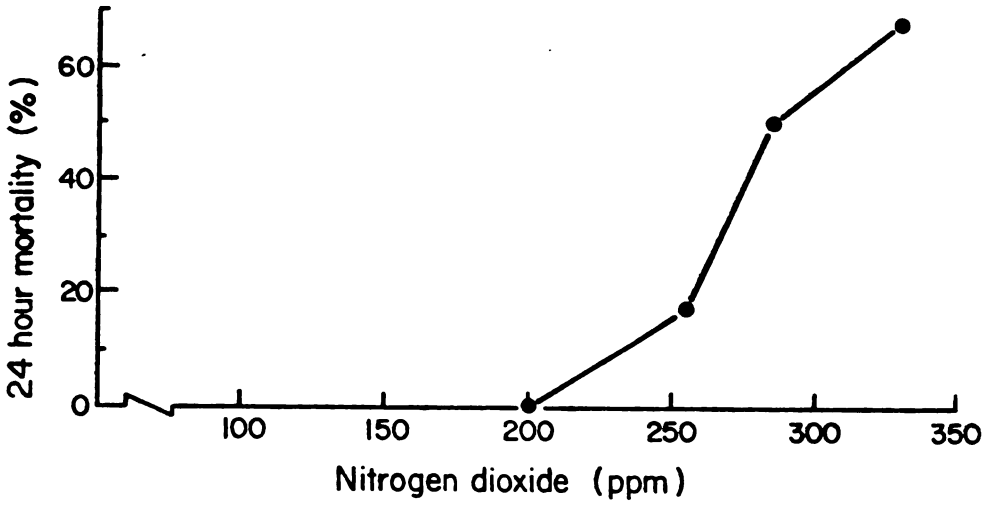
Twenty four hour mortality and wet:dry weight ratios are plotted against NO_2 concentration for 120 minute exposures on the following pages. In calculating Lung Wet/Dry and EVLW/dP ratios, all animals were considered; those dying of pulmonary edema prior to the 24 hour sacrifice time were assigned values of 10.0 for both of these ratios. These values were chosen on the basis of two animals (G 108-83 and G 211-83) fortuitously analysed at the moment of death, yeilding average values of 10.42 and 10.45 for Lung Wet/Dry and EVLW/dP respectively. It was also noted that literature values for both of these ratios approach 10.0 in severely edematous lungs, but rarely exceed this number (Guyton & Lindsey, 1959).

There is a steady, although gradual, increase in Lung Wet/Dry and EVLW/dP weight ratios up to 200 ppm NO_2. Above this concentration there is a much more rapid rise in these two ratios. This inflection point is also the concentration above which fractional mortality occurred within 24 hours.

A more detailed analysis of lungs from dying animals was not possible due to the difficulty of obtaining a blood sample representative of the living animal and the fact that lungs accumulate water after death (Boyd & Knight, 1963).

In the surviving animals it was possible to further analyze the accumulation of lung water by evaluating the individual components of the wet:dry weight ratios. In these animals the increases in Lung Wet/Dry and EVLW/dP weight ratios are approximately linear with increasing NO_2 concentration. However, there are dramatic increases in the

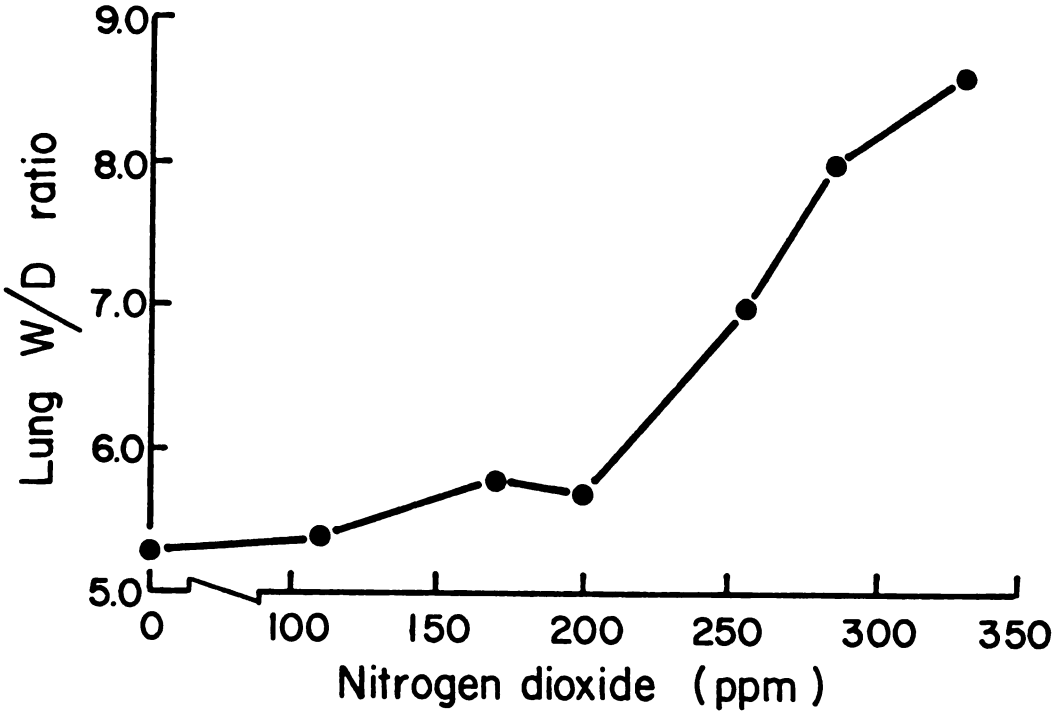
DOSE-RESPONSE: 24 HOUR (PI) MORTALITY



	Fractional Mortality (%)	
110 ppm	0/3	—
170 ppm	0/10	—
200 ppm	0/6	—
255 ppm	3/18	16.7
285 ppm	3/6	50.0
330 ppm	2/3	66.6

Exposures were carried out at the NO_2 concentrations listed for 120 minutes. Animals were observed over the subsequent 22 hours. When possible, time of death was noted. All survivors were sacrificed at 24 hours PI for lung water and blood analysis.

DOSE-RESPONSE: LUNG WET/DRY WEIGHT RATIOS (ALL ANIMALS)

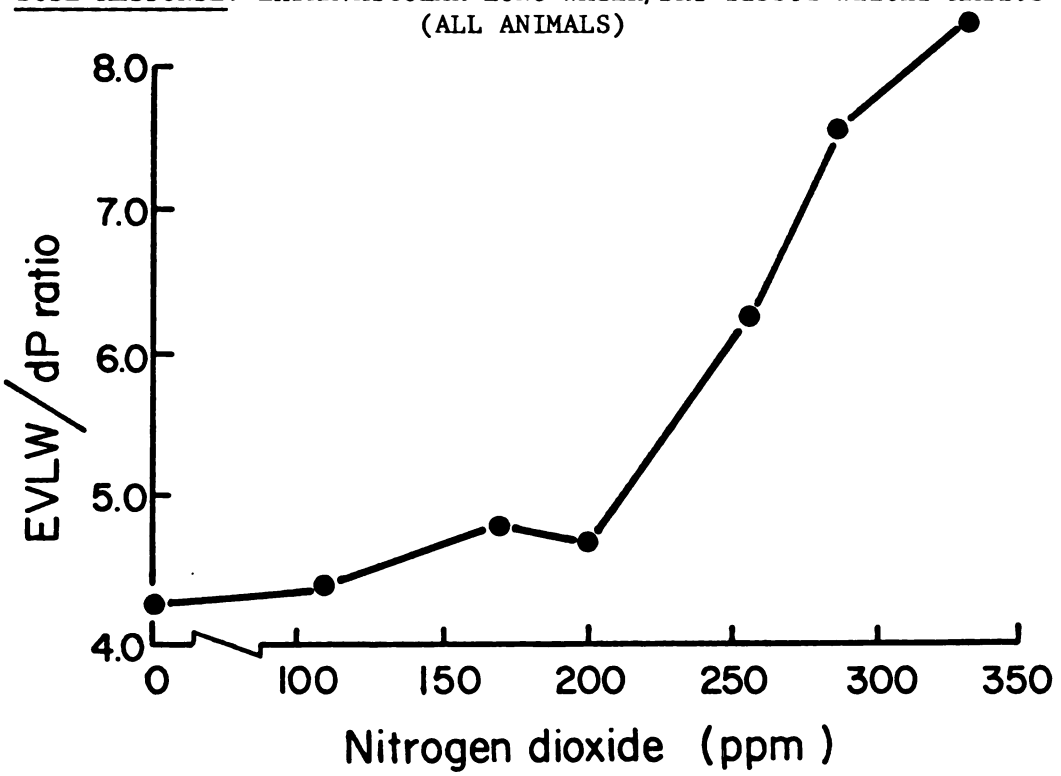


	Lung W/D†	% above control
control (22)	5.21 (0.04)	-
110 ppm (3)	5.42 (0.02)*	4.0
170 ppm (10)	5.77 (0.16)*	10.7
200 ppm (6)	5.70 (0.13)*	9.4
255 ppm (18)	6.77 (0.39)*	29.9
285 ppm (6)	8.02 (0.89)*	53.9
330 ppm (3)	8.62 (1.37)*	65.5

Exposures were carried out at the NO_2 concentrations listed for 120 minutes. Animals were sacrificed for lung water and blood analysis at 24 hours PI. Those animals dying early were assigned 10.0 for Lung Wet/Dry weight ratio. Data are expressed as mean (sem), with # of animals at each exposure level in parentheses following concentration.

* $p < .05$ (compared to controls)
† units are gm/gm

DOSE-RESPONSE: EXTRAVASCULAR LUNG WATER/DRY TISSUE WEIGHT RATIOS (ALL ANIMALS)



	EVLW/dP§	% above control
control (22)	4.18 (0.04)	-
110 ppm (3)	4.40 (0.03)*	5.3
170 ppm (10)	4.77 (0.18)*	14.1
200 ppm (6)	4.72 (0.14)*	12.9
255 ppm (18)	6.01 (0.47)*	43.8
285 ppm (6)	7.54 (1.11)*	80.4
330 ppm (3)	8.31 (1.69)*	98.8

Exposures were carried out at the NO_2 concentrations listed for 120 minutes. Animals were sacrificed for lung water and blood analysis at 24 hours PI. Those animals dying early were assigned 10.0 for EVLW/dP weight ratio. Data are expressed as mean (sem), with # of animals at each exposure level in parentheses following concentration.

* $p < .05$ (compared to controls)
§ units are gm/gm

DOSE-RESPONSE DATA

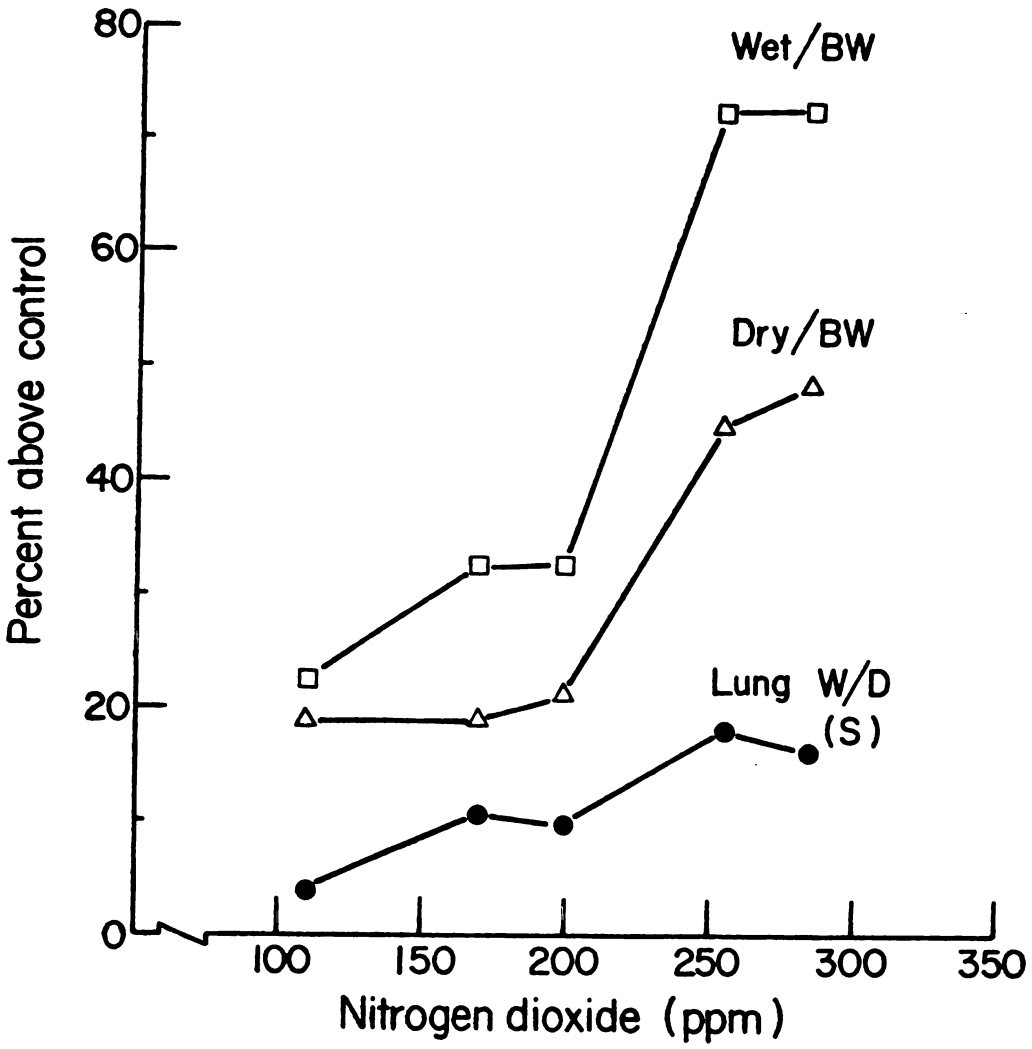
LUNG WET/DRY WEIGHT RATIOS: SURVIVORS ONLY

	Lung W/D†	Wet/BW§	Dry/BW§
control (22)	5.21 (0.04)	6.46 (0.13)	1.24 (0.03)
110 ppm (3)	5.42 (0.02)*	7.96 (0.20)*	1.47 (0.04)*
170 ppm (10)	5.77 (0.16)*	8.56 (0.55)*	1.47 (0.06)*
200 ppm (6)	5.70 (0.13)*	8.57 (0.40)*	1.50 (0.05)*
255 ppm (15)	6.13 (0.18)*	11.23 (0.83)*	1.79 (0.08)*
285 ppm (3)	6.04 (0.21)*	11.15 (1.39)*	1.84 (0.14)*
330 ppm	Only one animal survived for lung water analysis		

Exposures were carried out at the NO_2 concentrations listed for 120 minutes. Animals were sacrificed for lung water and blood analysis at 24 hours PI. Those animals dying early were deleted from lung water and blood analysis. Data are expressed as mean (sem), with # of animals at each exposure level in parentheses following concentration.

* $p < .05$ (compared to controls)
† units are gm/gm
§ units are gm/kg

DOSE RESPONSE: LUNG WET/DRY WEIGHT RATIOS (SURVIVORS)



Dose-response plot of Lung Wet/Dry weight ratios against NO_2 concentration. Lung Wet/Dry ratio data are for survivors only, ratios for all animals are presented in earlier graphs. In addition to the wet:dry ratios, the individual components, Lung Wet/BW and Lung Dry/BW are also plotted. Note the large increases in these component measures above 200 ppm NO_2. Due to proportionate increases in each of these components, there is little change in the Wet/Dry ratio above this concentration. The data plotted are percentages above control of the values in the table on the preceeding page.

DOSE-RESPONSE DATA

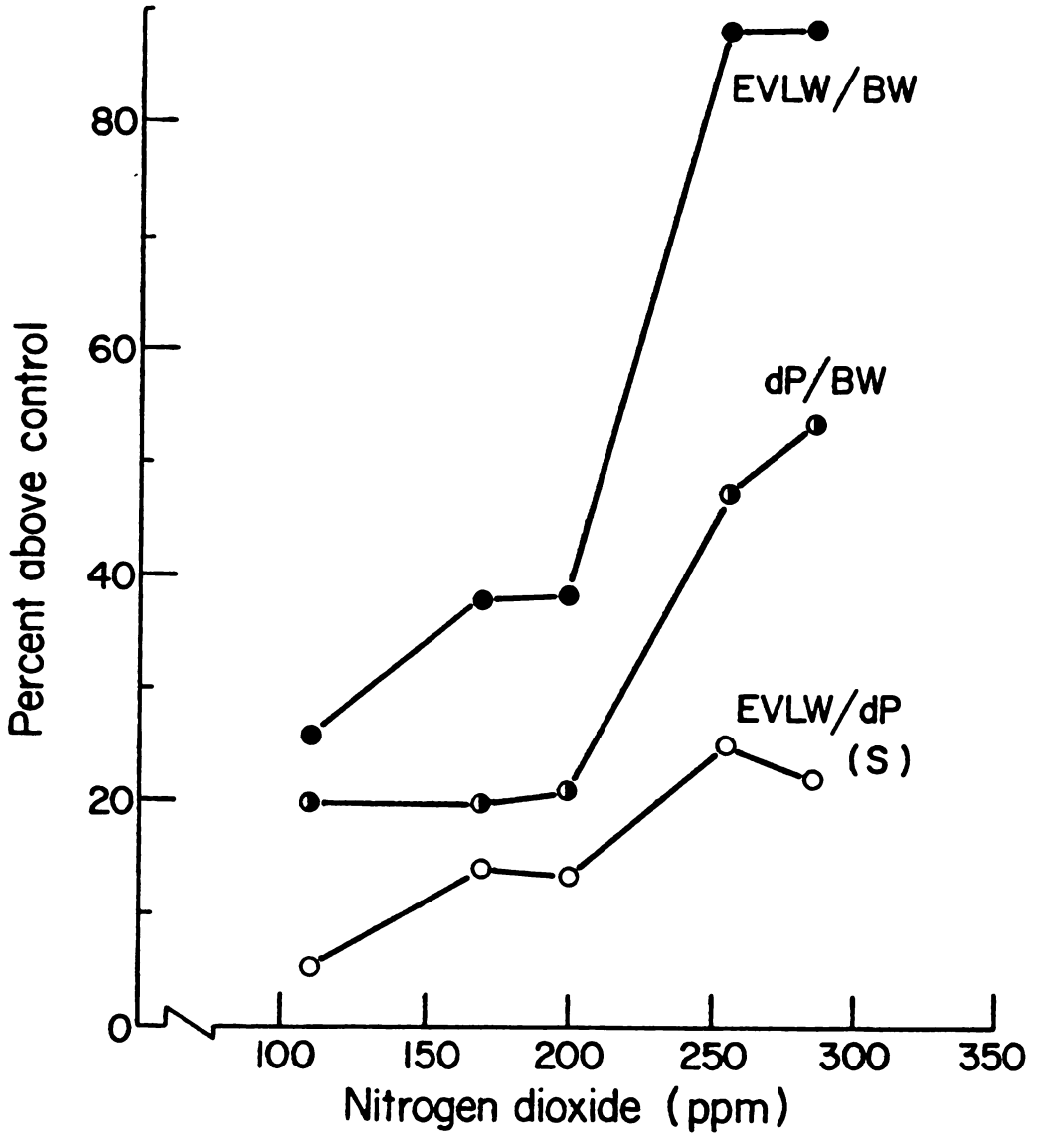
EXTRAVASCULAR LUNG WATER/DRY TISSUE WEIGHT RATIOS: SURVIVORS ONLY

	EVLW/dP†	EVLW/BW§	dP/BW§
control (22)	4.18 (0.04)	4.54 (0.12)	1.09 (0.03)
110 ppm (3)	4.40 (0.03)*	5.71 (0.21)*	1.30 (0.06)*
170 ppm (10)	4.77 (0.18)*	6.26 (0.48)*	1.30 (0.05)*
200 ppm (6)	4.72 (0.14)*	6.26 (0.30)*	1.32 (0.04)*
255 ppm (15)	5.21 (0.23)*	8.52 (0.71)*	1.60 (0.07)*
285 ppm (3)	5.09 (0.35)*	8.55 (1.21)*	1.67 (0.20)*
330 ppm	Only one animal survived for lung water analysis.		

Exposures were carried out at the NO_2 concentrations listed for 120 minutes. Animals were sacrificed for lung water and blood analysis at 24 hours PI. Those animals dying early were deleted from lung water and blood analysis. Data are expressed as mean (sem), with # of animals at each exposure level in parentheses following concentration.

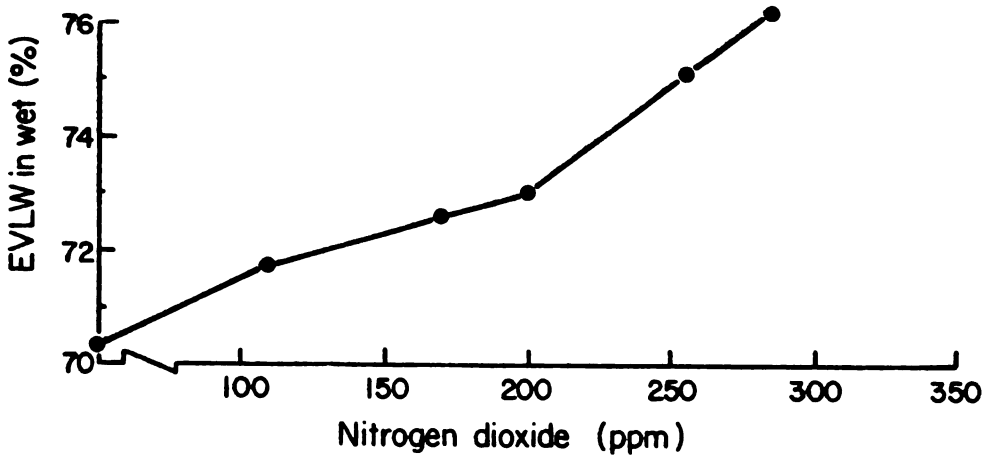
* $p < .05$ (compared to controls)
† units are gm/gm
§ units are gm/kg

DOSE RESPONSE: EVLW/dP WEIGHT RATIOS (SURVIVORS)



Dose-response plot of Extravascular Lung Water/dry Parenchymal tissue mass (both measures excluding contributions of residual blood in the lung). EVLW/dP ratio data are for survivors only, ratios for all animals are presented in earlier graphs. In addition to the wet:dry ratios, the individual components, EVLW/BW and dP/BW are also plotted. Note the large increases in these component measures above 200 ppm NO_2. Because of the proportionate increases in each of these components, there is little change in the EVLW/dP ratio above this concentration. The data plotted are percentages above control of the values listed in the table on the preceeding page.

DOSE RESPONSE: % EVLW IN LUNG WET WEIGHT
(SURVIVORS)



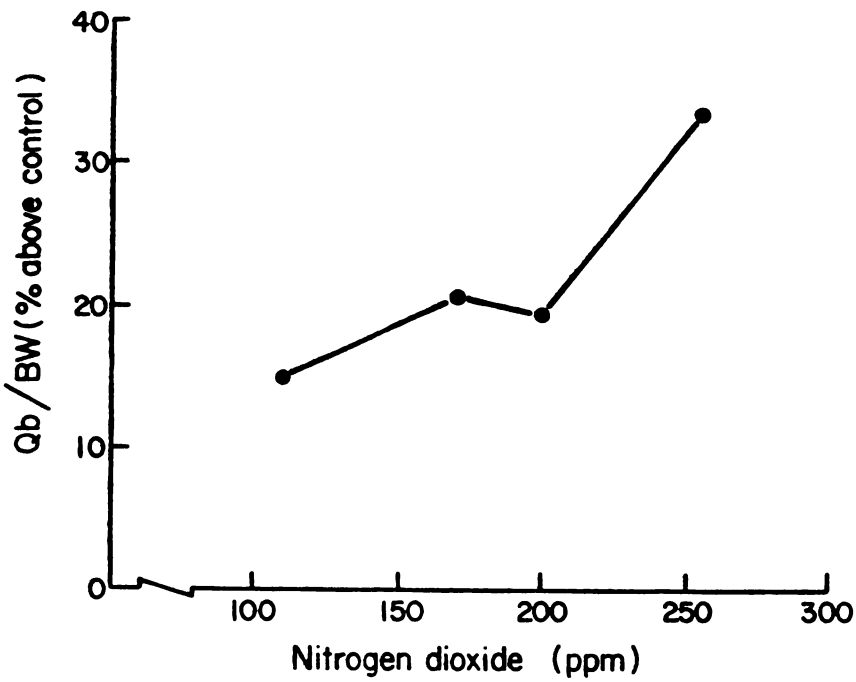
	EVLW/BW§	Wet/BW§	% EVLW in Wet
Controls (22)	4.54	6.46	70.3 (0.4)
110 ppm (3)	5.71	7.96	71.7 (0.8)
170 ppm (10)	6.26	8.56	72.6 (0.9)*
200 ppm (6)	6.26	8.57	73.0 (0.7)*
255 ppm (15)	8.52	11.23	75.1 (0.8)*
285 ppm (3)	8.55	11.15	76.2 (1.8)*

330 ppm Only one animal survived for lung water analysis.

Exposures were carried out at the NO_2 concentrations listed for 120 minutes. Animals were sacrificed for lung water and blood analysis at 24 hours PI. Those animals dying early were deleted from lung water and blood analysis. Data are expressed as mean (sem), with # of animals at each exposure level in parentheses following concentration.

* $p < .05$ (compared to controls)
§ units are gm/kg

DOSE RESPONSE: BLOOD/BW RATIOS (SURVIVORS)



	Blood/BW§	% above control
Controls (22)	0.83	—
110 ppm (3)	0.95	14.4
170 ppm (10)	1.00	20.5*
200 ppm (6)	0.99	19.3*
255 ppm (15)	1.11	33.7*

Exposures were carried out at the NO_2 concentrations listed for 120 minutes. Animals were sacrificed for lung water and blood analysis at 24 hours PI. Those animals dying early were deleted from lung water and blood analysis. Data are expressed as mean (sem), with # of animals at each exposure level in parentheses following concentration.

* $p < .05$ (compared to controls)
§ units are gm/kg

slopes of the lines describing increases of the individual components (Lung Wet/BW & Dry/BW and EVLW/BW & dP/BW weight measures) between 200 and 255 ppm NO_2 exposures. Since both wet and dry weight measures increase to a similar proportion, this trend is not observed in the weight ratios. These measures represent only those animals more resistent to NO_2, the survivors, and therefore must underestimate the typical reaction. This fact can be appreciated by comparing the wet:dry curves for all animals with those for survivors only. With the contributions from dying animals, these wet:dry curves exhibit dramatic increases above 200 ppm NO_2 exposure. The curves for survivors, representing apparently less susceptible animals, exhibit a more flattened appearance above 200 ppm NO_2.

Of all the measures of accumulation of excess water with increasing lung damage, as determined by exposure to increasing concentrations of NO_2, the most linear is that of % EVLW in Lung Wet Weight. This is determined by dividing the measure of extravascular lung water by the total wet weight of the lung. This parameter shows a linear increase with increasing NO_2 exposure at all levels above ambient. A trend such as this indicates that with increasing "dose" of NO_2 there is a proportional increase in the amount of free water that accumulates in the lung.

Residual blood content of the lung, expressed as Blood (Qb)/BW, exhibited a pattern similar to those of the wet and dry weight measures. Most notably, there was a dramatic increase occurring between 200 ppm and 255 ppm NO_2, in concert with the increases in both wet and dry measures. This excess blood contributed to the increases in Lung Wet and Dry weights.

RESULTS: TIME COURSE EXPERIMENTS

Traditionally, investigators have exposed animals to NO_2 and reported subsequent changes as occurring at various times "post-exposure." Early analysis of the present data indicated that it would be better to report findings as occurring at various time after the start of exposure. These results are therefore reported as occurring at various times "post-initiation", noted herein as X hours PI.

On the basis of the dose-response results, a 255 ppm NO_2 concentration for 120 minutes was chosen as the standard exposure. In the time course series of experiments, animals were exposed to this regimen and sacrificed at 2, 6, 12, 18 and 24 hours PI for analysis of lung water and blood content.

The accumulation of water in the lungs is detailed in the Lung Wet/Dry & EVLW/dP weight ratio charts and plotted as a function of time after the start of NO_2 exposure. These tables and graphs are on the following pages. Although some animals succumbed to pulmonary edema prior to 18 hours PI, only data for the survivors are plotted. A separate table includes Lung Wet/Dry and EVLW/dP data for those animals dying early.

When the Lung Wet/Dry and EVLW/dP weight ratios and their component parameters are plotted together, against time after initiation of exposure, a striking biphasic curve results. Both Lung Wet/Dry and EVLW/dP weight ratios peak early, at 6 hours PI, and then decline through the 18 hour PI time point. A second rise occurs between 18 and 24 hours PI with the latter value not quite reaching the magnitude of the early (6 hour PI) peak. If the animals dying prior to sacrifice are included,

TIME COURSE DATA

LUNG WET/DRY WEIGHT RATIOS: ALL ANIMALS

	Lung W/D†	% above control
control (22)	5.21 (0.04)	---
2 hours (4)	5.96 (0.23)*	14.4
6 hours (7)	6.38 (0.31)*	22.5
12 hours (7)	6.05 (0.17)*	16.1
18 hours (11)	6.97 (0.60)*	33.8
24 hours (18)	6.77 (0.39)*	29.9

Exposures were carried out at an NO_2 concentrations of 255 ppm for 120 minutes. Animals were sacrificed for lung water and blood analysis at the times listed (expressed as X hours PI). Those animals dying early were assigned 10.0 for Lung Wet/Dry weight ratio. Data are expressed as mean (sem), with # of animals at each time point in parentheses following time of sacrifice.

* $p < .05$ (compared to controls)
† units are gm/gm

TIME COURSE DATA

EXTRAVASCULAR LUNG WATER/DRY TISSUE WEIGHT RATIOS: ALL ANIMALS

	EVLW/dP§	% above control
control (22)	4.18 (0.04)	---
2 hours (4)	4.96 (0.24)*	18.7
6 hours (7)	5.39 (0.35)*	28.9
12 hours (7)	5.05 (0.18)*	20.8
18 hours (11)	6.24 (0.74)*	49.3
24 hours (18)	6.01 (0.47)*	43.8

Exposures were carried out at an NO_2 concentrations of 255 ppm for 120 minutes. Animals were sacrificed for lung water and blood analysis at the times listed (expressed as X hours PI). Those animals dying early were assigned 10.0 for EVLW/dP weight ratio. Data are expressed as mean (sem), with # of animals at each time point in parentheses following time of sacrifice.

* $p < .05$ (compared to controls)
§ units are gm/gm

TIME COURSE DATA

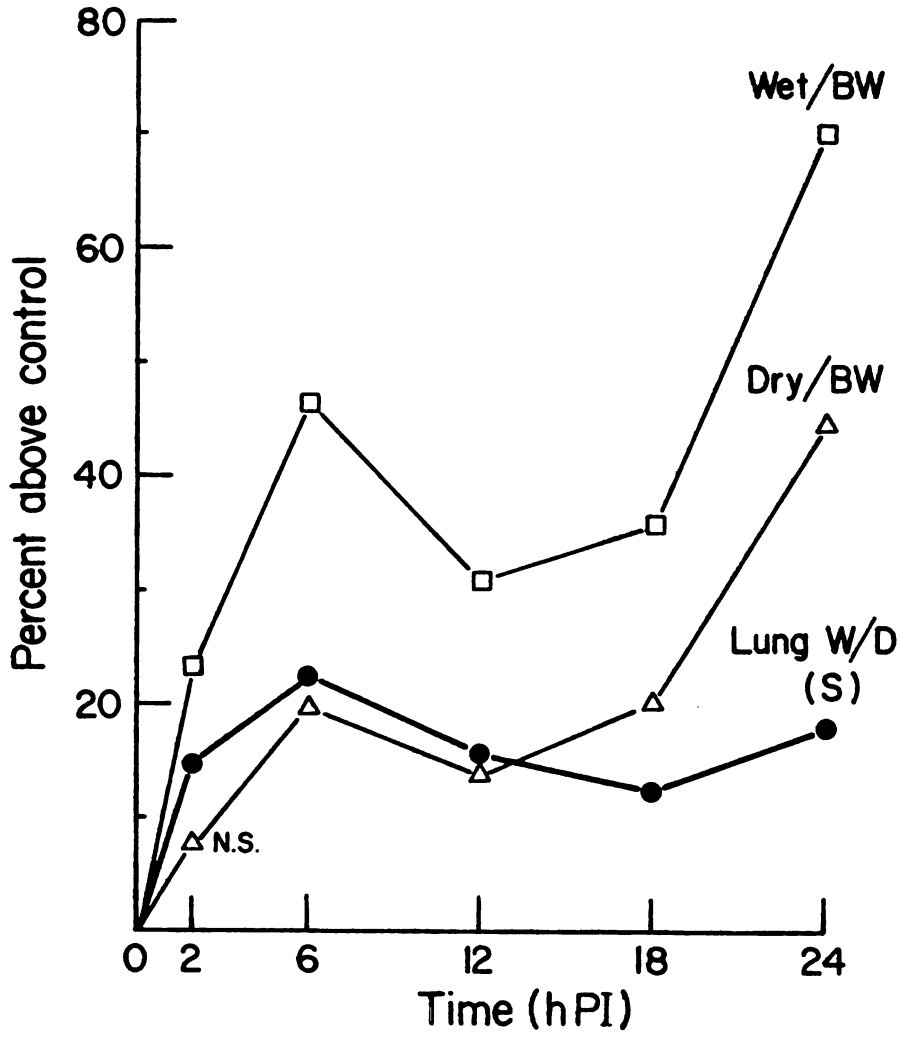
LUNG WET/DRY WEIGHT RATIOS: SURVIVORS ONLY

	Lung W/D†	Wet/BW§	Dry/BW§
control (22)	5.21 (0.04)	6.46 (0.13)	1.24 (0.03)
2 hours (4)	5.96 (0.23)*	7.95 (0.46)*	1.33 (0.04)
6 hours (7)	6.38 (0.31)*	9.48 (0.59)*	1.48 (0.02)*
12 hours (7)	6.05 (0.17)*	8.53 (0.32)*	1.41 (0.02)*
18 hours (8)	5.84 (0.17)*	8.76 (0.58)*	1.49 (0.05)*
24 hours (15)	6.13 (0.20)*	11.23 (0.83)*	1.79 (0.08)*

Exposures were carried out at an NO_2 concentrations of 255 ppm for 120 minutes. Animals were sacrificed for lung water and blood analysis at the times listed (expressed as X hours PI). Those animals dying early were deleted from lung water and blood analysis. Data are expressed as mean (sem), with # of animals at each time point in parentheses following sacrifice time.

* $p < .05$ (compared to controls)
† units are gm/gm
§ units are gm/kg

TIME COURSE: LUNG WET/DRY WEIGHT RATIOS
(SURVIVORS)



Time course plot of Lung Wet/Dry weight ratios for survivors. Data are listed in the table on the preceeding page and are plotted here as percentage above control for comparative purposes. Note the early increase in Wet/BW, with little corresponding rise in Dry/BW (the value at 2 hours PI is not significant), and the subsequent decline at 6 to 12 hours PI. Note also the later dramatic increases in both Wet/BW and Dry/BW at 18 to 24 hours PI. Due to the porportionate changes in both wet and dry measures, the Lung Wet/Dry weight ratio does not always reflect changes in lung water.

TIME COURSE DATA

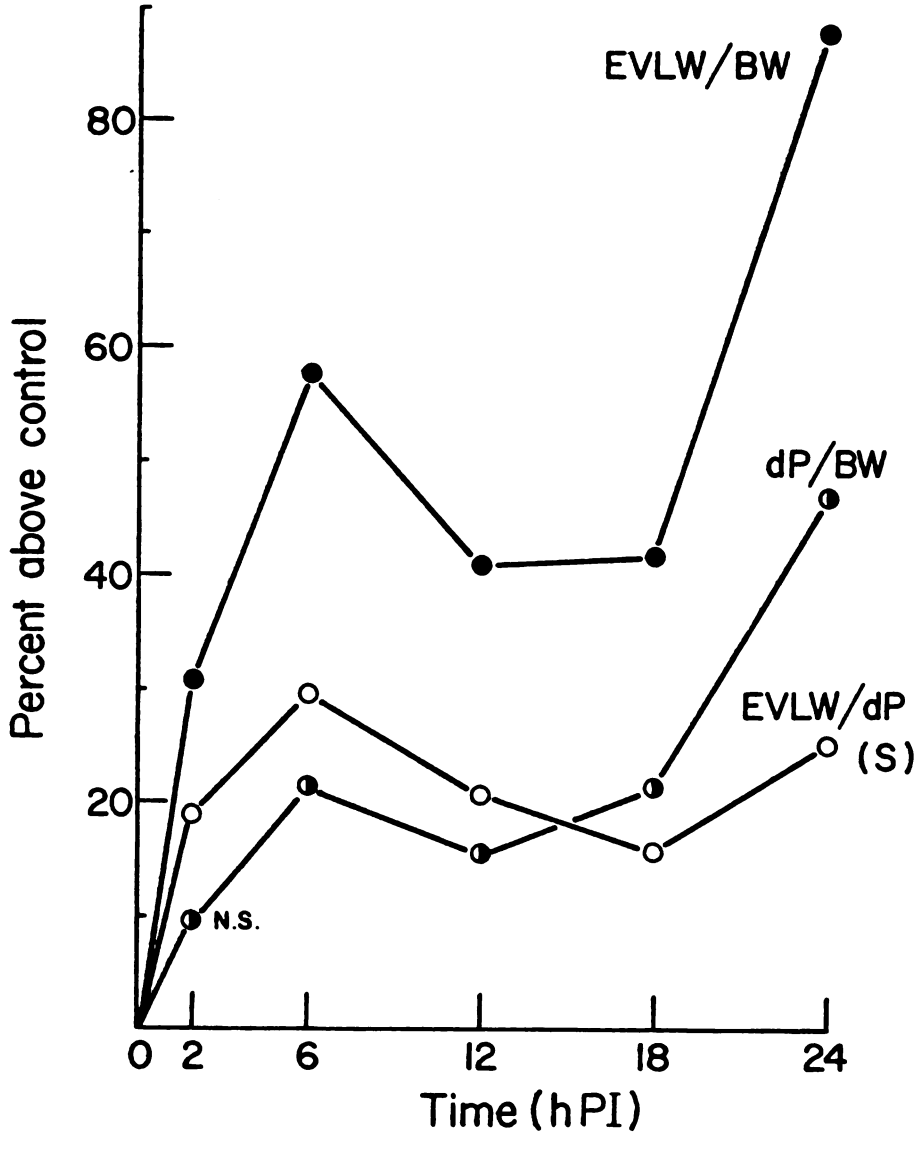
EXTRAVASCULAR LUNG WATER/DRY TISSUE WEIGHT RATIOS: SURVIVORS ONLY

	EVLW/dP†	EVLW/BW§	dP/BW§
control (22)	4.18 (0.04)	4.54 (0.12)	1.09 (0.03)
2 hours (4)	4.96 (0.24)*	5.94 (0.40)*	1.19 (0.04)
6 hours (7)	5.39 (0.35)*	7.16 (0.58)*	1.32 (0.02)*
12 hours (7)	5.05 (0.18)*	6.39 (0.28)*	1.26 (0.03)*
18 hours (8)	4.83 (0.20)*	6.43 (0.52)*	1.32 (0.05)*
24 hours (15)	5.21 (0.23)*	8.52 (0.71)*	1.60 (0.07)*

Exposures were carried out at an NO_2 concentrations of 255 ppm for 120 minutes. Animals were sacrificed for lung water and blood analysis at the times listed (expressed as X hours PI). Those animals dying early were deleted from lung water and blood analysis. Data are expressed as mean (sem), with # of animals at each time point in parentheses following sacrifice time.

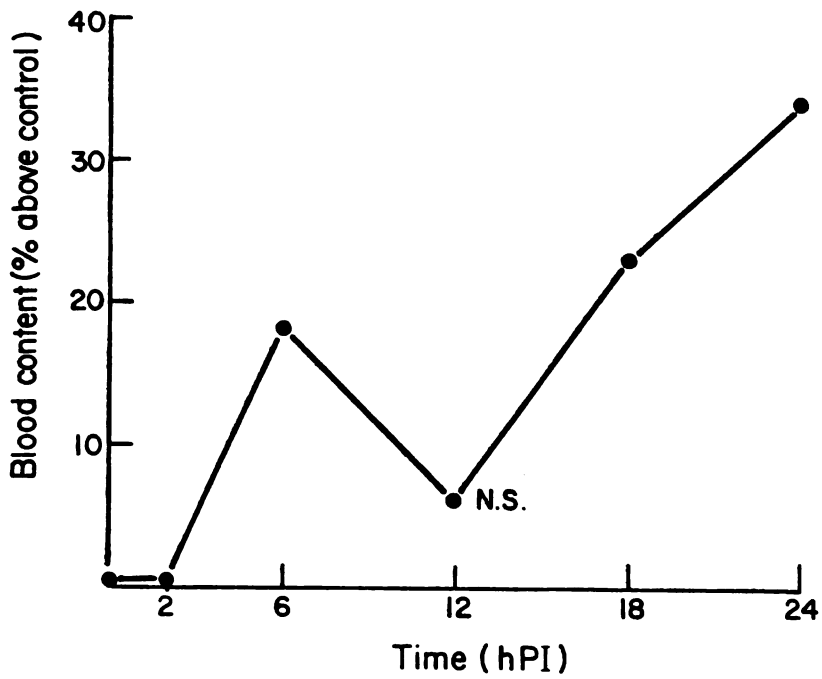
* $p < .05$ (compared to controls)
† units are gm/gm
§ units are gm/kg

TIME COURSE: EVLW/dP WEIGHT RATIOS
(SURVIVORS)



Time course plot of Extravascular Lung Water/dry Parenchymal tissue mass for survivors. Data are listed in the table on the preceeding page and are expressed here as percentage above control for comparative purposes. Note the dramatic early increase in EVLW/BW, with little increase in dP/BW (the 2 hour PI value is not significant), and the subsequent decline at 6 to 12 hours PI. Note also the later, very dramatic increases in both EVLW/BW and dP/BW at 18 to 24 hours PI. Due to proportionate increases, the ratio of these two measures does not always reflect the changes in lung water.

TIME COURSE: BLOOD/BW RATIOS (SURVIVORS)



	Blood/BW§	% above control
controls (22)	0.83 (.03)	—
2 hours (4)	0.82 (.03)	—
6 hours (7)	0.98 (.03) *	18.1
12 hours (7)	0.88 (.05)	(6.0)
18 hours (11)	1.02 (.03) *	22.9
24 hours (18)	1.11 (.07) *	33.7

Exposures were carried out at an NO_2 concentrations of 255 ppm for 120 minutes. Animals were sacrificed for lung water and blood analysis at the times listed (expressed as X hours PI). Those animals dying early were deleted from lung blood analysis. Data are expressed as mean (sem), with # of animals at each time point in parentheses following time of sacrifice.

* $p < 0.05$ (compared to controls)
§ units are gm/kg

the 18 and 24 hour PI values are much higher and the second rise much more dramatic.

Since both of these measures is a ratio, a clearer picture of the changes in lung water and tissue mass becomes apparent when the individual components (Lung Wet/BW & Dry/BW and EVLW/BW & dP/BW) of the ratios are considered. Both Lung Wet/BW and EVLW/BW rise sharply during the first 6 hours PI, reaching levels 45 to 55 % above control. There is then a decline between 6 and 12 hours PI, with little subsequent change to 18 hours PI. This 18 hour PI value is artificially low since the animals most sensitive to NO_2, those dying early, are not included. Between 18 and 24 hours PI there is a very dramatic increase in both Lung Wet and EVLW weights. This increase is approximately equal to the increase occurring during the initial 6 hours PI.

Thus there are 2 phases during which lung water increases dramatically following NO_2 exposure, the first at 0 to 6 hours PI and the second at 18 to 24 hours PI. These phases are separated by a period, 6 to 18 hours PI, with little change or even a decrease in lung water content. This biphasic nature in the response to NO_2 inhalation can be most easily appreciated in the Lung Wet/BW and EVLW/BW curves, although it is also apparent in the Lung Wet/Dry and EVLW/dP curves, especially those including animals dying prior to sacrifice.

Dry weight changes, as measured by Lung Dry and dP weights (expressed as the ratio to body weight) follow a somewhat different time course. There is little, if any, change during the first 2 hours, neither value at 2 hours PI is significantly different from control. By 6 hours PI there is an increase on the order of 20% above control. There is then a period between 6 and 18 hours PI, similar to that in

the lung water measures, with little change or even some decline. Another dramatic increase in dry tissue mass then occurs during the 12 to 24 hour PI period. This rise is especially large over the last six hours (18 to 24 hours PI) of this phase, the overall gain being greater than that of the first 6 hours PI.

The pulmonary blood measures for these experiments were accurate enough to allow comparison between the different time points. These data are summarized in the graph relating Blood/BW to time after start of exposure. Although there was a large increase in the wet weight measures, no change in blood mass was detected during the 2 hours of exposure. Between 2 and 6 hours PI a dramatic increase, on the order of 20%, occurs in pulmonary blood volume. This is followed by a sharp decrease between 6 and 12 hours PI. Subsequent to this decrease is another dramatic increase, again on the order of 20&, from 12 to 18 hours PI. A more modest increase then occurs between 18 and 24 hours PI.

RESULTS: PHARMACOLOGICAL INTERVENTION EXPERIMENTS

Results from the time course series of experiments suggested two potentially effective pharmacological strategies for treatment of NO_2-induced pulmonary edema. Agents with hemodynamic actions normally lowering blood pressure were predicted to effect the early course of edemagenesis. It was predicted that the later course of edemagenesis might respond to treatment with anti-inflammatory agents.

Animals in this series of experiments were pre-treated with the agent under consideration, at least two hours prior to the start of NO_2 exposure. The rationale behind choosing the dose to be administered differed for each agent and is given in the discussion for each. The two hour period between dosing and exposure to NO_2 allowed for onset of drug action before the animals experienced the effects of NO_2. Exposures to NO_2 were carried out under the standard conditions (255 ppm NO_2 for 120 minutes) determined in the dose-response series of experiments. Subsequent to the exposure, animals were again dosed at time intervals depending on the biologic half-life of the test agent. Any deaths occurring during this period were noted as soon as possible. At the pre-determined time point (either 6 or 24 hours after start of exposure) surviving animals were sacrificed and analyzed for lung water and blood content. The results of this analysis were compared to those of untreated animals similarly exposed to NO_2.

TERBUTALINE:

Terbutaline was the first drug tested. Although it was anticipated that terbutaline's major effect would be on events of the first 6 hours PI, the initial experiments were designed to run the full 24 hours in order to detect effects on the overall process and on survival. Animals were pre-treated with 0.1 mg/kg terbutaline (via IP injection) and reinjected every 8 hours thereafter until sacrifice or death. This dosing regimen was chosen on the basis of its attenuation of lung weight increases produced by aerosolized histamine (Persson et al., 1979). In two different experiments, a total of 8 guinea pigs were pre-treated, exposed to NO_2, sacrificed at 24 hours PI and analyzed for lung water and blood volumes.

Terbutaline dramatically and significantly increased 24-hour mortality compared to NO_2 exposure alone. Animals treated with terbutaline experienced 75% mortality during the 24 hour period following initiation of exposure to NO_2, as compared to only 17% in animals exposed to NO_2 without pre-treatment. Therefore, terbutaline treatment increased mortality secondary to NO_2 exposure by more than 3-fold.

Lung Wet/Dry weight ratios in terbutaline-treated animals averaged 9.22, an increase of 36% over the untreated-exposed animals. The increase in the EVLW/dP ratios, which averaged 9.02, was even more striking at 50%. Terbutaline, under these conditions, severely worsened the edemagenic response to NO_2. Only 2 animals survived until sacrifice to be analyzed for lung water and blood content. Even with only these 2 animals for statistics, significant increases on the order of 45% were detected in Lung Wet weight and EVLW.

PHARMACOLOGICAL INTERVENTION

Effect of TERBUTALINE Pre-Treatment on Mortality and Lung Wet:Dry Weight Ratios in Guinea Pigs Subsequent to NO_2 Exposure

24 HOURS PI

ALL ANIMALS

24 HOUR MORTALITY

	Mortality
Standard Exposure	(3/18) 16.7%
Terbutaline	(6/8) 75.0%*

* $p < .05$ (z test for proportions)

LUNG WET:DRY WEIGHT RATIOS

	Mean (sem)		% change from Standard Exps. (24 h PI)§
Lung Wet/Dry weight ratio (gm/gm)	9.22	(.51)	+ 36.2†
EVLW/dP weight ratio (gm/gm)	9.02	(.64)	+ 50.1†

These values represent the results of lung water and blood analysis of the Terbutaline-treated animals (n = 8). Animals were pre-treated with the agent under study, exposed to NO_2 and survivors sacrificed at 24 hours PI for lung water and blood analysis. Those animals dying prior to the sacrifice time were assigned 10.0 for both Lung Wet/Dry and EVLW/dP weight ratios. Values were compared to animals exposed under the standard conditions (255 ppm NO_2 for 120 minutes) and analyzed at 24 hours PI (§ all animals).

† $p < .005$

EVLW = extravascular lung water weight
dP = dry lung parenchymal tissue weight

PHARMACOLOGICAL INTERVENTION

Effect of TERBUTALINE + ATENOLOL Pre-Treatment on Lung Water and Blood in Guinea Pigs Subsequent to NO_2 Exposure

ALL ANIMALS

6 HOURS PI

	Mean	(sem)	% change from Standard Exps. (6 hours PI)
Lung Wet/Dry weight ratio (gm/gm)	8.86	(.75)	+ 38.9*
Wet/BW (gm/kg)	15.03	(1.70)	+ 58.5†
Dry/BW (gm/kg)	1.68	(.05)	+ 13.5†
EVLW/dP weight ratio (gm/gm)	8.28	(.90)	+ 53.6*
EVLW/BW (gm/kg)	12.49	(1.62)	+ 74.4†
dP/BW (gm/kg)	1.50	(.04)	+ 13.6†
% EVLW in Lung Wet weight	82.7	(1.45)	+ 10.0*
Blood/dP ratio (gm/gm)	0.69	(.02)	- 7.00†
Blood/BW (gm/kg)	1.04	(.06)	ns

These values represent the results of lung water and blood analysis of the Terbutaline + Atenolol-treated animals (n = 4). Animals were pre-treated with the agent under study, exposed to NO_2 and sacrificed at 6 hours PI for lung water and blood analysis. Results are compared to animals exposed under standard conditions (255 ppm NO_2 for 120 minutes) and sacrificed at 6 hours PI.

* $p < .05$
† $p < .005$

EVLW = extravascular lung water weight
dP = dry lung parenchymal tissue weight
BW = body weight

Because of the possibility that reflex tachycardia secondary to terbutaline may have been involved in worsening edemagenesis, a $beta_1$-blocker, atenolol, was added for the next set of experiments. These experiments were evaluated at the 6 hour PI time point so that only effects on the early course of edemagenesis would be examined.

Although no deaths occurred, there was again a dramatic worsening of NO_2-induced pulmonary injury over the no treatment group. Lung Wet/Dry weight ratio averaged 8.86, an increases over NO_2 alone of almost 40%. The increase in the EVLW/dP weight ratio, which averaged 8.28, was close to 55%. Even more dramatic were the increases in the wet weight measures. Lung Wet/BW increased almost 60% to 15.03 and EVLW/BW increased by approximately 75% to 12.49. Dry weight measures were much less effected, increasing only about 14%. This modest increase was responsible for an apparent decrease in one of the blood measures (Blood/dP).

These data, along with the 10% increase in % EVLW in Lung Wet weight, indicate a greater pulmonary accumulation of free water after NO_2 exposure subsequent to terbutaline than after NO_2 exposure alone.

RUTIN:

Rutin, a flavenoid, was tested in regard to its possible action on the early events in NO_2-induced pulmonary edema. A dose of 100 mg/kg (IP) was administered 2 hours prior to exposure to 255 NO_2 for 120 minutes. This dose was determined by prior experience in the laboratory. At the 6 hour PI time point the animals were sacrificed and their lung analyzed for water and blood content.

In general there was little effect of the rutin treatment on development of pulmonary edema secondary to NO_2 inhalation. Treatment produced changes, which did not reach significance, in the wet:dry weight ratios of less than 10%. Lung Wet/BW and EVLW/BW weight ratios, the measures of whole lung weights, appeared to decrease somewhat (about 10%) but these values were not significantly different from NO_2 alone. The only changes reaching statistical significance were the decreases in dry weight measures. Lung Dry/BW decreased to 1.34 equalling 9.5% lower than in the 6 hour PI, standard exposure animals. The dP/BW ratio averaged 1.16, a somewhat larger decrease to 12% below animals exposed to NO_2 alone. Only one other parameter posted a significant change, the Blood/dP ratio showed an apparent increase of almost 17%. Over 75% of this apparent increase can be attributed to the 12% decrease in dP. Because of this and the lack of change in the Blood/BW ratio, the increase in Blood/dP appears artifactual.

Although rutin produced no dramatic changes in the development of pulmonary edema following NO_2, the decreases in the dry weight measures indicate that less of the dry weight substances were deposited in the lungs. Thus there was some decrease in permeability.

PHARMACOLOGICAL INTERVENTION

Effect of RUTIN Pre-Treatment on Lung Water & Blood in Guinea Pigs Subsequent to NO_2 Exposure

ALL ANIMALS

6 HOURS PI

	Mean	(sem)	% change from Standard Exps. (6 hours PI)
Lung Wet/Dry weight rat (gm/gm)	6.36	(.35)	ns
Wet/BW (gm/kg)	8.53	(.63)	- 10.0
Dry/BW (gm/kg)	1.34	(.03)	- 9.5†
EVLW/dP weight ratio (gm/gm)	5.47	(.40)	ns
EVLW/BW (gm/kg)	6.37	(.63)	- 11.0
dP/BW (gm/kg)	1.16	(.04)	- 12.1†
% EVLW in Lung Wet weight	74.2	(1.95)	ns
Blood/dP ratio (gm/gm)	0.87	(.06)	+ 17.6*
Blood/BW (gm/kg)	1.01	(.05)	ns

These values represent the results of lung water and blood analysis of the Rutin-treated animals (n = 4). Animals were pretreated with the agent under study, exposed to NO_2 and sacrificed at 6 hours PI for lung water and blood analysis. Results are compared to animals exposed under standard conditions (255 ppm NO_2 for 120 minutes) and sacrificed at 6 hours PI.

* $p < .05$
† $p < .005$

EVLW = extravascular lung water weight
dP = dry lung parenchymal tissue weight
BW = body weight

INDOMETHACIN-6 HOURS PI:

In an attempt to learn the possible contribution of inflammation in the early course of edemagenesis, Indomethacin was used to pretreat one group of four animals. A dose of 20 mg/kg Indomethacin was chosen on the basis of the work of Giri et al. (1975) and administered intraperitoneally.

In contrast to the untreated animals, all of which survived 6 hours PI, one of the four died soon after the end of exposure. On the basis of this one death, Indomethacin certainly had a exacerbative effect on the early course of NO_2-induced pulmonary edema production. The assayed lung water and blood measures probably have little meaning in comparison to standard exposure animals as they were generated from the 75% of the animals least susceptable to NO_2 in this experiment.

In spite of loss of the animal most affected by NO_2, there were only 3 measures significantly decreased by Indomethacin pretreatment. The dry weight measures decreased approximately 10% each. Wet weight measures exhibited non-significant decreases of the same magnitude. The Blood/BW measure decreased (significantly) by 13%, although a similar decrease was not noted in the Blood/dP ratio, possibly due to a corresponding decrease in dP.

The one animal dying early, shortly after the end of exposure, was analyzed at death for lung water and blood (data not shown). Both Lung Wet/Dry and EVLW/dP weight ratios were over 10.0. The wet weight measures were 1.7 to 1.9 times those of the standard exposure animals at 6 hours PI, but the dry weight measures showed almost no change.

PHARMACOLOGICAL INTERVENTION

Effect of INDOMETHACIN Pre-Treatment on Lung Water & Blood in Guinea Pigs Subsequent to NO_2 Exposure

SURVIVORS ONLY§

6 HOURS PI

	Mean	(sem)	% change from Standard Exps. (6 hours PI)
Lung Wet/Dry weight rat (gm/gm)	6.31	(.66)	ns
Wet/BW (gm/kg)	8.41	(.64)	- 11.3
Dry/BW (gm/kg)	1.34	(.06)	- 9.5*
EVLW/dP weight ratio (gm/gm)	5.37	(.75)	ns
EVLW/BW (gm/kg)	6.36	(.69)	- 11.2
dP/BW (gm/kg)	1.19	(.05)	- 9.8*
% EVLW in Lung Wet weight	75.4	(2.48)	ns
Blood/dP ratio (gm/gm)	0.71	(.02)	ns
Blood/BW (gm/kg)	0.85	(.06)	- 13.3*

These values represent the results of lung water and blood analysis of the Indomethacin-treated animals (n = 3). Animals were pre-treated with the agent under study, exposed to NO_2 and sacrificed at 6 hours PI for lung water and blood analysis. Results are compared to animals exposed under standard conditions (255 ppm NO_2 for 120 minutes) and sacrificed at 6 hours PI.

§ 1 of 4 animals died prior to sacrifice

* $p < .05$

EVLW = extravascular lung water weight
dP = dry lung parenchymal tissue weight
BW = body weight

INDOMETHACIN-24 HOURS PI:

Indomethacin was expected to be have more effect on the later course of pulmonary edema following NO_2. The next experiment evaluated pretreatment with Indomethacin, exposure to NO_2 and subsequent retreatment with Indomethacin for 24 hours PI.

Although not reaching significance because of the number of animals involved, mortality increased 2.5-fold over these 24 hours as compared with no treatment. When all the animals were considered, the wet:dry ratios both increased 15 to 20%, although not significantly. As can be determined from the complete lung water data on survivors, these increases are probably due mainly to decreases in dry weight measures.

In the survivors, the wet:dry ratios did not change and there was no effect on either Lung Wet/BW or Dry/BW. EVLW/BW decreased about 10%, but not significantly. The only significant change associated with the wet:dry measures was an almost 12% decrease in dP/BW, indicating less deposition of non-blood dry weight material in the lung.

Both measures of blood content increased significantly. Blood/BW rose 20% and Blood/dP increased by 36%. The increase in blood in the lung is probably the reason for no changes being detected in the Lung Wet/Dry weight ratio and the individual components despite the decreases in EVLW/BW and dP/BW. Blood content contributes to the former, but not the latter.

Indomethacin certainly did not exert a protective effect with respect to the development of pulmonary edema after exposure to NO_2. If anything, treatment with Indomethacin worsened the outcome, resulting in mortality increases.

PHARMACOLOGICAL INTERVENTION

Effect of INDOMETHACIN Pre-Treatment on Mortality and Lung Wet:Dry Weight Ratios in Guinea Pigs Subsequent to NO_2 Exposure

24 HOURS PI

ALL ANIMALS

24 HOUR MORTALITY

	Mortality
Standard Exposure	(3/18) 16.7%
Indomethacin	(5/12) 41.7% [ns]

* $p < .05$ (z test for proportions)

LUNG WET:DRY WEIGHT RATIOS

	Mean	(sem)	% change from Standard Exps. (24 h PI)§
Lung Wet/Dry weight ratio (gm/gm)	7.85	(.57)	+ 16.0
EVLW/dP weight ratio (gm/gm)	7.31	(.71)	+ 21.6

These values represent the results of lung water and blood analysis of the Indomethacin-treated animals (n = 12). Animals were pre-treated with the agent under study, exposed to NO_2 and survivors sacrificed at 24 hours PI for lung water and blood analysis. Those animals dying prior to the sacrifice time were assigned 10.0 for both Lung Wet/Dry and EVLW/dP weight ratios. Values were compared to animals exposed under the standard conditions (255 ppm NO_2 for 120 minutes) and analyzed at 24 hours PI (§ all animals).

† $p < .005$

EVLW = extravascular lung water weight
dP = dry lung parenchymal tissue weight

PHARMACOLOGICAL INTERVENTION

Effect of INDOMETHACIN Pre-Treatment on Lung Water & Blood in Guinea Pigs Subsequent to NO_2 Exposure

SURVIVORS ONLY

24 HOURS PI

	Mean	(sem)	% change from Standard Exps. (24 hours PI)§
Lung Wet/Dry weight ratio (gm/gm)	6.32	(.28)	ns
Wet/BW (gm/kg)	10.38	(.77)	ns
Dry/BW (gm/kg)	1.63	(.06)	ns
EVLW/dP weight ratio (gm/gm)	5.38	(.35)	ns
EVLW/BW (gm/kg)	7.64	(.70)	- 10.3
dP/BW (gm/kg)	1.41	(.05)	- 11.9*
% EVLW in Lung Wet weight	73.1	(1.47)	ns
Blood/dP ratio (gm/gm)	0.95	(.05)	+ 36.0†
Blood/BW (gm/kg)	1.33	(.08)	+ 19.8*

These values represent the results of lung water and blood analysis of the Indomethacin-treated animals (n = 7). Animals were pre-treated with the agent under study, exposed to NO_2 and sacrificed at 24 hours PI for lung water and blood analysis. Results are compared to animals exposed under standard conditions (255 ppm NO_2 for 120 minutes) and sacrificed at 24 hours PI (§ survivors only).

* $p < .05$
† $p < .005$

EVLW = extravascular lung water weight
dP = dry lung parenchymal tissue weight
BW = body weight

DEXAMETHASONE-24 HOURS PI:

A possible protective effect of Dexamethasone was investigated in 24 hour PI experiments. Dexamethasone was administered (IP) in doses of 10 to 15 mg/kg before exposure and every 12 hours thereafter. It was noted that high dose steroids are often used to treat ARDS (Hopewell & Murray, 1976) and the Dexamethasone dose was set high relative to normal usage.

Dexamethasone had no effect on mortality with 3 of 12, or 25%, of treated animals dying within 24 hours PI. Neither were the wet:dry weight ratios affected by the treatment when all the animals were considered.

Animals surviving the full 24 hours PI exhibited some significant changes in lung and blood weights. Other measures were suggestive but did not reach significance. Because of relatively large, and significant, increases in pulmonary blood content, Lung Wet/BW and Dry/BW decreased less than EVLW/BW and dP/BW. Of these, the only decrease that was significant was the 14% lowering of dP/BW, the dry lung parenchymal mass. Extravascular lung water, as measured by EVLW/BW, decreased a relatively large 15% although this number was not significant. Therefore it appears that both excess non - vascular water and, definitely, non - blood dry weight substances decreased as a result of Dexamethasone treatment.

The response to Dexametnasone was equivacal. On the one hand, there was no significant effect on 24 hour mortality or extravascular lung water. However EVLW/BW did decrease as did dP/BW, significantly.

PHARMACOLOGICAL INTERVENTION

Effect of DEXAMETHASONE Pre-Treatment on Mortality and Lung Wet:Dry Weight Ratios in Guinea Pigs Subsequent to NO_2 Exposure

24 HOURS PI

ALL ANIMALS

24 HOUR MORTALITY

	Mortality
Standard Exposure	(3/18) 16.7%
Dexamethasone	(3/12) 25.0% [ns]

* $p < .05$ (z test for proportions)

LUNG WET:DRY WEIGHT RATIOS

	Mean (sem)		% change from Standard Exps. (24 h PI)§
Lung Wet/Dry weight ratio (gm/gm)	7.05	(.55)	ns
EVLW/dP weight ratio (gm/gm)	6.41	(.69)	ns

These values represent the results of lung water and blood analysis of the Dexamethasone-treated animals (n = 12). Animals were pre-treated with the agent under study, exposed to NO_2 and survivors sacrificed at 24 hours PI for lung water and blood analysis. Those animals dying prior to the sacrifice time were assigned 10.0 for both Lung Wet/Dry and EVLW/dP weight ratios. Values were compared to animals exposed under the standard conditions (255 ppm NO_2 for 120 minutes) and analyzed at 24 hours PI (§ all animals).

† $p < .005$

EVLW = extravascular lung water weight
dP = dry lung parenchymal tissue weight

PHARMACOLOGICAL INTERVENTION

Effect of DEXAMETHASONE Pre-Treatment on Lung Water and Blood in Guinea Pigs Subsequent to NO_2 Exposure

SURVIVORS ONLY

24 HOURS PI

	Mean	(sem)	% change from Standard Exps. (24 hours PI)§
Lung Wet/Dry weight ratio (gm/gm)	6.07	(.10)	ns
Wet/BW (gm/kg)	10.07	(.69)	- 10.3
Dry/BW (gm/kg)	1.65	(.02)	- 7.8
EVLW/dP weight ratio (gm/gm)	5.21	(.13)	ns
EVLW/BW (gm/kg)	7.18	(.20)	- 15.7
dP/BW (gm/kg)	1.38	(.01)	- 13.8*
% EVLW in Lung Wet weight	70.9	(.43)	- 5.6*
Blood/dP ratio (gm/gm)	1.11	(.04)	+ 60.0†
Blood/BW (gm/kg)	1.51	(.05)	+ 36.0*

These values represent the results of lung water and blood analysis of the Dexamethasone-treated animals (n = 9). Animals were pre-treated with the agent under study, exposed to NO_2 and sacrificed at 24 hours PI for lung water and blood analysis. Results are compared to animals exposed under standard conditions (255 ppm NO_2 for 120 minutes) and sacrificed at 24 hours PI (§ survivors only).

* $p < .05$
† $p < .005$

EVLW = extravascular lung water weight
dP = dry lung parenchymal tissue weight
BW = body weight

METHYL PREDNISOLONE-24 HOURS PI:

Although the data are not presented, four animals were pretreated with 30 mg/kg (IP) Methyl Prednisolone, exposed to NO_2 and retreated every 8 hours, until sacrifice at 24 hours PI. Methyl Prednisolone was found to exert no effect on either mortality or any of the measures of lung water or blood content in this experiment's assay.

CLOFIBRATE-24 HOURS PI:

Clofibrate, a drug with a number of potentially beneficial effects, was tested for its overall effect on NO_2-induced pulmonary injury in 24 hour PI latent period experiments. The dose of 10 mg/kg (IP) was based on a multiple of normal human usage (Goodman & Gilman, 1980). Animals were pretreated 2.5 to 3.0 hours prior to entering the inhalation chamber for exposure under the standard conditions of 255 ppm NO_2 for 120 minutes. After exposure, the animals were reinjected with the same dose every 12 hours until the 24 hour PI sacrifice time.

None of 11 animals treated with Clofibrate and exposed to NO_2 died prior to sacrifice. This 100% survival rate is in marked contrast to the 83% survival rate for untreated animals similarly exposed to NO_2 and represents the most dramatic exhibition of Clofibrate's protective effect.

Lung water and blood analysis was therefore possible on all the animals in this series of experiments. Neither the Lung Wet/Dry or the EVLW/dP weight ratio was different from untreated-exposed animals. However, there were significant decreases in all of the separate parameters contributing to both of these ratios. These decreases were greatest for the wet weight measures, Lung Wet/BW decreasing by 23% and EVLW/BW decreasing by over 25%, compared to standard exposure animals. The dry weight measures both decreased by about the same amount, Lung Dry/BW decreasing 16% and dP/BW decreasing 17%. Because of decreases in both numerators and denominators, the wet:dry ratios showed no overall decrease. Neither of the blood content measures reached a significant difference as defined by the $p < .05$ level. However, the

PHARMACOLOGICAL INTERVENTION

Effect of CLOFIBRATE Pre-Treatment on Lung Water & Blood in Guinea Pigs Subsequent to NO_2 Exposure

ALL ANIMALS

24 HOURS PI

	Mean	(sem)	% change from Standard Exps. (24 hours PI)§
Lung Wet/Dry weight rat (gm/gm)	5.77	(.08)	ns
Wet/BW (gm/kg)	8.62	(.23)	- 23.2*
Dry/BW (gm/kg)	1.50	(.04)	- 16.2*
EVLW/dP weight ratio (gm/gm)	4.77	(.08)	ns
EVLW/BW (gm/kg)	6.34	(.18)	- 25.6*
dP/BW (gm/kg)	1.33	(.04)	- 16.9*
% EVLW in Lung Wet weight	73.5	(.33)	ns
Blood/dP ratio (gm/gm)	0.72	(.02)	ns
Blood/BW (gm/kg)	0.96	(.03)	(- 13.5)**

These values represent the results of lung water and blood analysis of the Clofibrate-treated animals (n = 11). Animals were pretreated with the agent under study, exposed to NO_2 and sacrificed at 24 hours PI for lung water and blood analysis. Results are compared to animals exposed under standard conditions (255 ppm NO_2 for 120 minutes) and sacrificed at 24 hours PI (§ survivors only).

** $p = .06$
* $p < .05$
† $p < .005$

EVLW = extravascular lung water weight
dP = dry lung parenchymal tissue weight
BW = body weight

PHARMACOLOGICAL INTERVENTION

Effect of CLOFIBRATE Pre-Treatment on 24 Hour Survival in Guinea Pigs Subsequent to NO_2 Exposure

ALL ANIMALS

24 HOURS PI

	Survival	
Standard Exposure	(15/18)	83.3%
Clofibrate	(11/11)	100.0%

Animals were pre-treated with Clofibrate (10 mg/kg, IP) and exposed to NO_2 under the standard conditions (255 ppm NO_2 for 120 minutes). Animals were then observed for 24 hours, until lung water and blood analysis, and any deaths noted. Results are compared to animals exposed and observed similarly, but without Clofibrate pre-treatment.

Blood/BW ratio decreased by 13% in the Clofibrate animals, with a p value equal to 0.06.

Therefore, pretreatment with 10 mg/kg Clofibrate significantly protected against the production of pulmonary edema as a result of NO_2 exposure. This protection was evident as marked decreases in wet weight measures of the lung. Specifically, the excess accumulation of extravascular lung water, as measured by EVLW/BW, was markedly attenuated. The accumulation of dry weight substances, measured as dP/BW, was also decreased, although to a somewhat lesser extent. These are the measures most sensitive to fluid and protein accumulation in the lung.

The significant decreases in Lung Wet/BW and Dry/BW ratios, which include contributions from blood in the lung, and the moderate decrease in Blood/BW suggest that the hyperemic congestive component of the reaction to NO_2 was also decreased by Clofibrate.

The most dramatic demonstration of the protective effect exerted by Clofibrate in this experiment was on 24 hour mortality. Pretreatment of animals with Clofibrate at 10 mg/kg prevented the 1 to 2 deaths expected, on the basis of the experience with untreated-exposed animals, as a result of NO_2 exposure in a group of this size.

DISCUSSION

DISCUSSION: CONTROL ANIMALS

It is expected that pulmonary tissue and water masses would increase proportionately with body weight, and hence with age. However, the negative correlation between the ratios of wet:dry measures (Lung Wet/Dry and EVLW/dP weight ratios) and body weight indicates that the dry tissue mass increases at a somewhat faster rate than does the water content. This may be due to accumulation of cellular wastes or drying of connective tissue with aging

The Lung Wet/Dry weight ratio (5.21) in the control guinea pigs is the same as that (5.20) reported from biopsy specimens for human lungs (Staub, 1974). It is similar to the Lung Wet/Dry ratio for the rat (4.37), published by Giri et al. (1975).

The EVLW/dP weight ratio is very close to the published values for normal sheep, which range 3.95 to 4.09 (Selinger et al., 1975 and Erdmann et al., 1975). The normal ratio in humans appears to range somewhat lower than either the sheep or the guinea pig, published values ranging 3.57 to 4.08 (Gump, 1978 and Staub, 1974).

Although the ratios of Lung Wet/Dry and EVLW/dP weights are close to those of sheep, it is interesting to note that the individual parameters, Lung Wet/BW & Dry/BW and EVLW/BW & dP/BW, for the guinea pigs are only about half those of the sheep (Selinger et al., 1975 and Erdmann et al., 1975), as compared in the table on the next page . The human values calculated from the data of Staub (1974), assuming an average body weight of 70 kg, are closer to those of the guinea pig than of the sheep. Although Gump's (1975) data on human lungs are much different from Staub's, they also are closer to the guinea pig's than the sheep's.

COMPARISON OF LUNG WEIGHT RATIOS FROM CONTROL GUINEA PIGS WITH PUBLISHED VALUES FOR OTHER SPECIES

	Weight Ratios			Reference
	Lung Wet/Dry†	Wet/BW*	Dry/BW*	
Guinea Pig	5.21	6.46	1.24	This study.
Human	5.20	9.60	1.84	Staub (1974)
Sheep	-	14.28	-	Selinger et al. (1975)
Rat	4.37	3.54	0.81	Giri et al. (1975)
	EVLW/dP†	EVLW/BW*	dP/BW*	
Guinea Pig	4.18	4.54	1.09	This study.
Human	4.08	5.47	1.34	Staub (1974)
Human	3.57	2.83	0.79	Gump (1978)
Sheep	4.09	9.51	2.32	Erdmann et al. (1975)

* expressed as gm/kg
† expressed as gm/gm

There is a wider difference in the lung blood values between these guinea pigs and humans. In humans, the Blood/dp was reported by Staub (1974) to be 2.07 and the Blood/BW to be 2.79 gm/kg. Values for the same ratios in guinea pigs were found to be 0.77 and 0.83 gm/kg in the present study. These differences are not surprising in light of the lung's ability to contain variable amounts of blood in the normal state. A certain amount of the difference is no doubt also due to different methods of preparing the lung for analysis, as it is very easy to squeeze blood out of the major vessels of the lung.

A comparison of these data clearly show that the guinea pig is an acceptable model of the human with regard to lung water analysis. Thus, the selection of the guinea pig for these experiments is validated on the basis of comparison of normal lung water and blood contents with other common laboratory species and with the human.

HYDROSTATIC/PERMEABILITY PULMONARY EDEMA & WET:DRY RATIOS:

The basic assumption underlying interpretation of these data is that differences bewteen hydrostatic and permeability mechanisms of pulmonary edema are detectable by analysis of changes in wet and dry measures of the lung. Either type of edematous process will result in increases in Lung Wet weight and EVLW, as excess water accumulates in the lung. Only edemagemic processes that result in accumulation of dry weight materials, proteins or cells of extra-pulmonary origin, will acutely produce net increase in the dry weight measures, especially dP/BW. Although cellular proteins and other materials may be released from pulmonary cells by destructive processes, such as chemical attack on bronchiolar or alveolar epithelium, these do not lead to any net increase in the amount of dry weight material in the lung. It is only through increased deposition of material of non-pulmonary origin that the dry weight measures of the present study are increased. Therefore, it is by comparison of the increases in wet weights with concurrent changes in dry weights that a distinction between hydrostatic and permeability types of edema is possible.

Hydrostatic pulmonary edema is characterized as a high pressure-mediated accumulation of excess lung water with a low protein content. This type of edemagenic event results in increased levels of water, along with small electrolytes, in the lung. The important distinction is that, because of the integrity of the endothelial barrier to high molecular weight substances, plasma proteins do not accumulate along with the excess water. Therefore in hydrostatically-mediated types of pulmonary edema, the increases in wet weight measures will overwhelm

any increases in dry weight measures. The ratio of these changes, the "increase ratio", will be relatively large.

Loss or attenuation of the endothelial barrier permits leakage of plasma proteins, as occurs in permeability pulmonary edema, and will result in increased deposition of protein in the lung. This will be detected by the present assay as increased dry weight. In addition, increases in dry weight are also contributed by the accumulation of PMNs, and other cells and mediators of inflammation, as often happens with the permeability type of pulmonary edema. Although the present assay corrects for blood content, because of the means by which this is done (hemoglobin concentration measurement and correlation with whole blood water content), it will not correct for increased white blood cells either in the pulmonary vasculature or parenchyma. Concurrent with this increase in dry weight will, of course, be an increase in wet weight. With this type of edemagenic mechanism the increases in dry weight measures will more closely parallel the increases in wet weight than is the case with the hydrostatic type. The "increase ratio" will be correspondingly lower in permeability types of edema.

It is expected that no instance of pulmonary edema will be purely of one type or the other. Certainly driving pressure cannot be divorced from permeability edema and, since even the normal microvascular wall is not a perfect seive for proteins, there is some increase in protein leakage in hydrostatic edema. Therefore increases in both wet and dry lung weight measures will occur with either type of mechanism producing pulmonary edema. The distinction is made between the two types on relative increases in these measures.

In this analysis, conditions which produce substantial increases in lung wet weight measures accompanied by little or no increase in dry weight measures (high "increase ratio") will be considered primarily hydrostatic types of pulmonary edema. Lung wet weight increases occurring concurrently with substantial increases in dry weight measures (low "increase ratio") will be considered evidence of primarily a permeability type of pulmonary edema.

DISCUSSION: DOSE-RESPONSE EXPERIMENTS

Two important accomplishments were realized from the dose-response experiments. Firstly, standard exposure conditions for the remaining sets of experiments were delineated. Secondly, initial clues were obtained concerning the possible involvement of two distinct mechanisms in the edemagenic response of the lungs to NO_2.

After careful analysis of the dose-response data, it was determined the standard conditions for the remaining two series of experiments would be exposure to 255 ppm NO_2 for 120 minutes. Two criteria were fulfilled by the response of guinea pigs to this exposure regimen.

A standard exposure producing fractional mortality was desired. This would allow detection of any effect, of the pharmacological agents to be studied later, on mortality. It was necessary, however, to choose an exposure level with low enough mortality to allow enough survivors for complete lung water and blood content analysis. The 255 ppm exposure, resulting in approximately 17% mortality, met this goal. At this level any significant increases or decreases in mortality would be easily detectable. Additionally, enough animals (15 of 18 exposed) survived to yeild lung water and blood content measures with ranges small enough for ready comparison in the later experiments attempting to alter production of pulmonary edema.

Important also for the later experiments would be production of a level of pulmonary edema yeilding significantly high lung water and blood contents. For the time course experiments it would be necessary that the wet:dry weight ratios, and other measures of pulmonary water and blood, be high enough to easily detect the progression of the

edemagenic process over the preceeding 24 hours. For the pharmacological intervention series these measures also needed to be high enough to detect any ameliorative effects produced. At the same time, it was desirable that these wet:dry ratios not be so high that any increase, indicating an exacerbative action by the pharmacological agent under study, be undetectable. For this criteria also the 255 ppm concentration level produced just the right effect. The Lung Wet/Dry (6.77 in all animals & 6.13 in survivors) and the EVLW/dP (6.01 in all animals & 5.21 in survivors) weight ratios are in the middle of the ranges these ratios produced in the dose response experiments, as were their component measures. Setting standard conditions so that assay values are "midline" allows further experiments to detect changes in either direction, increases or decreases.

Exposure to 255 ppm NO_2 for 120 minutes therefore met the criteria for a standard exposure. These criteria could be summarized as production of injury severe enough to be easily quantified but not so severe as to be unalterable.

Valuable information concerning the nature of the pulmonary edema produced by NO_2 was also acquired from the dose-response data. Inspection of the dose-response curves for the individual components of the wet:dry ratios gave the first hint that the edemagenic response to NO_2 might involve two processes. As NO_2 concentration increases up to the 200 ppm exposure level, there are steady increases in wet weight measures (Lung Wet/BW and EVLW/BW) while dry weight measures (Lung Dry/BW and dP/BW) change little from the moderately increased values initially attained. This situation changes above 200 ppm. Both the wet weight

and dry weight measures increase dramatically between 200 and 255 ppm NO_2, indicating a change in the accumulation of lung fluid.

It therefore appears there is a "baseline" edemagenic process that starts at relatively low levels of NO_2 exposure. Lung wet weight measures progressively increase with increasing intensity of exposure, indicating accumulation of water in the lung. There is, however, no corresponding increase in the accumulation of the material yeilding increases in dry weight measures. This process has characteristics of a primarily hydrostatic-type edemagenic mechanism.

The change in fluid accumulation that occurs above 200 ppm NO_2 is a shift to more of a permeability-type edemagenic mechanism. There is a dramatic acceleration in the rate of excess lung water accumulation. In addition, there is a dramatic increase in accumulation of dry weight, indicating a large increase in deposition of dry weight substances from the pulmonary vasculature. It appears some barrier to accumulation of dry weight material has been compromised. This interpretation is reinforced by the observation that dry weight measures are increased further at the next higher exposure level.

Therefore, it appears there is a "baseline" increase in both lung water and dry weight substances for any NO_2 exposure producing an edematous response in the lungs. Below 200 ppm NO_2 there is a corresponding increase in excess lung water for increases in the severity of exposure, with little additional deposition of dry weight material. Above 200 ppm, the accumulation of excess lung water accelerates and there begins increased accumulation of dry weight substances over and above the moderate level "baseline" deposition.

That a critical change occurs between the 200 and 255 ppm exposures is even more apparent from the mortality data. At the 200 ppm exposure there was no mortality while a significant fraction of the animals exposed to 255 ppm died. It thus appears the greatly increased accumulation of dry weight material, charateristic of a permeability type of edemagenic mechanism, is associated with pulmonary injury serious enough to result in death.

The increase in wet weight measures appears to subside after this NO_2 level whereas the increases in dry weight measures continue at the 285 ppm level, resulting in decreased wet:dry weight ratios. These increases in deposition of dry weight material are indicative of a worsening permeability edemagenesis. With increasing severity of injury, at higher NO_2 exposures, it appears the endothelial barrier becomes even more compromised.

The apparent levelling-off of wet weight increases may be a reflection of the mortality at these NO_2 exposures. Animals dying appear, from observations in the literature and the experience with the two animals analyzed at death, to have wet:dry ratios close to 10.0. These "terminal wet:dry" ratios are much higher than those of the survivors from these NO_2 exposures. Alternatively, interstitial compliance, due to expansion by excess water, may have become so high that further increases in water accumulation require compromise of another barrier, one with an "all-or-none" character in regard to water permeability. The alveolar epithelium may have this characteristic.

DISCUSSION: TIME COURSE EXPERIMENTS

Evidence from the time course experiments indicates there are two distinct phases in the development of NO_2-induced pulmonary edema and hyperemic congestion. By analogy, there may also be two phases in the development of clinical ARDS. These two phases have characteristics of an initial hydrostatic and a later permeability mechanism producing pulmonary edema. The biphasic nature of edemagenesis is apparent from changes in wet and dry weights, as well as blood content, of the lungs over the first 24 hours PI after exposure to NO_2.

In the case of the wet weight measures, Lung Wet/BW and EVLW/BW, there was an initial peak, due to a marked increase over the first 6 hours PI. This was followed by a decline and plateau occurring between 6 and 18 hours PI. Following this was a second peak, a dramatic increase between 18 and 24 hours PI.

In contrast, the dry weight measures, Lung Dry/BW and dP/BW, did not show an early peak, rather they rose to a moderate plateau extending between 6 and 18 hours PI. Then, between 18 and 24 hours PI, there was a dramatic increase similar to that occurring in the wet weights.

The early changes, relatively greater increases in wet weight than in dry weight measures, are indicative of an initial (0 to 6 hours PI) edemagenic mechanism that is primarily hydrostatic. A levelling-off or even a diminution of edema formation is then indicated by the plateau between 6 and 18 hours PI. The dramatic increases in both wet and dry weight measures over the 18 to 24 hour PI period is evidence for a delayed, primarily permeability mechanism.

The changes in dry weight measures are important to the conclusion that a primarily hydrostatic mechanism is operative during the initial 6 hours PI. Increases in wet weight measures (Lung Wet/BW and EVLW/BW) that are much greater than corresponding increases in dry weight measures (Lung Dry/BW and dP/BW) constitute the important observation in distinguishing a primarily hydrostatically-mediated edema process. This is clearly the situation during these first 6 hours PI.

0 TO 2 HOURS PI:

By 2 hours PI, Lung Wet/BW was increased by 23% above normal. At the same time there was only a 7% apparent increase in Lung Dry/BW, this value having not reached significance may not be different from control. Assuming this apparent dry weight increase is real, the ratio of increases in Lung Wet/BW compared to Lung Dry/BW equals 3.2. Since there is no change from control in blood content at this time, these "whole lung" increases are most reflective of extravascular water.

The extravascular lung water (EVLW) and dry weight substance (dP) measures themselves show similar changes. Over the first 2 hours PI, EVLW/BW increased 31%. Again the apparent increase in the corresponding dry weight measure (dP/BW) was not significant, but equaled 9%. The ratio of these increases (EVLW/BW to dP/BW) is 3.4.

Therefore, component analysis of both wet:dry ratios shows that during the first 2 hours of the response to NO_2, excess lung water accumulated at least 3 times as fast as did the dry weight substances in the lung. A primarily hydrostatic mechanism is indicated by these types of changes, a conclusion reinforced by the lack of significance in the apparent dry weight increases.

Primary histological observations, as reported in a number of studies, during the first 2 hours after initiation of exposure to moderate to high levels of NO_2 are those of congestion of the pulmonary microvasculature. This is a finding typical of states in which vascular pressures are elevated and is termed hyperemic congestion or hemorrhagic consolidation or some combination of these terms.

Hine et al. (1970) exposed 5 species (mouse, rat, guinea pig, rabbit and dog) to various concentrations of NO_2 for 1 or 2 hours, after which the animals were sequentially sacrificed. Hyperemic consolidation and vascular congestion was most severe, and was accompanied by edema, in those animals dying after exposure to 40 to 75+ ppm NO_2. Survivors exhibited less severe hyperemic consolidation, vascular congestion and interstitial/alveolar edema. None of the animals at this early stage showed evidence of alveolar epithelial damage. Similar observations were made by Stephens et al. (1972). They noted pre- and post- capillary engorgement around alveoli in rats exposed to 17 ppm NO_2 for 2 hours.

In a group of beagles, Dowell et al. (1971) observed changes ranging from swelling and blebbing of endothelial walls, mitochondria and alveolar membranes to gross pulmonary edema with alveoli filled with fluid, cell debris and erythrocytes. These changes developed in response to a 1 hour exposure to concentrations between 3 and 16 ppm NO_2, surprisingly low levels for producing these types of changes when compared to the results of Guidotti (1980).

Notably lacking in these descriptions of early histological changes is mention of PMN infiltration or evidence of protein deposition in or around the alveoli. Dowell et al. (1971) did observe some margination of PMNs and platelets within the pulmonary vascualture, but the lung

appears to be a normal "storage site" for PMNs (Cohen & Rossi, 1983) and either increased margination or infiltration into septal or alveolar areas is necessary to conclude PMN activation. The histologic picture of the first 2 hours is one of microvascular congestion and some interstitial and alveolar edema characteristic of a hydrostatic type of mechanism (no protein deposition in alveoli or interstitium).

2 TO 6 HOURS PI:

Over the next 4 hours, accumulation of lung water slowed slightly and there was increased deposition of dry weight material. However, the overall events, as determined by the results of the lung water and blood analysis and the histologic observations, remain those of a primarily hydrostatic mechanism.

By 6 hours PI, Lung Wet/BW had reached its early peak at 47% above control. As can be seen from the change in slope on the time course graph, the rate of increase in Lung Wet/BW had slowed somewhat, possibly as a result of decreasing perimicrovascular compliance as the interstitium filled with fluid. At the same time, Lung Dry/BW had increased to 19% above control, reaching significance for the first time. The ratio of the increase in Lung Wet/BW compared to Lung Dry/BW equalled 2.5 at this early peak in the time course of the edemagenic response to NO_2.

The general behavior of the EVLW/BW and dP/BW parameters was similar to that of the "whole lung" wet and dry measures. However the magnitude of the changes was greater. At the 6 hour PI time point, EVLW/BW had increased to 58% above normal. With this measure also there was a decrease in the rate of accumulation, the change in slope being

somewhat greater than in the Lung Wet/BW case. This was no doubt due to a significant accumulation, at this time, of blood in the lung, which contributes to the Lung Wet/BW measure but not to EVLW/BW. The dP/BW measure peaked at 21% above control, indicating significant deposition of dry weight material. An "increase ratio" of 2.8 describes the relative difference in the increases in EVLW/BW and dP/BW.

Although the "increase ratios" were not as large as at 2 hours PI, the increase in wet weight measures continued to be much greater than the increase in dry weight measures at this 6 hour PI peak. These relative changes are again indicative of a primarily hydrostatic mechanism in the accumulation of excess lung water. The increases in dry weight measures and the lowering of the increase ratios gives the first hint that a permeability mechanism may also be making some contribution.

Histologically, the general picture during this time period also suggests a continuation of the primarily hydrostatic edemagenesis of the first 2 hours. In addition there is some evidence of the effect of a permeability type of edemagenic process.

In the 5 species exposed (40 to 75+ ppm NO_2, for 1 to 2 hours) by Hine et al. (1970), the hyperemic and edematous responses now become more severe in the animals that will eventually die, with survivors little different from earlier. Evidence of vascular congestion along with interstitial and alveolar edema is present in cats exposed to 80 ppm NO_2 for 3 hours (Langloss et al., 1977). The filled alveoli exhibited "a few" neutrophils. Beagles exposed intra-bronchially to 37 ppm NO_2 for 4 hours and sacrificed immediately also exhibit signs of interstitial edema and beginning breakdown of the endothelial wall at this time (Guidotti, 1980).

Rats exposed (17 ppm NO_2 for 4 hours) by Stephens et al. (1972) show the first signs of protein extravasation into the alveoli. In addition to progression of the congestive and edematous changes observed earlier, there is now sloughing of Type I cells from the alveolar wall. The bare areas of basement membrane left behind becomes covered with fibrin.

With the first evidence of PMN infiltration and protein deposition the primarily hydrostatic stage of edemagenesis appears to be drawing to a close during this period.

Early - Hydrostatic Phase:

Over the first 6 hours of the response to NO_2 inhalation, wet weight measures increase to a much greater extent than do dry weight measures. During this time there is, therefore, a much greater accumulation of excess water, as opposed to dry weight material, within the lung. Histological studies support this conclusion, reporting evidence of interstitial and alveolar edema with little evidence of protein deposition or cellular infiltration. Vascular congestion is evident from the microscopic descriptions by others and the Blood/BW measures in the present study. These changes are indicative of a primarily hydrostatic process in the development of pulmonary edema.

There is some evidence that this initial hydrostatic phase is not unique to the NO_2 model of ARDS. A number of investigators have used the chronically instrumented sheep to investigate potential mechanisms of ARDS. In this model, a thoracic lymph node is catheterized so that lung lymph may be continuously collected and analyzed for flow rate and protein concentration. In these sheep, lymph flow reflecting transendothelial fluid flux has traditionally been considered valid only for the

steady state, attained 1 to 2 hours after any experimental manipulation (Brigham et al., 1974). Therefore, most investigators have made no attempt to interpret their findings during the initial phases of response to their experimental models of ARDS. Examination of the data published for infusion of E. coli endotoxin (Brigham et al, 1979) and Pseudomonas bacteria (Brigham et al., 1974) indicates occurrence of an initial phase similar to that observed in the present study. In these infusion studies, lymph flow increased while pulmonary artery pressure increased and the lymph/plasma protein concentration decreased. This phase was transient, but showed the hallmarks of a hydrostatically-mediated increase in transendothelial fluid movement; increased vascular pressure producing increased lymph flow with decreased protein concentration. A similar conclusion is reached upon inspection of the data of Loyd et al. (1983) on the infusion of phorbol myristate acetate (PMA), which activates PMNs. In all of these studies, lymph flow and protein concentration measures more indicative of a permeability mechanism are present during the later, steady state period.

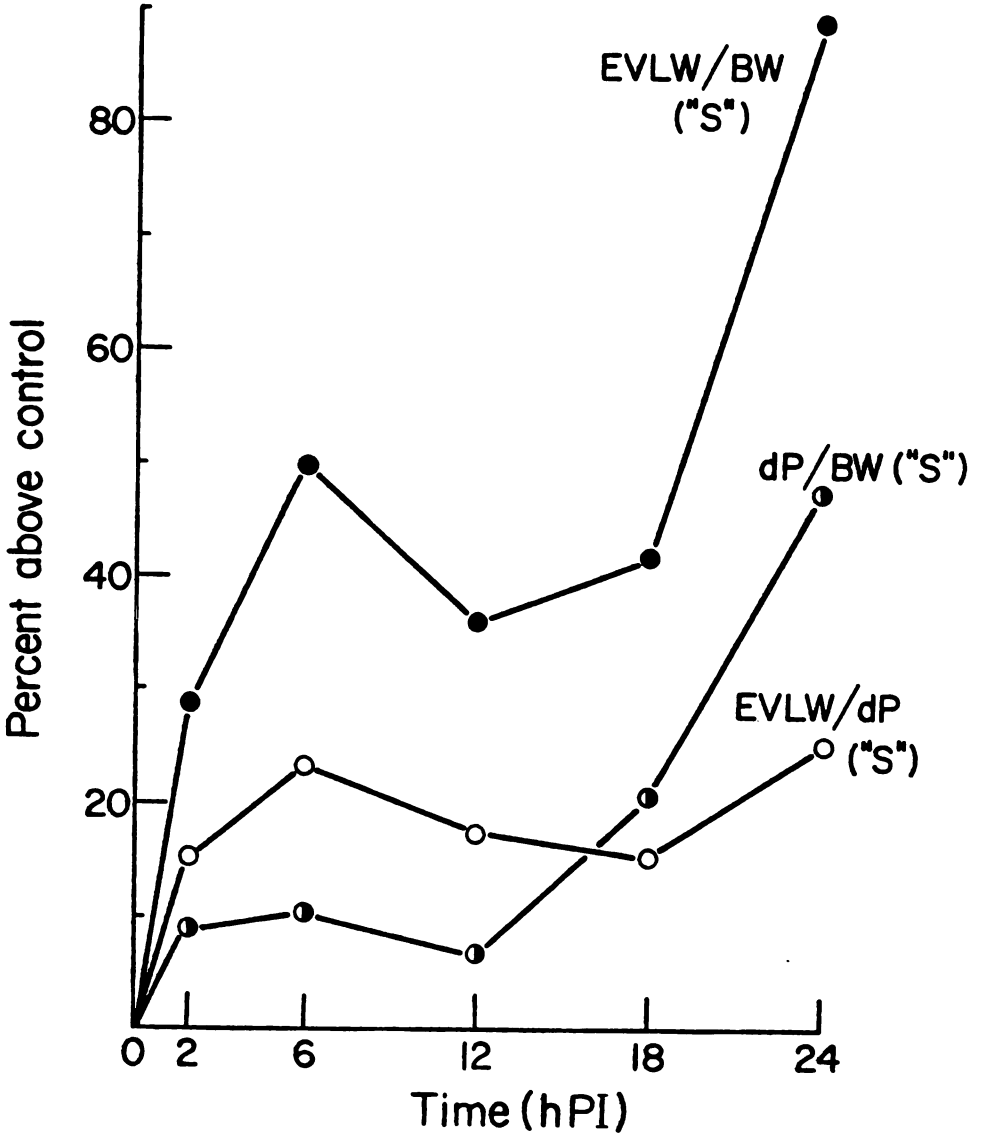
As discussed earlier, there appears also to be a "hydrostatic phase" in the dose-response curves generated in this study. This finding supports the idea that a hydrostatic mechanism is operative in the early response to NO_2. It is possible that at lower concentrations, the edematous response to NO_2 develops more slowly so that observations made 24 hours after 200 ppm NO_2 correspond to observations made 6 hours after 255 ppm NO_2. Alternatively, it is possible that the hydrostatic phase is a response that, at high concentrations, is superseded by continuing development of the pulmonary lesion. At low concentrations, the lesion may not progress beyond the hydrostatic component. Based on

observations of the effects of very low concentrations of NO_2, which produce a temporary lesion capable of healing (Evans et al., 1971), the second explanation seems the more likely.

This hydrostatic phase in the edemagenic and hyperemic response to NO_2 was not overwhelmingly large. In neither the dose-response nor the time course experiments were there any deaths during the phases with hydrostatic characteristics. All of the deaths in the dose-response experiments occurred above 200 ppm, where the lung water analysis indicated additional involvement of a permebility type mechanism. In the time course experiments, all deaths occurred well after the 6 hour PI time point, which appeared to be the end of the primarily hydrostatic phase. It is tempting to speculate that the protective effect produced by interstitial protein wash-out in hydrostatic forms of pulmonary edema was responsible for this lack of severity.

In considering the relative severity of the initial, primarily hydrostatic phase in this model of ARDS, it is interesting to consider the early lung weights in the "eventual survivors." To do this, the most severely affected animals were omitted from data analysis of the extravascular lung water and dry parenchymal tissue measures for the first 12 hours. After this time their deaths automatically omitted them from this consideration. The overall mortality rate was approximately 20% and the most consistent predictor of overall severity of the pulmonary lesion (as determined from both this study and that of Guyton & Lindsey, 1959) was a Lung Wet/Dry weight ratio close to 10.0. It was therefore decided that the best procedure for eliminating the "most severely affected" animals would be to eliminate the 20% of each time group with the highest Lung Wet/Dry weight ratio.

"EVENTUAL SURVIVORS": EVLW/dP WEIGHT RATIOS



Plot of the data from the "eventual survivors" analysis. Data plotted are the Extravascular Lung Water and dry Parenchymal tissue weight measures of those animals predicted to survive 24 hours PI after exposure to 255 ppm NO_2 for 120 minutes. Note that the general trends for EVLW/BW and EVLW/dP are very similar to those for all animals (see Results section). Note especially that the dP/BW measure is greatly attenuated over the initial 12 hours PI, as compared to all animals.

The time course of the changes in EVLW/dP and EVLW/BW & dP/BW weights obtained from this "eventual survivor" analysis is revealing. In general there is little change in either the EVLW/dP or EVLW/BW weight measures. The lack of change in EVLW/dP is surprising in light of the criteria used to include or exclude animals from this analysis. There is some decline in the average values of course, but the general trend remains the same as with analysis of all animals at the early time points. The striking difference between the "eventual survivors" and all animals is in the dP/BW weight ratio. Omission of those animals predicted to die prior to 24 hours PI noticeably decreases and flattens the early course of the dP/BW curve.

It therefore appears that in those animals eventually dying as a result of the exposure to NO_2 there is an earlier onset of a more permeability type of edemagenic mechanism. By this analysis there is certainly less early deposition of dry weight material in the "eventual survivors", more indicative of a hydrostatic phase than a permeability phase. It should be noted that even in the "eventual survivors" there is the later onset of a more severe edemagenic process with characteristics of a permeability type of mechanism.

Because of the lack of recognition of a possible early hydrostatic phase in ARDS, little attention has been given to mechanistic considerations. Two potential means exist for increasing hydrostatic pressure within the exchange area of a vascular bed. Inflow pressure can be increased by events "upstream" or outflow pressure can be increased by events "downstream" from the exchange vessels. In either case, the pressure increase can eventually be transmitted to the microvascular bed, increasing driving pressure for fluid flow into the interstitium.

Increases in hydrostatic pressure "upstream" from the pulmonary vascular bed can occur via cardiac stimulation. This has been shown responsible for the pulmonary edema occurring in epinephrine overdosage (Worthen et al., 1969). Nitrogen dioxide, however, has the opposite effect on the heart. Exposure to 40 ppm NO_2 has been shown to produce bradycardia and mild arrythmias (Tsubone et al., 1982), actions promoting lowered, not increased, pulmonary vascular pressure. It is interesting to note that these investigators found atropine could block the NO_2-induced bradycardia, suggesting its production was a parasympathetically mediated reflex in response to increased blood pressure of non-cardiac origin.

The alternative means of raising intravascular hydrostatic pressure, via increasing "downstream" resistance, seems more likely in the case of NO_2. Ivanhoe and Meyers (1964) have suggested a mechanism operative in other models of ARDS that may apply to this model. In their experiments on pulmonary edema resulting from inhalation of high concentrations of phosgene, which produces ARDS-like lung injury, they observed a sharp decrease in efferent sympathetic activity coincident with gassing. Sympathetic activity remained low for the duration of the exposure. In earlier experiments on another model of ARDS, pulmonary edema from vagal section, Schmitt & Meyers (1957) observed a protective effect from continuously administered isoproterenol. Previous reduction of sympathetic activity, produced by reserpine, resulted in faster development of a more severe pulmonary lesion after vagotomy. Toxic doses of a parasympatholytic agent, atropine, did not produce similar pulmonary edema or hyperemia suggesting that interruption of afferent, and not efferent, fibers is the crucial event in vagal section.

These observations were integrated by Ivanhoe & Meyers (1964) into a theory on the neuroparalytic basis of these types of pulmonary edema and hyperemic congestion. Basically, it states that a basal sympathetic tone is required to maintain the pulmonary circulation in a vasodilated state. Precipitating events, such as exposure to toxic gases or loss of afferent input via vagotomy, produce a reflex diminution of this basal sympathetic tone. When this happens, an intrinsic venular constriction occurs. Pressures within the pulmonary microvasculature are raised because of the "downstream" constriction and fluid is forced out into the parenchyma of the lung, producing pulmonary edema. This hypothesis is especially attractive because of its basis in experiments on a number of different models of ARDS, including oleic acid-induced permeability pulmonary edema (Dauber & Weil, 1981).

For the case of NO_2-induced pulmonary edema and hyperemic congestion, a somewhat related mechanism can be postulated based on the observation that NO_2 depresses prostacyclin synthesis in the lung (Kobayashi et al., 1981). Prostacyclin, or PGI_2, is a metabolic product of arachidonic acid similar to the prostaglandins and has been shown to dilate the pulmonary vasculature (Kadowitz & Hyman, 1980). It has also been shown that a "PGI_2-like" substance is released continually in the lung (Gryglewski et al., 1978) and it has been suggested that the pulmonary vasculature is normally kept in a vasodilated state by the production of just such a cyclooxygenase-derived vasodilator (Hyman & Kadowitz, 1979).

The results of Kobayashi et al. (1981) can be interpreted to indicate a possible vasoconstriction leading to increased hydrostatic pressure within the microvasculature. Because almost 50% of the pres-

sure drop within the pulmonary vasculature occurs within the capillary bed (Bhattacharya et al., 1982), non-specific vasoconstriction need not necessarily be confined to "upstream" vessels (which would not result in increases of microvascular hydrostatic pressure).

Either of the above mechanisms could potentially result in a hydrostatically-mediated edemagenesis in ARDS-type pulmonary injury. The neuroparalytic mechanism set forth by Ivanhoe & Meyers (1964) is more acceptable because of its broad experimental basis, and it is conceivable that the basal sympathetic tone of Ivanhoe & Meyers (1964) is the stimulus for the continual prostacyclin synthesis and release of Gryglewski et al. (1978). Support for this idea comes from the recent report (Ellsworth et al., 1983) that stimulation of the stellate ganglion results in increased synthesis and release of prostacyclin from isolated lungs in situ. Either case is additional supporting evidence for the existence of an initial hydrostatic phase common to many types of ARDS models.

6 TO 12 HOURS PI:

The early phase of the edematous response to NO_2 peaked at 6 hours PI and over the following 6 hour period there appeared to be some resolution of the injury. Between 6 and 12 hours PI there were declines in all measures of pulmonary injury; wet weights, dry weights and blood content.

As expected upon resolution of a primarily hydrostatic type of edema, the decrease in wet weight measures were greater than those of the dry weight measures. Lung Wet/BW weight decreased from its high of 47% above control to a level 32% above control. Likewise, the EVLW/BW

measure declined from its high point at 58% above control to 41% over control. Neither of these decreases completely eliminated the gains made between 2 and 6 hours PI.

Although slight, there were also decreases in the dry weight measures. The Lung Dry/BW measure decreased from 19% to 13% above control, while dP/BW declined from 21% to 16% over control.

On a relative basis, it is not surprising that the "whole lung" measures of Lung Wet/BW and Dry/BW decreased somewhat more than the more refined EVLW/BW and dP/BW measures. Since blood content of the lung decreased greatly during this period, the "whole lung" measures lose weight in two categories, extravascular water and blood.

The lung water and blood weight analysis therefore indicates that the 6 to 12 hour PI period was one of some resolution. It appears that the hydrostatic force driving fluid out of the microvasculature during the preceeding 6 hours abated and some of the excess fluid in the lung was cleared, probably by lymphatic drainage. Indeed, in the experiments in sheep catheterized for lung lymph collection, the initial hydrostatic phase was only a prelude to the steady state leakage characteristic of a permeability mechanism.

Although the lung water and blood analysis indicates some resolution of the accumulated excess water and dry weight material, the histologic observations at this time show that the injury is not abating. The most important event occurring during this period is the influx of inflammatory cells into the alveolar region, which just preceeds and may be responsible for, the full onset of a permeability edemagenesis.

Gardner et al. (1969) exposed rabbits to 40 ppm NO_2 for 3 hours and sequentially characterized the cell population of lung lavage fluid.

Beginning at 6 hours PI, they noted neutrophils recoverable in the lavage fluid, indicating the presence of these cells within the alveoli. The PMN infiltration continued and reached a plateau at 9 to 12 hours PI, with elevated levels present up to 24 hours. This time course is supported by the observations of Blank et al. (1978), who exposed rats to 30 to 40 ppm NO_2 for 5 hours. They noted PMN infiltration of the alveolar septa at 11 hours PI, a process which continued over the next 6 to 12 hours. Inflammatory cell infiltration of the alveolar septa was also first observed at this time in the 5 species exposed (40 to 75+ ppm NO_2 for 1 to 2 hours) by Hine et al. (1970).

Coincident with this evidence of beginning PMN involvement are observations of initial protein deposition both within the alveoli and the surrounding interstitium. These may be interpreted as initial signs of a permeability type of edema. Stephens et al. (1972) noted some interstitial and intra-alveolar fibrin after 8 hours exposure of rats to 17 ppm NO_2. At 11 hours PI, Blank et al. (1978) observed eosinophilic material in a few alveoli from rats exposed to 30 to 40 ppm NO_2 for 5 hours. In these animals, total lung protein had increased to approximately 70% above control levels.

At this time also are observations suggesting continuing endothelial breakdown. The rats of Stephens et al. (1972) showed subendothelial damage, associated with interstitial edema.

Therefore with the break in lung water accumulation comes evidence of impending damage more serious than the initial hydrostatic pressure increases. Infiltration of PMNs and the possible recruitment of other components of inflammation and coagulation portend lung injury more

closely approximating the severe pathology of ARDS in its fully developed clinical form.

12 TO 18 HOURS PI:

This was a period of relative stability in lung water measures. There was no change at all in the EVLW/BW measure, indicating a balance between accumulation of extravascular water and its clearance from the lung. Lung Wet/BW weight increased somewhat during this period, this increase was due to the accumulation of blood as illustrated by the Blood/BW increase. Blood content of the lung, as measured by Blood/BW weight, rose to 23% above control at 18 hours PI from a value not different from control at 12 hours PI.

Small increases occurred in dry weights over this time, although in general the plateau achieved at 6 hours PI was maintained. Contributing to the increase in Lung Dry/BW was the accumulation of blood, as noted earlier for Lung Wet/BW. Part of the increase in dP/BW, which neglects whole blood, was no doubt due to influx of PMNs and proteins associated with the developing inflammatory reaction observed in the histological descriptions.

During this time period, the histologic picture is qualitatively similar to that at the end of the 6 to 12 hour PI period. Most changes are somewhat more severe, as the permeability mechanism of edema becomes more established.

Stephens et al. (1972) described increased areas of denuded basement membrane within the alveoli of their exposed rats (17 ppm NO_2 for 16 hours). Fibrin was associated with these areas and bits of free cytoplasm were sometimes attached to the fibrin in the alveoli. The

areas of interstitial edema were increasing in size. The rats exposed by Blank et al. (1978) to 40 ppm NO_2 for 5 hours showed similar histology at 17 hours PI. The extent of PMN infiltration of the interstitium and the presence of eosinophilic (indicating high protein content) fluid within the alveoli were more pronounced than at 11 hours PI, and some free PMNs were now present in the alveoli. Total lung protein had also increased at this time.

Langloss et al. (1977) observed similar histologic effects in their cats (exposed to 80 ppm NO_2 for 3 hours) at 15 hours PI. Intra-alveolar fibrin started to appear at this time and was seen emanating from damaged capillaries, thus providing visual evidence of the onset of a permeability type of edema process. PMNs were present in the alveoli along with the edema fluid.

Although there were no changes in lung water, both the small increases in dry weight and the histological observations are indicative of a mounting inflammatory reaction. PMNs were accumulating in the alveolar septa and the alveoli themselves. In addition, the fluid accumulating in the alveoli showed evidence of high protein concentration as both plasma proteins and mediators of inflammation entered the parenchymal and gas exchange areas of the lung.

18 TO 24 HOURS PI:

Both physiologically and histogically this was the period of the most severe damage to the lung. Most of the deaths in the exposed animals, regardless of concentration, occurred during this period. There were dramatic increases in all of the measures of lung damage,

and the appearance of the lung was one of maximal pulmonary edema and hyperemic congestion.

Lung wet:dry weight ratios changed little between 18 and 24 hours PI. Masked by this observation is the fact there were large increases in both the wet and dry components of these ratios.

Lung Wet/BW weight increased from 36% above control to its maximal level at 74% over normal. Even this very high value is artificially low because of the death of those animals with the greatest accumulation of lung water. The increase in EVLW/BW was even more dramatic, increasing from 42%, at 18 hours PI, to 88% above control at the end of the period. Again, this value was kept low by death of the more severely affected animals. Since a Lung Wet/Dry weight ratio equal to 10.0 was found, from the data of Guyton & Lindsey (1959) and this study, to be the parameter most indicative of impending death, these large increases in EVLW represent a condition of utmost severity.

Coincident with these increases in lung wet weights were equally dramatic increases in dry weights, representing the first real departure from the plateau attained at 6 hours PI. The Lung Dry/BW weight increased from 20%, at 18 hours PI, to 44% above control at 24 hours PI. The dry parenchymal tissue mass (dP/BW) increased from 21% to 47% above control during this period of dramatic increases. These increases were due to accumulation of blood, evidenced by the rise in Blood/BW and contributing to the "whole lung" dry weight increase, inflammatory cells and plasma proteins entering through compromised endothelial walls and depositing within the parenchyma.

Analysis of the increases in wet and dry measures indicates that a permeability type of mechanism was primarily responsible for the lung

water accumulation between 18 and 24 hours PI. The increase ratio for Lung Wet/BW and Lung Dry/BW is 1.7 at the 24 hour PI point. For the EVLW/BW and dP/BW the increase ratio is 1.9 at this time. These increase ratios are in marked contrast to those at 2 hours PI, when Lung Wet/BW:Dry/BW increases equalled 3.2 and EVLW/BW:dP/BW increases were 3.4. These markedly lower increase ratios at 24 hours PI are indicative of a primarily permeability mechanism producing the increases in lung water during this period.

A primarily permeability edemagenesis is also evident from the histological appearances in animals exposed to NO_2 and sacrificed at corresponding times. The histological reports are of maximal pulmonary edema and vascular congestion.

Hine et al. (1970) reported their most severe congestion and edema scores in the 5 species they exposed to 40 to 75+ ppm NO_2 for 1 to 2 hours. The inflammatory response also peaked during this period, with proteinaceous material in the airways. In the series of cats exposed to 80 ppm NO_2 for 3 hours (Langloss et al., 1977), fibrin meshworks were observed inside alveoli and the number of PMNs there reached a maximum. Gardner el al. (1969), in rabbits exposed to 40 ppm NO_2 for 3 hours, noted alveolar PMNs remaining at approximately peak levels throughout this phase. Subsequently, the alveolar PMN population began to decline.

Stephens et al. (1972) described maximal interstitial and alveolar edema with intra-alveolar cell debris and fibrin "easily observed" in rats exposed to 17 ppm NO_2 for 24 hours. There were also increased numbers of macrophages in alveoli and further swelling of subendothelial tissue within the alveolar wall. Erythrocyte extravasation into alveoli and tissue spaces was also noted.

Rats previously exposed to 40 ppm NO_2 for 5 hours (Blank et al., 1977) displayed further progression of edematous and inflammatory reactions. Alveolar exudate was present and increased numbers of inflammatory cells were noted in alveolar walls. The total lung protein remained elevated.

Squirrel monkeys, whose respiratory anatomy is similar in many ways to that of humans, also demonstrate this typical reaction at 18 to 24 hours PI. Bils (1974) exposed squirrel monkeys to 30 ppm NO_2 for 3 hours and observed hyperemia with some centroacinar hemorrhage, submucosal edema with inflammatory cell infiltrates and intra-alveolar serous transudates and inflammatory exudates.

The overall picture at this time, in animals exposed to edemagenic concentrations of NO_2, is one of fulminant pulmonary edema primarily due to decreased integrity of the microvasculature, permeability pulmonary edema. Documenting this interpretation are the histological observations of protein and inflammatory cells within the interstitium and the alveoli, as well as the increases in dry weight in the present study.

Delayed - Permeability Phase:

This massively edematous condition in the lungs comprises the second phase of the pulmonary reaction to inhalation of high concentrations of NO_2, the Delayed-Permeability phase. In contrast to the Early-Hydrostatic phase, this is a life-threatening development. All of the animals dying as a result of their exposure did so during the Delayed-Permeability phase. Additionally, the analysis of "eventual survivors" indicates that the animals predicted to eventually die accumulation of

water in the lung eventually leads to alveolar flooding, which is responsible for deaths occurring during this period.

Many of the mechanisms potentially responsible for this type of pulmonary injury were discussed earlier in regard to permeability pulmonary edema in general. The histologic studies cited strongly support the involvement of PMNs and possibly other mediators of inflammation. With respect to NO_2-induced permeability pulmonary edema, the major concern becomes the initiating event in this reaction.

It is clear from a number of studies that the initial observable alterations from NO_2 exposure occur in the vasculature, not the alveolus as might be expected from an inhaled toxic agent. Guidotti (1980) exposed beagles to 37 ppm NO_2 for 4 hours via tracheal cannula, resulting in delivery of a functionally higher NO_2 concentration to the alveoli, and sacrificed them at the end of exposure. Ultrastructural observations by electron microscopy consisted of a normal alveolar epithelium, but with initial signs of damaged capillary endothelium. Another study in dogs by Dowell et al. (1971), also observed endothelial changes as the first signs of damage. These investigators reported the presence of blisters or blebs in endothelial walls, decreasing capillary lumen diameter. Similar observations have been made in rats exposed for 8 hours to 17 ppm NO_2 (Stephens et al., 1972). In the series of cats exposed by Langloss et al. (1977) the first sign of structural damage was breakdown of the endothelium in the alveolar wall.

In addition to the findings of damage initially confined to the capillary endothelium, the delayed nature of the toxic reaction argues against direct attack, as some have suggested, on the alveolar epi-

thelium as the major event. It therefore appears that the basis of the toxic effect of NO_2 inhalation lies in some indirect physiological action in response to NO_2 rather than a direct effect of NO_2 itself.

There are many lines of evidence that activation of PMNs is the crucial event in NO_2-induced pulmonary toxicity. It is clear from the histological studies that PMN infiltration of the lung just preceeds the severe damage of the permeability phase. DeNicola et al. (1981) reported the earliest observable response to NO_2 inhalation was a 10-fold increase in the number of PMNs recoverable by bronchoalveolar lavage (BAL). These increased numbers of PMNs in the air spaces occurred prior to any sign of histologic damage or any increase in a number of biochemical indicies. These included lactate dehydrogenase, a marker of cell death and glutathione reductase & peroxidase, antioxidant enzymes which are increased with the cell's response to oxidant attack.

A number of potential means exist by which NO_2 could activate PMNs specifically or an inflammatory reaction in general. It is possible that direct damage by NO_2 releases activators of inflammation from injured cells. The data of DeNicola et al. (1981) argues against the possibility that NO_2 initially produces damage severe enough to release inflammatory stimuli from within the cell. If this were the case, elevated levels of lactate dehydrogenase would be expected early in the course of NO_2-induced injury. Their investigation found no such evidence of lactate dehydrogenase release.

Much more likely is the possibility that subtle damage to the cellular membrane, produced by NO_2, generates activators of the inflammatory reaction. Free radicals such as NO_2 may interact with lipid membranes to produce a variety of lipid peroxides or lipid free radicals.

In a model system, Pryor & Lightsley (1981) observed production of the initiation product of lipid peroxidation from the reaction between NO_2 and membrane-type lipids. Similar model studies were performed by Rowlands & Gause (1971), resulting in production of long-lived lipid free radicals from membrane-type lipids. Evidence of possible lipid peroxidation in the lung as a result of exposure to NO_2 has been reported (Thomas et al., 1968 and Sagai et al., 1982). However, another study utilizing similar exposures and measuring pentane expiration, a more sensitive indicator than used in the previous studies, found no evidence of such lipid peroxidation (Dillard et al., 1980) in the lung.

Free radical reactions between NO_2 and membrane lipids are potentially important to the initiation of inflammation because of two observations. Firstly it has been shown that chemotactic substances and other mediators of inflammation can be generated from the reaction of free radicals with lipids. Oxidation, via a free radical/lipid peroxidation type mechanism, of arachidonic acid can non-enzymatically generate prostaglandins or hydroxy- and hydroperoxyeicosatetraenoic acids (HETEs, HPETEs) of biological significance (Porter et al., 1981). Also, Perez et al. (1980) have shown that exposure of arachidonic acid to a free radical generating system results in non-enzymatic production of a lipid with potent chemotactic activity for human PMNs. It is therefore possible that the free radical interaction of NO_2 with membrane lipids, if such a reaction occurs in vivo, could result in generation of biologically active compounds that direct an inflammatory attack on the lung. Secondly, it has been shown that fatty acid hydroperoxides, such as may be generated from the interaction of free radicals and lipid membranes, can produce an ARDS-type reaction in

animals (Cortesi & Privett, 1972). Injection of methyl linoleate hydroperoxide in rats produced edematous, hyperemic lungs which were increased in weight. The mechanism for this reaction was not investigated. It has subsequently been shown (Wenzel & Hale, 1978/a) that certain free fatty acids are directly toxic to cultured cardiac and endothelioid cells.

Another possible means by which NO_2 initiates lung inflammation is suggested by its effects on cyclic GMP. Guanylate cyclase can be activated by a number of compounds either containing or capable of generating nitroso groups (Gruetter et al., 1980) and it has recently been shown that exposure to NO_2 greatly increases lung cGMP levels, with no significant effect on cAMP levels (Kobayashi et al., 1982). In this regard it is noteworthy that PMNs, collected via catheterization of the pulmonary artery, from ARDS patients have an elevated cGMP to cAMP content ratio. (Zimmerman et al., 1983). These PMNs were also shown to have a much greater chemotactic and respiratory burst activity than those from critically ill, non-ARDS patients.

Mast cell degranulation is controlled by a balance between intracellular levels of these two cyclic nucleotides. Elevations in cGMP, as occur with NO_2, promote degranulation. Along with histamine, a number of other active compounds are released upon mast cell degranulation. Most notably, platelet activating factor (PAF/AGEPC) is also released. By increasing synthesis of cGMP relative to cAMP, NO_2 may stimulate mast cell degranulation and PAF/AGEPC release in the lung. Mast cell degranulation has been reported following a one hour NO_2 exposure (Thomas et al., 1967) and antihistamines have been reported to counter the bronchospasm that sometimes occurs at the start of exposure

(von Nieding & Krekeler, 1971). It therefore appears that mast cell degranulation and histamine release occur early in the reaction to NO_2. Conurrent PAF/AGEPC release may contribute to initiation of an ensuing inflammatory reaction. Camussi et al. (1983) have recently shown that intratracheal instillation of PAF/AGEPC results in an edematous, hyperemic lung injury similar to that caused by NO_2 exposure or ARDS. This injury was characterized acutely by PMN infiltration and edema of the alveoli and alveolar septum in addition to intralumenal lesions of the capillary wall. Similar to both ARDS and the pulmonary reaction to NO_2, this injury showed chronic progression to pulmonary fibrosis.

Alveolar macrophages are the first inflammatory cells encountered by NO_2. As such, they may play an important role in initiating the inflammatory reaction that eventually develops. Macrophages elaborate at least two mediators with chemotatic activity specific for PMNs. The first of these is a low molecular weight (<1000) substance which is resistant to inactivation by trypsin, proteases and heat. This factor contains lipid, it is thought to be an arachidonic acid metabolite via lipoxygenase (Hunninghake et al., 1980). The other PMN-specific chemotaxin elaborated by alveolar macrophages is a heat-sensitive peptide with a molecular weight of approximately 9,000 (Merrill et al., 1980). Of possible significance to the timing of the delayed, permeability phase of NO_2-induced pulmonary injury is the observation that peak chemotactic activity occurred at 22 hours after stimulation of these macrophages in culture.

In addition to these PMN-specific chemotaxins, alveolar macrophages can secrete a number of other mediators capable of initiating or directing an inflammatory reaction within the alveolar wall. These

include components of compliment and metabolites of arachidonic acid, notably leukotrienes (Reynolds, 1983). Alveolar macrophages are sensitive to NO_2. Exposure in vivo to NO_2 results in decreased production of superoxide anion in response to PMA stimulation (Amoruso et al., 1981) and unusual changes in redox potential, $NAD^+/NADH_2$ ratio is increased in cytoplasm and decreased in mitochondria (Simons et al., 1974). It is therefore likely that these cells within the alveolus make some contribution to mounting or directing the inflammatory attack associated with the Delayed-Permeability phase of NO_2 pulmonary injury.

A multitude of other mechanisms could also conceivably be involved in the mediation of the PMN infiltration and overall inflammatory reaction that is associated with the late phase of NO_2 precipitated respiratory distress. Too little is known about inflammation in general and its specific course in respiratory injury to reasonably assign a definite causative role to one or another of the mechanisms discussed. An important point is that the delayed, permeability phase of both NO_2-induced hemorrhagic pulmonary edema and ARDS are associated with intense inflammatory activity within the gas exchange areas of the lung.

RELATION TO ARDS:

Undoubtedly, not all of the various conditions predisposing to development of ARDS initiate the events leading to lung injury in exactly the same manner. It does appear however, that a common pathway exists, leading to full development of the characteristic pulmonary damage. The usefulness of ARDS models, such as NO_2 toxicity, are mainly in elucidating new aspects of this common pathway.

The occurrence of two phases, separate in both time and mechanism, is the most important finding in the present series of experiments. As originally stated, the purpose of these studies was to determine if the delayed onset in respiratory symptoms, from inhalation of high concentrations of NO_2 modelling clinical ARDS, was due to a delay in onset of the edemagenic process. It was alternatively hypothesized that an edemagenic mechanism became operative almost immediately, but that symptoms of distressed respiration developed only later after some "threshold" in lung water accumulation had been exceeded. As is the case with many scientific studies, it turned out there was an element of truth in both preconceptions. Analysis of the accumulation of lung water and dry weight substances clearly indicates an almost immediate onset of a hydrostatic phase of edemagenesis in the lung. This hydrostatically-mediated increase in lung water is not severe enough to produce death or even noticable signs or symptoms of respiratory difficulty, it is therefore not apparent from casual observation. After the early peak in lung water accumulation, is a "latent period" during which some of this excess water leaves the lung. There is however, evidence of a mounting inflammatory assault at this time. Symptoms of distressed respiration finally develop later, in concert with the onset of a permeability mechanism resulting in accumulation of large amounts of water and dry weight material within the gas exchange areas of the lung. Associated temporally, and possibly causally, with the delayed, permeability edemagenesis is a severe inflammmatory reaction, with PMNs presumably producing much of the damage leading to fluid and protein loss, within the alveolar walls and spaces.

The pulmonary changes of the Delayed-Permeability phase of NO_2-induced lung injury have been cited as the basis for the utility of NO_2 as a model of ARDS. Consideration must also be given to the possible existence of two phases, not just in NO_2-induced lung injury, but in all forms of ARDS. Support for universal occurrence of two phases comes from the fact that other models of ARDS also present some evidence for a two phase edemagenic course.

Loyd et al. (1983) infused phorbol myristate acetate (PMA), an activator of PMNs, into sheep catheterized for lymph collection. They reported an early increase in pulmonary artery pressure coincident with increased lung lymph flow, conditions characteristic of a hydrostatic mechanism producing increased transendothelial fluid flow. Pulmonary artery pressure remained elevated for 4 hours in these sheep. By 3 hours after infusion of PMA, lymph protein concentration had returned to normal in spite of a continuing high flow rate. These later events are characteristic of a permeability mechanism responsible for increased microvascular fluid loss. They concluded that PMA infusion, presumably causing PMN activation, produced "initial pulmonary hypertension and a later period of apparently increased pulmonary vascular permeability."

As noted earlier, the data presented on experiments with infusion of E. coli endotoxin (Brigham et al., 1979) and Pseudomonas bacteria (Brigham et al., 1974) also appear to show an early hydrostatic phase and a later permeability phase in the increased lung lymph flow produced by these models of septic ARDS. Both of these studies show an early transient phase of increased flow of lymph with a relatively low protein concentration, concurrent with increaes in pulmonary artery pressure. All of these are changes characteristic of a hydrostatic process respon-

sible for the increased formation of lymph. Later development of a permeability mechanism is evident during the "steady state" part of the experiments.

Data published from studies on lymph flow subsequent to infusion of microemboli (Binder et al., 1979) do not present as clear an indication of an early hydrostatic phase. During the microembolization procedure there were large increases in both pulmonary vascular resistance and lymph flow. Increased pulmonary vascular resistance may be evidence of increased hydrostatic pressure, although these data are not reported. Protein concentration of the lung lymph appeared not to decrease, however. In another study of microembolization (Vaage et al., 1976), produced in this case by in vivo stimulation of platelet aggregation, increases in fluid filtration were clearly associated with increases in pulmonary artery pressure. These concurrent increases strongly suggest a hydrostatic basis for the increased fluid flow, although protein concentrations were not measured.

Therefore many, if not all, forms of experimental ARDS include an early phase of hydrostatically-mediated edemagenesis. This phase hydrostatic phase is usually not severe enough to produce life-threatening symptoms, although significant accumulation of excess water can occur in the lung. The permeability phase of edemagenesis, characteristic of the pathophysiology of fully developed ARDS, develops later, after the classical "latent period" of ARDS, in association with an intense inflammatory reaction. During this phase, accumulation of excess water and dry weight substances compromises the structural and functional integrity of the lung. At present it cannot be determined to what

extent, if any, development of the Delayed-Permeability phase is dependent on, or influenced by, occurrence of the Early-Hydrostatic phase.

Should the occurrence of two phases be confirmed as a universal feature of ARDS a number of implications suggest themselves. There are clinical ramifications with regard to both diagnosis and treatment.

Early diagnosis is beneficial in almost any disease process. The results of the analysis of "eventual survivors" in this study indicates that early diagnosis may be capable of indentifying those patients who will eventually develop the most severe form of the Delayed-Permeability phase of ARDS. From these results it is expected that those patients will manifest an increase in lung dry weight material early in the course of their predisposing medical condition. It may be possible to clinically monitor these patients. Determination of protein concentration in bronchoalveolar lavage fluid may be a means by which this is accomplished. Early elevation of BAL fluid protein may portend the coming development of a severe episode of the Delayed-Permeability phase of ARDS.

Those patients found by monitoring to show early development of the Delayed-Permeability phase of ARDS may benefit from treatment specific for the Early-Hydrostatic phase. The edemagenic process in this early phase is the same, on the microvascular level, as that operative in congestive heart failure (CHF). It should be noted that the mechanism, on an organ level, is different however. The true "cardiogenic" pulmonary edema of CHF is a result of increased hydrostatic pressure "backing up" into the pulmonary circulation from the left ventricle of the failing heart. In the Early-Hydrostatic phase

of ARDS, microvascular pressure is elevated due to increases in local vascular resistance. A number of modes of therapy are available for this "cardiogenic"-like pulmonary edema.

Based on the possible mechanisms discussed earlier, loss of basal sympathetic modulation and/or prostaglandin-mediated local control, it is expected that vasodilator therapy may prove beneficial in this phase. With regard to this suggestion, a report on the ameliorative effect of furosemide in oleic acid-induced respiratory distress should be considered (Ali et al., 1979). Oleic acid infusion produces an edematous, hyperemic pulmonary injury similar to that of other models of ARDS. Administration of furosemide early, 2 hours after oleic acid, in the development of the pulmonary injury markedly improved the condition. Furosemide resulted in decreases in EVLW/BW, Lung Wet/BW and Dry/BW, in addition to decreasing shunt fraction (fraction of blood being shunted through non-ventilated areas of the lung and therefore not being oxygenated). Although furosemide is traditionally considered a diuretic, in the treatment of acute pulmonary edema its major beneficial effect is due to vasodilatation (Meyers et al., 1980).

Based on this study, and that of Ali et al. (1979) it is predicted that optimal vasodilator treatment can decrease the severity of this early phase and possibly the overall course.

SUMMARY:

Time course experiments have shown that there are two phases to the lung injury of the NO_2-induced model of ARDS. Accumulations of lung water and blood peak during early, hydrostatically-mediated and again during later, permeability-mediated phases of edemagenesis. The early phase is characterized by accumulation of a "watery" fluid with little accompanying dry weight material. This phase produces few or no overt symptoms and resolves somewhat during the subsequent "latent period." The Delayed-Permeability phase produces accumulation of large amounts of fluid and dry weight material, enough to seriously compromise lung function and produce clinical symptoms of respiratory distress. This phase is associated with an intense inflammatory reaction, featuring massive PMN infiltration, within the alveolar zone of the lung. It is this delayed phase that develops both the symptoms and pathology characteristic of the fully developed lesion in ARDS.

Based on data from this and other studies, it is concluded that this two-phase development may be a feature of all types of ARDS. It is not unexpected that the Early-Hydrostatic phase is not apparent clinically and has therefore been largely ignored. Specific treatment of the Early-Hydrostatic phase may influence the development of the Delayed-Permeability phase. The results here indicate that early diagnosis of potentially severe ARDS cases by BAL may be possible.

DISCUSSION: PHARMACOLOGICAL INTERVENTION EXPERIMENTS

In a series of experiments on pharmacologic intervention, a number of different therapeutic agents were administered in an attempt to influence the development of the pulmonary edema and hyperemic congestion resulting from exposure to NO_2. The measures used to evaluate the effects of these agents on NO_2-induced lung injury were Lung Wet/Dry and EVLW/dP weight ratios and their spearate components, Lung Wet/BW, Dry/BW, EVLW/BW and dP/BW.

Two time points were studied in the pharmacological intervention series of experiments, 6 and 24 hours PI. Results from the time course experiments indicated that 6 hours PI was the height of the Early-Hydrostatic phase of edemagenesis and 24 hours PI corresponded to intense involvement of the Delayed-Permeability phase. It was felt that these times of maximal involvement of the two phases of edemagenesis would provide discriminating tests for any pharmacological effects.

The Early-Hydrostatic phase produces changes characteristic of the mechanism for which it is named. Accumulation of excess lung water in amounts far exceeding excess dry weight indicates the primary edemagenic process is most likely an increased microvascular hydrostatic pressure. As discussed earlier, the most reasonable explanation for an increased microvascular hydrstatic pressure is increased vascular resistance within or downstream of the fluid exchange vessels. There is also some evidence in the dry weight changes that a permeability process makes some contribution late in this phase. Corresponding histological studies suggest that initial stages of inflammation may

be responsible. Optimal drug for use against this edemagenic process would therefore appear to be vasodilators. Reductions in hydrostatic driving force in the microvascular lumen would be achieved with relaxation of the downstream vessels, and would theoretically have a major impact on production of edema fluid. Another desirable action would be some effect on the cells or mediators of inflammation, which appear to be making a minor contribution to edema development at this time.

The Delayed-Permeability edemagenic phase is associated with and may be the result of an intense inflammatory reaction with the lung. Deposition of significant amounts of dry weight material, presumably proteins and leukocytes, in addition to accumulation of large amounts of excess water are characteristic of this phase. These changes could conceivably be due to microvascular damage produced by the PMNs infiltrating the gas and fluid exchange areas of the lung. For this reason, it was felt that anti-inflammatory agents held the most promise for attenuating this phase.

Although agents were chosen for their effects on these two probable mechanisms of edemagenesis, other actions of these drugs could influence the development of NO_2-induced pulmonary injury. For example, drug-induced alteration of respiratory rate could effectively change the "dose" of NO_2 administered. Specific measurements of respiratory rate were not made on the animals in the inhalation chamber, but they were observed during exposures. With the possible exception of terbutaline and a transient period of gasping in an occasional animal (lasting less than 5 minutes and having no effect severity of pulmonary injury), no grossly apparent changes occurred in respiratory pattern before, during or after exposure to NO_2.

TERBUTALINE:

A $beta_2$ sympathetic agonist, Terbutaline, was the initial agent tested in this series of experiments. Terbutaline appeared a promising drug for a number of reasons. This $beta_2$ agonist is capable of producing vasodilatation and inhibiting permeability increases at the same time.

Isoproterenol, a less specific beta agonist, was shown by Schmitt & Meyers (1957) to provide some protection against the development of pulmonary edema and hyperemic congestion in another model of ARDS, vagal section in the guinea pig. Its protective action was ascribed to relaxation of smooth muscle made hyperactive by loss of basal sympathetic inhibition of intrinsic activity. Smooth muscle relaxation is a $beta_2$ function. Terbutaline, posessing more selectivity for $beta_2$ over $beta_1$ receptors, was anticipated to produce less cardiac stimulation as a side effect, and therefore lower intravascular pressure to a greater degree. It was felt the beneficial actions of Terbutaline would occur via $beta_2$-mediated vasodilatation, lowering microvascular hydrostatic pressure and with it, the driving force for fluid out of the pulmonary capillaries.

Important also in the choice of Terbutaline, were the results of Persson et al. (1978 & 1979). This group reported that Terbutaline, injected sub-cutaneously prior to exposure of guinea pigs, prevented the increase in lung water normally occurring with histamine aerosol.

Contrary to these expectations, Terbutaline dramatically worsened the course of pulmonary injury produced by inhalation of NO_2. In the first experiments, assessing the effect of Terbutaline alone on the overall development of pulmonary edema and hyperemic congestion, there

was a significant increase in 24 mortality in the Terbutaline treated animals. A possibility that was considered was that Terbutaline-induced decreases in systemic vascular resistance, and therefore mean blood pressure, had resulted in reflex stimulation of the heart. This reflex increase in contractility and rate may actually have increased vascular pressures within the pulmonary circulation, thereby raising the microvascular hydrostatic driving gradient and worsening edema development.

A second experiment was planned, adding a $beta_1$ blocker, Atenolol, to the treatment. Addition of a $beta_1$ blocker should block the reflex cardiac stimulation, mediated through $beta_1$ receptors on the heart. This experiment was designed to run 6 hours PI to more accurately evaluate the effect of Terbutaline on the Early-Hydrostatic phase. Although no deaths occurred in the treatment group, lung water analysis showed again a dramatic worsening of edema development. Increases were especially large for the lung water measurements themselves and the wet:dry weight ratios. EVLW/BW increased 74% over control, while dP/BW increased only 14%, yeilding an increase ratio of 5.3 for these two measures.

The results from both experiments are indicative of a heightened, not attenuated, hydrostatic edemagenic process. The Terbutaline treated animals developed much more excess lung water, but little more excess dry weight material, than similarly exposed, untreated animals. Reflex cardiac stimulation is a logical explanation for the first set of experiments. Terbutaline, when injected, produces some direct cardiac stimulation (Danilo & Rosen, 1982), although not as much as isoproterenol. This effect is probably due to some "residual" $beta_1$ stimulant

action in Terbutaline. It may be that the dose of Atenolol used to supposedly block both the reflex and direct stimulation of cardiac $beta_1$ receptors was inadequate.

Another possibility is that Terbutaline-induced vasodilatation overcame a vasoconstrictive compensatory action in the pulmonary vasculature. One of the characteristics of NO_2-induced pulmonary edema and hyperemic congestion is a patchy distribution of damage within the lung. Histologic and gross observations show focal areas of greater involvement than the surrounding tissue. It is possible that in the early stages, hypoxic vasoconstriction shunts blood away from the most severely damaged areas, thus limiting fluid loss and non-productive perfusion. The smooth muscle relaxant effect of Terbutaline may have prevented this protective vasoconstriction, allowing blood access to sites of high fluid loss.

A contributary effect may be due to the bronchodilation produced by $beta_2$ stimulants. Inhalation of an irritant gas, such as NO_2, produces bronchoconstriction in normal animals, probably serving to limit penetration of the noxious gas into the lung. Treatment with a $beta_2$ stimulant would counter or reverse this bronchoconstrictive action and could conceivably result in deeper penetration of higher concentrations of the toxic gas. Therefore, the increased mortality and morbidity in these animals could be due, in part, to an effective increase in the dose delivered.

The dose of terbutaline used in this experiment was high enough to produce some noticable excitation in the animals. It is therefore likely that they also experienced some respiratory stimulation due to this CNS excitation. This increased respiratory rate would also contri-

bute to increasing the effective dose of NO_2 as the animals breathed faster while in the exposure chamber.

A number of cases have been reported in which Terbutaline is suspected of having caused pulmonary edema directly (Benedetti et al., 1981). Most of these have concerned treatment of pregnant women in order to delay labor and involved concurrent steroids. Benedetti et al. report two cases not involving steroids and one in which invasive cardiovascular monitoring detected a large increase in pulmonary vascular resistance at one point during the development of pulmonary edema. It is not clear from the descriptions in the literature if there could be any relation to the heightened development of pulmonary injury in the present experiments, but the possibility exists.

In spite of its predicted effect, based on the known features of its pharmacology, Terbutaline markedly worsened the development of pulmonary edema in this NO_2 model of ARDS. This effect appeared specific for the Early-Hydrostatic phase of edemagenesis and is therefore likely related to effects on cardiovascular function. An exhaustive study of this effect was not performed, so reasons for this exacerbative effect must remain specualtive.

RUTIN:

Prior experience in our laboratory indicated that a flavonoid, Rutin, might prove effective in treating the Early-Hydrostatic phase. This flavonoid has been shown in vivo to decrease capillary permeability in the skin (Ambrose & DeEds, 1947/1949). In these studies, Rutin decreased the leakage of Evans blue from normal capillaries. Another effect of Rutin could potentially prove beneficial in countering the inflammatory contribution to edmagenesis after NO_2 exposure is inhibition of lipoxygenase (Baumann et al., 1980). Rutin was found to exert an inhibitory effect on this arachidonic acid metabolizing enzyme that was equal to that of mefenamic acid, an anti-inflammatory agent. There was no detectable inhibition of "PG synthetase" in these experiments. Lipoxygenase is mainly responsible for production of HETEs & HPETEs and the leukotrienes from arachidonic acid, "PG synthetase" is involved with production of prostaglandins.

Rutin pretreatment (100 mg/kg, IP) did not result in a major effect on NO_2-induced pulmonary injury during the first 6 hours PI. There were no differences in wet:dry weight ratios in the treated animals as compared to untreated-exposed controls. Lung wet weights (Lung Wet/BW & EVLW/BW) were both decreased approximately 10%, but the difference was insignificant. Significant decreases were observed however in the dry weight measures. Lung Dry/BW decreased 9.5% and dP/BW decreased 12%. These decreases indicate there was some effect on the deposition of dry weight material in the lung. It may be, therefore, that Rutin exerted a mild anti-inflammatory effect during these initial 6 hours PI resulting in some decrease in permeability.

INDOMETHACIN:

Indomethacin is an effective, clinically useful non-steroidal anti-inflammatory agent. Of the common agents of its class, Indomethacin is the most potent at inhibition of cyclooxygenase, the first enzyme in the pathway converting arachidonic acid to prostaglandins. In addition, Indomethacin inhibits the motility of PMNs (Shen & Winter, 1977). This drug therefore has the potential for interfering mainly with the Delayed-Permeability phase of pulmonary edema and hyperemic congestion. It was tested against both phases.

Indomethacin did not provide any protection against NO_2 toxicity at either 6 or 24 hours PI. Overall, the drug appeared to worsen the development of pulmonary injury, as measured at both time points.

At 6 hours, there was one death in the four animals pretreated and then exposed to NO_2. No deaths occurred in the untreated-exposed animals at this time point. Animals surviving to the 6 hour sacrifice for lung water and blood analysis showed little change from controls. Wet:dry weight ratios were not different from normal. Wet weight measures, Lung Wet/BW and EVLW/BW, both decreased approximately 11%, but not significantly. Similar to the results with Rutin, significant decreases were achieved in the dry weights. Both Lung Dry/BW and dP/BW decreased by about 9.5%, both significantly. In considering these values, it should be kept in mind that one treated animal died prior to inclusion in this analysis, although none of the controls died. This animal had developed severe pulmonary edema and hyperemic congestion and, presumably, would have significantly increased average lung water, and possibly dry weight, measures for the group as a whole.

Because of the probable role that inflammation plays in the Delayed -Permeability phase of edemagenensis, it was expected that Indomethacin might exert more of a protective effect over 24 hours PI. Although the one death prior to 6 hours PI did not support this expectation, it was possible this one animal expressed and anomolous reaction or that Indomethacin hastened death in an animal otherwise mortally affected by NO_2. In spite of apparently increasing mortality at 6 hours PI, Indomethacin could conceivably decrease the overall severity of pulmonary injury in the animals that eventually survive.

The results at 24 hours PI of Indomethacin treatment are not indicative of any such protection. Mortality at 24 hours PI increased, although not of statistical significance, more than 2-fold. Aside from changes in blood contents, there were almost no changes in the analysis for lung edema and hyperemia. Blood content increased significantly, Blood/dP increasing by 36% and Blood/BW by 20%. Lung Wet and Dry weights were no different from control, although EVLW/BW and dP/BW, the corresponding non-blood measures, both decreased. Deposition of dry weight material decreased significantly, as noted by the significant 12% decrease in dP/BW. The large increase in blood content prevented any decrease in the "whole lung" (blood inclusive) measures, Lung Wet/BW and Dry/BW, in spite of the reduction in lung water and dry material noted in the blood exclusive measures.

In spite of its potent anti-inflammatory activity, Indomethacin proved ineffective at inhibiting to any degree the pulmonary injury in response to NO_2 inhalation. It is possible that Indomethacin exerted an anti-inflammatory effect, via inhibition of production of mediators

through cyclooxygenase or inhibition of PMN function, and at the same time exerted an additional negative effect. These two actions, one protective and the other exacerbative, could have cancelled each other. For example, it has been shown that treatment with Indomethacin appears to increases permeability in the E. coli endotoxin infusion model (Ogletree & Brigham, 1982). It is thought this exacerbative effect is due to decreased synthesis of prostacyclin, which may counter lung water loss by producing maximal vasodilatation when there is injury to the pulmonary circulation. Additionally, this worsening could be due to increased production of arachidonic acid metabolites from the "other", non-cylcooxygenase pathway. Some of these lipoxygenase products, produced in greater quantities since the competing cylcooxygenase pathway is blocked, can increase vascular permeability. Unfortunately, the analysis of "negative" results is often as difficult, or more so, than with "positive" results.

Therefore, the situation with respect to Indomethacin is unclear, except for the observation that mortality was definately increased as a result of treatment.

DEXAMETHASONE/METHYL PREDNISOLONE:

Glucocorticoids are widely used for their anti-inflammatory actions and are administered in ARDS. A number of the effects of steroids could potentially be responsible for their anti-inflammatory acitivity.

Anti-inflammatory steroids induce the synthesis of a factor that inhibits phospholipase A_2, the enzyme responsible for the production of arachidonic acid from phospholipids (Flower & Blackwell, 1979) in the cell. Arachidonic acid metabolites (prostaglandins, leukotrienes and thormboxanes) are responsible for mediating many of the effects of inflammation, and it has long been appreciated that steroids interfere with release of arachidonic acid. This action of steroids has classically been thought responsible for most of their anti-inflammatory properties.

Amoung their anti-inflammatory actions, steroids reduce increased capillary permeability, leukocyte diapedesis and blood extravasation (Baxter & Forsham, 1972). All of these are actions potentially beneficial in the Delayed-Permeability phase of pulmonary injury subsequent to NO_2.

Both Dexamethasone and Methyl Prednisolone were tested against NO_2-induced pulmonary edema and hyperemic congestion. Methyl Prednisolone (30 mg/kg, "high dose") was found to exert no effect on either mortality or accumulation of excess lung water and blood at 24 hours PI. The amounts of Dexamethasone administered (10 to 15 mg/kg) were also in the high dose range. Dexamethasone also produced no important changes in the parameters measured. Mortality and wet:dry weight ratios were no different in treated animals than controls. Decreases, which did not

reach statistical significance, were noted in the wet weights. Lung Wet/BW decreased 10% and EVLW/BW was 16% lower than controls. Dry weights decreased also, with the 14% decrease in dP/BW being significant while the 8% decrease in Lung Dry/BW was not significant. The % EVLW in Lung Wet weight also decreased, by almost 6%. The decrease in this parameter, as well as the lack of significance in the "whole lung" weights can be explained by the large increases in blood in the lung. The Blood/dP increased by 60% and Blood/BW by 36% over control values. Therefore the lung accumulated much less extravascular water, but in addition accumulated much more blood than in untreated animals. Lung Wet/BW did not fall to near the extent of EVLW/BW because of the increased influx of blood.

Dexamethasone produced some provocative trends, especially the 16% decrease in EVLW/BW and decreased % EVLW in Lung Wet weight. However, the drug was not effective overall, mortality was not lower and may have been higher than in controls. This may actually have produced the apparent lowering of extravascular lung water, via death, and elimination from analysis, of the more severely affected animals.

Although widely used in the treatment of ARDS, glucocorticoids have produced results comparable with this study. In a recent reveiw of the effect of steroids in shock and ARDS, Nicholson (1982) concluded that steroid use was "not proven", but stated that this did not necessarily mean "not beneficial."

CLOFIBRATE:

Clofibrate is traditionally classified as a hypolipidemic agent, used for treating hypercholesterolemia. In this regard, it has a number of effects on lipid metabolism and transport. Clofibrate lowers serum triglyceride and cholesterol levels, especially in cases where elevated. These effects are partly due to decreased Very Low Density Lipoproteins (VLDLs) and Low Density Lipoproteins (LDLs), which transport triglycerides and cholesterol in the blood, and partly to alterations in hepatic and adipose tissue metabolism of these and other lipids (Meyers et al., 1980).

In addition to its effects on lipid transport and metabolism, Clofibrate has a number of other pharmacological actions. Clofibrate inhibits platelet aggregation, lowers serum fibrinogen levels and increases fibrinolysis (Meyers et al., 1980), in addition to other effects that will be discussed. Many of the actions of Clofibrate have the potential for interfering with some of the mechanisms discussed earlier as possibly being responsible for the Delayed-Permeability phase of ARDS-type pulmonary injury. It was for this reason, and the protective effect of this agent in paraquat lung injury (Frank et al., 1982), that Clofibrate was tested in these experiments.

In contrast to the other drugs tested for pharmacological intervention, Clofibrate was clearly effective at reducing NO_2-induced pulmonary edema and hyperemic congestion. This effectiveness was manifested as reduction of both severity of lung damage, as determined by lung wet and dry weight gains, and lethality after exposure to high concentrations of NO_2.

Neither of the wet:dry weight ratios were affected by Clofibrate, this was due to approximately proportionate changes in both numerators and denominators of these ratios. The most striking decreases were in the lung water measures. Lung Wet/BW was decreased to 23% below that of untreated animals exposed to NO_2 and analyzed at 24 hours PI. Lung extravascular water content was decreased even further by Clofibrate, EVLW/BW was 25.6% lower in the treated animals. Since, as has been discussed earlier, wet:dry weight ratios approaching 10.0 are the most reliable predictors of a terminal outcome, decreases in excess lung water appear very important in avoiding mortality.

Lung dry weight measures also were decreased in the Clofibrate animals, albeit to a somewhat lesser extent. Lung Dry/BW was reduced to 16% and the dP/BW measure to 17% below that of the untreated-exposed animals. These measures indicate that in addition to less excess water in the lungs, there was less deposition of excess dry weight material during the development of injury. These reductions indicate the therapeutic effect of Clofibrate was exerted primarily on the Delayed-Permeability phase of NO_2 pulmonary toxicity, this being the phase most responsible for deposition of dry weight materials in the lung.

The lung dry weight measures were reduced to the levels attained at 6 hours PI, at the height of the Early-Hydrostatic phase of edemagenesis. Excess water measures were decreased to approximately the levels at 12 hours PI, after the peak in this early phase. These values raise the intriguing possibility that Clofibrate prevented the Delayed-Permeability phase entirely. A more likely explanation is that Clofibrate interfered with both phases of NO_2-induced pulmonary injury.

Although barely missing statistical significance, blood content of the lung was also reduced by Clofibrate. The Blood/BW measure was 13.5% lower in the treated animals. Had the dP measure not decreased by about the same extent, Blood/dP would most likely have decreased also. Clofibrate therefore exerted an effect on NO_2-induced accumulation of all of the major components in ARDS-type lung injury: water, dry weight materials and blood.

The most dramatic demonstration of the protective action of Clofibrate was on 24 hour mortality. Of the untreated, exposed animals, only 83% survived until sacrifice at 24 hours PI. Clofibrate animals experienced no premature deaths. These results bear out the significance of the reductions in excess lung water.

PROTECTIVE SCHEMES:

Clofibrate has an extensive and varied pharmacology. Many of its therapeutic, as well as secondary, effects could conceivably be responsible for interference with production of the pulmonary edema and hyperemic congestion of the lung characteristic of ARDS-type lung injury. Unfortunately, there is little evidence in the literature, or in the results of the present experiment, to strongly suggest one or another action as primarily responsible. Most of these actions are more suited for affecting the Delayed-Permeability phase of injury, but it is more likely that both phases are affected.

Vascular Smooth Muscle:

An action of Clofibrate reported by Fairhurst et al. (1981) has the potential for countering hydrostatically-mediated edema production more

than altering permeability. In this study, Clofibrate was shown to inhibit norepinephrine, histamine or angiotensin II stimulated contraction of vascular smooth muscle. This action was specific for receptor-mediated contraction as Clofibrate did not affect K^+-mediated contractions. It is conceivable the Early-Hydrostatic phase of edemagenesis is produced via receptor-mediated constriction of vessels "downstream" from the microvascular areas participating in fluid exchange. This post-microvascular constriction could raise hydrostatic pressure within these exchange vessels enough to increase fluid loss. If, as suggested earlier, prostacyclin mediates this constriction according to the theory of Ivanhoe and and Meyers (1964) on sympathetic attenuation, Clofibrate may also interfere with the response to stimulation of its receptor. Normal synthesis of prostacyclin in vascular walls of the rabbit, minipig and human is inhibited by Clofibrate (Jager et al., 1982).

These observations may also have relevance to the Delayed-Permeability phase. Due to loss of the protective effect from interstitial protein "wash out", elevations of hydrostatic pressure contribute more to edema formation in increased permeability than in increased pressure conditions. If there are receptor-mediated pressure increases during the Delayed-Permeability phase, any effect of Clofibrate decreasing this pressure could noticably affect edemagenesis.

Free Fatty Acids:

At least one of Clofibrate's effects on lipid transport and metabolism initially appears capable of contributing to its protective effect in NO_2-induced lung injury. Clofibrate decreases serum free

fatty acid (FFA) levels, even in the normolipidemic state (Priego et al., 1979). It has been shown that FFAs are toxic to heart and endothelioid cells in culture (Wenzel & Hale, 1978/a). In this study, the unsaturated arachidonic (20:4) and linolenic (18:2) acids were the most toxic, producing major injury to the cultured cardiovascular cells. Arachidonic acid most likely exerted this effect via active metabolites from the action of cyclo- or lipo- oxygenase. Whole lung linolenate levels are decreased, and BAL fluid linolenate levels are increased as a result of NO_2 exposure (Roehm et al., 1971). It therefore appears that NO_2 might be releasing FFAs from membranes within the parenchyma of the lung. These FFAs could then enter the alveoli, causing epithelial damage or the pulmonary circulation, causing endothelial damage leading to increased loss of fluid and protein. In this scheme, Clofibrate's protective effect could be due to lowering serum FFA transport to potential sites of vascular damage.

However, a couple of other observations argue strongly against this scheme. Clofibrate lowers serum FFA levels partly due to displacement of FFAs from albumin binding sites (Thorp, 1963) and partly due to increased liver uptake and metabolism of FFAs, an effect occuring acutely in hepatocyte cultures (Capuzzi et al., 1983). It would therefore seem that Clofibrate should worsen local FFA toxicity, since it would inhibit removal from the site of generation, due to decreased capacity of albumin to bind and transport FFAs. In addition, the stimulatory effect of Clofibrate on cellular uptake of FFAs can actually increase their toxicity. Gordon (1978) reported potentiation of FFA toxicity to cultured mammalian cells when Clofibrate was added to the culture and attributed this effect to increased FFA uptake by the cells.

Free Radical/Lipid Interactions:

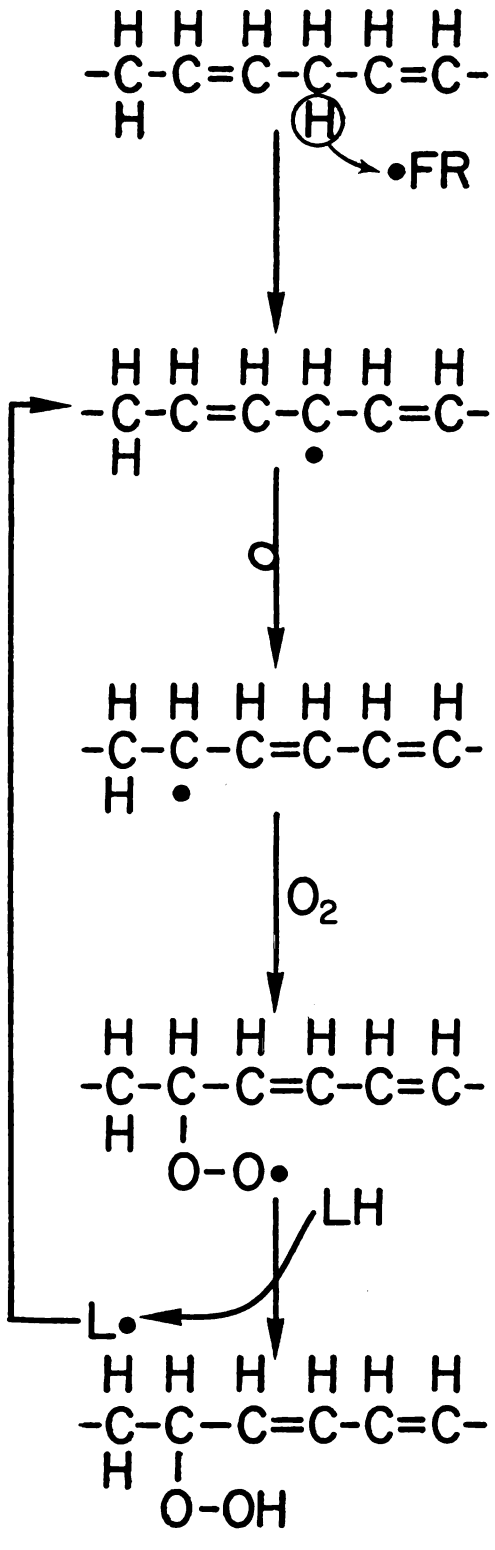
As discussed earlier, toxic effects of NO_2 on the lung may be due in part to a free radical interaction with cellular lipids. Conceivable products of this type of reaction include species that are direct cellular toxins, enzymatically generated mediators of inflammation or non-enzymatically generated chemotaxins.

Any agent, such as NO_2, with the potential to form a free radical also has the potential to initiate lipid peroxidation (Symposium, 1973), the basic scheme for which is shown on the next page. A free radical (FR•) first abstracts an allylic hydrogen atom from an alpha-methylene carbon in a polyunsaturated fatty acid, such as linoleic acid. The alpha-methylene is a carbon atom situated between two double bonded carbons, to each of which it is connected by a single bond. Because of the electron-withdrawing nature of the double bonds, the C-H bond in the alpha-methylene carbon is weaker than others in the molecule. It is therefore more susceptible to abstraction by free radicals.

Abstraction of the allylic hydrogen leaves behind a fatty acid free radical, which may rearrange to form a conjugated system. In the event of two double bonds this is a conjugated diene, which characteristically absorbs radiation at 235 nm. Increased absorption at this wavelength in an organ homogenate is sometimes interpreted as evidence of lipid peroxidation, but this conclusion should be supported by more definative evidence.

Due to its diradical nature, O_2 can react with and bind to this lipid free radical, forming a peroxy free radical. This peroxy free radical can then abstract an allylic hydrogen from an alpha-methylene

FREE RADICAL-LIPID INTERACTIONS:



POLYUNSATURATED FATTY ACID

Free Radical (FR•) abstracts allylic H from alpha-methylene carbon.

LIPID FREE RADICAL

Internal rearrangement.

CONJUGATED DIENE

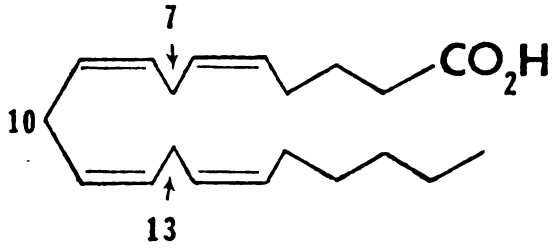
Addition of oxygen.

PEROXY FREE RADICAL

Abstraction of allylic H from another polyunsaturated fatty acid--chain initiation.

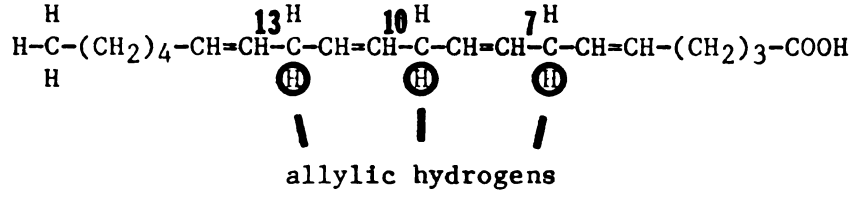
LIPID HYDROPEROXIDE

ARACHIDONIC ACID PEROXIDATION:



ARACHIDONIC ACID

Molecular Formula:



LIPID PEROXIDATION :

Position 10 Abstraction:
- abstraction of allylic H from C_{10}, with subsequent addition of O_2, yeilds the arachidonic acid hydroperoxide, HPETE (hydroperoxyecosatetraenoic acid).
- this mechanism mimics the action of lipoxygenase.

Position 7 Abstraction:
- abstraction of allylic H from C_7, with subsequent formation of a lipid free radical and internal cyclization, can form an endoperoxide involving C_9 and C_{11}.
- subsequent abstraction at C_{13}, with rearrangement and addition of O_2, then yeilds a hydroperoxide at C_{15}.
- result of these two auto-oxidations is formation of PGG_2.
- this mechanism mimics the action of cyclooxygenase.

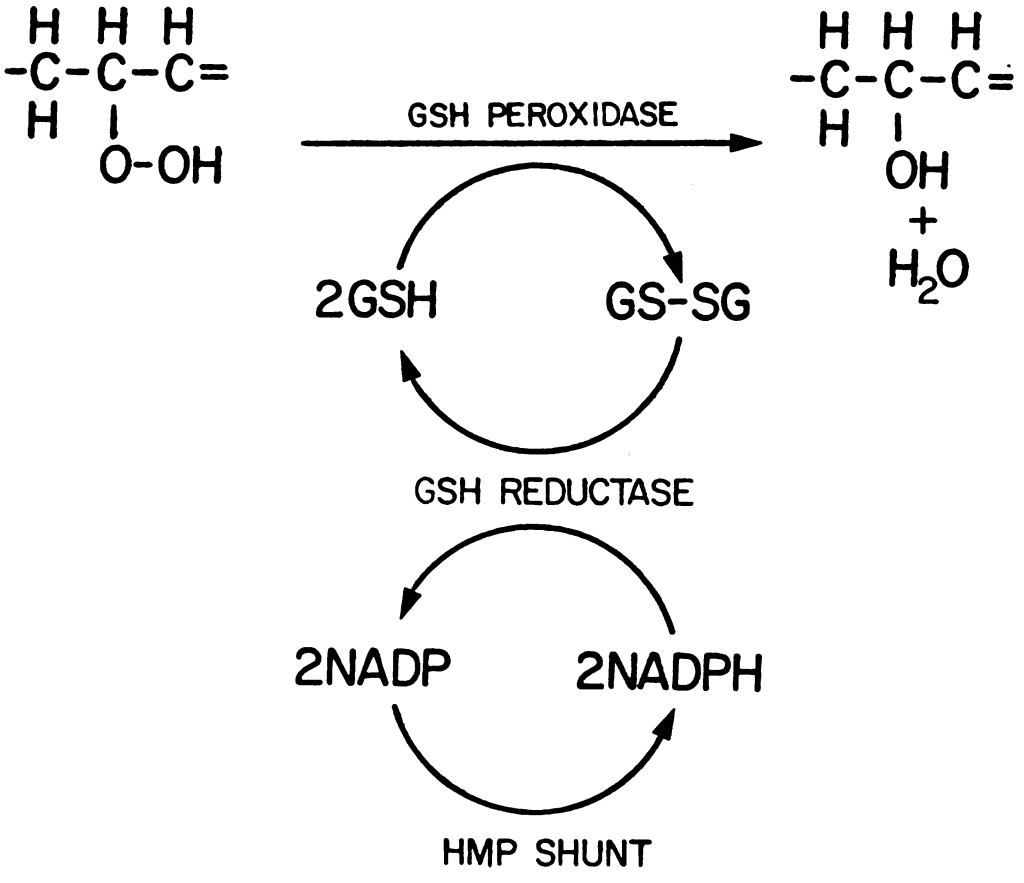
These two mechanisms illustrate the general mechanism by which the auto-oxidation of arachidonic acid, via free radical/lipid peroxidative-type reactions, can non-enzymatically generate compounds similar or identical to mediators of inflammation.

carbon of another polyunsaturated fatty acid. This produces a lipid hydroperoxide, and may initiate a chain reaction. Such reactions can have severe consequences on cellular integrity and function. Membranes are particularly sensitive to the effects of lipid peroxidation. Formation of lipid hydroperoxides and rearrangement of double bonds within membrane lipids can dramatically alter membrane fluidity. Free radicals generated within the membranes can attack proteins, thus altering their structures and functions. Lipid hydroperoxides, one of the products of this scheme, are potentially important in the development of ARDS-type pulmonary injury.

Lipid hydroperoxides, as discussed earlier, have the capability to either produce or mediate lung damage similar to that observed in ARDS and NO_2 toxicity. Cortesi & Privett (1972) produced edematous, hyperemic lungs in rabbits by injection of methyl linoleate hydroperoxide, a possible free radical metabolite of one of the fatty acids mobilized by NO_2 (Kobayashi et al., 1980). Peroxidative-type reactions with arachidonic acid may generate "false mediators" of inflammation (Perez et al., 1980 & Porter et al., 1981).

Cellular "antioxidant defenses" exist to protect cells against the disruptive effects of lipid peroxidation (see next page). Lipid hydroperoxides are converted by glutathione peroxidase to lipid alcohols. In the process of this reaction, two molecules of glutatione (GSH = reduced and GSSG = oxidized forms) are oxidized to one of GSSG. GSH is then regenerated by GSH reductase with reducing equivalents from the Hexose Monophosphate (HMP) Shunt in the form of $NADPH_2$. Two enzymes of the HMP Shunt, glucose-6-phosphate dehydrogenase (G-6-PDH) and

"ANTIOXIDANT DEFENSE":



Lipid hydroperoxides are metabolized to their corresponding alcohols by the action of glutathione (GSH) peroxidase. In this reaction, reducing equivalents are directly supplied by two molecules of GSH, which are oxidized to one of GSSG. Reduction back to GSH is accomplished by GSH reductase with reducing equivalents supplied by $NADPH_2$. This $NADPH_2$ is produced by the Hexose Monophosphate (HMP) shunt, specifically by glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-P-GDH). Both G-6-PDH and 6-P-GDH may be induced by Clofibrate.

6-phospho-gluconate dehydrogenase (6-P-GDH), are responsible for most of this $NADPH_2$ (Chow & Tappel, 1973).

Another enzyme active in detoxifying free radicals is catalase. Superoxide anion (O_2^-), a free radical of oxygen, can be produced by interaction with any of the free radicals so far generated. Because of the lung's high O_2 concentration, this formation of O_2^- may be more likely than in other organs (Boyd, 1980). This O_2^- is metabolized by superoxide dismutase (SOD) to H_2O_2 and O_2. The H_2O_2 is subsequently deactivated to H_2O by catalase. Both O_2^- and H_2O_2 are themselves highly reactive species and as such have the potential to interact with lipids, proteins and other components to produce cellular damage.

Clofibrate has the potential for enhancing the antioxidant defense against lipid peroxidation at two different points in the scheme. Induction of both catalase and one of the enzymes of the HMP shunt has been shown to occur with Clofibrate.

Peroxisomes are small, membrane-bound, cytoplasmic organelles which contain a number of oxidases in addition to catalase (DeDuve & Baudhuin, 1966). Svoboda and Azarnoff (1966), among others, have reported greatly increased amounts of microbodies (peroxisomes) in the livers of animals treated with Clofibrate. After three days of feeding Clofibrate, they observed a marked increase in the number of microbodies and a proliferation of smooth endoplasmic reticulum. Correlated with these structural changes was increased activity of catalase in liver homogenates from these animals. Peroxisomes are also present in the lung, notably in the alveolar epithelium in Type II cells (Petrik, 1971). This location is ideally suited for an enzyme system that could interfere with the toxicity of an inhaled edemagenic agent. If Clofibrate increases

catalase levels in these cells, the enhanced breakdown of H_2O_2 may contribute to decreasing peroxidative lung damage.

As attractive as this hypothesis appears, there is evidence against it. The protective effect in the present experiments was exerted over a period of a little more than 24 hours. Jones & Masters (1978) studied the kinetics of catalase induction in the liver by Clofibrate and observed a bi-phasic effect. Over the initial 48 hours, catalase activity actually decreased, although there was a later rapid increase to levels 3-fold over control. Such a lag period would occur at just the time catalase should be exerting its protective effect in the present experiment. There is also evidence that Clofibrate does not induce all types of peroxisomes, at least within the lung. Drath et al. (1982) studied the effect of Clofibrate on alveolar macrophages. Although liver weight increased and serum triglyeride levels decreased (two indications of Clofibrate's effectiveness) over two weeks of dosing, there was no proliferation of peroxisomes in alveolar macrophages. These reports certainly raise questions about the potential role of elevated catalase levels as a mechanism of Clofibrate's protective action in NO_2-induced lung injury. In the protection against paraquat toxicity, lung injury very similar to that of NO_2, Clofibrate produced no effect on lung catalase or GSH peroxidase activity levels (Frank et al., 1982).

Another means by which Clofibrate may interfere with peroxidative mechanisms involves increased activity of an HMP shunt enzyme. Metabolic conversion of glucose-6-phosphate to 6-phospho-gluconate, the initial step in the HMP shunt, results in generation of reducing equivalents in the form of $NADPH_2$. This reaction is catalyzed by G-6-PDH, an

enzyme induced by Clofibrate. Platt & Cockrill (1966) fed Clofibrate to rats and measured redox enzyme levels in their livers at the end of two weeks. In addition to increased levels of G-6-PDH, increases in both NADP and $NADPH_2$ were also noted. It is not known with certainty whether G-6-PDH activity and NADP & $NADPH_2$ levels in the lung are also increased by Clofibrate treatment. In the previously cited study (Drath et al., 1982) on the effects of Clofibrate on alveolar macrophages, "HMP shunt activity" was also measured. In this case, two weeks of Clofibrate treatment did not affect normal HMP shunt activity. However, in alveolar macrophages taken from the treated animals and stimulated with either Zymosan (a particulate activator) or PMA (a soluble activator), HMP shunt activity was definately increased over that in similarly stimulated macrophages from untreated animals.

The antioxidant capability, in the form of $NADPH_2$, of this system can be utilized according to the scheme of Chow & Tappel (1973) to reduce any lipid hydroperoxides formed by free radical-lipid interactions. With these elevations in $NADPH_2$, GSH reductase can cycle enough GSH to allow the peroxidase to reduce lipid hydroperoxides to the corresponding alcohols.

The time course of these experiments is much longer than in the present study. The effect of Clofibrate on HMP shunt-derived reducing potential may not be manifested within the 24 hour time frame critical to NO_2 toxicity in the present experiments. In this regard, it should be noted that six days of pretreatment with Clofibrate did not increase total lung activity of G-6-PDH in the paraquat protection experiments of Frank et al. (1982). This lack of elevation of G-6-PDH activity and the finding of Drath et al. (1982) of increased HMP shunt activity in

alveolar macrophages from animals treated for two weeks may mean that a week or more is needed for increased enzyme activities to develop in the lung in response to Clofibrate. Cofibrate stimulation of liver enzyme activity requires a much shorter time to develop (Svoboda & Azarnoff, 1966 and Jones & Masters, 1978).

Coagulation System:

At least two components of the coagulation system are affected by Clofibrate. Both of these, platelets and fibrin, have been suggested as important in the pathogenesis of ARDS.

Clofibrate has been shown to inhibit platelet aggregation and secretion. This action was well characterized by Huzoor-Akbar et al. (1981) in a comparison of activation of platelets from Clofibrate treated patients and normal volunteers. Treatment with Clofibrate inhibited platelet activation, determined by aggregation and secretion of serotonin. Platelet activation in response to ADP, epinephrine, collagen and thrombin, all potent activators of platelets, was significantly decreased in a concentration dependent manner in platelets from treated patients.

Clofibrate's effects on platelets appeared due to inhibition of prostaglandin synthesis. The thrombin-induced liberation of arachidonic acid (AA) from platelet phospholipids and the subsequent generation of malondialdehyde, associated with AA oxidation to prostaglandin and thromboxane compounds, were inhibited by Clofibrate. However, AA-induced aggregation of platelets was not inhibited, indicating the block was likely at the level of the phospholipase releasing AA from

the cellular membrane. Subsequent metabolism of AA, yielding the pro-activation species PGG_2, PGH_2 and TXA_2, was not blocked by Clofibrate.

As noted earlier, the evidence implicating platelet activation in the pathogenesis of ARDS is conflicting. Local activation of platelets in the lung does produce a syndrome with all of the pathologic features of fully developed ARDS (Camussi et al., 1983). However, increases in pulmonary microvascular permeability that follow platelet embolization are shortlived. Potentially, platelet acivation could play an important role in the initiation of an ARDS-type pulmonary reaction. Clofibrate could possibly affect this pathology in proportion to the involvement of platelet activation. However, the transient nature of the permeability responses to platelet embolization (Binder et al., 1980) argue against this being the major effect of Clofibrate in the present experiments. Since the studies cited above involved durations of treatment in excess of a few days the question is again raised of whether there was adequate time in the present experiments for manifestation of these actions of Clofibrate.

Clofibrate also appears to have some effects on fibrin and fibrinolysis, although these effects may not be manifested in normal (ie non-hyperlipidemic) individuals. For this reason, the relationship of these effects to the pathologic condition subsequent to inhalation of high concentrations of NO_2 is unclear.

Cotton & Wade (1966) reported a study of hypercholesterolemic patients treated with Clofibrate. They observed Clofibrate-induced decreases in serum fibrinogen only in patients with initially elevated levels. Fibrinogen levels decreased steadily over the initial four to

five months of treatment, suggesting this action of Clofibrate develops too slowly to contribute to the present results. However, fibrinogen determinations were reported only on a monthly basis so it is possible a significant immediate effect was obscured, although not likely.

Increases in fibrinolytic activity in plasma occur with Clofibrate treatment only in patients in whom this activity was previously depressed (Srivastava et al., 1963 and Goodhart & Dewar, 1966). The same caveats discussed with respect to fibrinogen levels apply to this effect of Clofibrate.

A number of intreguing speculations on a possible association between fibrin(ogen) and ARDS have been advanced and some of these were discussed earlier. Observation of these materials in the lung in conjunction with ARDS-type injury is not surprising, indeed their absence would be more significant. Similar to the situation with the experiments on platelet activation, increases in microvascular permeability are produced by fibrin(ogen) or degradation products (Manwaring et al., 1978). However, defibrinogenation experiments produce data in conflict with the idea of a major role for this part of the coagulation system in ARDS (Binder et al., 1980).

Given the questionable involvement of fibrin(ogen) in ARDS, the lack of kinetic data concerning Clofibrate's effect on fibrinogen and the uncertainty of its effect in the normal state it is difficult to conceive a proctective effect for Clofibrate due to its effects on this system. However, too many questions remain unanswered to definately rule out a major effect of Clofibrate here.

Thyroxine:

Early studies (Thorp, 1963) on the action of Clofibrate indicated a displacement of thyroxine (T_4) from its plasma binding sites, with consequently lowered plasma concentrations. Platt & Thorp (1966) reported that Clofibrate produced an alteration in the distribution of thyroid hormones between the liver and plasma. They characterized this shift as producing a condition of relative peripheral hypothyroidism and hepatic hyperthyroidism. Ruegamer et al. (1969) clarified the situation by observing the response of labelled T_4 to Clofibrate administered to rats. Clofibrate produced an apparent displacement of T_4 from the plasma into the liver, rendering the liver "hyperthyroid." However, the rest of the animal remained euthyroid, there was no change in basal metabolic rate and the activity of thyroid-sensitive enzymes in other tissues remained at normal levels. Although the total T_4 in plasma did not change, the total protein bound iodine in plasma decreased by 29% in response to Clofibrate.

These changes in plasma thyroid hormone and iodine levels are of especial interest with regard to NO_2 toxicity. Fairchild & Graham (1963) investigated the effect of altered thyroid hormone levels on NO_2 toxicity. They pharmacologically or surgically altered thyroid hormone levels in mice, then exposed them to NO_2 (30 to 50 ppm for 3 to 4 hours) and reported on mortality. Pretreatment with thyroid blocking agents, methimazole, K^+-perchlorate and others produced significant protection against acute lethality. In the corresponding experiment, pretreatment with thyroid hormones, T_3 and T_4, dramatically increased mortality from NO_2 exposure. This potentiation of NO_2 toxicity was not due to the pro-

duction of a hypermetabolic state per se as metabolic stimulants had no effect on NO_2 toxicity in other experiments.

These results may be due to the utilization of plasma iodine (from either T_3, T_4 or protein bound) by the myeloperoxidase system of PMNs. The myeloperoxidase (MPO) enzyme produces a highly reactive, toxic intermediate from H_2O_2 and an electron donor. In the reaction, H_2O_2 is reduced and the electron donor is oxidized to a highly reactive metabolite, the nature of which depends on the identity of the donor. Although chloride is often the donor, forming hypochlorous acid, other halides and thiocyanate ions may also participate. In this role, iodide is actually much more effective than cloride (Klebanoff, 1968).

This system is relatively non-selective with respect to targets. When the reactive metabolite is generated, it will attack the closest structure, usually a bacterium inside a phagosome within the PMN. However, under some conditions this system can direct its toxic reactive species at external structures, such as adjacent microvascular endothelium. This is a circumstance known as "frustrated phagocytosis," which occurs when a PMN attempts ingestion of a structure too big, for example a capillary endothelial wall, to be effectively phagocytized.

As alluded to earlier, plasma sources of iodine (T_3, T_4 or that bound to proteins) can replace iodide as the electron donor in the MPO system (Klebanoff, 1967). Decreases in total plasma iodine, as may occur with Clofibrate, could conceivably interfere with extracellular release from PMNs of reactive metabolites in an inflammatory focus, produced in response to inhalation of NO_2 for instance.

Leukocytes:

Clofibrate has the capability to directly inhibit the function of leukocytes. Some of its other pharmacological actions also have the potential to indirectly interfere normal leukocyte function. Given the evidence discussed earlier implicating PMNs in ARDS, these actions may be especially relevant to the pathogenesis of pulmonary edema and hyperemic congestion of the lung following NO_2 inhalation.

Counts of circulating white blood cells (WBCs) were determined in the early clinical trials on Clofibrate (Engstrom, 1966). Mean PMN counts decreased by 21% during Clofibrate therapy and total white cell counts decreased by 19%. Circulating mononuclear cells experienced the largest decrease, a 27% fall during treatment.

These were long term studies and the first WBC counts taken were at least two weeks after initiation of Clofibrate therapy. However, these initial post-treatment counts exhibited sharp declines in circulating WBCs in all of the cases illustrated. These initial values were close to, or below, the means attained over several months. It is therefore possible that a sharp decrease occurs in the number of circulating WBCs within the initial 24 hours after Clofibrate administration.

This study did not attempt to determine the fate of WBCs no longer in circulation. It is likely the lowered WBC counts reflected decreased release from bone marrow. However, it is also possible there was early sequestration or inactivation of WBCs. The data presented do not allow any interpretation regarding the likelihood that the decreased WBC counts reflect an attenuated ability to mount an inflammatory reaction in the lung.

Clofibrate may also be able to inhibit leukocyte migration, at least in response to specific stimuli. Horvath et al. (1982) studied the migration of cultured human leukocytes in response to vascular antigens. They reported decreased migration in Clofibrate-treated leukocytes as compared to those not receiving any pretreatment.

One of the most potent means the PMN has of producing cellular (whether exogenous or endogenous) injury is generation of O_2 radicals. During activation, an NADH oxidase on the external membrane (Briggs et al., 1975) of the PMN generates superoxide anions (O_2^-). As discussed earlier, these highly reactive free radicals are capable of oxidizing lipids, proteins, nucleic acids or other cellular constituents. With membrane invagination during phagocytosis, this enzyme and its reactive products become incorporated into the interior of the phagosome. Conversion of O_2^- to H_2O_2 readily takes place within the phagosome due to the low pH in the interior (Jenson & Bainton, 1973). Interaction between these two active oxygen species produces a third, the hydroxyl radical (HO•) via the Haber-Weiss reaction (Haber & Weiss, 1934). All of these active oxygen species are inter-related and can interconvert. These active oxygen species generated within the phagosome attack the ingested material but are normally retained therein. However, as discussed earlier, in "frustrated phagocytosis", leakage of these active oxygen species, in addition to proteases may result in destruction of adjacent tissue. Clofibrate's ability to induce catalase may increase levels of this enzyme within the PMN and interfere with phagocytosis before it becomes "frustrated."

The results of Drath et al. (1982) suggest however that catalase induction in WBCs may not occur with Clofibrate. Peroxisomes, the site

of inducible catalase in hepatocytes, are not increased in alveolar macrophages as a result of Clofibrate treatment. An additional observation, that total lung weight was not increased by Clofibrate treatment, may mean that lung peroxisomes, and their associated catalase, are not inducible, as are those of the liver.

Analogous to its effects on platelets (Huzoor-Akbar et al., 1981), Clofibrate may interfere with generation, by the PMN, of mediators of inflammation from AA. Activated PMNs produce prostaglandins from AA at a rate up to 10-fold above that of quiescent PMNs (Higgs et al., 1975).

A number of the actions of PMNs, including elaboration of activated O_2 species, free radicals and arachidonic acid metabolites, may be involved in the pulmonary injury produced by NO_2. Clofibrate is a drug with numerous effects, many of which have the potential for interacting with the metabolic and regulatory systems involved in activating and mediating the inflammatory actions of PMNs. Therefore a seemingly endless list can be generated of means whereby Clofibrate potentially affects PMN function to inhibit possible mediation of the edemagenic response to NO_2.

SUMMARY:

In spite of strong theoretical bases for their choice, many of the agents tested were ineffective at influencing NO_2-induced pulmonary injury. Rutin, Indomethacin and the steroids belong in this category. Any of these may still be found to posses beneficial actions that were not elaborated because of pharmacokinetic inadequecies, or they all may be directed at the wrong mechanism.

Terbutaline dramatically worsened pulmonary injury from NO_2, even at 6 hours when its action as vasodilator should have exerted some protective effect. Addition of a $beta_1$ blocker did not affect Terbutaline's negative effect, indicating cardiac stimulation was not responsible for increasing lung water.

Clofibrate demonstrated a striking protective effect in these experiments. Expected mortality did not occur in response to NO_2 inhalation and there was significant attenuation of the expected increases in both wet weights and dry weights. These findings are indicative of interference mainly with the Delayed-Permeability phase of edemagenesis, the more serious of the two phases in this model of ARDS. In addition, Clofibrate inhibited the accumulation of blood in the lung, indicating interference also with the hyperemic component of the reaction to NO_2.

The results from these experiments, taken with those of Frank et al. (1982) with a model of ARDS produced by paraquat, indicate that Clofibrate may exert a pharmacological action significantly interfering with the development of ARDS-type lung injury. Too little evidence exists to do more than speculate about the nature of this action.

ABBREVIATIONS & REFERENCES

ABBREVIATIONS

AA	Arachidonic Acid
Abs	Absorbance
AGEPC	Acetyl Glyceryl Ether Phosphorylcholine (PAF)
ARDS	Adult Respiratory Distress Syndrome
BAL	Bronchoalveolar Lavage
BW	Body Weight
cAMP	Cyclic 3'-5'-Adenosine Monophosphate
cf	Cubic Feet
CF	Correction Factor (Blacker Assay)
cGMP	Cyclic 3'-5'-Guanosine Monophosphate
CO	Carbon Monoxide
CT	Concentration x Time
dl	Deciliter
dP	Weight of dry Parenchymal tissue of lung (excludes blood)
EVLW	Extravascular Lung Water
FFA	Free Fatty Acid
FR•	Free Radicals
Fwb	Fractional Water, Blood
Fwh	Fractional Water, Homogenate
Fws	Fractional Water, Supernatant
G-6-PDH	Glucose-6-Phosphate Dehydrogenase
GSH	Glutathione (reduced)
GSSG	Glutathione (oxidized)
$[Hb]_b$	Hemoglobin concentration, peripheral blood
$[Hb]_s$	Hemoglobin concentration, supernatant
HCT	Hematocrit
HETE	Hydroxyeicosatetraenoic Acid
HMP	Hexose Monophosphate
HPETE	Hydroperoxyeicosatetraenoic Acid
K_f	Microvascular Filtration Coeficient

LDL	Low Density Lipoprotein
L/P	Lymph to Plasma Protein Concentration Ratio
NADP(H)	Nicotinamide Adenine Dinucleotide Phosphate
$NaNO_2$	Sodium Nitrite
NEDA	N-1-naphthylethylenediamine
ns	Not Significant and Less Than 10%
O_2^-	Superoxide Anion
OH•	Hydroxyl Radical
Q_b	Residual Lung Blood Weight
Q_f	Net Transvascular Fluid Flow
Q_h	Lung Homogenate Weight
Q_l	Lung (- Blood) Wet Weight
Q_{lb}	Lung Wet Weight
Q_w+	Hongenization Water Weight
PAF	Platelet Activating Factor
$PaCO_2$	Arterial Partial Pressure of Carbon Dioxide
PaO_2	Arterial Partial Pressure of Oxygen
6-P-GD	6-Phosphogluconate Dehydrogenase
PI	Post-Initiation
P_{la}	Left Atrial Hydrostatic Pressure
PMA	Phorbol Myristate Acetate
PMN	Polymorphonuclear Leukocyte
P_{mv}	Microvascular Hydrostatic Pressure
P_{pa}	Pulmonary Artery Hydrostatic Pressure
ppm	Parts-per-Million
P_{pmv}	Perimicrovascular Hydrostatic Pressure
II_{mv}	Microvascular Oncotic Pressure
II_{pmv}	Perimicrovascular Oncotic Pressure
R	Microvascular Reflection Coeficient
S/"S"	Survivors Only
SG_b	Specific Gravity, Peripheral Blood
SG_s	Specific Gravity, Supernatant
TEA	Triethanolamine
V_b	Residual Lung Blood Volume

REFERENCES:

Abraham WM, Welker M, Oliver W, Mingle M, Januszkiewicz AJ, Wanner A & Sackner MA: Cardiopulmonary Effects of Short-Term Nitrogen Dioxide Exposure in Conscious Sheep. Environ Res 22: 61, 1980.

Adams FH, Fujiwara T, Emmanouilides GC & Raiha N: Lung Phospholipids of Human Fetuses and Infants With and Without Hyaline Membrane Disease. J Pediatr 77: 833, 1970.

Ali J, Chernicki W & Wood LDH: Effect of Furosemide in Canine Low-Pressure Pulmonary Edema. J Clin Invest 64: 1494, 1979.

Ambrose AM & DeEds F: Effect of Rutin on Permeability of Cutaneous Capillaries. J Pharmacol Exptl Ther 90: 363, 1947.

Amoruso MA, Witz G & Goldstein BD: Decreased Superoxide Production by Alveolar Macrophages Following Inhalation of Ozone or Nitrogen Dioxide. Life Sci 28: 2215, 1981.

Anderson RR, Holliday RL, Driedger AA, Lefcoe M, Reid B & Sibbald J: Documentation of Pulmonary Capillary Permeability in the Adult Respiratory Distress Syndrome Accompanying Sepsis. Amer Rev Resp Dis 119: 869, 1979.

Ashbaugh DG, Bigelow DB, Petty TL & Levine BE: Acute Respiratory Distress in Adults. Lancet ii: 319, 1967.

Avery ME & Mead J: Surface Properties in Relation to Atelectasis and Hyaline-Like Membrane Disease. Amer J Dis Child 97: 517, 1959.

Badway JA & Karnovsky ML: Active Oxygen Species and the Functions of Phagocytic Leukocytes. Ann Rev Biochem 49: 695, 1980.

Baumann J, Bruchhausen Fv & Wurm G: Flavonoids and Related Compounds as Inhibitors of Arachidonic Acid Peroxidation. Prostaglandins 20: 627, 1980.

Baxter JD & Forsham PH: Tissue Effects of Glucocorticoids. Amer J Med 53: 573, 1982.

Beil M & Ulmer WT: Effect of NO_2 in Workroom Concentrations on Respiratory Mechanics and Bronchial Sensitivity to Acetylcholine in Normal Persons. Int Arch Occup Environ Health 38: 31, 1976.

Benedetti TJ, Hargrove JC & Rosene KA: Maternal Pulmonary Edema During Premature Labor Inhibition. Obstet Gynecol 59: 33S, 1982.

Bhattacharya J, Nanjo S & Staub NC: Micropuncture Measurement of Lung Microvascular Pressure During 5-HT Infusion. J Appl Physiol 52: 634, 1982.

Bils RF:
stru

Binder AS
Depl
J Ap

Binder AS
in
Emb

Blacker
Ran

Blair WH
II:
18:

Blank M
que
Lu
19

Boyd EM
of

Boyd MR
of

Bowers
i
D

Briggs
O
N

Brigha
b

Brigha

Brigh

Camus

Bils RF: Effects of Nitrogen Dioxide and Ozone on Monkey Lung Ultrastructure. Pneumonologie 150: 99, 1974.

Binder AS, Kageler N, Perel A, Flick MR & Staub NC: Effect of Platelet Depletion on Lung Vascular Permeability after Microemoli in Sheep. J Appl Physiol 48: 414, 1980.

Binder AS, Nakahara K, Ohkuda K, Kageler N & Staub NC: Effect of Heparin or Fibrinogen Depletion on Lung Fluid Balance in Sheep after Emboli. J Appl Physiol 47: 213, 1979.

Blacker JH: Triethanolamine for Collecting Nitrogen Dioxide in the TLV Range. Amer Indust Hygiene Assoc J 34: 390, 1973.

Blair WH, Henry MC & Ehrlich R: Chronic Toxicity of Nitrogen Dioxide-II: Effect on Histopathology of Lung Tissue. Arch Environ Health 18: 186, 1969.

Blank ML, Dalbey W, Nettesheim P, Price J, Creasia D & Snyder F: Sequential Changes in Phospholipid Composition and Synthesis in Lungs Exposed to Nitrogen Dioxide. Amer Rev Resp Dis 117: 273, 1978.

Boyd EM & Knight LM: Postmortem Shifts in the Weight and Water Levels of Body Organs. Toxicol Appl Pharmacol 5: 119, 1963.

Boyd MR: Biochemical Mechanisms in Chemical-Induced Lung Injury: Roles of Metabolic Activation. CRC Crit Rev Toxicol 8: 103, 1980.

Bowers R, Brigham K & Owen P: Salicylate Pulmonary Edema: The Mechanism in Sheep and Review of the Cinicial Literature. Amer Rev Resp Dis 115: 261, 1977.

Briggs RT, Drath DB, Karnovsky ML & Karnovski MJ: Localization of NADH Oxidase on the Surface of Human Polymorphonuclear Leukocytes by a New Cytochemical Method. J Cell Biol 67: 566, 1975

Brigham KL & Owen PJ: Increased Sheep Lung Vascular Permeability Caused by Histamine. Circ Res 37: 647, 1975.

Brigham KL, Woolverton WC, Blake LH & Staub NC: Increased Sheep Lung Permeability Caused by Pseudomonas Bacteremia. J Clin Invest 54: 792, 1974.

Brigham KL, Bowers RE & Haynes J: Increased Sheep Lung Vascular Permeability by Escherichia coli Endotoxin. Circ Res 45: 292, 1979.

Camussi G, Pawlowski I, Tetta C, Roffinello C, Alberton M, Brentjens J & Andres G: Acute Lung Inflammation Induced in the Rabbit by Local Instillation of 1-o-Octadecyl-2-acetyl-sn-glyceryl-3-phosphoryl-choline or of Native Platelet-Activating Factor. Amer J Pathol 112: 78, 1983.

Capuzzi DM, Intenzo CM, Lackman RD, Whereat AF & Scott DM: Stimulation of Fatty Acid Utilization in Rat and Monkey Hepatocytes. Biochem Pharmacol 32: 2195, 1983.

Carp H & Janoff A: Potential Mediator of Inflammation: Phagocyte Derived Oxidants Suppress the Elastase-Inhibitory Capacity of alpha$_1$-Proteinase Inhibitor. J Clin Invest 66: 987, 1980.

Carson TR, Rosenholtz MS, Wilinski FT & Weeks MH: The Responses of Animals Inhaling Nitrogen Dioxide for Single, Short-Term Exposures. Amer Indust Hyg Assoc J 23: 457, 1962.

Chow CK & Tappel AL: Activities of Pentose Shunt and Glycolytic Enzymes in Lungs of Ozone-Exposed Rats. Arch Environ Health 26: 205, 1973.

Clark JM & Lambertson CJ: Pulmonary Oxygen Toxicity: A Review. Pharmacol Rev 23: 37, 1971.

Cohen AB & Rossi M: Neutrophils in Normal Lungs. Amer Rev Resp Dis 127: S3, 1983.

Committee on Medical and Biological Effects of Air Pollutants: Nitrogen Oxides (series on Medical and Biological Effects of Environmental Pollutants). Natl Acad Sci Publ Office, Washington, D.C., 1977.

Cortesi R & Privett OS: Toxicity of Fatty Ozonides and Peroxides. Lipids 7: 715, 1972.

Cotton RC & Wade EG: Further Observations on the Effect of Ethyl Chlorophenoxyisobutyrate + Androsterone (Atromid) on Plasma Fibrinogen and Serum Cholesterol in Patients with Ischemic Heart Disease. J Atheroscler Res 6: 98, 1966.

Craddock PR, Fehr J, Brigham KL, Kronenberg RS & Jacob HS: Complement and Leukocyte-Mediated Pulmonary Dysfunction in Hemodialysis. New Eng J Med 296: 769, 1977.

Danilo P & Rosen TS: Effects of Terbutaline on Cardiac Automaticity and Contractility. J Clin Pharmacol 22: 223, 1982.

Dauber IM & Weil J: Pulmonary Edema Due to Lung Injury Causes Sympathetically-Mediated Pulmonary Vasoconstriction Which Increases Edema. Fed Proc 40: 402, 1981.

DeDuve C & Baudhuin P: Microbodies (Peroxisomes) and Related Particles. Physiol Rev 46: 323, 1966.

DeNicola DB, Rebar AH & Henderson RF: Early Damage Indicators in the Lung V. Biochemical and Cytological Response to NO_2 Inhalation. Toxicol Appl Pharmacol 60: 301, 1981.

Dillard
Viv
uns
Dic

Douglas
Fa
71

Dowell
Di

Drabkin
fr
bi

Drath
ou
A

Ehrlic
I

Engstr
(
A

Erdman

Evans

Fairc

Fairh

Fein

Fent

Flic

Dillard CJ, Sagai M & Tappel AL: Respiratory Pentane: A Measure of in Vivo Lipid Peroxidation Applied to Rats Fed Diets Varying in Polyunsaterated Fats, Vitamine E and Selenium and Exposed to Nitrogen Dioxide. Toxicol Lett 6: 251, 1980.

Douglas ME & Downs JB: Pulmonary Function Following Severe Respiratory Failure and High Levels of Positive End-Expiratory Pressure. Chest 71: 18, 1977.

Dowell AR, Kilburn KH & Pratt PC: Short-term Exposure to Nitrogen Dioxide. Arch Int Med 128: 74, 1971.

Drabkin DL & Austin JH: Spectrophotometric studies II. Preparations from Washed Blood Cells; Nitric Oxide Hemoglobin and Sulfhemoglobin. J Biol Chem 112: 51, 1935.

Drath DB, Davies P, Shorrey JM & Simpson P: The Effects of Clofibrate on Alveolar Macrophage Peroxisome Content and Oxygen Metabolism. Agents & Actions 12: 365, 1982.

Ehrlich R: Effect of Nitrogen Dioxide on Resistance to Respiratory Infection. Bacteriol Rev 30: 604, 1966.

Engstrom J: Clinical Trial with Ethyl-a-p-Chlorophenoxyisobutyrate (Atromid-S) with Special Reference to White Blood Cell Counts. Acta Med Scand 180: 519, 1966.

Erdmann J, Vaughan T, Brigham K, Woolverton W & Staub NC: Effect of Increased Vascular Pressure on Lung Fluid Balance in Unanesthetized Sheep. Circ Res 37: 271, 1975.

Evans MJ, Stephans RJ & Freeman G: Effects of Nitrogen Dioxide on Cell Renewal in the Rat. Arch Int Med 128: 57, 1971.

Fairchild EJ & Graham ST: Thyroid Influence on the Toxicity of Respiratory Irritant Gases, Ozone and Nitrogen Dioxide. J Pharmacol Exptl Ther 139: 177, 1963.

Fairhurst AS, Kent G & Purdy RE: Clofibrate and Vascular Smooth Muscle: Actions on Rabbit Aorta. Eur J Pharmacol 72: 323, 1981.

Fein A, Grossman RF, Jones JG, Overland E, Pitts L, Murray JF & Staub NC: The Value of Edema Fluid Protein Measurement in Patients with Pulmonary Edema. Amer J Med 67: 32, 1979.

Fenters JD, Ehrlich J, Findlay J, Spangler J & Tolkacz V: Serologic Response in Squirrel Monkeys Exposed to Nitrogen Dioxide and Influenza Virus. Amer Rev Resp Dis 104: 448, 1971.

Flick MR, Perel A, Kageler W & Staub NC: Regional Extravascular Lung Water in Normal Sheep. J Appl Physiol 46: 932, 1979.

Flick MR, Perel A & Staub NC: Leukocytes are Required for Increased Lung Microvascular Permeability after Microembolization in Sheep. Circ Res 48: 344, 1981.

Flower RJ & Blackwell GJ: Anti-inflammatory Steroids Induce Biosynthesis of a Phospholipase A_2 Inhibitor Which Prevents Prostaglandin Generation. Nature 278: 456, 1979.

Fowler AA, Giclas PC & Hyers TM: Protein and Cell Count of Bronchoalveolar Lavage Fluid from Patients with Adult Repsiratory Dis tress Syndrome. Amer Rev Resp Dis 123: S247, 1981.

Frank L, Neriishi K, Sio R & Pascual D: Protection from Paraquat-Induced Lung Damage and Lethality in Adults Rats Pretreated with Clofibrate. Toxicol Appl Pharmacol 66: 269, 1982.

Freeman G, Furiosi NJ & Haydon GB: Effects of Continuous Exposure to 0.8 ppm NO_2 on Respiration of Rats. Arch Environ Health 13: 454, 1966.

Freeman G, Crane SC, Stephens RJ & Furiosi NJ: Environmental Factors in Emphysema and a Model System with NO_2. Yale J Biol Med 40: 566, 1968.

Freeman G, Crane SC, Stephens RJ & Furiosi NJ: Pathogenesis of the Nitrogen Dioxide - Induced Lesion in the Rat Lung: A Review and Presentation of New Observations. Amer Rev Resp Dis 98: 429, 1968a.

Gage JC: Experimental Inhalation Toxicology in Methods in Toxicology, (Ed. Hayes), Blackwell Scientific Publ, Oxford & London, 1970.

Gardner DE, Holzman RS & Coffin DL: Effects of Nitrogen Dioxide on Pulmonary Cell Population. J Bacteriol 98: 1041, 1969.

Giri SN, Benson J, Siegel DM, Rice SA & Schiedt M: Effects of Pretreatment with Anti-Inflammatory Drugs on Ozone-Induced Lung Damage in Rats. Proc Soc Exptl Biol Med 150: 810, 1975.

Goodhart JM & Dewar HA: Effect of Atromid-S on Fibrinolytic Activity in Patients with Ischemic Heart Disease and Normal Blood Cholesterol Levels. Brit Med J 1966 I: 325.

Goodman & Gilman's The Pharmacological Basis of Therapeutics, 6th Ed. (AG Gilman, LS Goodman & A Gilman, eds.), Macmillan, New York, 1980.

Gordon GB: Saturated Free Fatty Acid Toxicity III: Potentiation by Chlorophenoxyisobutyrate (Clofibrate) in Strain L Cells. Res Comm Chem Pathol Pharmcol 20: 79, 1978.

Gruet

Grygl

Guido

Gump

Guyto

Guyto

Habe

Hamme

Hayn

Higg

Hine

Hohn

Hope

Gruetter DY, Gruetter CA, Barry BK, Baricos WH, Hyman AL, Kadowitz PJ & Ignarro LJ: Activation of Coronary Arterial Guanylate Cyclase by Nitric Oxide, Nitroprusside and Nitrosoguanidine-Inhibition by Calcium, Lanthanum and Other Cations, Enhancement by Thiols. Biochem Pharmacol 29: 2943, 1980.

Gryglewski RJ, Korbut R, Ocetkiewicz A, Spawinski J, Wojtaszek B & Sweis J: Lungs as a Generator of Prostacyclin-Hypothesis on Physiological Significance. Naunyn-Schmiedeberg's Arch Pharmacol 304: 45, 1978.

Guidotti TL: Toxic Inhalation of Nitrogen Dioxide: Morphologic and Functional Changes. Exp Mol Pathol 33: 90, 1980.

Gump FE: Lung Fluid and Solute Compartments in Lung Water and Solute Exchange. (NC Staub, ed.), Marcel Dekker, N.Y., 1978.

Guyton AC: Interstitial Fluid Pressure II: Pressure-Volume Curves of the Interstitial Space. Circ Res 16: 452, 1965.

Guyton AC & Lindsey AW: Effects of Elevated Left Atrial Pressure and Decreased Plasma Protein Concentration on the Development of Pulmonary Edema. Circ Res 7: 649, 1959.

Haber F & Weiss J: The Catalytic Decomposition of Hydrogen Peroxide by Iron Salts. Proc Roy Soc Lond A 147: 332, 1934.

Hammerschmidt DE, Weaver LJ, Hudson LD, Craddock PR & Jacobs HS: Association of Compliment Activation and Elevated Plasma-C5a with Adult Respiratory Distress Syndrome: Pathophysiologic Relevance and Possible Prognostic Value. Lancet 1: 947, 1980.

Haynes JB, Hyers TM, Giclas PC, Franks JJ & Petty TL: Elevated Fibrin-(ogen) Degradation Products in Adult Respiratory Distress Syndrome. Amer Rev Resp Dis 122: 841, 1980.

Higgs GA, McCall E & Youlten LJF: A Chemotatic Role for Prostaglandins Released from Polymorphonuclear Leukocytes During Phagocytosis. Brit J Pharmacol 53: 539, 1975.

Hine CH, Meyers FH & Wright RW: Pulmonary Changes in Animals Exposed to Nitrogen Dioxide, Effects of Acute Exposures. Toxicol Appl Pharmacol 16: 201, 1970.

Hohn DC, Meyers MJ, Gherini ST, Beckmann A, Markison RE & Churg AM: Production of Acute Pulmonary Injury by Leukocytes and Activated Compliment. Surgery 88: 48, 1980.

Hopewell P & Murray J: The Adult Respiratory Distress Syndrome. Ann Rev Med 27: 343, 1976.

Horvath M, Varsanyi M, Ladanyi A & Josfay A: The Effects of Azathioprine, Clofibrate and Tribenoside on Leukocyte Migration Inhibition Induced by Vascular Antigens in Vascular Disease. Allergol Immunopathol 10: 25, 1982.

Hunninghake GW, Gadek JE, Fales HM & Crystal RG: Human Alveolar Macrophage-Derived Chemotactic Factor for Neutrophils. J Clin Invest 66: 473, 1980.

Huzoor-Akbar, Patel S, Kokrady SS, Witiak DT, Newman HAI & Feller DR: Effects of Clofibrate and 6-Substituted Chroman Analogs on Human Platelet Function. Biochem Pharmacol 30: 2013, 1981.

Hyman AL & Kadowitz PJ: Pulmonary Vasodilator Activity of Prostacyclin (PGI_2) in the Intact Cat. Circ Res 45: 404, 1979.

Iliff LD: Extra-alveolar Vessels and Edema Development in Excised Dog Lungs. Circ Res 28: 524, 1971.

Ingram RH: Adult Respiratory Distress Syndrome in Harrison's Principles of Internal Medicine, 8th ed., (ed. Thorn et al.). McGraw - Hill, N.Y., 1977.

Ivanhoe F & Meyers FH: Phosgene Poisoning as an Example of Neuroparalytic Acute Pulmonary Edema: The sympathetic reflex involved. Dis of the Chest 46: 211, 1964.

Jager E, Kefalides A, Clopath P & Sinzinger H: The Effect of Clofibrate on Plasma Factor and Vascular Prostacyclin Synthesis. Pharmcol Res Comm 14: 815, 1982.

Janoff A, White R, Carp H, Harel S, Dearing R & Lee D: Lung Injury Induced by Leukocytic Proteases. Amer J Pathol 97: 111, 1979.

Jenson MS & Bainton DF: Temporal Changes in pH within the Phagocytic Vacuole of the Polymorphonuclear Neutrophilic Leukocyte. J Cell Biol 56: 379, 1973.

Jones GL & Masters CJ: On the Synthesis and Degradation of the Multiple Forms of Catalase in Mouse Liver. Arch Biochem Biophys 187: 431, 1978.

Kadowitz PJ & Hyman AL: Vasoconstrictor Effects of Thromboxane B_2 on the Canine and Feline Pulmonary Vascular Bed. J Pharmacol Exptl Ther 213: 300, 1980.

Kapanci Y, Assimacopoulos A, Zwahlen A, Irle C & Gabbiani G: "Contractile Interstitial Cells" in Pulmonary Alveolar Septa. J Cell Biol 60: 375, 1975.

King TKC, Weber B, Okinaka A, Friedman SA, Smith JP & Briscoe WA: Oxygen Transfer in Catastrophic Respiratoy Failure. Chest 65 (Suppl): 40S, 1974.

Kleb

Kleb

Klei

Koba

Koba

Lang

La T

Lave

Lee

Loyd

Lung

MacF

Malil

Manw

McGu

Klebanoff SJ: Iodination of Bacteria: A Bacteriocidal Mechanism. J Exptl Med 126: 1063, 1967.

Klebanoff SJ: Myeloperoxidase-Halide-Hydrogen Peroxide Anti-Bacterial System. J Bacteriol 95: 2131, 1968.

Kleinerman J & Cowdrey CR: The Effects of Continuous High Level Nitrogen Dioxide on Hamsters. Yale J Biol Med 40: 579, 1968.

Kobayashi T, Morita I & Murota S-I: Effect of Nitrogen Dioxide Exposure on the Prostacyclin Synthesis in Lung. Toxicol Lett 9: 373, 1981.

Kobayashi T, Miki N, Oda H, Akiyama M, Kubota K, Takahashi H & Nagasawa S: Effect of Nitrogen Dioxide Exposure on Cyclic GMP in Rat Lung. Toxicol Lett 13: 35, 1982.

Langloss JM, Hoover EA & Kahn DE: Diffuse Alveolar Damage in Cats Induced by Nitrogen Dioxide or Feline Calicivirus. Amer J Pathol 89: 637, 1977.

La Towsky LW, MacQuiddy EL & Tollman JP: Toxicology of Oxides of Nitrogen I: Toxic Concentrations. J Indust Hyg Toxicol 23: 129, 1941.

Laver MB, Hallowell P & Goldblatt A: Pulmonary Dysfunction Secondary to Heart Disease: Aspects Relevant to Anesthesia and Surgery. Anesthesiol 33: 161, 1970.

Lee CT, Fein AM, Lippmann M, Holtzman H, Kimble P & Weinbaum G: Elastolytic Activity in Pulmonary Lavage Fluid from Patients with Adult Respiratory Distress Syndrome. New Eng J Med 304: 192, 1981.

Loyd JE, Newman JH, English D, Olgetree ML, Meyrick BO & Brigham KL: Lung Vascular Effects of Phorbol Myristate Acetate in Awake Sheep. J Appl Physiol 54: 267, 1983.

Lung Program, Natl Heart & Lung Institute: Respiratory Diseases: Task Force on Problems, Research Approaches & Needs. U.S. Dept Health, Education & Welfare Publ # (NIH) 73 - 432, 1972.

MacFarland HN: Respiratory Toxicology. Essays in Toxicol 7: 121, 1976

Malik AB & van der Zee H: Thrombin Induced Pulmonary Insufficiency. Thromb Res 11: 497, 1977.

Manwaring D, Thorning D & Curreri PW: Mechanisms of Acute Pulmonary Dysfunction Induced by Fibrinogen Degradation Product D. Surgery 84: 45, 1978.

McGuire WW, Spragg RG, Cohen AB & Cochrane CG: Studies on the Pathogenesis of the Adult Respiratory Distress Syndrome. J Clin Invest 69: 543, 1982.

McNamee JE & Staub NC: Pore Models of Sheep Lung Microvascular Barrier Using New Data on Protein Tracers. Microvasc Res 18: 229, 1979.

Merrill WW, Naegel GP, Matthay RA & Reynolds HY: Alveolar Macrophage-Derived Chemotactic Factor-Kinetics of in vitro Production and Partial Characterization. J Clin Invest 65: 268, 1980.

Meyers FH, Jawetz E & Goldfien A: Review of Medical Pharmacology (7^{th} Ed), Lange Medical Publ, Palo Alto, 1980.

Milne J: NO_2 Inhalation and Bronchiolitis Obliterans. J Occup Med 11: 538, 1969.

Murray JF: Mechanisms of Acute Respiratory Failure (Conference Report). Amer Rev Resp Dis 115: 1071, 1977.

Nash G, Blennerhassett JB & Pontoppidan H: Pulmonary Lesions Associated with Oxygen Therapy and Artificial Ventilation. N Eng J Med 276: 368, 1967.

National Air Quality Criteria Advisory Committee: Air Quality Criteria for Nitrogen Oxides, U.S. Govt. Printing Office, Washington, D.C., 1971.

Nelson GO: Controlled Test Atmospheres-Principles and Techniques, Ann Arbor Science Publ, 1971.

Nicholson DP: Glucocorticoids in the Treatment of Shock and the Adult Respiratory Distress Syndrome. Clin Chest Med 3: 121, 1982.

Nicolayson G, Nicolayson A & Staub NC: A Quantitative Radioautographic Comparison of Albumin Concentration in Different Sized Lymph Vessels in Normal Mouse Lungs. Microvasc Res 10: 138, 1975.

Niewoehner DE & Hoidal JR: Lung Fibrosis and Emphysema: Divergent Responses to a Common Injury? Science 217: 359, 1982.

NIOSH: Criteria for a Recommended Standard....Occupational Exposure to Oxides of Nitrogen. Dept of Health, Education & Welfare, 1976.

Ogletree M & Brigham KL: Effects of Cyclooxygenase Inhibitors on Pulmonary Vascular Responses to Endotoxin in Unanesthetized Sheep. Prostaglandins Leukotrienes Med 8: 489, 1982.

Paintal AS: The Mechanism of Excitation of Type J Receptors and the J Reflex in Breathing: Hering-Breuer Centenary Symposium (Ciba Foundation Symposium, R Porter, ed), J & A Churchill, London, 1970.

Pattle RE: Properties, Function and Origin of the Alveolar Lining Layer. Nature (London) 175: 1125, 1955.

Pattle RE: Surface Lining of the Lung Alveoli. Physio Rev 45: 48, 1965.

Pearce ML, Yamashita J & Beazell BA: Measurement of Pulmonary Edema. Circ Res 16: 482, 1965.

Perez HD, Weksler BB & Goldstein IM: Generation of a Chemotactic Lipid from Arachidonic Acid by Exposure to a Superoxide-Generating System. Inflammation 4: 313, 1980.

Persson CGA, Ekman M & Erjefalt I: Terbutaline Preventing Permeability Effects of Histamine in the Lung. Acta Pharmacol Toxicol 42: 395, 1978.

Persson CGA, Ekman M & Erjefalt I: Vascular Anti-Permeability Effects of Beta-Receptor Agonists and Theophylline in the Lung. Acta Pharmacol Toxicol 44: 216, 1979.

Petrik P: Fine Structural Identification of Peroxisomes in Mouse and Rat Bronchiolar and Alveolar Epithelium. J Histochem Cytochem 19: 339, 1971.

Petty TL: Adult Respiratory Distress Syndrome in Pathophysiology of Respiration. (ed. MH Kryger), Wiley, N.Y., 1981.

Petty TL & Ashbaugh DG: The Adult Respiratory Distress Syndrome-Clinical Features, Factors Affecting Prognosis and Principles of Management. Chest 60: 233, 1971.

Petty TL, Reiss OK, Paul GW, Silvers GW & Elkins ND: Characteristics of Pulmonary Surfactant in Adult Respiratory Distress Syndrome Associated with Trauma and Shock. Amer Rev Resp Dis 115: 531, 1977.

Phillips RA, van Slyke DD, Hamilton PB, Dole VP, Emerson K & Archibald RM: Measurement of Specific Gravities of Whole Blood and Plasma by Standard Copper Sulfate Solutions. J Biol Chem 183: 305, 1950.

Pietra GG, Szidon JP, Levanthal MM & Fishman AP: Histamine and Interstitial Pulmonary Edema in the Dog. Circ Res 29: 323, 1971

Pietra GG, Magno M, Johns L & Fishman AP: Bronchial Veins and Pulmonary Edema, in Pulmonary Edema, (ed AP Fishman & EM Renkin), Amer Physio Soc, Bethesda, 1979.

Pietra GG, Ruttner JR & Wust W: The Lung After Trauma and Shock: Fine Structure of the Alveolar Capillary Barrier in 23 Autopsies. J Trauma 21: 454, 1981.

Platt DS & Cockrill BL: Changes in the Liver Concentrations of the NAD Coenzymes and in the Activities of Oxidoreductase Enzymes Following Treatment of the Rat with Ethyl Chlorophenoxyisobutyrate. Biochem Pharmacol 15: 927, 1966.

Porter NA, Lehman LS, Weber BA & Smith KJ: Unified Mechanism for Polyunsaturated Fatty Acid Autoxidation. Competition of Peroxy Radical Hydrogen Atom Abstraction, beta-Scission and Cyclization. J Amer Chem Soc 103: 6447, 1981.

Priego JG, Maroto ML, Pina M & Catalan RE: Effects of Etiofibrate, Clofibrate and Nicotinic Acid on Lipid Metabolism in Normolipemic Rats. Gen Pharmacol 10: 215, 1979.

Pryor WA & Lightsley JW: Mechanisms of NO_2 Reactions: Initiation of Lipid Peroxidation and the Production of Nitrous Acid. Science 214: 435, 1981.

Public Health Monograph #57: Exposure Chambers for Research in Animal Inhalation, US Dept Health, Education & Welfare.

Rapaport E, Kuida H, Haynes FW & Dexter L: Pulmonary Red Cell and Plasma Volumes and Pulmonary Hematocrit in the Normal Dog. Amer J Physiol 185: 127, 1956.

Ruegamer WR, Ryan NT, Richert DA & Westerfeld WW: The Effects of p-Chlorophenoxyisobutyrate on the Turnover Rate and Distribution Thyroid Hormone in the Rat. Biochem Pharmacol 18: 613, 1969.

Reynolds HY: Lung Inflammation: Role of Endogenous Chemotactic Factors in Attracting Polymorphonuclear Granulocytes. Amer Rev Resp Dis 127: S16, 1983.

Riddick JH, Campbell KI & Coffin DL: Histopathologic Changes Secondary to Nitrogen Dioxide Exposure in Dog Lungs. Amer J Clin Pathol 49: 239, 1969.

Robertson B: Review of Experimental Hyaline Membrane Disease. Diag Histopathol 4: 49, 1981.

Robin ED, Carey LC, Grenwik A, Glauser F & Gaudio R: Capillary Leak Syndrome with Pulmonary Edema. Arch Int Med 130: 66, 1972.

Robin ED: Permeability Pulmonary Edema, in Pulmonary Edema (AP Fishman & EM Renkin, eds), Amer Physiol Soc, Bethesda, 1979.

Roehm JN, Hadley JG & Menzel DB: Antioxidants vs Lung Disease. Arch Intern Med 128: 88, 1971.

Rowlands JR & Gause EM: Reaction of Nitrogen Dioxide with Blood and Lung Components. Arch Int Med 128: 94, 1971.

Sacks T, Moldow CF, Craddock PR, Bowers TK & Jacob HS: Oxygen Radicals Mediate Endothelial Cell Damage by Compliment-Stimulated Granulocytes. J Clin Invest 61: 1161, 1978.

Said

Salt

Schn

Schm

Seli

Shar

Shas

Shas

Shen

Sher

Sher

Shy

Simo

Snas

Said SI, Avery ME, Davis RK, Banerjee CM & El-Gohar M: Pulmonary Surface Activity in Induced Pulmonary Edema. J Clin Invest 44: 458, 1965.

Saltzman BE: Colorimetric Microdetermination of Nitrogen Dioxide in the Atmosphere. Anal Chem 26: 1949.

Schneider RC, Zapol WM & Carvalho AC: Platelet Consumption and Sequestration in Severe Acute Respiratory Failure. Amer Rev Resp Dis 122: 841, 1980.

Schmitt GH & Meyers FH: Characterization of the Acute Pulmonary Edema that Follows Vagal Section in the Guinea Pig. Am J Physiol 190: 89, 1957.

Selinger SC, Bland RD, Demling RH & Staub NC: Distribution Volumes of (^{131}I)Albumin, (^{14}C)Sucrose & ^{36}Cl in Sheep Lung. J Appl Physiol 39: 773, 1975.

Sharp JT, Bunnell IL, Griffith GT & Greene DG: The Effects of Therapy on Pulmonary Mechanics in Human Pulmonary Edema. J Clin Invest 40: 665, 1961.

Shasby DM, Fox RB, Harada RN & Repine JE: Reduction of the Edema of Acute Lung Injury by Granulocyte Depletion. J Appl Physiol 52: 1237, 1982.

Shasby DM, Vanbenthuysen KM, Tate RM, Shasby SS, McMurtry I & Repine JE: Granulocytes Mediate Actue Edematous Lung Injury in Rabbits and in Isolated Rabbit Lungs Perfused with Phorbol Myristate Acetate: Role of Oxygen Radicals. Amer Rev Resp Dis 125: 443, 1982a.

Shen TY & Winter CA: Chemical and Biological Studies on Indomethacin, Sulindac and Their Analogues. Adv Drug Res 12: 90, 1977.

Sherwin RP & Carlson A: Protein Content of Lung Lavage Fliud from Guinea Pigs Expsoed to 0.4 ppm Nitrogen Dioxide. Arch Environ Health 27: 90, 1973.

Sherwin RP & Richters V: Lung Capillary Permeability-Nitrogen Dioxide Exposure and Leakage of Tritiated Serum. Arch Intern Med 128: 61, 1971.

Shy CM & Love GJ: Recent evidence on the human health effects of NO_2. In: Nitrogen oxides and Their Effects on Health (ed. SD Lee), Ann Arbor Science, 1980.

Simons JR, Theodore J & Robin ED: Common Oxidant Lesion of Mitochondrial Redox State Produced by Nitrogen Dioxide, Ozone and High Oxygen in Alveolar Macrophages. Chest 66 (Suppl): 9S, 1974.

Snashall PD & Hughes JMB: Lung Water Balance. Rev Physio Biochem Pharmacol 89: 5, 1981.

Snashall PD, Nakahara K & Staub NC: Estimation of Perimicrovascular Fluid Pressure in Isolated Perfused Dog Lung Lobes. J Appl Physiol 46: 1003, 1979.

Srivastava SC, Smith MJ & Dewar HA: The Effect of Atromid on Fibrinolytic Activity of Patients with Ischemic Heart Disease and Hypercholesterolemia. J Atheroscler Res 3: 640, 1963.

Starling EH: On the Absorption of Fluids from the Connective Tissue Spaces. J Physiol (London) 19: 312, 1896.

Staub NC: Pathogenesis of Pulmonary Edema. Amer Rev Resp Dis 109: 358, 1974.

Staub NC: Pulmonary Edema. Physiological Reviews 54: 678, 1974a.

Staub NC, Bland R, Brigham KL, Demling R, Erdmann JR & Woolverton WA: Preparation of Chronic Lung Lymph Fistulas in Sheep. J Surg Res 19: 315, 1975.

Staub NC, Gee M & Vreim C: Mechanism of Alveolar Flooding in Acute Pulmonary Edema in Lung Liquids, (Ciba Foundation Symposium # 38) Elsevier, Amsterdam, 1976.

Staub NC, Nagano H & Pearce ML: Pulmonary Edema in Dogs, Especially Sequence of Fluid Accumulation. J Appl Physiol 22: 227, 1967.

Stephens RJ, Freeman G & Evans MJ: Early Response of Lungs to Low Levels of Nitrogen Dioxide. Arch Environ Health 24: 160, 1972.

Studer R & Potchen J: The Radioisotopic Assessment of Regional Microvascular Permeability to Macromolecules. Microvasc Res 3: 35, 1971.

Svoboda DJ & Arzanoff DL: Response of Hepatic Microbodies to a Hypolipidemic Agent, Ethyl Chlorophenoxyisobutyrate (CPIB). J Cell Biol 30: 442, 1966.

Symposium: Free Radical Pathology. Federation Proc 32: 1859, 1973.

Taylor FB Jr & Abrams ME: Effect of Surface Active Lipoprotein on Clotting and Fibrinolysis, and of Fibrinogen on Surface Tension of Surface Active Lipoprotein. Amer J Med 40: 346, 1966.

Thomas HV, Mueller PK & Lyman RL: Lipoperoxidation of Lung Lipids in Rats Exposed to Nitrogen Dioxide. Science 159: 532, 1968.

Thomas HV, Mueller PK & Wright R: Response of Rat Lung Mast Cells to Nitrogen Dioxide. J Air Pollut Control Assoc 17: 33, 1967.

Thorp JM: An Experimental Approach to the Problem of Disordered Lipid Metabolism. J Atheroscler Res 3: 351, 1963.

Tsubone H, Oda H, Suzuki AK & Kubota K: Electrocardiographic Abnormalities in Rats by Acute Exposure to Nitrogen Dioxide. Toxicol Lett 12: 125, 1982.

Vaage J: Intravascular Platelet Aggregation and Acute Respiratory Insufficiency. Circ Shock 4: 279, 1977.

Vaage J, Nicolaysen G & Waaler BA: Aggregation of Platelets and Increased Hydraulic Conductivity of Pulmonary Exchange Vessels. Acta Physiol Scand 98: 175, 1976

Vaughan TR Jr, Erdmann AJ III, Brigham KL, Woolverton WC & Staub NC: Pulmonary Transcapillary Albumin Flow: Effect of High Pressure and Increased Permeability. Amer Rev Resp Dis 109: 692, 1974.

von Nieding G & Krekeler H: Pharmakologische Beeinflussung der akuten NO_2-Wirkung auf die Lungfunktion von Gesunden und Kranken mit einer chronischen Bronchitis. Int Arch Arbeitsmed 29: 55, 1971.

von Nieding G, Krekeler H & Fuchs R: Studies of the Acute Effects of NO_2 on Lung Function: Influence on Diffusion, Perfusion and Ventilation in the Lungs. Int Arch Arbeitsmed 31: 61, 1973.

vonOettingen WF: The Toxicity & Potential Dangers of Nitrous Fumes. U.S.P.H. Bull. #272, 1941.

Wagner WD: Experimental Study of Threshold Limit of NO_2. Arch Environ Health 10: 455, 1965.

Wallace PGM & Spence AA: Adult Respiratory Distress Syndrome. Br Med J 286: 1167, 1983.

Weibel ER & Bachofen H: Structural Design of the Alveolar Septum and Fluid Exchange, in Pulmonary Edema (ed AP Fishman & EM Renkin), Amer Physiol Soc, Bethesda, 1979.

Wenzel DG & Hale TW: Toxicity of Free Fatty Acids for Cultured Rat Heart Muscle and Endothelioid Cells. I. Saturated Long-Chain Fatty Acids. Toxicol 11: 109, 1978.

Wenzel DG & Hale TW: Toxicity of Free Fatty Acids for Cultured Rat Heart Muscle and Endothelioid Cells. II. Unsaturated Long-Chain Fatty Acids. Toxicol 11: 119, 1978a.

Worthen M, Placik B, Argano B, Mac Canon DM & Luisada AA: On the Mechanism of Epinephrin-Induced Pulmonary Edema. Jap Heart J 10: 133, 1969.

Yockey CC, Eden BM & Byrd RB: The McConnell Missile Accident. JAMA 244: 1221, 1980.

Zimmerman GA, Renzetti AD & Hill HR: Functional and Metabolic Activity of Granulocytes from Patients with Adult Respiratory Distress Syndrome. Amer Rev Resp Dis 127: 290, 1983.

Zuwaylif, FH: General Applied Statistics (2nd Ed), Addison-Wesley, Reading, Mass., 1974.