XYLAZINE-INDUCED PULMONARY EDEMA

By

HAMID R. AMOUZADEH

Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
1982

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1986

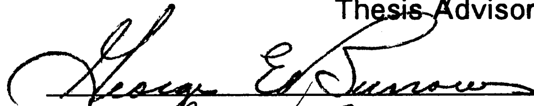
Submitted to the faculty of the Graduate College
of Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1991

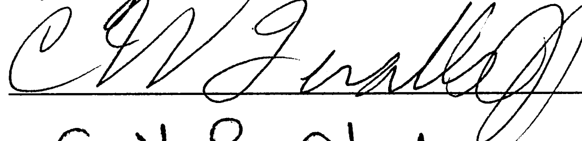
Oklahoma State Univ. Library

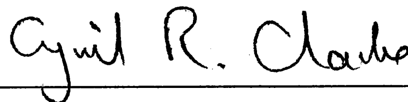
XYLAZINE-INDUCED PULMONARY EDEMA

Thesis Approved:

Thesis Advisor







Dean of the Graduate College

1407615

To

Elaheh & Elham

for their courage and patience against incredible odds

Experience does not ever err, it is only your judgment that errs in promising itself results which are not caused by your experiment.

Leonardo Da Vinci (C. 1510)

PREFACE

This dissertation is the outcome of a chance observation. During a study on the interaction between some commonly used drugs and anesthetics we noticed the pulmonary toxicity of xylazine. A few investigator have reported pulmonary edema and death after xylazine or xylazine-ketamine administration. However, the cause was not determined. This work is an attempt in elucidating the mechanism of the pulmonary toxicity of xylazine. The initial findings, presented in Chapter II, have been published in *Veterinary and Human Toxicology*. In Chapter III, pathophysiology of xylazine-induced pulmonary edema and initial attempts in determination of its etiology is described. This part has been published in *Toxicology and Applied Pharmacology*. Chapter IV further describe the pathophysiology of xylazine-induced pulmonary edema. In chapter V the direct toxicity of xylazine on pulmonary endothelium was investigated. These chapters have been submitted for publication in *Toxicology and Applied Pharmacology* and *Toxicology in vitro*, respectively.

Although the findings of this study identifies and characterizes the pulmonary toxicity of xylazine, the underlying pathophysiology of xylazine-induced pulmonary edema remains unclear. The results presented here provide clues and directions for further studies in the elucidation of the mechanism of xylazine-induced pulmonary edema.

This work is the culmination of a 14 year endeavor during which I have been overcome many obstacles and benefitted from the help of many individuals. However, I am mostly indebted to Dr. Subbiah Sangiah, my major professor. I wish to express

my sincere gratitude to Dr. Sangiah, without whose continuous support and concern this work would have not been possible. He provided an environment conducive to learning and research. I also would like to thank him and his family for their trust and friendship. I appreciate the sustained help and guidance of Dr. C. W. Qualls Jr. I also thank Dr. G. E. Burrows and Dr. C. R. Clarke for their participation in my committee and their help and guidance.

Financial support for this work was provided by Dr. Fred Radd of Ponca City, Oklahoma and the College of Veterinary Medicine Seed Grants for which I am most grateful. I also would like to thank the Department of Physiological Sciences for providing me with much needed financial support through teaching assistanship. Many individuals have helped me during my stay at Oklahoma State University. I would like to express my appreciation to the followings: Dr. C.L. Ownby for her support and encouragement, Dr. E. C. Short for help with enzyme assays, Dr. L. E. Stein for providing cell culture facilities and expertise, Dr. J.W. Wyckoff for IL-1 and TNF assays, Dr. A. Mauromoustakos for excellent advice in statistical analysis, Dr. G. Dzata for help with TNF assay, Dr. L. Faulkner for editorial help, Freidoon Alavi for his friendship and support, Colleen Marshall for help with cell culture, Dr. Dan sherban for xanthine oxidase assay, and Glen Bailey for the initial experiments. To my friend, Deanna M. Bohlen, thanks for making a trying time easier.

My family deserves my deepest appreciation. I am thankful to Mansour, Elaheh and Elham for their support and encouragement and my parents for their continuous moral and financial support, especially my mother for being the source of my inspirations.

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION AND REVIEW OF LITERATURE 1

Pharmacology of Xylazine 1
Metabolism of Xylazine 3
Toxicity of Xylazine 4
Biotransformation and Toxicity 4
Pulmonary Edema 7
Cellular and Biochemical Mediators of Acute Lung Injury 8
Leukocytes 8
Oxygen Radicals 9
Arachidonic Acid Metabolites 10
Cytokines 11
Pulmonary Vascular Endothelium and Acute Lung Injury 14
Statement of Dissertation Problem 15
References 18

II. EFFECTS OF SOME HEPATIC MICROSOMAL ENZYME INDUCERS AND INHIBITORS ON XYLAZINE-KETAMINE ANESTHESIA 30

Introduction 30
Materials and Methods 31
Drugs 32
Results 32
Duration of Anesthesia 32
Pathological Findings 33
Discussion 33
References 38

III. XYLAZINE-INDUCED PULMONARY EDEMA IN RATS 40

Introduction 40
Methods 42
Animals 42
Drugs 42
Experiment One: Determination of the Cause of Pulmonary Edema 43

Chapter Page

Experiment Two: Selection of the Edemagenic Dose of Xylazine 43
Experiment Three: Characterization of Pulmonary Edema 44
Experiment Four: Mechanism of Pulmonary edema 45
Data Analysis 45
Results 46
Determination of the Cause of Pulmonary Edema 46
Selection of the Edemagenic Dose of Xylazine 46
Characterization of Pulmonary Edema 47
Behavioral Observations 47
Pathological Findings 47
Mechanism of Pulmonary Edema 54
Discussion 54
References 62

IV. BIOCHEMICAL AND ULTRASTRUCTURAL ALTERATIONS IN XYLAZINE-INDUCED PULMONARY EDEMA 66

Introduction 66
Methods 70
Animals 70
Drugs and Chemicals 70
Assessment of Pulmonary Edema (PE) 71
Experiment One: Determination of Protein, Lactate Dehydrogenase (LDH), Xanthine oxidase (XO), Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF) in Bronchoalveolar Lavage Fluid (BALF) . . . 71
Protein Assay 71
LDH Assay 71
Xanthine Oxidase Assay 72
IL-1 Assay 72
TNF Assay 73
Experiment Two: Light and Electron Microscopy 74
Data Analysis 74
Results 74
Gross Pathological Findings 74
Pulmonary Edema 75
Protein, LDH, XO, IL-1 and TNF 75
Light and Electron Microscopy 75
Discussion 76
References 87

Chapter | Page

V. EFFECTS OF XYLAZINE ON BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS IN CULTURE ... 94

Introduction ... 94
Materials and Methods ... 95
Endothelial Cells ... 95
Chemicals and Cell Culture Medium ... 96
Experimental Procedure ... 96
Data Analysis ... 96
Results ... 97
Discussion ... 97
References ... 105

VI. SUMMARY AND CONCLUSIONS ... 106

LIST OF TABLES

Table Page

CHAPTER II

1 EFFECTS OF SOME HEPATIC MICROSOMAL ENZYME INHIBITORS AND INDUCERS ON XYLAZINE (21 mg/kg, i.m.)-KETAMINE (45 mg/k,i.m.) ANESTHESIA IN RATS 34

CHAPTER III

1 THE EFFECTS OF XYLAZINE (42 mg/kg, im) ON THE PLEURAL FLUID TO SERUM PROTEIN RATIO 51

2 SUMMARY OF SELECTED WHOLE BLOOD AND SERUM CLINICOPATHOLOGY FROM XYLAZINE (42 mg/kg, im)-TREATED RATS 52

3 THE EFFECTS OF ANTU (5 mg/kg, ip) ON THE PLEURAL FLUID TO SERUM PROTEIN RATIO 53

LIST OF FIGURES

Figure Page

CHAPTER I

1. Xylazine 1

2. Flow chart of the hypothetical mechanism of xylazine-induced pulmonary edema. MFO = Mixed function oxidases, AA = Arachidonic acid metabolites. IL-1 = Interleukin-1 and TNF = Tumor necrosis factor 17

CHAPTER II

1. Lung from a rat pretreated with SK&F 525-A (25 mg/kg, i.p.) 30 min. before administration of xylazine (21 mg/kg, i.m.) and ketamine (45 mg/kg, i.m.). Note both the perivascular (arrow) and intra-alveolar (*) edema. H & E, 1100x 36

CHAPTER III

1. Effects of (B) xylazine (21 mg/kg, im, n=6); (C) ketamine (45 mg/kg, im, n=6); (D) xylazine (42 mg/kg, im) plus ketamine (45 mg/kg, im, n=6); (E) SK&F 525-A (25 mg/kg, ip, n=6); (F) SK&F 525-A (25 mg/kg, ip) plus xylazine (21 mg/kg, im, n=6); (G) SK&F 525-A (25 mg/kg, ip) plus ketamine (45 mg/kg, im, n=6); (H) SK&F 525-A (25 mg/kg, ip) plus xylazine (21 mg/kg, im) and ketamine (45 mg/kg, im, n=6) on % LW/BW ratio. The treatment schedule is given in experiment one of Methods. (*) Significantly ($p < 0.05$) different from control (A, n=5) 48

2. Effect of xylazine (42 mg/kg, im) on % LW/BW ratio and PLE at various times. (*) Significantly ($p < 0.05$) different from control (C) 49

3. Effect of ANTU (5 mg/kg, ip) on % LW/BW ratio and PLE at various times. (*) Significantly ($p < 0.05$) different from control (C) 50

Figure Page

4. Light micrograph of lung from a rat twelve hr after xylazine (42 mg/kg, im). Note the perivascular (arrows) and intra-alveolar (*) edema (H & E, 80x) 56

5. Effects of pretreatment with adrenergic and cholinergic antagonists on xylazine-induced PE and PLE. (A) xylazine (42 mg/kg, im, n=23), (B) yohimbine (4.2 mg/kg, ip, n=6), (C) prazosin (20 mg/kg, ip, n=6), (D) tolazoline (20 mg/kg, ip, n=6), (E) yohimbine (4.2 mg/kg, ip) plus prazosin (20 mg/kg, ip, n=6), and (F) atropine (20 mg/kg, ip, n=6). The pretreatment schedules is given in experiment three of Methods. Animals were given 42 mg/kg xylazine, im after pretreatment period. (*) Significantly ($p < 0.05$) different from xylazine group. (+) Significantly ($p < 0.05$) different from control % LW/BW ratio (0.47 ± 0.01, n=16) 57

6. Effects of pretreatment with blockers of various mediators of acute lung injury on xylazine-induced PE and PLE. (A) xylazine (42 mg/kg, im, n=23), (B) dimethyl sulfoxide (7.8 g/kg, n=6), (C) allopurinol (50 mg/kg, po), (D) superoxide dismutase (20,000 U/kg, ip), (E) catalase (20,000 U/kg, ip, n=6), (F) BW755C (50 mg/kg, ip, n=6), (G) ibuprofen (50 mg/kg, ip, n=6), and (H) cystathionine (100 mg/kg, ip) plus taurine (100 mg/kg, ip, n=6). The pretreatment schedule is given in experiment three of Methods. Animals were given 42 mg/kg xylazine, im after pretreatment period. (*) Significantly ($p < 0.05$) different from xylazine group. (+) Significantly ($p < 0.05$) different from control % LW/BW ratio (0.47 ± 0.01, n=16). 59

CHAPTER IV

1. Percent lung to body weight (% LW/BW) ratio and pleural effusion volume in rats given 42 mg/kg xylazine (im, n=31). C = Control (Saline, im, n=13). (*) Significantly different from control ($p < 0.05$). 77

2A. Protein concentration in BALF from rats given 42 mg/kg xylazine (im, n=24). C = Control (saline, im, n=9). (*) Significantly different from control ($p < 0.05$) 78

Figure | Page

2B. Lactate dehydrogenase levels (LDH) in BALF from rats given 42 mg/kg xylazine (im, n=24). C = Control (saline, im, n=9). (*) Significantly different from control ($p < 0.05$) 79

2C. Xanthine oxidase levels in BALF from rats given 42 mg/kg xylazine (im, n=24). C = Control (saline, im, n=9). (*) Significantly different from control ($p < 0.05$) 80

2D. Interleukin-1 (IL-1) activity in BALF from rats given 42 mg/kg xylazine (im, n=24). C = Control (saline, im, n=9). (*) Significantly different from control ($p < 0.05$) 81

2E. Tumor necrosis factor (TNF) activity in BALF from rats given 42 mg/kg xylazine (im, n=24). C = Control (saline, im, n=9). (*) Significantly different from control ($p < 0.05$) 82

3. Light micrograph of the lung from a rat given 42 mg/kg, xylazine (im, n=4) and sacrificed 12 hr later. Note perivascular (arrowheads), peribronchiolar (arrows) and alveolar edema (*). H&E x37 83

4. Electron micrograph of the lung of a rat given 42 mg/kg, xylazine (im, n=4) and sacrificed 12 hr later. Note endothelial thinning, detachment from basement membrane or blabbing (arrowheads). Uranyl acetate and lead citrate. x9000 84

CHAPTER V

1A. Phase-contrast micrograph of the morphology of the BPAEC monolayer 0.5 hr (A) and 3.0 hr (B) after treatment with distilled water (control) 98

1B. Phase-contrast micrograph of the morphology of BPAEC monolayer 0.5 hr (A) and 3 hr (B) after treatment with 0.3 μg/ml xylazine 99

1C. Phase-contrast micrograph of the morphology of BPAEC monolayer 0.5 hr (A) and 3 hr (B) after treatment with 3 μg/ml xylazine 100

1D. Phase-contrast micrograph of the morphology of BPAEC monolayer 0.5 hr (A) and 3 hr (B) after treatment with 30 μg/ml xylazine 101

Figure		Page

1E. Phase-contrast micrograph of the morphology of BPAEC monolayer 0.5 hr (A) and 3 hr (B) after treatment with 300 μg/ml xylazine. Note elongation and retraction of cells 102

2. Effect of various concentration of xylazine (0.3 - 300 μg/ml) on viability of BPAEC after 0.5 and 3 hr incubation 103

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Xylazine, 2(2,6-dimethyl phenylamino)-4-H5,6-dihydro-1,3-thiazine hydrochloride (Bay Va 1470 or Rompun) was developed as an antihypertensive drug in Germany by Bayer, AG. The pronounced central nervous system (CNS) depressive effect of xylazine during clinical trials in human led to its introduction as sedative, analgesic and muscle relaxant for veterinary use.

In veterinary medicine, xylazine is used alone or in combination with other anesthetics and tranquilizers. The combination of xylazine and ketamine is commonly used for short surgical procedures. Ketamine, a dissociative anesthetic, produces anesthesia characterized by marked analgesia (Wright, 1982).

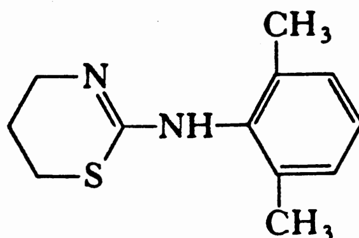


Figure 1. Xylazine

Pharmacology of Xylazine

Xylazine is an α_2-adrenergic agonist with sedative, analgesic and muscle-relaxant properties (Greene and Thurmon, 1988). The CNS effects of xylazine are

mediated mainly through α_2-adrenergic receptors (Hsu, 1981). The analgesia is mediated by peripheral and central α_2-adrenoceptor stimulation (Schmitt et al., 1974). Xylazine also has local anesthetic effects which is thought to be due to blockade of action potential and conduction velocity (Aziz and Martin, 1978). The muscle relaxant property of xylazine is due to blockade of intraneuronal impulse transmission in CNS (Booth, 1982).

Xylazine causes a transient hypertension followed by hypotension, bradycardia and heart block. The hypertension is caused by peripheral vasoconstriction mediated by postsynaptic α_2-adrenoceptors (Schmidtt et al., 1970; Antonaccio et al., 1973; Langer, 1980). Hypotension is due to a centrally mediated decrease in sympathetic outflow (Schmidtt et al., 1970; Antonaccio et al., 1973; Van Zwieten and Timmermans, 1983). The bradycardia and heart block are due to vagal stimulation, decreased CNS sympathetic output and decreased norepinephrine release in cardiac nerves by stimulation of presynaptic α_2-adrenoceptors (Schmidtt et al., 1970; Antonaccio, et al., 1973 and Langer, 1980).

The effect of xylazine on the respiratory system is minimal. However, some effects such as hypoxia and reduced respiratory rate have been observed in sheep and horses, respectively (Waterman et al., 1987; Fessel, 1970; Burns and McMullan, 1972; Hoffman, 1974; McCashin and Gabel, 1975).

Gastrointestinal transit time is prolonged in mice (Hsu, 1982), dogs (Hsu and McNeel, 1983; McNeel and Hsu, 1984), sheep (Seifelnasr et al., 1984), bears and tigers (Cooke and Kane, 1980) after administration of xylazine. This effect is attributed to the inhibitory effect of xylazine on the release of acetylcholine from Auerbach's plexus (Vizi, 1974).

The most pronounced effects of xylazine on the urinary system are glucosuria

and diuresis. These effects have been reported in cattle (Thurmon et al., 1978), cats (Hartsfield, 1980), ponies (Trim and Hanson, 1986), horses (Greene et al., 1987; Thurmon et al., 1978) and rats (Mohammad et al., 1989). The glucosuria is secondary to elevated blood glucose levels as a consequence of decreased insulin release. The increased urinary output caused by xylazine, similar to clonidine, appears to be mediated through central and renal α_2-adrenoceptors (Roman et al., 1979; Gellai and Ruffalo, 1987).

Plasma insulin levels are decreased after administration of xylazine in horses (Thurmon et al., 1982; Tranquilli et al., 1984; Greene et al., 1987), cattle (Symond and Mallinson, 1978; Eichner et al., 1979), sheep (Brockman, 1981), and dogs (Goldfine and Areiff, 1979; Benson et al., 1984). This effect is attributed to the inhibitory effects of xylazine on the α_2-adrenoceptor of pancreatic ß cells (Hsu and Hummel, 1981). Xylazine increased plasma levels of growth hormone in dogs (Hampshire and Attszuler, 1981), decreased antidiuretic hormone in horses (Greene, et al., 1986) and decreased prolactin in rats (Fayez et al., 1989).

Metabolism of Xylazine

The metabolic fate of xylazine is not well understood. In rats, xylazine is rapidly metabolized, yielding approximately twenty metabolites (Duhm et al., 1969). The major metabolite of xylazine, 1, amino-2-6-dimethylbenzene, is thought to result from oxidative or hydrolytic breakdown of the thiazine ring (Putter and Sanger, 1973). Garcia-Villar et al. (1981) found that xylazine is rapidly distributed and eliminated in horses, cattle, sheep and dogs. They suggested that the rapid elimination may be due to intense metabolism rather than renal excretion, evidenced by a lack of significant amounts of unchanged xylazine in the urine of sheep.

Toxicity of Xylazine

Untoward cardiovascular effects of xylazine, such as transient hypertension followed by prolonged hypotension, bradycardia and heart block are among the most important side effects of xylazine. However, these effects are often not fatal, and xylazine toxicity, at times life-threatening, is attributed to pulmonary edema and respiratory distress. There are few cases of xylazine toxicity reported in literature; however, most lethalities after xylazine or xylazine-ketamine anesthesia are attributed to underlying diseases and are not reported. Cases of pulmonary edema and/or death after xylazine or xylazine-ketamine administration have been reported in sheep (Uggla and Lindqvist, 1983), dogs (Kirkpatrick, 1978; Kommonen and Koskinen, 1984), a bull (Newey, 1977), a stallion (Fuentes, 1978), a pony (Clarke and Hall, 1969), rabbits (Palmore , 1990), rats (Kanniappan and Ramaswamy, 1979), and grey seals (Baker and Gatesman, 1985). Xylazine toxicity has also been reported in humans (Poklis et al., 1978; Carruthers et al., 1979; Gallanosa et al., 1981; Spoerke, et al., 1986).

Biotransformation and Toxicity

Biotransformation is the process by which the body handles endogenous chemicals and xenobiotics. Biotransformation is the sum of two separate phases through which chemicals are prepared for elimination from the body. The final outcome of these two phases is the modification of drugs or xenobiotics to a more polar molecule suitable for elimination.

In phase I, drugs or xenobiotics are made more polar by exposing or adding functional groups. The addition of hydrophilic moieties is achieved by two oxidative enzyme systems, cytochrome P-450 and flavine-monooxygenase, both of which add a hydroxyl group to drugs or xenobiotics. Other enzymes, such as hydrolases,

esterases and amidases, expose existing functional groups (Sipes and Gandolfi, 1986). In phase II, drugs or xenobiotics are conjugated by moieties such as glucuronides, sulfate, acetylates and amino acids in order to make them more suitable for excretion and elimination from the body.

The cytochrome P-450 system plays a major role in the phase I reactions. One of the most important characteristics of the cytochrome P-450 enzyme system is its ability to be induced or inhibited. A wide range of drugs and xenobiotics can alter the activity of cytochrome P-450 by induction or inhibition. Phenobarbital (PHB) and polycyclic aromatic hydrocarbons such as 3-methylcholantherene (3-MC) are two of well-studied enzyme inducers with different patterns of induction (Sipes and Gandolfi, 1986). Each of these inducers affects different isozymes of cytochrome P-450 and forms the basis of the classification of the isozymes of cytochrome P-450. Hence, isozymes of cytochrome P-450 are classified as either PHB- or 3-MC-inducible.

In most circumstances, the induction of the cytochrome P-450 reduces the toxicity of a compound, except when cytochrome P-450 metabolism leads to the formation of a reactive metabolite. In this case, inducers augment the toxicity. Mansour et al. (1988) have shown that inducers of cytochrome P-450 such as 3-MC, ß-naphthoflavone and PHB protect rats from lung injury and pulmonary edema due to oxygen toxicity. This protection was attributed to an increased pulmonary cytochrome P-450 component and peroxidase activity. In contrast, induction by PHB or 3-MC augments the toxicity of acetaminophen, which is metabolized to a reactive intermediate by cytochrome P-450 (Mitchell et al., 1973). In general, however, inducers of the cytochrome P-450 reduce the toxic effect of chemicals; whereas, inhibitors enhance the response to a drug or its toxic effects.

Inhibitors of cytochrome P-450 are important because they include a large

number of drugs used in human and veterinary therapeutics. Cimetidine (Speeg et al., 982), chloramphenicol (Christensen and Skovsted, 1969; Haplert and Neal, 1980) and ketoconazole (Mosca et al., 1985), are examples of commonly used therapeutic agents that inhibit cytochrome P-450. Cimetidine alters the pharmacokinetics of many drugs by inhibition of the hepatic cytochrome P-450 microsomal enzyme system (Bauman and Kimelblatt, 1982). Recent studies indicate that cimetidine inhibits the metabolism of drugs by reversible binding to hepatic cytochrome P-450 (Knodell et al., 1982; Speeg et al., 1982). Chloramphenicol, a broad spectrum antibiotic, inhibits the hepatic P-450 metabolism of barbiturates and prolong the anesthetic effect in various animals including mice, rats, dogs, cats, and monkeys (Adams, 1970; Adams and Dixit, 1970; Adams et al., 1977). In contrast to cimetidine, inhibition by chloramphenicol is due to irreversible binding of chloramphenicol to cytochrome P-450 (Halpert and Neal, 1980). Similar to induction, inhibition of cytochrome P-450 can have toxic consequences either by augmenting the drug effect or by causing a shift to a toxifying pathway capable of generating reactive metabolites.

In addition to toxicity resulting from drug interactions, it is also possible that biotransformation can result in the formation of reactive metabolites capable of binding to cellular proteins, causing damage. Reactions mediated by both phases of biotransformation can generate reactive metabolites (Sipes and Gandolfi, 1986; Monk, 1991). Many drugs and toxicants exert their toxic effects through the formation of reactive species. A classical example of the formation of reactive metabolites by cytochrome P-450 is the N-hydroxylation product of acetaminophen, which is conjugated by glutathione (Booth, 1982). In hepatic glutathione depletion, such as that caused by acetaminophen overdose, this highly reactive intermediate can cause hepatic necrosis. Speeg et al. (1985) have shown that inhibition of the oxidation of

acetaminophen by cimetidine, a known inhibitor of cytochrome P-450, reduces the extent of acetaminophen-induced hepatic injury in rats. With regard to acute lung injury, α-naphthylthiourea, paraquat and 4-ipomeanol cause lung injury through reactive species generated by cytochrome P-450 (Boyd, 1980).

Another mechanism for biotransformation-mediated toxicity is the possibility of a shift to a toxifying pathway. Large doses of xenobiotics may deplete cellular defense mechanisms and saturate major nontoxic pathways so that minor pathways capable of producing reactive intermediates become prominent (Boyd, 1980; Sipes and Gandolfi, 1986).

Considering the growing list of drugs and xenobiotics capable of affecting cytochrome P-450, it is important to understand the interaction between cytochrome P-450 and drugs and xenobiotics, as it could have important implications in therapeutics, where several drugs are often administered concomitantly.

Pulmonary Edema

Pulmonary edema (PE) is thought to be caused by increased pressure or increased permeability (Staub, 1984). Increased-pressure PE is characterized by the lack of cellular damage, low-protein edema fluid and prolonged course of development. In contrast, increased-permeability PE is characterized by the presence of cellular damage, protein-rich edema fluid and rapid onset (Staub, 1984). Although there might not be a clear distinction between these two forms of PE in some experimental models of acute lung injury and PE, the ratio of proteins in the edema fluid to the serum or plasma can serve as an indicator of the nature of PE. The protein concentration of the edema fluid in increased-permeability PE is usually greater that 70% (Sprung et al., 1981; Staub, 1984). A number of drugs including analgesics, antiarrhythmic agents

and opiates have been reported to cause pulmonary damage through non-cardiogenic factors, such as direct toxicity or indirect augmentation of an inflammatory reaction (Cooper et al., 1986a and 1986b). Also, toxicants such as α-naphthylthiourea, paraquat and 4-ipomeanol cause lung injury through reactive species generated by cytochrome P-450 (Boyd, 1980) which can cause endothelial injury leading to PE. The mechanism of pulmonary toxicity of these agents is not well-understood and it is possible that inflammatory mediators such as leukocytes, oxygen radicals, arachidonic acid metabolites, and cytokines in some way play a role in the etiology of acute lung injury and PE.

Cellular and Biochemical Mediators of Acute Lung Injury

Increasing evidence indicates that acute lung injury is the culmination of the activity of leukocytes and a host of inflammatory mediators of cellular origin which together cause pulmonary vascular endothelial injury and result in increased-permeability PE. Leukocytes (neutrophils and eosinophils), oxygen reactive metabolites, arachidonic acid metabolites such as prostaglandins (PG), leukotrienes (LT) and thromboxanes (TX), interleukin-1 (IL-1) and tumor necrosis factor (TNF) have been shown to be involved in various experimental models of acute lung injury.

Leukocytes. Neutrophils mediate an inflammatory response by releasing proteolytic enzymes and generating oxygen-derived free radicals which are capable of damaging pulmonary vascular endothelium and destroying lung tissues (Varani et al., 1985). Superoxide dismutase, catalase and dimethyl sulfoxide partially prevented the acute lung injury by scavenging reactive oxygen metabolites such as superoxide anion, hydrogen peroxide, and hydroxyl radicals (Varani et al., 1985; Fox et al., 1983).

Depletion of neutrophils with agents such as cyclophosphamide, nitrogen mustard and hydroxyurea reduce the severity of acute lung injury in experimental animals (Stephens et al., 1988; Shasby et al., 1982a; Johnson and Malik, 1980; Heflin and Brigham, 1981). There are also cases such as ischemia-reperfusion (Grosso et al., 1990; Deeb et al., 1990) where lung injury is independent of neutophils.

Eosinophilic pleural effusion in patients with various underlying diseases (Kokkola and Valta, 1974; Veress et al., 1979) suggests a role for eosinophils in acute lung injury. The effects of activated eosinophils on pulmonary vessels is biphasic; an initial, intense vasoconstriction followed by increased vascular permeability and PE (Hoidal, 1990). Rowen et al. (1990) have shown that eosinophils can cause acute edematous injury mediated partially by oxygen radicals in isolated perfused rat lung. Transmembrane pores formed by eosinophils' cationic proteins have been implicated in cell damage (Young et al., 1986). Activated eosinophils cause pulmonary vasoconstriction, bronchoconstriction, and vascular endothelial injury (Fujimoto et al., 1990). These results indicate that injurious effects of neutrophils and eosinophils are caused by their cytolytic proteins and oxygen radicals.

Oxygen Radicals. Oxygen radicals, generated by leukocytes, derived from xanthine oxidase, resulting from lipid peroxidation or produced during metabolism of xenobiotics, have been implicated in acute lung injury. Oxygen radicals cause oxidative stress which could cause cellular injury if cellular defense mechanisms such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (enzymatic), glutathione, vitamin E and vitamin C (non-enzymatic) are overwhelmed. Oxygen radicals can cause lipid peroxidation, which can increase membrane permeability, oxidize sulfhydryl group of proteins, disturb cellular enzyme functions, and damage

nucleic acids. Oxygen radicals derived from xanthine oxidase play an important role in ischemia-reperfusion injury in small intestine, stomach, liver, kidney, lung, heart and brain (Korthuis and Granger, 1986). Xanthine dehydrogenase is converted to xanthine oxidase upon reperfusion of a hypoxic area. Xanthine oxidase uses oxygen as substrate to generate reactive oxygen species (Grisham and McCord, 1986). It is also hypothesized that upon injury, endothelial xanthine dehydrogenase (a cytoplasmic enzyme) is converted to xanthine oxidase capable of generating oxygen radicals (Jarasch et al., 1986). Grosso et al. (1990), in a pulmonary hypoperfusion/ischemia-reperfusion model, have shown that xanthine oxidase-generated oxygen radicals are partially responsible for pulmonary capillary endothelial damage and PE. Oxygen radicals generated by neutrophils have cause acute lung injury and PE (Repine et al., 1982; Shasby et al., 1982b; McDonald et al., 1987; Patterson et al., 1989). The pulmonary toxicity of paraquat and bleomycin has been proposed to be caused by oxygen radicals generated from their metabolism (Frank, 1985). It is also likely that oxygen free radicals are generated as the result of membrane lipid peroxidation and breakdown of membrane arachidonic acids.

Arachidonic Acid Metabolites. Oxidative stress and lipid peroxidation result in the breakdown of arachidonic acid through cyclooxygenase and lipoxygenase pathways. The breakdown products of these pathways, such as prostaglandins (PG), thromboxanes (TX) and leukotrienes (LT), have been implicated in lung inflammation. Cyclooxygenase products PGE_2 and PGI_2 have vasodilatory effects, and TX cause pulmonary vasoconstriction, bronchoconstriction, and platelet and neutrophil aggregation (Henderson, 1987). Leukotrienes, which are lipoxygenase-derived products, increase vascular permeability and cause granulocyte accumulation and

adherence (Henderson, 1987). Lung injury caused by amphotericin B in rats is thought to be associated with oxidant stress and eicosanoid production but independent of neutrophils (McDonnell et al., 1988). LT were found in the bronchoalveolar lavage fluid (Stephenson et al., 1988) and PE fluid from patients with adult respiratory distress syndrome (Matthay et al., 1984). In an isolated rabbit lung model, Littner and Lott (1989) have shown that the cyclooxygenase product, thromboxane A_2 from endogenous arachidonic acid breakdown caused increased-pressure pulmonary edema. Cyclooxygenase and lipoxygenase products are released from endothelial cells *in vitro* under hyperoxic conditions (Jackson et al., 1986). The increased permeability caused by LTB_4 is thought to be neutrophil-independent (Burgess et al., 1990). LT have also been shown to stimulate the human umbilical endothelial cells to synthesize platelet-activating factor and bind neutrophils (McIntyre et al., 1986).

Cytokines. Cytokines such as IL-1 and TNF may also play a role in acute lung injury. IL-1 is produced primarily by monocytes/macrophages in response to infection and injury. Vascular endothelium, smooth muscle cells, astrocyte and microglial cells of the brain and B lymphocytes also produce IL-1 (Dinarello, 1988). Overall, IL-1 causes a number of cellular and biochemical effects which lead to congestion of vessels, formation of clot, infiltration of inflammatory cells and increased endothelial permeability (Dinarello, 1988). *In vitro*, IL-1 directly affects the vascular endothelium, causing increased adhesiveness for granulocytes (Bevilacqua et al, 1985; Cotran et al., 1986), increased synthesis of platelet-activating factor (Bussolino et al., 1986), and increased prostaglandin E_2, a vasodilator (Albrightson et al., 1985). IL-1 depresses both the levels and the activity of hepatic cytochrome P-450 in mice (Shedlofsky et al., 1987). The level of IL-1 is increased in bronchoalveolar fluid in patients with

bacterial pulmonary infection (Wilmott, 1990), in alveolar macrophages from patients with Adult Respiratory Distress Syndrome (ARDS, Jacobs, 1989), and in ventricular fluid from patients with head injury (McClain et al., 1987). IL-1 and TNF levels are increased in patients with fulminant hepatic failure (Muto et al., 1988). Elevated levels of IL-1 have also been reported in women after ovulation (Cannon and Dinarello, 1985). IL-1 bioactivity was increased in monocrotaline-treated rats (Gillespie et al., 1988). A monokine preparation containing IL-1 caused pulmonary edema and albumin leakage in rats (Gillespie et al., 1989). Goldblum et al. (1988) have shown that human IL-1 causes acute pulmonary vascular endothelial injury and lung edema in rabbits. It is suggested that the cytotoxic effects of IL-1 are caused indirectly through stimulating the release of oxygen radicals from endothelial cells (Matsubara and Ziff, 1986).

Tumor necrosis factor (TNF) is also produced mainly by monocytes/ macrophages (Fong and Lowry, 1990). TNF induces IL-1 production by the vascular endothelium (Dinarello, 1986; Nawroth et al., 1986), activates polymorphonuclear cell functions (Shalaby et al., 1985) and induces procoagulant activity of vascular endothelium (Bevilacqua et al., 1986; Nawroth and Stern, 1986). Although the production of TNF is associated with infection, elevated levels of TNF have been reported in non-infectious conditions such as cancer (Balkwill et al., 1987; Aderka, et al., 1985), thermal injury (Marano et al., 1990), renal allograft rejection (Maury and Teppo, 1987), head injury (Goodman et al., 1990), heart failure (Levine et al., 1990), hepatic failure (Muto et al., 1988) and ARDS (Roten et al., 1991). TNF production by peripheral blood mononuclear cells from anorexia nervosa patients (Schattner et al., 1990) and alveolar macrophages from patients with rheumatoid arthritis (Gosset et al., 1991) is increased. It appears that elevation of TNF is not exclusive to infectious diseases. Infusion of TNF into rats caused hypotension, metabolic acidosis, elevated

hematocrit and potassium levels, and hyperglycemia leading to death from respiratory arrest (Tracy et al., 1986). At necropsy, prominent hemorrhagic lesions were found in the lung and gastrointestinal tract (Tracy et al., 1986). In mice, administration of TNF caused peripheral blood lymphopenia and neutrophilia accompanied by necrosis in the small intestine due to endothelial injury (Remick et al., 1987). Systemic administration of TNF can cause pulmonary vascular endothelial injury and pulmonary edema in guinea pigs and rabbits (Stephens et al., 1988; Goldblum et al., 1989). This effect of TNF appears to be granulocyte-dependent, as granulocyte depletion prevents acute lung injury (Stephens et al., 1988). TNF caused generation of superoxide anion from neutrophils (Tsujimoto et al., 1986). TNF also increases endothelial cell permeability *in vitro* (Horvath et al., 1988; Henning et al., 1988; Brett et al., 1989; Shinjo et al., 1989; Royall et al., 1989; Goldblum and sun, 1990). This effect is thought to be independent of neutrophils (Horvath et al., 1988) and due endothelial cytoskeletal alteration involving G protein (Brett et al., 1989). Hocking et al. (1990) found that TNF caused neutrophil-dependent pulmonary edema in isolated, perfused guinea pig lung. The edema was attributed to increased pulmonary capillary pressure caused by thromboxanes, platelet activating factor (PAF), and increased capillary permeability mediated by PAF (Hocking, 1990). Results from studies involving endothelial cytotoxicity of TNF are contradictory and depend on the model used. TNF was not cytotoxic to human endothelium (Schuger et al., 1989, Pober and Cotran, 1990), except when TNF-pretreated human umbilical endothelial cells were transferred to a balanced salt solution (Schuger et al., 1989). It appears that TNF does not have direct endothelial cytotoxicity; rather, it increases the susceptibility of endothelium to injury. Pre-exposure of rat arterial endothelial cells to TNF increases the toxicity of activated neutrophils towards these cells (Varani et al., 1988). This is perhaps due to

generation of cytotoxic superoxide anion from neutrophils stimulated by TNF (Tsujimoto et al., 1986). TNF-stimulated eosinophils are cytotoxic to human umbilical vein endothelium, possibly through peroxidase activity of eosinophils (Slungaard et al., 1990). Inhibition of protein synthesis in bovine pulmonary endothelial cells by cycloheximide makes these cells susceptible to lysis by TNF (Nolop and Ryan, 1990).

Oxygen radicals, leukocytes, arachidonic acid metabolites and cytokines appear to cause acute lung injury; commonly the injury is due to the sum of the effects of several of these mediators. Whatever the mechanism, the ultimate target tissue of these mediators is the vascular endothelium.

Pulmonary Vascular Endothelium and Acute Lung Injury

Endothelial cells have important synthetic and metabolic functions. In addition to providing a barrier, endothelial cells synthesize prostaglandins, prostacyclin, factor VIII-related antigen and fibronectin. They also inactivate norepinephrine and adenosine and convert angiotensin I into angiotensin II (Hammersen and Hammersen, 1985). Acute lung injury caused by xenobiotics such as α-naphthylthiourea, paraquat, 4-ipomeanol, nitrofurantoin and bleomycin is thought to be the result of pulmonary epithelial or vascular endothelial oxidant injury (Boyd, 1980; Martin et al., 1985; Martin and Kachel, 1987). Injury is due to the formation of oxygen radicals during metabolism by cytochrome P-450. The isolation and culture of endothelial cells from pulmonary vessels provides a useful model to elucidate the mechanism of action of many well-known pulmonary toxicants and the role of mediators such as leukocytes, oxygen radicals, arachidonic acid metabolites and cytokines in acute lung injury. In addition, endothelial cell culture will provide a suitable *in vitro* model for the study of interrelationships among these mediators with respect to etiology of acute lung injury

caused by drugs and xenobiotics.

Statement of Dissertation Problem

Important drug interactions occur during anesthesia when a number of drugs are administered. Many toxicities and deaths have been reported after administration of xylazine or a xylazine-ketamine combination. However, the causes were not determined or were attributed to an underlying disease. Unexplained death or PE after xylazine or xylazine-ketamine administration have been reported in sheep (Uggla and Lindqvist, 1983), dogs (Kirkpatrick, 1978; Kommonen and Koskinen, 1984), a bull (Newey, 1977), a stallion (Fuentes, 1978), a pony (Clarke and Hall, 1969), rabbits (Palmore , 1990), rats (Kanniappan and Ramaswamy, 1979), and grey seals (Baker and Gatesman, 1985). These toxic effects could be the result of impaired metabolic capability of individual patients.

The purpose of the first part of this study was to assess the effects of inducers and inhibitors of cytochrome P-450 on the duration of xylazine-ketamine anesthesia. Inhibition of cytochrome P-450 was accompanied with respiratory distress, PE, pleural effusion and death. It appears that PE is one of the side effects of xylazine or xylazine-ketamine, probably xylazine. PE can be caused directly by xylazine or by metabolites resulting from the biotransformation of xylazine by cytochrome P-450. Whatever the cause, the outcome is PE and respiratory distress. The most likely target of xylazine toxicity is the lung vascular endothelium. Xylazine or its metabolites could damage pulmonary endothelial cells and cause leakage of plasma proteins into the interstitium and eventually into the pleural space. Endothelial injury is one of the common features of increased-permeability PE caused by drugs and chemicals. Alternatively, PE can be caused by xylazine-induced hemodynamic changes resulting in pulmonary

hypertension. The initial hypertensive effects of xylazine caused by peripheral vasoconstriction could result in pulmonary hypertension leading to increased vascular permeability. It is also likely that xylazine or its metabolites directly damage pulmonary endothelium and cause increased-permeability PE. Other possible mechanisms include the involvement of mediators of acute lung injury such as leukocytes, oxygen radicals, arachidonic acid metabolites, and cytokines such as IL-1 and TNF.

The purpose of this study was to confirm that xylazine causes PE, to characterize xylazine-induced PE, and to elucidate the role of each of above factors in the etiology of xylazine-induced PE (Figure 2). The results of this study will provide insight into the mechanism of xylazine-induced PE which could be useful in the therapeutic management of xylazine toxicity in animals and humans.

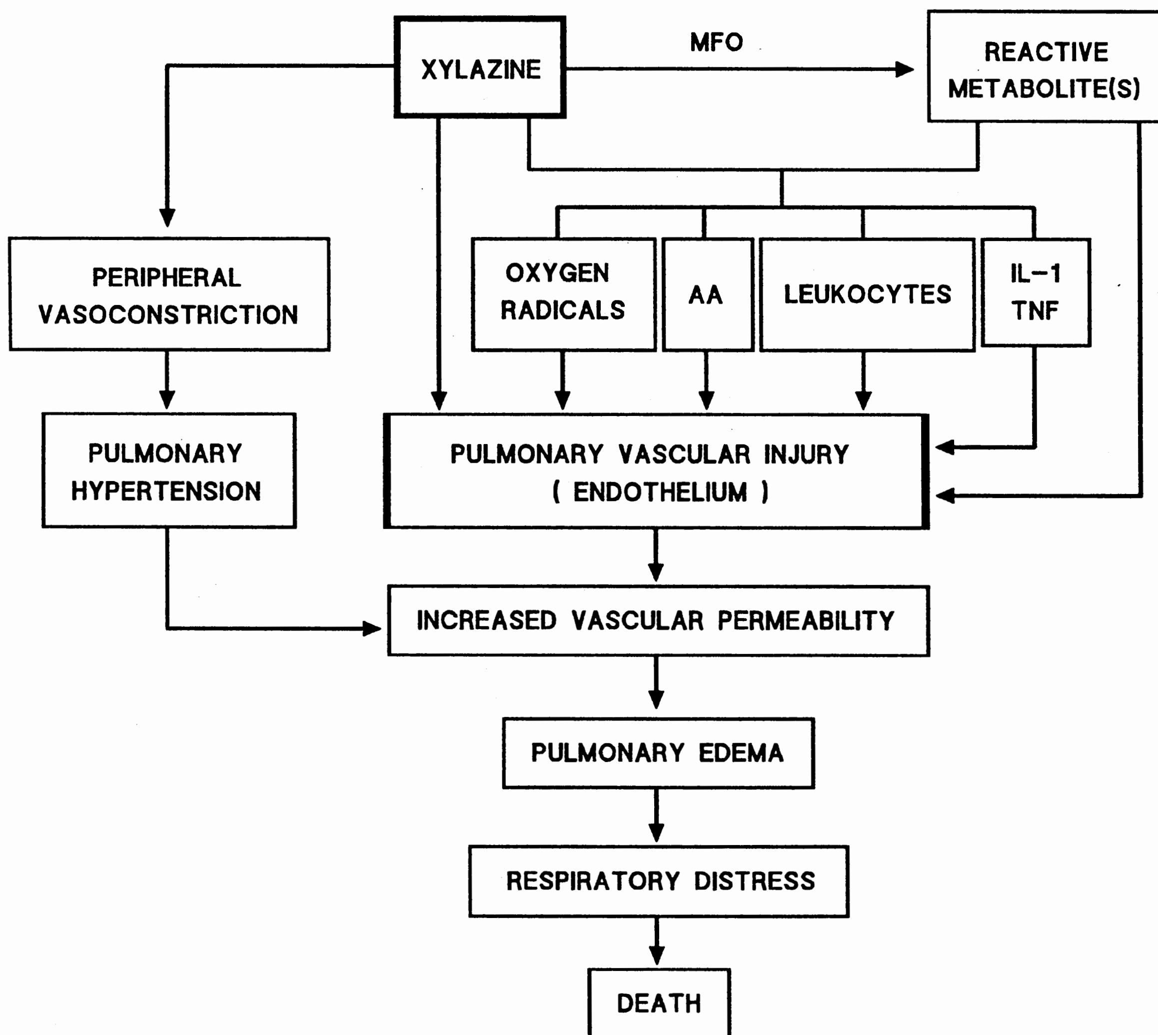


Figure 2. Flow chart of the hypothetical mechanism of xylazine-induced pulmonary edema. MFO = Mixed function oxidases, AA = Arachidonic acid metabolites. IL-1 = Interleukin-1 and TNF = Tumor necrosis factor.

References

ADAMS, H.R. (1970). Prolongation of barbiturate anesthesia by chloramphenicol in laboratory animals. *J. Am. Vet. Med. Assoc.* 157, 1908-1913.

ADAMS, H.R. AND DIXIT, B.N. (1970). Prolongation of pentobarbital anesthesia by chloramphenicol in dogs and cats. *J. Am. Vet. Med. Assoc.* 156, 902-905.

ADAMS, H.R., ISAACSON, E.L. AND MASTERS, B.S.S. (1977). Inhibition of hepatic microsomal enzymes by chloramphenicol. *J. Pharmacol. Exp. Ther.* 203, 388-396.

ADERKA, D., FISHER, S., LEVO, Y., HOLTMAN, HAHN, T. AND WALLACE, D. (1985). Cachectin/tumor-necrosis-factor production by cancer patients. *Lancet* 2, 1190.

ALBRIGHTSON, C.R., BAENZIGER, N.L. AND NEEDLEMAN, P. (1985). Exaggerated human vascular cell prostaglandin biosynthesis mediated by monocytes: role of monokines and interleukin-1. *J. Immunol.* 135, 1872-1877.

ANTONACCIO, M.J., ROBSON, R.D. AND KERWIN, L. (1973). Evidence for increased vagal tone and enhancement of baroreceptor activity after xylazine (2(2,6-dimethyl phenylamino)-4-H5,6-dihydro-1,3-thiazine) in anesthetized dogs. *Eur. J. Pharmacol.* 23, 311-315.

AZIZ, M.A. AND MARTIN, R.J. (1978). α Agonist and local anesthetic properties of xylazine. *Zbl. Vet. Med.* 25, 181-188.

BALKWILL, F., OSBORNE, R., BURKE, F., NAYLOR, S., TALBOT, D., DURBIN, H., AVERNIER, J. AND FRIES, W. (1987). Evidence for tumor necrosis factor/ cachectin production in cancer. *Lancet* 1, 1229-1230.

BAKER, J.R. AND GATESMAN, T.J. (1985). Use of carfentanil and ketamine-xylazine mixture to immobilize wild grey seals (*Halichoerus grypus*). *Vet. Rec.* 116, 208-210.

BAUMAN, J.H. AND KIMELBLATT, B.J. (1982). Cimetidine as an inhibitor of drug metabolism: Therapeutic implications and review of the literature. *Drug Intel. Clin. Pharmacol.* 16, 380-386.

BENSON, G.J., THURMON, J.C., NEFF-DAVIS, C.A., CORBIN, J.E., DAVIS, L.E., WILKINSON, B. AND TRANQUILLI, W.J. (1987). Effect of xylazine hydrochloride upon plasma glucose and serum insulin concentrations in adult pointer dogs. *J. Am. Anim. Hosp. Assoc.* 20, 791-794.

BEVILACQUA, M.P., POBER, J.S., MAJEAU, G.R., COTRAN, R.S. AND GIMBRONE, M.A. (1986). Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the action of interleukin 1. *Proc. Natl. Acad. Sci.* 83, 4533-4537, 1986.

BEVILACQUA, M.P., POBER, J.S., WHEELER, M.E., COTRAN, R.S. AND GIMBRONE, M.A. (1985). Interleukin-1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. *J. Clin. Invest.* 76, 2003-2011.

BOOTH, N.H. (1986). Non-narcotic analgesics. In *Veterinary Pharmacology and Therapeutics.* 6th Ed., (N.H. Booth and L.E. McDonald, Eds.), pp. 311-318. Iowa State University Press, Ames Iowa.

BOYD, M.R. (1980). Biochemical mechanisms in chemical-induced lung injury: role of metabolic activation. *CRC Critical Reviews in Toxicology.* 7, 103-176.

BRETT, J., GERLACH, H., NAWROTH, P., STEINBERG, S., GODMAN, G. AND STERN, D. (1989). Tumor necrosis factor/cachectin increases permeability of endothelial cell monolayers by a mechanism involving regulatory G protein. *J. Exp. Med.* 169, 1977-1991.

BROCKMAN, R.P. (1981). Effect of xylazine on plasma glucose, glucagon and insulin concentration in sheep. *Res. Vet. Sci.* 30, 383-383.

BURGESS, C.A., MCCANDLESS, B.K., COOPER, J.A. AND MALIK, A.B. (1990). Leukotriene B_4 increases pulmonary transvascular filtration by a neutrophil-independent mechanism. *J. Appl. Physiol.* 68, 1260-1264.

BURNS, S.J. AND MCMULLEN, W.C. (1972). Clinical application of BAY VA 1470 in the horse. *Vet. Med. Small Anim. Clinician* 67, 77-79.

BUSSOLINO, F., BREVIARIO, F., TETTA, C., MASSIMO, A., MANTOVANI, A. AND DEJANA, E. (1986). Interleukin-1 stimulates platelet activating factor production in cultured human endothelial cells. *J. Clin. Invest.* 77, 2027-2033.

CANNON, J.G. AND DINARELLO, C.A. (1985). Increased plasma interleukin-1 activity in women after ovulation. *Science* 227, 1247-1248.

CARRUTHERS, S.G., NELSON, M., WEXLER, H.R., AND STILLER, C.R. (1979). Xylazine hydrochloride (Rompun) overdose in man. *Clin. Toxicol.* 15, 281-285.

CHRISTENSEN, L.K. AND SKOVSTED, L. (1969). Inhibition of drug metabolism by chloramphenicol. *Lancet* 2, 1397-1399.

CLARK, K.W. AND HALL, L.W. (1969). Xylazine - A new sedative for horses and cattle. *Vet. Rec.* 85, 512-517.

COOK, C.S. AND KANE, K.K. (1980). Apparent suppression of gastrointestinal motility due to xylazine - A comparative study. *J. Zool. Anim. Med.* 11, 46-48.

COOPER, J.A.D., WHITE, D. AND MATTHAY, R.A. (1986a). Drug-induced pulmonary disease, Part 1: Cytotoxic drugs. *Am. Rev. Respir. Dis.* 133, 321-340.

COOPER, J.A.D., WHITE, D. AND MATTHAY, R.A. (1986b). Drug-induced pulmonary disease, Part 2: Non-cytotoxic drugs. *Am. Rev. Respir. Dis.* 133, 488-505.

COTRAN, R.S., GIMBRONE, M.A., BEVILACQUA, M.P., MENDRICK, D.L. AND POBER, J.S. (1986). Induction and detection of a human endothelial activation antigen in vivo. *J. Exp. Med.* 164, 661-666.

DEEB G.M., GRUM, C.M., LYNCH, M.J., GUYNN, T.P., GALLAGHER, K.P., LJUNGMAN, A.G., BOLLING, S.F. AND MORGANROTH, M.L. (1990). Neutrophils are not necessary for induction of ischemia-reperfusion lung injury. *J. Appl. Physiol.* 68, 374-381.

DINARELLO, C.A., CANNON, J.G., WOLFF, S.M., BRENHEIM, H.A., BEUTLER, B., CERAMI, A., FIGARI, I.S., PALLADINO, M.A. AND O'CONNER, J.V. (1985). Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J. Exp. Med.* 163, 1433-1450.

DINARELLO, C.A. (1988). Biology of interleukin 1. *FASEB J.* 2, 108-115.

DUHM, B., MAUL, W., MEDENWALD, M., PATZCHKE, K., AND WEGNER L.A. (1969). Untersuchungen mit radioaktiv markieterm BAY Va 1470 an Ratten. *Berlinerund Munchener Tierarztliche Wochenchrift* 82, 104-109.

EICHNER, R.D., PRIOR, R.L. AND KVASNICKA, W.G. (1979). Xylazine-induced hyperglycemia in beef cattle. *Am. J. Vet. Res.* 40, 127-129.

FAYEZ, M., AHMED, H.H. and EL-BADRY, A.A. (1989). The effect of xylazine on basal serum prolactin concentration in male rats. *Arch. Exp. Veterinarmed.* 43, 405-408.

FESSEL, L. (1970). Clinical experience with BAY VA 1470 (Rompun). *Wiener Tieraztliche Monatsschrift*, 57, 199-210.

FONG, Y. AND LOWRY, S.F. (1990). Tumor necrosis factor in the pathophysiology of infection and sepsis. *Clin. Immunol. Immunopathol.* 55, 157-170.

FOX, R.B., HARADA, R.N., TATE, R.M. AND REPINE, J.E. (1983). Prevention of thiourea-induced pulmonary edema by hydroxyl-radical scavengers. *J. Appl. Physiol.* 55, 1456-1459.

FRANK, L. (1985). Oxygen toxicity in eukaryotes. In *Superoxide Dismutase VIII*, (L.W. Oberley, Ed.), pp. 1-43. CRC press, Boca Raton, FL.

FUENTES, V.O. (1978). Sudden death in a stallion after xylazine medication. *Vet. Rec.* 102, 106.

FUJIMOTO, K., PARKER, J.C. AND KAYES, S.G. (1990). Activated eosinophils increase vascular permeability and resistance in isolated perfused rat lungs. *Am. Rev. Respir. Dis.* 142, 1414-1421.

GALLANOSA, A.G., SPYKER, D.A., SHIPE, J.R., AND MORRIS, D.L. (1981). Human xylazine overdose: A comparative review with clonidine, phenothiazine and tricyclic antidepressants. *Clin. Toxicol.* 18, 663-678.

GARCIA-VILLAR, R., TOUTAIN, P.L., ALVINERIE, M. AND RUCKEBUSCH, Y. (1981). The pharmacokinetics of xylazine hydrochloride: an interspecific study. *J. Vet. Pharmacol. Therap.* 4, 87-92.

GILLESPIE, M.N., GOLDBLUM, COHEN, D.A., AND MCCLAIN, C.J. (1988). Interleukin 1 bioactivity in the lung of rats with monocrotaline-induced pulmonary hypertension. *Proc. Soc. Exp. Biol. Med.* 187, 26-32.

GILLESPIE, M.N., OLSON, J.W., HENNING, B., COHEN, D.A., MCCLAIN, C.J. AND GOLDBLUM, S.E. (1989). Monokine-induced lung injury in rats: Similarities to monocrotaline-induced pneumotoxicity. *Toxicol. Appl. Pharmacol.* 98, 134-143.

GOLDBLUM, S.E., YONEDA, K., COHEN, D.A. AND MCCLAIN, C.J. (1988). Provocation of pulmonary vascular endothelial injury in rabbits by human recombinant interleukin-1ß. *Infec. Immun.* 56, 2255-2263.

GOLDBLUM, S.E., HENNING, B., JAY, M., YONEDA, K. AND MCCLAIN, C.J. (1989). Tumor necrosis factor α-induced pulmonary vascular endothelial injury. *Infec. Immun.* 57, 1218-1226.

GOLDBLUM, S.E. AND SUN, W.L. (1990). Tumor necrosis factor-α augments pulmonary arterial transendothelial albumin flux in vitro. *Am. J. Physiol.* 258, L57-L67.

GOLDFINE, I.D. AND ARIEFF, A.I. (1979). Rapid inhibition of basal and glucose-stimulated insulin release by xylazine. *Endocrinol.*, 105, 920-922.

GOODMAN, J.C., ROBERTSON, C.S., GROSSMAN, R.G. AND NARAYAN, R.K. (1990). Elevation of tumor necrosis factor in head injury. *J. Neuroimmunol.* 30, 213-217.

GOSSET, P., PEREZ, T., LASSALLE, P., DUQUESNOY, B., FARRE, J.M., TONNEL, A.B. AND CAPRON, A. (1991). Increased TNF-α secretion by alveolar macrophages from patients with rheumatoid arthritis. *Am. Rev. Respir. Dis.* 143, 593-597.

GREENE, S.A., THURMON, J.C., TRANQUILLI, W.J., AND BENSON, G.J. (1986). Decreased antidiuretic hormone concentration is associated with xylazine-induced diuresis in mares. *Vet. Surg.* 15, 459.

GREENE, S.A., THURMON, J.C., TRANQUILLI, W.J., AND BENSON, G.J. (1987). Yohimbine prevents xylazine-induced hypoinsulinemia and hyperglycemia in mares. *Am. J. Vet. Res.* 48, 676-678.

GREENE, S.A., AND THURMON, J.C. (1988). Xylazine-a review of its pharmacology and use in veterinary medicine. *J. Vet. Pharmacol. Therap.* 11, 295-313.

GRISHAM, M.B. AND MCCORD, J.M. (1986). Chemistry and cytotoxicity of reactive oxygen metabolites. In *Physiology of Oxygen Radicals* (A.E. Taylor, S. Matalon and P. Ward, Eds.) pp. 1-18. American Physiological Society. Bethesda, Maryland.

GROSSO, M.A., BROWN, J.M., VIDERS, D.E., MULVIN, D.W., BANERJEE, A. VELASCO, S.E., REPINE, J.E. AND HARKEN, H. (1989). Xanthine-oxidase-derived oxygen radicals induce pulmonary edema via direct endothelial cell injury. *J. Surg. Res.* 46, 355-360.

HALPERT, J. AND NEAL, R.A. (1980). Inactivation of purified rat liver cytochrome P-450 by chloramphenicol. *Molec. Pharmacol.* 17, 427-431.

HAMMERSEN, F. AND HAMMERSEN, E. (1985). Some structural and functional aspects of endothelial cells. *Basic Res. Cardiol.* 80, 491-501.

HAMPSHIRE, J. AND ATTSZULER, N. (1981). Clonidine or xylazine as provocative tests for growth hormone secretion in dog. *Am. J. Vet. Res.* 42, 1073-1076.

HARTSFIELD, S.M. (1980). The effects of acetylpromazine, xylazine, and ketamine on urine production in cats. Presented at the American college of Veterinary Anesthesiologists Annual Scientific Meeting, St. Louis, MO.

HEFLIN, A.C. AND BIRGHAM, K.L. (1981). Prevention by granulocyte depletion of increased vascular permeability of sheep lung following endotoxemia. *J. Clin. Invest.* 68, 1253-1260.

HENDERSON, W. R. (1987). Eicosanoids and lung inflammation. *Am. Rev. Respir. Dis.* 135, 1176-1185.

HENNING, B., HONCHEL, R., GOLDBLUM, S.E. AND MCCLAIN, C.J. (1988). Tumor necrosis factor-mediated hypoalbuminemia in rabbits. *J. Nutr.* 118, 1586-1590.

HOCKING, D.C., PHILLIPS, P.G. FERRO, T.J. AND JOHNSON, A. (1990). Mechanism of pulmonary edema induced by tumor necrosis factor-α. *Cir. Res.* 67, 68-77.

HOFFMAN, P.E. (1974). Clinical evaluation of xylazine as a chemical restraining agent, sedative, and analgesic in horses. *J. Am. Vet. Med. Assoc.* 164, 42-45.

HOIDAL, J.R. (1990). The eosinophil and acute lung injury. *Am. Rev. Respir. Dis.* 142, 1245-1246.

HORVATH, C. J., FERRO, T.J., JESMOK, G. AND MALIK, A.B. (1988). Recombinant tumor necrosis factor increases pulmonary vascular permeability independent of neutrophils. *Proc. Natl. Acad. Sci.* 85, 9219-9223.

HSU, W.H., LEE, P. AND BETTS, D.M. (1981). Xylazine-induced mydriasis antagonism by α-adrenergic blocking agents. *J. Vet. Pharmacol. Ther.* 4, 97-101.

HSU, W.H. AND HUMMEL, S.K. (1981). Xylazine-induced hyperglycemia in cattle: A possible involvement of α-adrenergic receptors regulating insulin release. *Endocrinol.* 109, 825-829.

HSU, W.H. (1982) Xylazine-induced delay of gastrointestinal transit in mice. *Eur. J. Pharmacol.* 83, 55-60.

HSU, W.H. AND HEMBROUGH, F.B. (1982). Intravenous glucose tolerance test in cats: Influence by acetylpromazine, ketamine, morphine, thiopental and xylazine. *Am. J. Vet. Res.* 43, 2060-2061.

HSU, W.H. AND MCNEEL, S.V. (1983). Effect of yohimbine on xylazine-induced prolongation of gastrointestinal transit in dogs. *J. Am. Vet. Med. Assoc.* 183, 297-300.

JACKSON, R.M., CHANDLER, D.B. AND FILMER, J.D. (1986). Production of arachidonic acid metabolites by endothelial cells in hyperoxia. *J. Appl. Physiol.* 61, 584-591.

JACOBS, R.F., TABOR, D.R., BURKS, A.W. AND CAMPBELL, D. (1989). Elevated interleukin-1 release by human alveolar macrophages during the adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* 140, 1686-1692.

JARASCH, E.D., BRUDER, G. AND HEID, H.W. (1986). Significance of xanthine oxidase in capillary endothelial cells. *Acta Physiol. Scand.* Suppl. 548, 49-46.

JOHNSON, A. AND MALIK, A.B. (1980). Effect of granulocytopenia on extravascular lung water content after microembolization. *Am. Rev. Respir. Dis.* 22, 561-566.

KANNIAPPAN, M. AND RAMASWAMY, V.M. (1979). A study on the toxic effects of morphine, metamidazole and xylazine. *Cheiron* 8, 159-163.

KIRKPATRICK, R.M. (1978). Use of xylazine and ketamine as a combination anesthetic. *Canine Practice* 5, 53-57.

KOKKOLA, K. AND VALTA, R. (1974). Aetiology and findings in eosinophilic pleural effusion. *Scand. J. Respir. Dis.* Suppl. 89, 159-165.

KNODELL, R.G., HOLTZMAN, J.L., CRANKSHAW, D.L., STEELE, N.M., STANLEY, L.N. (1982). Drug metabolism by rat and human hepatic microsomes in response to interaction with H_2-receptor antagonists. *Gastroentrol.* 82, 84-88.

KOMMONEN, B., KOSKINEN, L. (1984). Fluorescein angiography of the canine ocular fundus in ketamine-xylazine anesthesia. *Acta. Vet. Sacnd.* 25, 346-351.

KORTHUIS, R.J. AND GRANGER, N. (1986). Ischemia-Reperfusion Injury: Role of Oxygen-Derived Free Radicals. In *Physiology of Oxygen Radicals*, (A.E. Taylor, S. Matalon and P. Ward, Eds.), pp. 217-249. American Physiological Society. Bethesda, Maryland.

LANGER, S.Z. (1980). Presynaptic regulation of release of catecholamine. *Phramacol. Rev.* 32, 337-364.

LEVINE, B., KALMAN, J., MAYER, L., FILLIT, H.M. AND PACKER, M. (1990). Elevated circulating levels of tumor necrosis factor in sever chronic heart failure. *New Eng. J. Med.* 323, 236-241.

LITTNER, M.R. AND LOTT, F.D. (1989). Edema from cyclooxygenase products of endogenous arachidonic acid in isolated lung. *J. Appl. Physiol.* 67, 846-855.

LONIGRO, A.J., SPRAGUE, R.S., STEPHENSON, A.H., DAHMS, T.E. AND HAYEK, D.A. (1989). Leukotrienes and the microvascular permeability of acute lung injury. in *Advances in Prostaglandin, Thromboxane, and Leukotriene Research* Vol. 19, (B. Samuelssen, P.Y.K Wong and F.F. Sun, Eds.), pp. 317-320, Raven Press, New York.

MANSOUR, H., BRUN-PASCAUD, M., MARQUETTY, C., GOUGERT-POCIDALO, M.A., HAKIM, J. AND POCIDALO J.J. (1988). Protection of rat from oxygen toxicity by inducers of cytochrome P-450 system. *Am. Rev. Respir. Dis.* 137, 688-694.

MARANO, M.A., FONG, Y., MOLDAWER, L.L., WEI, H., CALVANO, S.E., TRACEY, K.J., BARIE, P.S., MANOGUE, K., CERAMI, A., SHIRES, G.T. AND LOWRY, S.F. (1990). Serum cachectin/tumor necrosis factor in critically ill patients with burns correlates with infection and mortality. *Surg. Obst. Gynecol.* 170, 32-38.

MARTIN, W.J., POWIS, G.W. AND KACHEL, D.L. (1987). Nitrofurantoin-stimulated oxidant production in pulmonary endothelial cells. *J. Lab. Clin. Med.* 105, 23-29.

MARTIN, W.J. AND KACHEL, D.L. (1987). Bleomycin-induce pulmonary endothelial cell injury: Evidence for the role of iron-catalyzed toxic oxygen-derived species. *J. Lab. Clin. Med.* 110, 153-158.

MATSUBARA, T. AND ZIFF, M. (1986). Increased superoxide anion release from human endothelial cells in response to cytokines. *J. Immunol.* 137, 3295-3298.

MATTHAY, M.A., ESCHENBACHER, W.L., GOETZL, E.J. (1984). Elevated concentrations of leukotriene D_4 in pulmonary edema fluid of patients with adult respiratory distress syndrome. *J. Clin. Immunol.* 4, 479-483.

MAURY, C.P.J. AND TEPPO, A.M. (1987). Raised serum levels of cachectin/tumor necrosis factor α in renal allograft rejection. *J. Exp. Med.* 166, 1132-1137.

MCCASHIN, F.B. AND GABEL, A.A. (1975). Evaluation of xylazine as a sedative and preanesthetic agent in horses. *Am. J. Vet. Res.* 36, 1421-1429.

MCCLAIN, C.J., COHEN, D., OTT, L., DINARELLO, C.D. AND YOUNG, B. (1987). Ventricular fluid interleukin-1 activity in patient with head injury. *J. Lab. Clin. Med.* 110, 48-54.

MCDONALD, R.J., BERGER, E.M. AND REPINE, J.E. (1987). Neutrophil-derived oxygen metabolites stimulate thromboxane release, pulmonary artery pressure increases, and weight gain in isolated perfused rat lungs. *Am. Rev. Respir. Dis.* 135, 957-959.

MCDONNELL, T.J., CHANG, S.W., WESTCOTT, J.Y. AND VOELKEL, N.F. (1988). Role of oxidant, eicosanoids, and neutrophils in amphotericin B lung injury in rats. *J. Appl. Physiol.* 65, 2195-2206.

MCINTYRE, T.M., ZIMMERMAN, G.A. AND PRESCOTT S.M. (1986). Leukotrienes C_4 and D_4 stimulate human endothelial cells to synthesize platelet-activating factor and bind neutrophils. *Proc. Natl. Acad. Sci.* 83, 2204-2208.

MCNEEL, S.V. AND HSU, W.H. (1984). Xylazine-induced prolongation of gastrointestinal transit in dogs: Reversal by yohimbine and potentiation by doxapram. *J. Am. Vet. Med. Assoc.* 185, 878-881.

MITCHELL, J.R., JOLLOW, D.J., POTTER, W.Z., DAVIS, D.C., GILLETTE, J.R. AND BORODIE, B.B. (1973). Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. *J. Pharmacol. Exp. Ther.* 187, 185-194.

MOHAMMAD, F.K., AHMED, F.A. and AL-KASSIM, N.A.H. (1989). Effect of yohimbine on xylazine-induced diuresis in rats. *Vet. Hum. Toxicol.* 31, 13-15.

MONKS, T.J., ANDERS, M.W., DEKANT, W., STEVENS, J.L. LAU, S.S. AND VAN BLADEREN, P.J. (1990). Glutathione conjugate mediated toxicities. *Toxicol. Appl. Pharmacol.* 106, 1-19.

MOSCA, P., BONAZZI, P., NOVELLI, G., JEZEQUEL, A.M. AND ORLANDI, F. (1985). *In vivo* and *in vitro* inhibition of hepatic microsomal drug metabolism by ketoconazole. *Br. J. Exp. Pathol.* 66, 737-742.

MUTO, Y., NOURI-ARIA, K.T., MEAGER, A., ALEXANDER, G.J., EDDELSTON, A.L.W.F. AND WILLIAMS, R. (1988). Enhanced tumor necrosis factor and interleukin-1 in fulminant hepatic failure. *Lancet* 2, 72-74.

NAWROTH, P.P. AND STERN, D.M. (1986). Modulation of endothelial cell hemostatic properties by tumor necrosis factor. *J. Exp. Med.* 163, 740-745.

NAWROTH P.P., BANK, I., HANDLEY, D., CASSIMERIS, J., CHESS, J. AND STERN D. (1986). Tumor necrosis factor/cachectin interacts with endothelial receptors to induce release of interleukin 1. *J. Exp. Med.* 163, 1363-1375.

NEWEY, R.M. (1977). Death of a dehorned charolais. *Vet. Rec.* 101, 335-336.

NOLPO, K.B. AND RYAN, U.S. (1990). Enhancement of tumor necrosis factor-induced endothelial cell injury by cycloheximide. *Am. J. Physiol.* 259, L123-L129.

PALMORE, W.P. (1990). A fatal response to xylazine and ketamine in group of rabbits. *Vet. Res. Comm.* 14, 91-98.

PATTERSON, C.E., BARNARD, J.W., LAFUZE, J.E., HULL, M.T., BALDWIN, S.J. AND RHODES, R.A. (1989). The role of activation of neutrophils and microvascular pressure in acute pulmonary edema. *Am. Rev. Respir. Dis.* 140, 1052-1062.

POBER, J.S. AND COTRAN, R.S. (1990). Cytokines and endothelial cell biology. *Physiol. Rev.* 70, 427-451.

POKLIS, A., MACKELL, M.A. AND CASE, E.S. (1985). Xylazine in human tissue and fluid in a case of fatal drug abuse. *J. Anal. Toxicol.* 9, 234-236.

PUTTER, J. AND SANGER, G. (1973). Chemical studies to detect residues of xylazine hydrochloride. *Vet. Med. Rev.* 2, 145-158.

REMICK, D.G., KUNKEL, R.G., LARRICK, J.W. AND KUNKEL, S.L. (1987). Acute *in vivo* effects of human recombinant tumor necrosis factor. *Lab. Invest.* 56, 583-590.

REPINE, J.E., BOWMAN, M. AND TATE, R.M. (1982). Neutrophils and lung edema. *chest* 81, 47S-50S.

ROTEN, R., MARKERT, M., FEIHL, F., SCHALLER, M., TAGAN, M. AND PERRET, C. (1991). Plasma levels of tumor necrosis factor in adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* 143, 590-592.

ROWEN, J.L., HYDE, D.M. AND MCDONALD, R.J. (1990). Eosinophils cause acute edematous injury in isolated rat lungs. *Am. Rev. Respir. Dis.* 142, 215-220.

ROYALL, J.A., BERKOW, R.L., BECKMAN, J.S., CUNNINGHAM, M.K., MATALON, S. AND FREEMAN, B. (1989). Tumor necrosis factor and interleukin 1α increase vascular endothelial permeability. *Am. J. Physiol.* 257, L399-L410.

SCHATTNER, A., STEINBOCK, M., TEPPER, R., SCHONFELD, A., VAISMAN, N. AND HAHN, T. (1990). Tumor necrosis factor production and cell-mediated immunity in anorexia nervosa. *Clin. Exp. Immunol.* 79, 62-66.

SCHMIDTT, H., FOURNADJIEV, G. AND SCHMIDTT, H. (1970). Central and peripheral effects of 2-(2,6-dimethylphenyl-amino)-4-H-5, 6-dihydro-1, 3-thiazine (Bayer 1470) on the sympathetic system. *Eur. J. Pharmacol.* 10, 230-238.

SCHMITT, H., LEDOUREC, J.C. AND PETILLOT, N. (1974). Antagonism of antinocioceptive action of xylazine, a sympathomimetic agent, by adrenoceptor and cholinoceptor blocking agents. *Neuropharmacology* 13, 295-303.

SCHUGER, L., VARANI, J., MARKS, R.M., KUNKEL, S.L., JOHNSON, K.J. AND WARD, P.A. (1989). Cytotoxicity of tumor necrosis factor-*α* for human umbilical vein endothelial cells. *Lab. Invest.* 61, 62-68.

SEIFELNASR, E., SALEH, M. AND SOLIMAN, F.A. (1974). *In Vitro* investigations on the effect of Rompun on rumen motility in sheep. *Vet. Med. Rev.* 2, 158-165.

SHALABY, M.R., AGGARWAL, B.B., RINDERKNECHT, E., SVEDERSKY, L.P., FINKLE, B.S. AND PALLADINO, M.R. (1985). Activation of human polymorphonuclear neutrophil functions by interferon- and tumor necrosis factor. *J Immunol* 135, 2069-2073.

SHASBY, M.D., FOX, R.B., HRADA, R.N. AND REPINE, J.E. (1982a). Reduction of the edema of acute hyperoxic lung injury by granulocyte depletion. *J. Appl. Physiol.* 52, 1237-1244.

SHASBY, D.M., VANBENTHUYSEN, K.M., TATE, R.M., SHASBY, S.S., MCMURTURY, I. AND REPINE, J.E. (1982b). Granulocyte mediate acute edematous lung injury in rabbit and isolated rabbit lung perfused with phorbol myristate acetate: role of oxygen radicals. *Am. Rev. Respir. Dis.* 125, 443-447.

SHEDLOFSKY, S.I., SWIM, A.T., ROBINSON, J.M., GALLICCHIO, V.S., COHEN, D.A. AND MCCLAINE, C.J. (1987) Interleukin-1 (IL-1) depresses cytochrome P-450 levels and activities in mice. *Life Sci.* 40, 2331-2336.

SHINJO, K., TSUDA, S., HAYAMI, T., ASAHI, T. AND KAWAHARADA, H. (1989). increase in permeability of human endothelial cell monolayer by recombinant lymphotoxin. *Biochem. Biophys. Res. Commun.* 162, 1431-1437.

SIPES, I.G. AND GANDOLFI, A.J. (1986). Biotransformation of toxicants. In *Casarett and Doull's Toxicology* (C.D. Klaassen, M.O. Amdur and J. Doull, Eds.), pp. 64-98. Macmillan Publishing Company, New York.

SLUNGAARD, A., VERCELLOTTI, G.M., WALKER, G., NELSON, R.D. AND JACOBS, H.S. (1990). Tumor necrosis factor *α*/cachectin stimulates eosinophil oxidant production and toxicity towards human endothelium. *J. Exp. Med.* 171, 2025-2041.

SPEEG, K.V., MITCHELL, M.C. AND MALDONADO, A.L. (1985). Additive protection of cimetidine and N-acetylcysteine treatment against acetaminophen-induced hepatic necrosis in the rat. *J. Pharmacol. Exp. Ther.* 234, 550-554.

SPOERKE, D.G., HALL, A.H., GRIMES, M.J., HONEA, B.N. AND RUMACK, B.H. (1986). Human overdose with the veterinary tranquilizer xylazine. *Am. J. Emerg. Med.* 4, 222-224.

SPRUNG, C.L., RACKOW, E.C., FEIN, I.A., JACOB, A.I. AND ISIKOFF, S.K. (1981). The spectrum of pulmonary edema: Differentiation of cardiogenic, intermediate, and noncardiogenic forms of pulmonary edema. *Am. Rev. Respir. Dis.* 124, 718-722.

STAUB, N.C. (1984). Pathophysiology of pulmonary edema. In *Edema* (N.C. Staub and A.E. Taylor, Eds.), pp. 719-746. Raven Press, New York.

STEPHENS, K. E., ISHIZAKA, A., WU, Z., LARRICK, J.W. AND RAFFIN T.A. (1988). Granulocyte depletion prevents tumor necrosis factor-mediated acute lung injury in guinea pigs. *Am. Rev. Respir. Dis.*, 138, 1300-1307.

STEPHENS, K. E., ISHIZAKA, A., LARRICK, J.W. AND RAFFIN T.A. (1988). Tumor necrosis factor causes increased pulmonary permeability and edema. *Am. Rev. Respir. Dis.*, 137, 1364-1370.

STEPHENSON, A.H., LONGRO, A.J., HYERS, T.M., WEBSTER, R.O. AND FOWLER, A.A. (1988). Increased concentration of leukotrienes in bronchoalveolar lavage fluid of patients with ARDS or at risk for ARDS. *Am. Rev. Respir. Dis.* 138, 714-719.

SYMOND, H.W., AND MALLINSON, C.B. (1978) The effect of xylazine and xylazine followed by insulin on blood glucose and insulin in dairy cow. *Vet. Rec.* 102, 27-29.

THURMON, J.C., NEFF-DAVIS, C.A., DAVIS, L.E., STOKER, R.A., BENSON, G.J. AND LOCK, T.F. (1982). Xylazine hydrochloride-induced hyperglycemia and hyperinsulinemia in thoroughbred horsed. *J. Vet. Pharmacol. Ther.* 5, 241-245.

THURMON, J.C., NELSON, D.R., HARTSFIELD, S.M. AND RUMORE, C.A. (1978). Effects of xylazine hydrochloride on urine in cattle. *Aust. Vet. J.* 54, 178-180.

TRACY, K.J., BEUTLER, B., LOWRY, S.F., MERRYWEATHER, J., WOLPE, S., MILSARK, I.W., HARIRI, R.J., FAHEY, T.J., ZENTELIA, A., ALBERT, J.D., SHIRES, G.T. AND CERAMI, A. (1986). Shock and tissue injury induced by recombinant human cachectin. *Science* 234, 470-473.

TRANQUILLI, W.J., THURMON, J.C., CORBIN, J.E., BENSON, G.J. AND DAVIS, L.E. (1984). Halothane-sparing effect of xylazine in dogs and subsequent reversal with tolazoline. *J. Vet. Pharmacol. Ther.* 7, 23-28.

TRIM, C.M. AND HANSON, R.R. (1986). Effect of xylazine on renal function and plasma glucose in ponies. *Vet. Rec.* 118, 65-67.

TSUJIMOTO, M., YOKOTA, S., VILCEK, J. AND WEISSMANN, G. (1986). Tumor necrosis factor provokes superoxide anion generation from neutrophils. *Biochem. Biophys. Res. Commun.* 137, 1094-1100.

UGGLA, A. AND LINDQVIST, A. (1983). Acute pulmonary oedema as an adverse reaction to the use of xylazine in sheep. *Vet. Rec.* 111, 42.

VAN ZWIETEN, P.A. AND TIMMERMANS, P.B.M.W.M. (1983). Cardiovascular α_2-receptors. *J. Molec. Cell. Pharmacol.* 224, 501-507.

VARANI, J., FLIGIEL, S.E.G., TILL, G.O., KUNKEL, R.G., RYAN, U.S. AND WARD, P.A. (1985). Pulmonary endothelial cell killing by human neutrophils. Possible involvement of hydroxyl radical. *Lab. Invest.* 53, 565-663.

VARANI, J., BENDELOW, M.J., SEALY, D.E., KUNKEL, S.L., GANNON, D.E., RYAN, U.S. AND WARD, P.A. (1988). Tumor necrosis factor enhances susceptibility of vascular endothelial cells to neutrophil-mediated killing. *Lab. Invest.* 59, 292-295.

VERESS, J.F., KOSS, L.G. AND SCHREIBER, K. (1979). Eosinophilic pleural effusions. *Acta Cytol.* 23, 40-44.

VIZI, E.S. (1974). Inhibitory effect of morphine and noradrenaline on neurochemical transmission. In *Proceedings, First Congress of the Hungarian Pharmacological Society*. Eds. Knoll, J. and Vizi, E.S. pp. 19-38. Budapest Akademiai Kiado.

WATERMAN, A.F. (1983). Influence of premedication with xylazine on the distribution of and metabolism of intramuscularly administered ketamine in cats. *Res. Vet. Sci.* 35, 285-290.

WILLMOTT, R.W., KASSAB, J.T., KILIAN, P.L., BENJAMIN, W.R., DOUGLAS, S.D. AND WOOD, R.E. (1990). Increased levels of interleukin-1 in bronchoalveolar washings from children with bacteria pulmonary infection. *Am. Rev. Respir. Dis.* 142, 365-368.

WRIGHT, M. (1982). Pharmacologic effects of ketamine and its use in veterinary medicine. *J. Am. Vet. Med. Assoc.* 180, 1462-1471.

YOUNG, J.D.E., PETERSON, G.B., VENGE, P. AND COHN, Z.A. (1986). Mechanism of membrane damage mediated by human eosinophil cationic protein. *Nature* 321, 613-616.

CHAPTER II

EFFECTS OF SOME HEPATIC MICROSOMAL ENZYME INDUCERS AND INHIBITORS ON XYLAZINE-KETAMINE ANESTHESIA

Introduction

Xylazine-ketamine combination is a commonly used anesthetic in veterinary medicine. Xylazine is an alpha 2-adrenergic agonist with analgesic, sedative and muscle-relaxant properties (Greene and Thurmon, 1988). Ketamine, a dissociative anesthetic, produces anesthesia characterized by marked analgesia (Wright, 1982). The combination of xylazine and ketamine produces adequate anesthesia for most short surgical procedures. The metabolic fate of xylazine is not well understood. In rats, xylazine is rapidly metabolized, yielding about twenty metabolites (Duhm, et al., 1969). Garcia-Villar et al. (1981) found that xylazine is rapidly distributed and eliminated in horses, cattle, sheep and dogs. They suggested that the rapid elimination may be due to intense metabolism rather than renal excretion, as evidenced by a lack of significant amounts of unchanged xylazine in the urine of sheep. These studies suggest that xylazine may be metabolized by hepatic cytochrome P-450, similar to the metabolism of ketamine. A major pathway of ketamine biotransformation is N-demethylation by cytochrome P-450 enzymes (White et al., 1982). The extent of metabolism varies among species (Chang and Glazko, 1974). In rats and cats, a large portion of ketamine is excreted unchanged in urine (Wright, 1982). In contrast, in humans and monkeys, most of the ketamine is metabolized (Wright, 1982).

Many commonly used drugs such as chloramphenicol, cimetidine, ketoconazole

and phenobarbital affect hepatic microsomal enzyme systems and alter the metabolism of other drugs (Haplert and Neal, 1980; Knodell et al., 1981; Sheet and Mason, 1984; Park and Breckenridge, 1981). Such drugs interactions could profoundly alter the duration and outcome of anesthesia. The purpose of this study was to investigate the effects of some inducers and inhibitors of hepatic microsomal enzyme systems on xylazine-ketamine anesthesia in rats.

Materials and Methods

Male Sprague-Dawley rats (Sasco Labs., Omaha, NE) of similar age (250-350 g) were housed with temperature, humidity and air circulation maintained at optimal levels according to the Guide for the Care and Use of Laboratory Animals. They were allowed free access to food and water and kept on a 12-hour light-dark cycle. One week after arrival, the rats were divided into pre-anesthetic treatment groups of 8 to 10 each. The control group received saline (i.p.) pretreatment. The other groups were pretreated as follows: chloramphenicol (CHPC), 100 mg/kg, i.p., 60 minutes; phenobarbital (PHB), 40 mg/kg, i.p., once daily for 4 days; SK&F 525-A, 25 mg/kg, i.p., 30 minutes; ketoconazole (KCZ), 40 mg/kg, p.o., 2 hours and cimetidine (CIM), 100 mg/kg, i.p. 60 minutes prior to intramuscular administration of xylazine (21 mg/kg) and ketamine 45 mg/kg). The duration of anesthesia was measured as the time from the loss of righting reflex to the time of return of righting reflex. The animals that died in SK&F 525-A and ketoconazole pretreated groups were necropsied. Animals showing less severe signs of respiratory distress recovered but were euthanatized and necropsied. Gross pathological changes were noted, and the lungs were removed and fixed in buffered formalin. After fixation, tissues were processed, sectioned at 6μ and stained with H & E. Mean ± SE of the duration of anesthesia for each group was

calculated and compared to control using Dunnett's test. Differences were considered significant at $P < 0.05$.

Drugs. Chloramphenicol (Chloromycetin sodium succinate, Park-Davis, Morris Plains, NJ), phenobarbital (Elsin-Sinn Inc., Cherry Hill, NJ), cimetidine (Tagamet, SK&F, Carolina, PR), ketoconazole (Nizoral, Janssen, New Brunswick, NJ), xylazine (Rompun, Mobay, Shawnee, KS) and ketamine (Ketaset, Bristol-Myers, Syracuse, NY) were purchased commercially. Ketoconazole tablets were dissolved in distilled water prior to administration with an oral needle. The combination of xylazine and ketamine was a mixture of 7 ml of 2% xylazine and 3 ml of 10% ketamine. SK&F 525-A (proadefen hydrochloride) was a gift from Smith Kline & French Labs., Swedenland, PA.

Results

Duration of Anesthesia. The animals lost their righting reflex 2-3 minutes after administration of xylazine-ketamine. All animals showed marked polyuria during anesthesia. In all animals, the urine contained a substantial amount of glucose, as indicated by strip glucose test. Pretreatment with CHPC, CIM, KCZ and SK&F 525-A significantly ($P < 0.05$) increased the duration of anesthesia to 143.7 ± 6.9, 127.6 ± 10.1, 138.7 ± 9.5 and 183.8 ± 11.4 min., respectively, as compared to that of control group (94.1 ± 5.5 min.). However, pretreatment with PHB did not affect the duration of anesthesia (101.9 ± 6.6 min.) significantly (Table 1). Animals pretreated with SK&F 525-A and ketoconazole showed respiratory distress about 6 hours after recovery from anesthesia, and the severity of respiratory distress increased within the next 24 hours, leading to death from respiratory arrest in some animals (Table 1).

Pathological Findings. Rats that died from 12 to 24 hours after pretreatment with SK&F 525-A and ketoconazole had extensive serous pleural effusion (5-10 ml). The fluid was typically straw colored and sometimes contained numerous intact red blood cells. These animals also had severe pulmonary edema affecting the alveoli and perivascular stroma (Figure 1). There were no significant lesions in animals that survived, except for increased numbers of macrophages in alveoli and the alveolar stroma.

Discussion

Pretreatment with SK&F 525-A, chloramphenicol, ketoconazole and cimetidine significantly increased the duration of anesthesia, as expected. However, phenobarbital, a known inducer of cytochrome P-450, did not affect the duration of anesthesia. The increase in duration of anesthesia in animals pretreated with known cytochrome P-450 inhibitors SK&F 525-A, CHPC, KCZ and CIM indicates that cytochrome P-450 enzymes are involved in the metabolism of xylazine-ketamine (Haplert and Neal, 1980; Knodell et al., 1981; Sheet and Mason, 1984; Park and Breckenridge, 1981). The lack of a significant change in the duration of anesthesia with phenobarbital pretreatment suggests that phenobarbital inducible cytochrome P-450 isoenzymes are not involved in the metabolism of xylazine-ketamine.

Death due to pulmonary edema after xylazine and xylazine-ketamine administration has been reported. Uggla and Lindqvist (1983) reported a case of xylazine-induced pulmonary edema and death in sheep. They also noted eight cases of acute pulmonary edema among fifty sheep sedated with xylazine. A case of death due to pulmonary edema has been reported in a Poodle anesthetized with xylazine-ketamine for angiography (Kommonen and Koskinen, 1984). Two deaths occurred in

TABLE 1

EFFECTS OF SOME HEPATIC MICROSOMAL ENZYME INHIBITORS AND INDUCERS ON XYLAZINE (21 mg/kg, i.m.)-KETAMINE (45 mg/kg,i.m.) ANESTHESIA IN RATS

Pretreatment	Dose (mg/kg)	Route	Time#	Duration of anesthesia (min)	n	Dead
Control (saline)	___	i.p.	_____	94.1 ± 5.5	10	0
Chloramphenicol	100	i.p.	60 min	143.7 ± 6.9*	10	0
Cimetidine	100	i.p.	60 min	127.6 ± 10.1*	10	0
Ketoconazole	40	p.o.	2 hrs	138.7 ± 9.5*	10	2
SK&F 525-A	25	i.p.	30 min	183.8 ± 11.4*	8	7
Phenobarbital§	40	i.p.	4 days	101.9 ± 6.6	9	0

* Significantly different from control at $P < 0.05$.

§ Once daily

Pretreatment time before administration of xylazine-ketamine.

German Shepards within 24 hours of receiving xylazine and ketamine (Kirckpatrick, 1978). But the cause of death was not reported. Kanniappan and Ramaswamy (1979) noted death due to severe dyspnea within 30 hours after injection of xylazine in rats, whereas no similar death occurred in mice receiving xylazine. In our study 7 out of 8 and 2 out of 10 rats pretreated with SK&F 525-A and ketoconazole, respectively, died from pulmonary edema within 24 hours. The reported cases of death and those found in our studies are perhaps due to impairment of metabolism of xylazine and ketamine or species variation in metabolic disposition.

The impaired metabolism could lead to increased plasma half-life of the parent drug and/or formation of reactive metabolites which could both play a role in pathophysiology of pulmonary edema. Pulmonary edema is classified according to its etiology. Hemodynamic edema is caused by increased vascular pressure and permeability edema is caused by increased vascular permeability (Staub, 1984). Hemodynamic edema is characterized by a relatively slow course in contrast to permeability edema which is characterized by rapid progression (Staub, 1984). Pulmonary edema observed in our study could have resulted from the hemodynamic effects of xylazine and/or ketamine. Alternatively, xylazine and/or ketamine or their potential reactive metabolites could damage pulmonary vascular endothelium leading to increased permeability.

Xylazine is known to have deleterious cardiovascular effects such as bradycardia, hypotension and varying degrees of heart block (Greene, and Thurmon, 1988). On the other hand ketamine has been shown to produce an elevation of mean aortic pressure, pulmonary arterial pressure and central venous pressure (Wright, 1982).

If pulmonary edema results from the cardiovascular effects of xylazine and/or

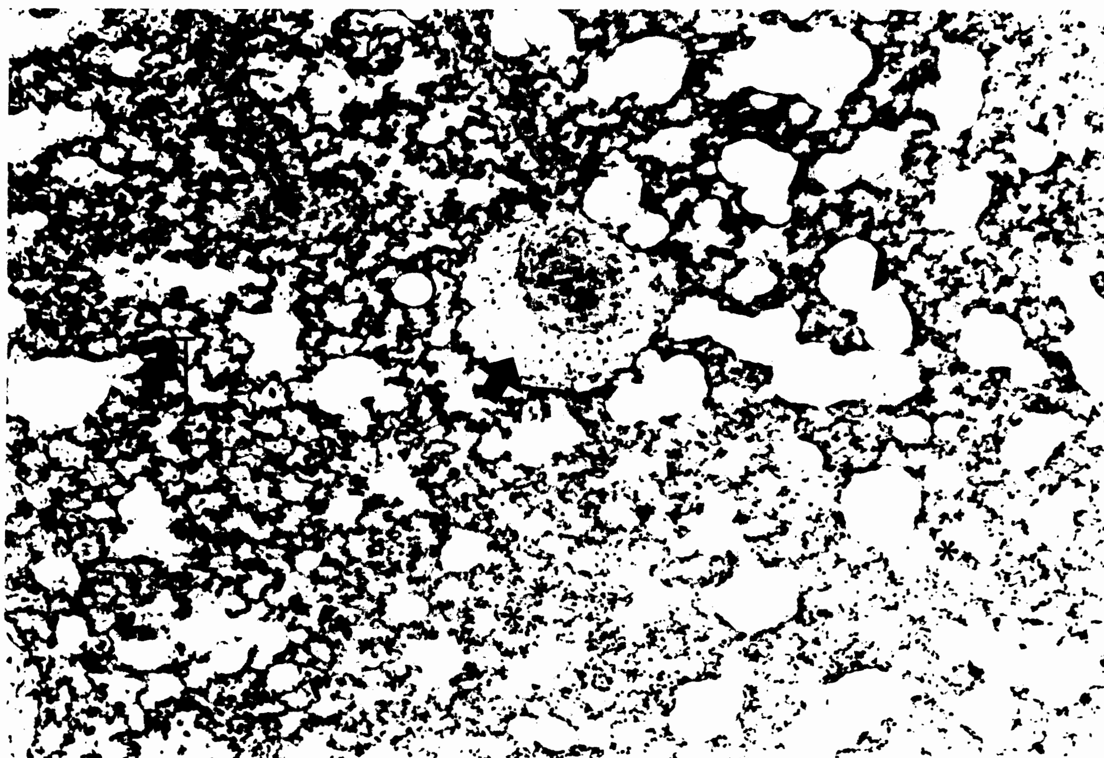


Figure 1. Lung from a rat pretreated with SK&F 525-A (25 mg/kg, i.p.) 30 min. before administration of xylazine (21 mg/kg, i.m.) and ketamine (45 mg/kg, i.m.). Note both the perivascular (arrow) and intra-alveolar (*) edema. H & E, 1100x.

ketamine due to the inhibition of their metabolism and increased plasma half-life, there should be some cases of pulmonary edema in the CHPC and CIM pretreated groups. We did not observe any signs of respiratory distress and death in the CHPC and CIM pretreated group. This suggests that hemodynamic changes as the cause of pulmonary edema are unlikely. Also, it appears that the hemodynamic pulmonary edema follows a slower course as compared to permeability pulmonary edema (Staub, 1984). The presence of large amount of edema fluid and death within 24 hours indicates an acute course of events which perhaps are the result of vascular endothelial damage by potential reactive metabolites similar to that caused by α-naphthylthiourea (Cunningham and Hurley, 1971; Boyd and Neal, 1976). The lack of respiratory distress and death in CHPC and CIM pretreated groups could mean that CHPC and CIM inhibit specific isoenzymes of cytochrome P-450 which are not involved in the toxicity while SK&F 525-A and KCZ inhibit isoenzymes which are involved in xylazine-ketamine toxicity.

References

BOYD, M.R. AND NEAL, R.A. (1976). Studies on the mechanism of toxicity and of development of tolerance to the pulmonary toxin α-naphthylthiourea (ANTU). *Drug Metab. Disp.* 4, 314-322.

CHANG, T, AND GLAZKO, A.J. (1974). Biotransformation and disposition of ketamine. *Int. Anesthesiol. Clin.* 12, 157-177.

CUNNINGHAM, A.L. AND HURLEY, J.V. (1971). Alpha-naphthyl-thiourea-induced pulmonary oedema in the rat: A topographical and electron microscope study. *J. Path.* 106, 25-35.

DUHM, B., MAUL, W., MEDENWALD, M., PATZCHKE, K., AND WAGNER L.A. (1969). Untersuchungen mit radioaktiv markieterm BAY Va 1470 an Ratten. *Berlinerund Munchener Tierarztliche Wochenchrift* 82, 104-109.

GARCIA-VILLAR, R., TOUTAIN, P.L., AND ALVINERIE, M. (1981). The pharmacokinetics of xylazine hydrochloride: an interspecific study. *J. Vet. Pharmacol. Therap.* 4, 87-92.

GREENE, S.A., AND THURMON, J.C. (1988). Xylazine-a review of its pharmacology and use in veterinary medicine. *J. Vet. Pharmacol. Therap.* 11, 295-313.

HALPERT, J. AND NEAL, R.A. (1980). Inactivation of purified rat liver cytochrome P-450 by chloramphenicol. *Molec. Pharmacol.* 17, 427-431.

KANNIAPPAN, M. AND RAMASWAMY, V.M. (1979). A study on the toxic effects of morphine, metamidazole and xylazine. *Cheiron* 8, 159-163.

KIRKPATRICK, R.M. (1978). Use of xylazine and ketamine as a combination anesthetic. *Canine Practice* 5, 53-57.

KNODELL, R.G., HOLTZMAN, J.L., CRANKSHAW, D.L., STEELE, N.M., STANLEY, L.N. (1982). Drug metabolism by rat and human hepatic microsomes in response to interaction with H_2-receptor antagonists. *Gastroentrol.* 82, 84-88.

KOMMONEN, B., KOSKINEN, L. (1984). Fluorescein angiography of the canine ocular fundus in ketamine-xylazine anesthesia. *Acta. Vet. Sacnd.* 25, 346-351.

PARK, B.K. AND BRECKENRIDGE, A.M. (1981). Clinical implication of enzyme induction and enzyme inhibition. *Clin. Phamacokin.* 6, 1-24.

SHEET, J.J., MASON, J.I. (1984). Ketoconazole: a potent inhibitor of cytochrome P-450-dependent drug metabolism in rat liver. *Drug Metab. Dispo.* 12, 603-606.

STAUB, N.C. (1984). Pathophysiology of pulmonary edema. In *Edema* (N.C. Staub and A. E. Taylor, Eds.), pp. 719-746. Raven Press, New York.

UGGLA, A. AND LINDQVIST, A. (1983). Acute pulmonary oedema as an adverse reaction to the use of xylazine in sheep. *Vet. Rec.* 111, 42.

WHITE, P.F., WAY, W.L., TREVOR, A.J. (1982). Ketamine-Its pharmacology and therapeutic uses. *Anesthesiology* 56, 119-136.

WRIGHT, M. (1982). Pharmacologic effects of ketamine and its use in veterinary medicine. *J. Am. Vet. Med. Assoc.* 180, 1462-1471.

CHAPTER III

XYLAZINE-INDUCED PULMONARY EDEMA IN RATS

Introduction

Xylazine-ketamine combination is a commonly used anesthetic in veterinary medicine. Xylazine is an α_2-adrenergic agonist with analgesic, sedative and muscle-relaxant properties (Greene et al., 1988). Ketamine, a dissociative anesthetic, produces anesthesia characterized by marked analgesia (White, 1982). In a recent study (Amouzadeh et al., 1989) on the effects of inhibitors and inducers of cytochrome P-450 on the duration of xylazine-ketamine anesthesia, pretreatment of rats with SK&F 525-A and ketoconazole prolonged the duration of anesthesia, but caused acute lung injury leading to death from pulmonary edema (PE), pleural effusion (PLE) and respiratory distress. Cases of PE and/or death after xylazine or xylazine-ketamine administration have been reported in sheep (Uggla and Lindqvist, 1983), dog (Kirkpatrick, 1978; Kommonen and Koskinen, 1984), and rat (Kanniappan and Ramaswamy 1979; Amouzadeh, et al., 1989). Xylazine toxicity has also been reported in humans (Poklis et al., 1978; Carruthers et al., 1979; Gallanosa et al., 1981). Xylazine causes a transient hypertension followed by hypotension, bradycardia and heart block. The hypertension is caused by peripheral vasoconstriction mediated by postsynaptic α-adrenoceptors (Schmidtt et al., 1970; Antonaccio et al., 1973; Langer, 1980). Hypotension is due to centrally mediated decrease in sympathetic outflow (Schmidtt et al., 1970; Antonaccio et al., 1973; Van Zwieten and Timmermans, 1983). Bradycardia and heart block are due to vagal stimulation,

decreased CNS sympathetic output and decreased norepinephrine release in cardiac nerve by stimulation of presynaptic α_2-adrenoceptor (Schmidtt et al., 1970; Antonaccio, et al., 1973 and Langer, 1980). It appears that the cause of PE and death is xylazine.

PE is thought to be caused by either increased pressure (cardiogenic) or increased permeability (non-cardiogenic). Increased-pressure PE in characterized by lack of cellular damage, low protein edema fluid and benign course of development. In contrast, increased-permeability PE is characterized by presence of cellular damage, protein-rich edema fluid and rapid onset (Staub, 1984). A number of drugs including analgesics, antiarrhythmic agents and opiates have been reported to cause pulmonary damage through non-cardiogenic factors such as direct toxicity or indirect augmentation of an inflammatory reaction (Cooper et al., 1986a and 1986b). Inflammatory mediators such as arachidonic acid breakdown products and oxygen radicals generated by neutrophils or xanthine oxidase have been implicated in experimental lung injury (Shasby et al., 1982; Birgham, 1985; Grosso et al., 1989). In addition, toxicants such as α-naphthyltiourea, paraquat and 4-ipomeanol cause lung injury through reactive species generated by cytochrome P-450 (Boyd, 1980). The role of reduced glutathione (GSH) in protection of lung from injury caused by oxidant stress or reactive species is well-established (Adams et al., 1983; Minchin and Boyd et al., 1983). GSH has been shown to protect lung against injury induced by paraquat and 4-ipomeanol (Boyd et al., 1982; Hagen et al., 1986).

The pulmonary toxicity of xylazine could be caused directly by xylazine itself or its metabolite(s) or indirectly through inflammatory mediators of acute lung injury such as arachidonic acid breakdown products and oxygen radicals.

The objectives of this study were to confirm that xylazine causes PE, to

characterize the pathological changes by comparison to α-naphthylthiourea (ANTU), a known edemagenic agent, and to elucidate roles of hemodynamic factors, metabolites, arachidonic acid breakdown products and oxygen radicals in etiology of xylazine-induced PE.

Methods

Animals:

Specific virus antibody-free male Sprague-Dawley rats (Sasco Labs., Omaha, NE) of similar age and weight (225-250 g) were housed with temperature, humidity and air circulation maintained according to the Guide for the Care and Use of Laboratory Animals (NIH, 1985). They were allowed free access to food and water and were kept on a 12-hour light-dark cycle. One week after arrival, the rats were randomly assigned to various treatment groups.

Drugs:

Allopurinol, yohimbine, polyethylene glycol-conjugated superoxide dismutase and catalase, dimethyl sulfoxide (DMSO), ibuprofen, prazosin, tolazoline, atropine sulfate, cystathionine, taurine (Sigma Chemical Company, St. Louis, MO), xylazine (Gemini , Rockville centre, NY), ketamine (Ketaset , Labs, Syracuse, NY) and α-naphthylthiourea (Eastman Kodak, Rochester, NY) were purchased commercially. SK&F 525-A and BW755C were gifts from Smith French & Kline Labs, Swedenland, PA and Wellcome Research Laboratories, Beckenham Kent, UK, respectively. The combination of xylazine and ketamine was a mixture of 7 ml of 2 % xylazine and 3 ml of 10% ketamine. α-naphthylthiourea (ANTU) suspension was prepared in olive oil. DMSO was diluted to a 50 % solution with distilled water before administration.

Ibuprofen was dissolved in propylene glycol. The other compounds were dissolved in distilled water.

Experiment One: Determination of the Cause of Pulmonary Edema

Animals were randomly assigned to eight groups of six. Each group received one of the following treatments: saline; xylazine (21 mg/kg, im); ketamine (45 mg/kg, im); xylazine (21 mg/kg, im) plus ketamine (45 mg/kg, im); SK&F 525-A (50 mg/kg, ip); SK&F 525-A (50 mg/kg, ip) 30 min before xylazine (21 mg/kg, im); SK&F 525-A (50 mg/kg, ip) 30 min before ketamine (45 mg/kg, im); and SK&F 525-A (50 mg/kg, ip) 30 min before xylazine (21 mg/kg, im) plus ketamine (45 mg/kg, im). Animals were killed by decapitation 24 hr after the administration of drugs. The lungs and hearts were removed *en block*. The lungs were separated from the hearts, trimmed of fat and connective tissues, blot-dried with gauze and their wet-weight was measured. Percent LW/BW ratios were compared as an indicator of PE.

Experiment Two: Selection of the Edemagenic Dose of Xylazine

Preliminary studies with 42 mg/kg xylazine indicated that this may be the appropriate dose to cause PE and PLE. In order to select an appropriate dose which caused PE and PLE with least number of acute death, twelve rats were randomly assigned to two groups of six. They received intramuscular injection of either 42 mg/kg or 63 mg/kg of xylazine and were killed by decapitation 24 later. The lungs and hearts were removed *en block*. The lungs were separated from the hearts, trimmed of fat and connective tissues, blot-dried with gauze and their wet weight was measured. Pleural fluid was collected and its volume was measured.

Experiment Three: Characterization of Pulmonary Edema

Animals were randomly assigned to two groups of 24 each. One group received xylazine (42 mg/kg, im) and the other group received α-naphthylthiourea (5 mg/kg, ip). The control group consisted of six animals which received vehicles (saline or olive oil). After administration of drugs the animals were observed for signs of behavioral changes such as convulsion, sedation, respiratory distress, polyuria (qualitatively) and glucosuria (glucose strip test, Keto-Diastix , Miles Inc., Elkhart, IN). They were killed by decapitation at 3, 6, 12 and 24 hr after administration of xylazine or ANTU. Trunk blood was collected with and without EDTA. Gross pathological changes were noted. The volume of pleural fluid and wet-lung weight were measured. The blood and pleural fluid were centrifuged at 2000 X g for 15 min and serum and supernatant, respectively, were collected and frozen. Lung tissues were fixed in Carson's modified buffered formalin. After fixation, tissues were routinely processed, sectioned at 6 μm and stained with H & E for histological examination.

Total and differential white blood cell counts (WBC) and hematocrit (HCT) were measured. Serum and pleural fluid protein electrophoresis was done using Helena Serum Protein Electrophoresis Procedure (Helena Labs., Beaumont, TX). Total protein, lactate dehydrogenase (LDH), albumin, Na, K, and Cl were determined using Roche COBAS-MIRA diagnostic instrument (Roche Diagnostic Systems, Nutley, NJ). Total protein was determined according to Biuret method. Albumin was determined by modification of bromocresol green colorimetric method. LDH level was determined using Dri-STAT Reagent (Beckman, Carlsbad, CA). Na, K, and Cl were measured by ion-specific electrodes.

Pulmonary edema was assessed by comparison of percent lung to body weight ratios (% LW/BW) of treated groups to that of control. The volume of pleural fluid,

where present, was measured. The ratios of total protein and various electrophoretic protein fractions in pleural fluid to those in serum were used as an indicator of the nature of pulmonary edema.

Experiment Four: Mechanism of Pulmonary Edema

Animals were randomly assigned to pretreatment groups of six. Each group received one of the following pretreatments: yohimbine (4.2 mg/kg, ip, 10 min); prazosin (20 mg/kg, ip, 10 min); tolazoline (20 mg/kg, ip, 10 min); yohimbine (4.2 mg/kg, ip, 10 min) plus prazosin (20 mg/kg, ip, 10 min); atropine (20 mg/kg, ip, 10 min); dimethyl sulfoxide (7.8 g/kg, ip, 30 min); allopurinol (50 mg/kg, po, 24, 12 and 2 hr); superoxide dismutase (20,000 U/kg, ip, 30 min); catalase (20,000 U/kg, ip, 30 min); BW755C (50 mg/kg, po, 30 min before xylazine and every 6 hr for 24 hr); ibuprofen (50 mg/kg, ip, 30 min before xylazine and every 6 hr for 24 hr); cystathionine (100 mg/kg, ip, 30 min) plus taurine (100 mg/kg, ip 30 min). After pretreatment period, the animals were given 42 mg/kg xylazine intramuscularly. Control animals received either vehicles (propylene glycol or distilled water, $n=16$) or 42 mg/kg xylazine ($n=24$). Animals were killed by decapitation 24 hr after the administration of drugs. The lungs and hearts were removed *en block*. The lungs were separated from the hearts, trimmed of fat and connective tissues, blot-dried with gauze and their wet-weight was measured. Pleural fluid was collected and its volume was measured.

Data Analysis:

The mean $\pm$ SE of percent lung to body weight ratio, PF/S total and individual protein ratios, pleural fluid volume, total and differential WBC counts, HCT, Na, K, Cl

and serum LDH were calculated. In experiment one the effects of xylazine, ketamine and SK&F 525-A were evaluated by analysis of variance for 2^3 factorial experiment using SAS General Linear Model (GLM) procedure. Data from experiment two were analyzed using unpaired Student t-test. In experiments three and four the data were analyzed by one-way analysis of variance using SAS General Linear Model (GLM) procedure. The significance of treatment means as compared to control values was determined by Dunnett's test. A probability level of $P < 0.05$ was considered significant for all experiments.

Results

Determination of the Cause of Pulmonary Edema

Xylazine, SK&F 525-A plus xylazine, and SK&F 525-A plus xylazine and ketamine increased ($p < 0.05$) the % LW/BW ratio as compared to control. Ketamine, SK&F 525-A, SK&F 525-A plus ketamine, and xylazine plus ketamine did not affect the % LW/BW ratio. There was no significant interaction ($p > 0.05$) between xylazine and ketamine with regards to % LW/BW ratios. However, xylazine-induced changes in %LW/BW ratios were enhanced by SK&F 525-A (Figure 1).

Selection of the Edemagenic Dose of Xylazine

At 42 mg/kg, % LW/BW ratio was 0.89 ± 0.05, and PLE was 5.7 ± 1.5 ml. At 63 mg/kg, % LW/BW ratio was 0.85 ± 0.03, and PLE was 4.3 ± 1.0 ml. The mortality was 50 % in each group. Since there was no difference between the 42 and 63 mg/kg doses of xylazine with regard to % LW/BW ratio and PLE, the lower dose of xylazine (42 mg/kg, im) was used in experiments two, three and four.

Characterization of Pulmonary Edema

Percent LW/BW ratio was increased ($p < 0.05$) in both xylazine- (Figure 2) and ANTU-treated (Figure 3) rats as compared to control. PLE in xylazine-treated rats increased to a maximum at 24 hr (Figure 2). In ANTU-treated rats, PLE reached a maximum at 6 hr (Figure 3). PF/S protein ratios for xylazine- and ANTU-treated groups are presented in Table 1 and 3, respectively. In xylazine-treated rats, WBC, neutrophil, and monocyte counts were increased ($p < 0.05$) at 12 hr (Table 2). Hematocrit was increased ($p < 0.05$) at 3, 6, 12, and 24 hr after xylazine. Serum sodium and chloride concentration were decreased ($p < 0.05$) at 6 and 12 hr and potassium concentration was increased ($p < 0.05$) at 6 hr. Serum LDH was increased ($p < 0.05$) at 3 hr (Table 2). In ANTU-treated rats, neutrophil count was increased ($p < 0.05$) at 6 hr. Monocyte count was decreased ($p < 0.05$) at 6 and 24 hr. Serum chloride concentration was increased ($p < 0.05$) at 12 hr. Potassium concentration was decreased ($p < 0.05$) at 12 hr and increased ($p < 0.05$) at 24 hr. Serum LDH was increased ($p < 0.05$) at 3 and 6 hr (data not shown).

Behavioral Observations. Xylazine-treated rats (42 mg/kg, im) convulsed and lost their righting reflex within 5 min, followed by pronounced polyuria and glucosuria. 6-12 hr the animals became dyspneic and showed signs of nasal and orbital bleeding as evidenced by blood clots around orbits and nostrils. Subsequently, dyspnea increased in severity and lead to death associated with respiratory distress in some animals. ANTU-treated rats became dyspneic within 6-12 hr; dyspnea increased in severity, but the animals survived.

Pathological Findings. Xylazine-treated rats (42 mg/kg, im) had extensive serous PLE.

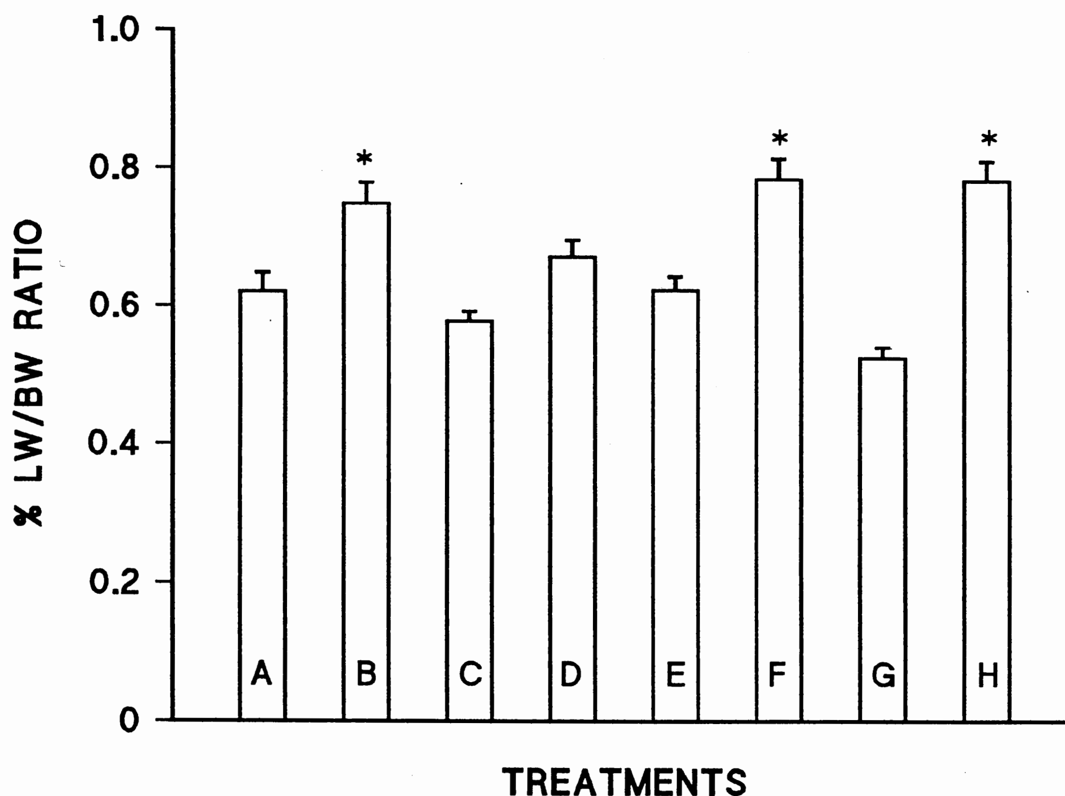


Figure 1. Effects of (B) xylazine (21 mg/kg, im, n = 6); (C) ketamine (45 mg/kg, im, n = 6); (D) xylazine (42 mg/kg, im) plus ketamine (45 mg/kg, im, n = 6); (E) SK&F 525-A (25 mg/kg, ip, n = 6); (F) SK&F 525-A (25 mg/kg, ip) plus xylazine (21 mg/kg, im, n = 6); (G) SK&F 525-A (25 mg/kg, ip) plus ketamine (45 mg/kg, im, n = 6); (H) SK&F 525-A (25 mg/kg, ip) plus xylazine (21 mg/kg, im) and ketamine (45 mg/kg, im, n = 6) on % LW/BW ratio. The treatment schedule is given in experiment one of Methods. (*) Significantly ($p < 0.05$) different from control (A, n = 5).

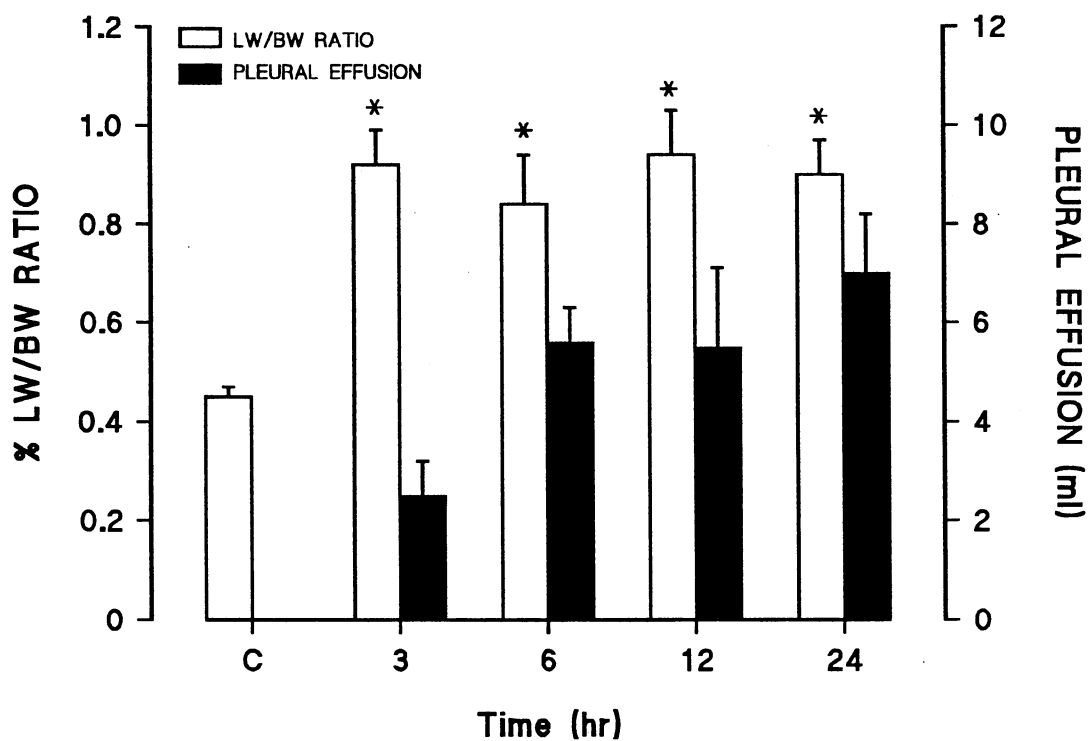


Figure 2. Effect of xylazine (42 mg/kg, im) on % LW/BW ratio and PLE at various times. (*) Significantly ($p < 0.05$) different from control (C).

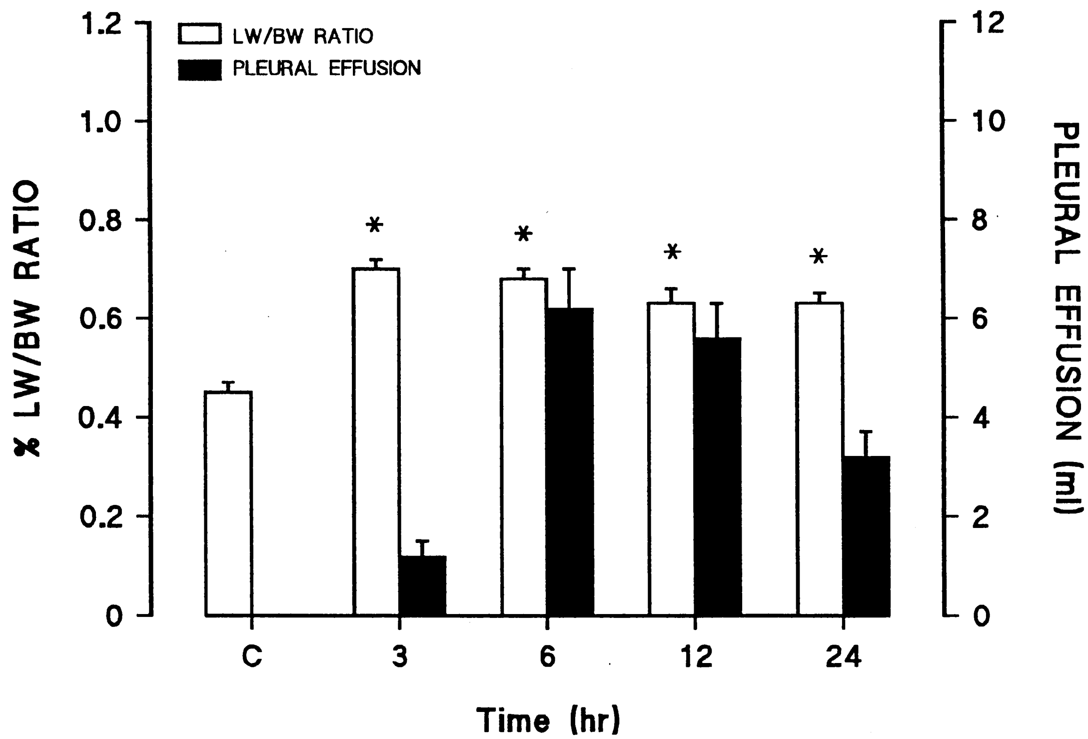


Figure 3. Effect of ANTU (5 mg/kg, ip) on % LW/BW ratio and PLE at various times. (*) Significantly ($p < 0.05$) different from control (C).

TABLE 1

THE EFFECTS OF XYLAZINE (42 mg/kg, im) ON THE PLEURAL FLUID TO SERUM PROTEIN RATIO

	Time (hr)			
PROTEIN	3	6	12	24
TOTAL	0.86 ± 0.02[a] (4)[b]	0.90 ± 0.01 (4)	0.85 ± 0.04 (2)	0.68 ± 0.05 (2)
ALBUMIN	1.08 ± 0.02 (4)	1.08 ± 0.02 (4)	1.02 ± 0.05 (2)	1.08 ± 0.02 (2)
α_1	0.77 ± 0.14 (4)	0.50 ± 0.06 (4)	0.52 ± 0.00 (2)	0.57 ± 0.07 (2)
α_2	0.50 ± 0.08 (4)	0.74 ± 0.10 (4)	1.08 ± 0.02 (2)	0.72 ± 0.08 (2)
β	1.06 ± 0.05 (4)	1.22 ± 0.05 (4)	1.18 ± 0.24 (2)	0.99 ± 0.10 (2)
γ	0.77 ± 0.20 (4)	0.93 ± 0.14 (4)	1.47 ± 1.47 (1)	1.53 ± 0.04 (2)

[a] All values are expressed as mean ± SE.

[b] The number of animals is given in parantheses.

TABLE 2

SUMMARY OF SELECTED WHOLE BLOOD AND SERUM CLINICOPATHOLOGY FROM XYLAZINE (42 mg/kg, im)-TREATED RATS

Parameter[a]	Control	Time (hr) 3	6	12	24
WBC	9100.0 ± 696.6 (7300-11000)[b] (6)[c]	9983.3 ± 1235.4 (6100-13100) (6)	14516.7 ± 2535.8 (4900-23200) (6)	31333.3 ± 3883.4* (24100-37400) (3)	11220.0 ± 2147.5 (5400-16900) (5)
NEUTROPHIL	3079.7 ± 358.4 (1950-4280) (6)	3646.2 ± 412.8 (2074-4585) (6)	8510.2 ± 1664.9 (2107-13920) (6)	20742.0 ± 3484.7* (15424-27302) (3)	6424.4 ± 2420.6 (1944-14872) (5)
EOSINOPHIL	115.8 ± 40.2 (0-219) (6)	172.5 ± 28.3 (122-282) (6)	86.2 ± 50.0 (0-296) (6)	401.7 ± 401.7 (0-1205) (3)	42.6 ± 42.6 (0-213) (5)
LYMPHOCYTE	5633.2 ± 567.0 (3358-7038) (6)	6075.0 ± 833.7 (3843-8253) (6)	5830.7 ± 1065.2 (2695-9280) (6)	9149.3 ± 1159.7 (7471-11375) (3)	4562.4 ± 850.3 (1859-6384) (5)
MONOCYTE	253.0 ± 74.3 (78-408) (6)	89.7 ± 38.7 (0-252) (6)	89.7 ± 80.3 (49-489) (6)	1040.3 ± 521.5* (0-1625) (3)	176.4 ± 40.2 (71-268) (5)
HCT (%)	41.1 ± 0.5 (6)	50.4 ± 2.8* (6)	55.4 ± 1.2* (4)	52.0 ± 0.5* (3)	54.6 ± 3.8* (5)
Na (mEq/L)	140.8 ± 0.6 (6)	138.6 ± 0.6 (5)	135.5 ± 0.6* (4)	124.5 ± 2.5* (2)	137.5 ± 3.5 (2)
K (mEq/L)	6.8 ± 0.2 (6)	6.4 ± 0.2 (5)	10.2 ± 1.1* (4)	9.5 ± 0.1 (2)	9.2 ± 2.5 (2)
Cl (mEq/L)	100.5 ± 0.8 (6)	96.6 ± 1.3 (5)	93.2 ± 1.0* (4)	90.5 ± 1.5* (2)	94.5 ± 4.5 (2)
SERUM LDH (U/L)	1190.0 ± 112.2 (6)	1951.8 ± 172.8* (5)	1252.2 ± 229.2 (4)	1437.5 ± 36.5 (2)	1904.5 ± 687.5 (2)

* Significantly different from control at $p < 0.05$.

[a] All values are expressed as mean ± S.E.

[b] The range of values is given in parantheses.

[c] The nunber of animals is given in parantheses.

WBC = Total white blood cell count, HCT = Hematocrit, LDH = Lactate dehydrogenase.

TABLE 3

THE EFFECTS OF ANTU (5 mg/kg, ip) ON THE PLEURAL FLUID TO SERUM PROTEIN RATIO

PROTEIN	Time (hr)			
	3	6	12	24
TOTAL	0.84 ± 0.03[a] (6)[b]	0.88 ± 0.01 (6)	0.77 ± 0.01 (6)	0.70 ± 0.01 (6)
ALBUMIN	1.11 ± 0.03 (6)	1.03 ± 0.01 (6)	1.03 ± 0.03 (6)	1.08 ± 0.01 (6)
α_1	0.83 ± 0.29 (6)	0.51 ± 0.08 (4)	0.60 ± 0.08 (6)	0.64 ± 0.04 (6)
α_2	0.58 ± 0.09 (6)	0.66 ± 0.11 (4)	0.69 ± 0.09 (6)	0.91 ± 0.16 (6)
β	0.96 ± 0.12 (6)	1.26 ± 0.11 (6)	1.08 ± 0.08 (6)	0.89 ± 0.09 (6)
γ	0.63 ± 0.10 (6)	1.47 ± 0.37 (6)	1.35 ± 0.23 (6)	1.22 ± 0.16 (6)

[a] All values are expressed as mean ± S.E.

[b] The number of animals is given in parantheses.

The fluid was typically straw-colored and clotted upon exposure to air. It also contained numerous intact red blood cells (RBC) which settled from fluid upon centrifugation. The lungs were congested and edematous. Petechia and ecchymoses were commonly seen in the lung parenchyma of xylazine-treated animals. These animals also had severe PE affecting the alveoli and perivascular stroma (Figure 4). ANTU-treated rats (5 mg/kg, ip) had edematous lungs and extensive serous PLE which was colorless and clotted upon exposure to air and did not contain any RBC. There were no gross pathological changes in other organs in either group.

Mechanism of Pulmonary Edema

Pretreatment with yohimbine, prazosin, tolazoline, prazosin plus yohimbine, atropine, DMSO, allopurinol, superoxide dismutase, catalase, BW755C, ibuprofen and cystathionine plus taurine did not affect the % LW/BW ratio. PLE was increased ($P < 0.05$) by yohimbine, yohimbine plus prazosin, and allopurinol, was reduced ($p < 0.05$) by DMSO, and was not changed in other groups (Figure 5 & 6).

Discussion

The results of this study indicate that xylazine causes PE characterized by rapid onset, cellular damage and protein-rich pleural fluid. The increase in PF/S protein ratio to above 0.7 and the near unity of the ratio of albumin, β-globulins and γ-globulins in pleural fluid to that in serum indicate that large protein molecules gain access to pleural space, most likely through injured pulmonary vascular endothelium. The increase in PF/S protein ratio in xylazine-treated rats is similar to that in ANTU-treated rats. PE caused by ANTU is the result of increased permeability due to endothelial injury (Teplitz, 1979). Therefore, xylazine-induced PE can be classified as increased-

permeability PE (Staub, 1984).

The elevated hematocrit was attributed to intravascular fluid loss secondary to extensive pleural effusion, severe diuresis and glucosuria. The cause of hyponatremia is unknown. The mild hyperkalemia may be due to a mild buildup of acids, as the sum of the total measured cations remained fairly constant. The increased serum potassium level may have resulted from severe acidosis and cell damage. Increased serum LDH levels indicates cellular damage.

Adverse cardiovascular effects of xylazine such as transient hypertension followed by hypotension, bradycardia and heart block, mediated by central and peripheral α-adrenoceptors and cardiac muscarinic receptors (Schmidtt et al., 1970; Antonaccio et al., 1973; Langer, 1980; Greene, et al., 1986), suggest that hemodynamic factors may play a role. However, failure of α-adrenergic antagonists such as yohimbine (α_2-receptor), tolazoline (α_1- and α_2-receptors) and prazosin (α_1-receptor) and cholinergic antagonist (atropine) to prevent xylazine-induced PE and PLE indicates that receptor-mediated hemodynamic changes may not be the cause of PE.

We have recently shown that the anesthetic dose of xylazine (21 mg/kg) caused PE and PLE when the metabolism of xylazine was inhibited by inhibitor of cytochrome P-450 such as SK&F 525-A (Amouzadeh et al., 1989). This effect is similar to that seen after administration of a higher dose of xylazine (42 mg/kg) indicating that xylazine may directly injure pulmonary vascular endothelium and cause PE and PLE. The direct effect of xylazine on pulmonary vascular endothelium needs to be investigated. However, xylazine is rapidly metabolized in various species including rat (Garcia-Villar et al., 1981; Duhm et al., 1969) making the possibility of direct effect of xylazine unlikely. It is possible that the direct endothelial injury is caused by

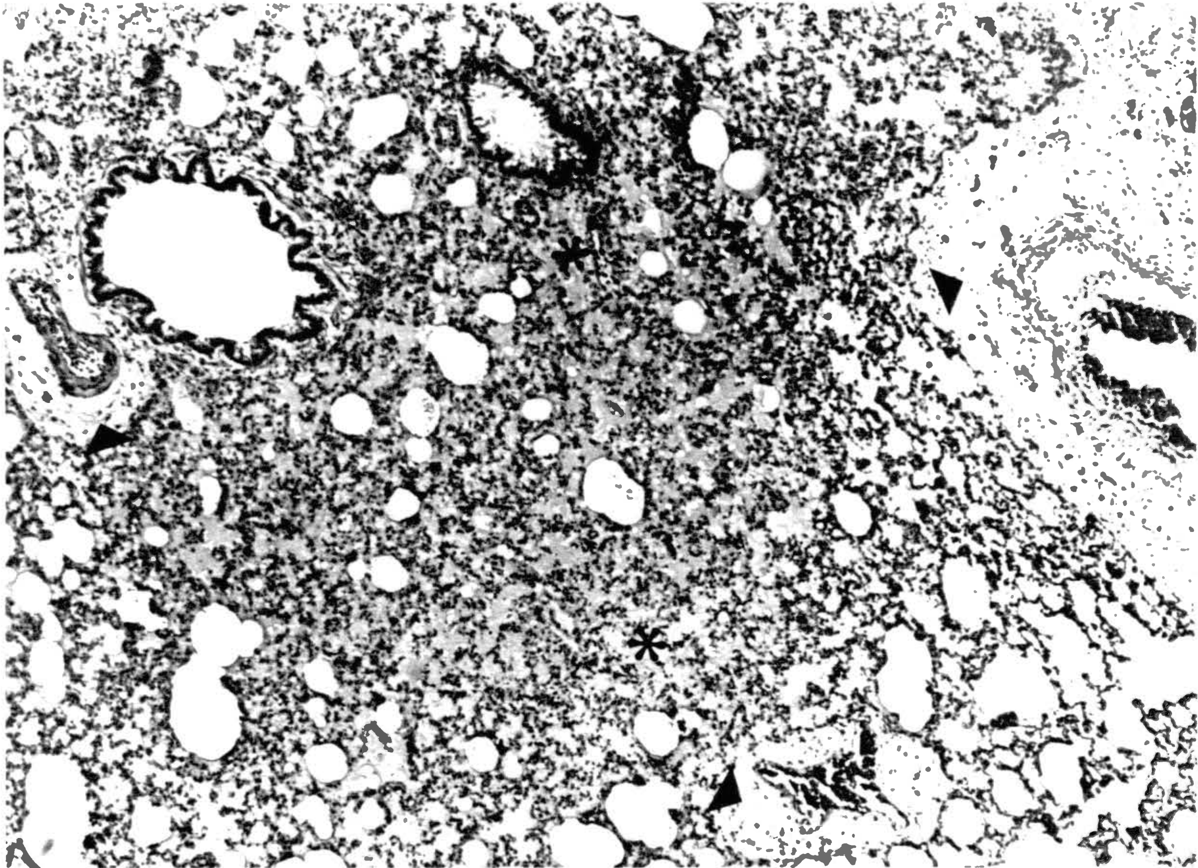


Figure 4. Light micrograph of lung from a rat 12 hr after xylazine (42 mg/kg, im). Note the perivascular (arrowheads) and intra-alveolar (*) edema (H & E, x 80).

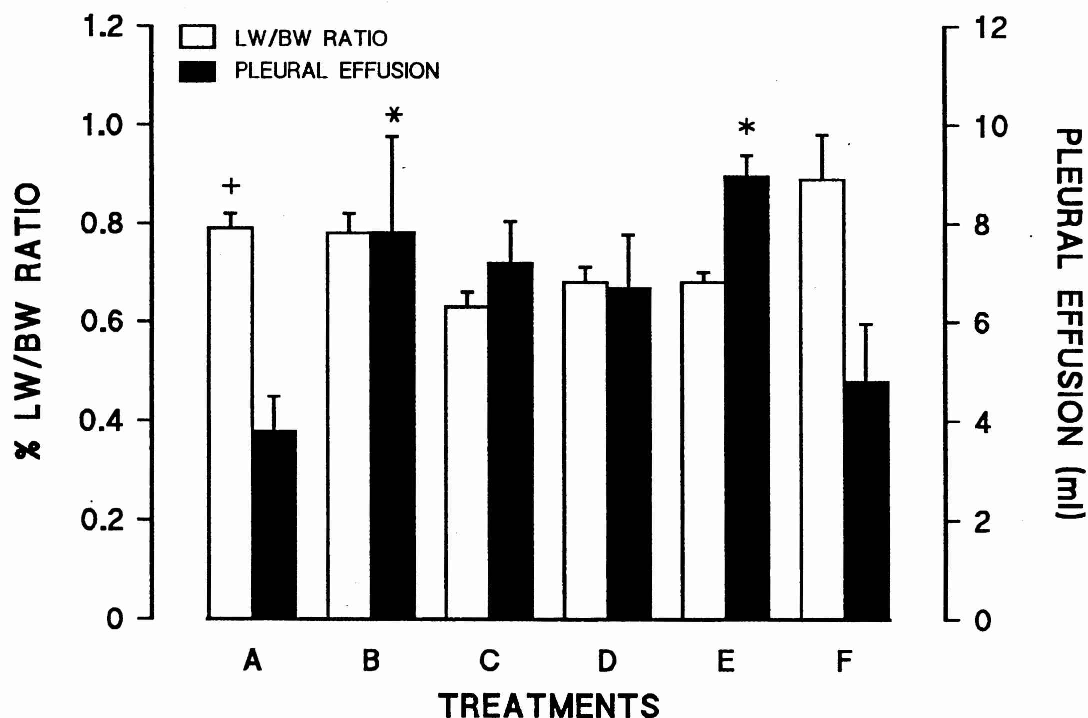


FIGURE 5. Effects of pretreatment with adrenergic and cholinergic antagonists on xylazine-induced PE and PLE. (A) xylazine (42 mg/kg, im, n=23), (B) yohimbine (4.2 mg/kg, ip, n=6), (C) prazosin (20 mg/kg, ip, n=6), (D) tolazoline (20 mg/kg, ip, n=6), (E) yohimbine (4.2 mg/kg, ip) plus prazosin (20 mg/kg, ip, n=6), and (F) atropine (20 mg/kg, ip, n=6). The pretreatment schedules is given in experiment three of Methods. Animals were given 42 mg/kg xylazine, im after pretreatment period. (*) Significantly ($p < 0.05$) different from xylazine group. (+) Significantly ($p < 0.05$) different from control % LW/BW ratio (0.47 ± 0.01, n=16).

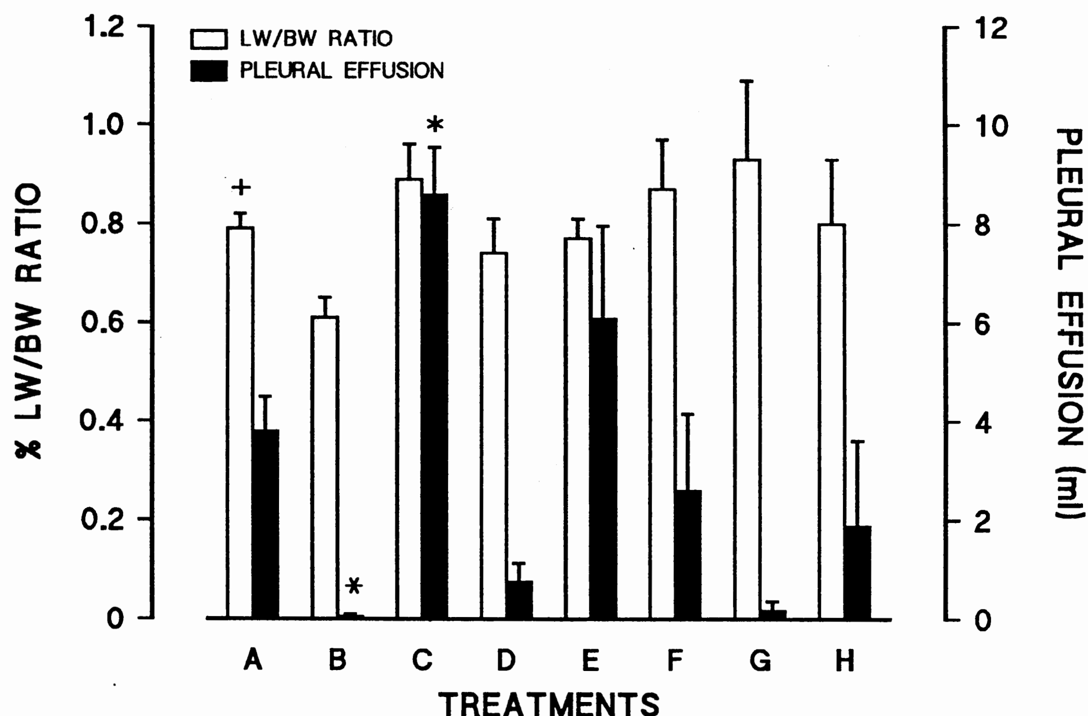


Figure 6. Effects of pretreatment with blockers of various mediators of acute lung injury on xylazine-induced PE and PLE. (A) xylazine (42 mg/kg, im, n = 23), (B) dimethyl sulfoxide (7.8 g/kg, n = 6), (C) allopurinol (50 mg/kg, po), (D) superoxide dismutase (20,000 U/kg, ip), (E) catalase (20,000 U/kg, ip, n = 6), (F) BW755C (50 mg/kg, ip, n = 6), (G) ibuprofen (50 mg/kg, ip, n = 6), and (H) cystathionine (100 mg/kg, ip) plus taurine (100 mg/kg, ip, n = 6). The pretreatment schedule is given in experiment three of Methods. Animals were given 42 mg/kg xylazine, im after pretreatment period. (*) Significantly ($p < 0.05$) different from xylazine group. (+) Significantly ($p < 0.05$) different from control % LW/BW ratio (0.47 ± 0.01, n = 16).

potential reactive metabolite(s). The combination of cystathionine and taurine has been shown to supplement the hepatic glutathione level and protect the liver against acetaminophen toxicity (Kitamura et al., 1989). Although administration of cystathionine and taurine combination, shown to supplement glutathione pool (Kitamura et al., 1989), did not affect PE and PLE in this study, the animals appeared more active, and respiratory distress was not so severe as in the xylazine-treated group. This result suggests that glutathione conjugation might be important in elimination of the metabolites of xylazine.

Large doses of xenobiotics can deplete cellular defense mechanisms and saturate major nontoxic pathways so that minor pathways capable of producing reactive intermediates become prominent (Sipes and Gandolfi, 1986). It is possible that inhibition of metabolism by cytochrome P-450 or increasing the dose saturates the detoxifying pathway and shifts the metabolism of xylazine to an alternate pathway. This could result in the formation of reactive metabolites capable of injuring pulmonary endothelium.

It is also possible that mediators of acute lung injury such as arachidonic acid breakdown products and oxygen radicals generated by neutrophils or xanthine oxidase play a role in xylazine-induced PE. Oxygen radicals, generated by xanthine oxidase, caused PE by direct endothelial damage in a pulmonary hypoperfusion/ischemia-reperfusion model (Grosso et al., 1989). Inhibition of xanthine oxidase by allopurinol prevented ischemic injury in cat small intestine (Parks et al., 1982). In contrast, allopurinol failed to prevent lung damage caused by ANTU (Martin, 1986). In this study, allopurinol augmented xylazine-induced PE and PLE. This is, perhaps, due to an inhibitory effect of allopurinol on hepatic drug metabolizing enzymes (Vessel et al., 1970; McInnes and Brodie, 1988) which could enhance the direct effects of xylazine

on pulmonary vascular endothelium. Alternatively, inhibition of hepatic metabolizing enzymes by allopurinol could shift the metabolism of xylazine to a toxifying pathway resulting in the formation of reactive metabolite(s) capable of damaging pulmonary vascular endothelium. DMSO, an effective scavenger of hydroxyl radicals, has been shown to prevent PE and acute lung injury (Fox et al., 1983, Kimura et al., 1988). The reduction in the extent of PE and PLE by DMSO in this study indicates that reactive oxygen radicals, such as hydroxyl radicals, may be involved in xylazine-induced PE. Superoxide dismutase and catalase did not affect % LW/BW ratio and PLE in spite of their increased half-life by conjugation to polyethylene glycol (Abuchowski et al., 1977; McCord and English, 1981). This could be due to inadequate doses of superoxide dismutase and catalase or lack of accessibility of these scavengers to the source of reactive species which may be intracellular (Downey, 1990).

Neutrophils are thought to be involved in PE (Repine et al., 1982). They mediate an inflammatory response by releasing proteolytic enzymes and generating oxygen-derived free radicals which are capable of damaging pulmonary vascular endothelium and destroying lung tissue (Varani et al., 1985). The elevation of neutrophil counts in xylazine-treated rats suggests that they are possibly involved. This increase at a later period (12 hr) indicates that neutrophils are responding to an acute injury rather than being the cause themselves. However, the role of neutrophils needs further investigation.

The role of metabolites of arachidonic acid in experimental lung injury is well-established (Birgham, 1985). Ibuprofen, a blocker of the cyclooxygenase pathway and BW755C, a blocker of both cyclooxygenase and lipoxygenase pathways had no protective effect. This lack of protection by ibuprofen and BW755C indicates that arachidonic acid breakdown products may not be involved in xylazine-induced PE.

In summary, the results indicate that xylazine causes increased-permeability PE characterized by rapid onset, cellular damage and protein-rich pleural fluid. Xylazine-induced PE is not mediated by hemodynamic factors through peripheral and/or central α-adrenergic and myocardial cholinergic receptors, and oxygen radicals are possibly involved in its etiology. Elucidation of the exact mechanism of xylazine-induced PE can be useful in therapeutic management of xylazine toxicity in animals and man.

References

ABUCHOWSKI, A., MCCOY, J., PALCZUK, N.C., VAN E.S., T. AND DAVIS, F. (1977). Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. *J. Biol. Chem.* 252, 3582-3586.

ADAMS, J.D. JR., LAUTERBURG, B.H. AND MITCHELL, J.R. (1983). Plasma glutathione and glutathione disulfide in the rat: regulation and response to oxidative stress. *J. Pharmacol. Exp. Ther.* 227, 749-754.

AMOUZADEH, H.R., SANGIAH, S. AND QUALLS, C.W. (1989). Effects of some hepatic microsomal enzyme inducers and inhibitors on xylazine-ketamine anesthesia. *Vet. Hum. Toxicol.* 31, 532-534.

ANTONACCIO, M.J., ROBSON, R.D. AND KERWIN, L. (1973). Evidence for increased vagal tone and enhancement of baroreceptor activity after xylazine in anesthetized dogs. *Eur. J. Pharmacol.* 23, 311-315.

BIRGHAM, K.L. (1985). Metabolites of arachidonic acid in experimental lung vascular injury. *Fed. Proc.* 44, 43-45.

BOYD, M.R. (1980). Biochemical mechanisms in chemical-induced lung injury: role of metabolic activation. *CRC Critical Reviews in Toxicology.* 7, 103-176.

BOYD, M.R., STIKO, A., STATHAM, C.N. AND JONES, R.B. (1982). Protective role of endogenous glutathione and other sulfhydryl compounds against lung damage by alkylating agents. Investigation with 4-ipomeanol in the rat. *Biochem. Pharmacol.* 31, 1579-1583.

CARRUTHERS, S.G., WEXLER, H.R., AND STILLER, C.R. (1979). Xylazine hydrochloride (Rompun) overdose in man. *Clin. Toxicol.* 15, 281-285.

COOPER, J.A.D., WHITE, D. AND MATTHAY, R.A. (1986a). Drug-induced pulmonary disease, Part 1: Cytotoxic drugs. *Am. Rev. Resp. Dis.* 133, 321-340.

COOPER, J.A.D., WHITE, D. AND MATTHAY, R.A. (1986b). Drug-induced pulmonary disease, Part 2: Noncytotoxic drugs. *Am. Rev. Resp. Dis.* 133, 488-505.

DOWNEY, J.M. (1990). Free radicals and their involvement during lung-term myocardial ischemia and reperfusion. *Annu. Rev. Physiol.* 52, 487-504.

DUHM, B., MAUL, W., MEDENWALD, M., PATZCHKE, K., AND WAGNER L.A. (1969). Untersuchungen mit radioaktiv markieterm BAY Va 1470 an Ratten. *Berliner Und Munchener Tierarztliche Wochenchrift* 82, 104-109.

FOX, R.B., HARADA, R.N., TATE, R.M. AND REPINE, J.E. (1983). Prevention of thiourea-induced pulmonary edema by hydroxyl-radical scavengers. *J. Appl. Physiol.* 55, 1456-1459.

GALLANOSA, A.G., SPYKER, D.A., SHIPE, J.R., AND Morris, D.L. (1981). Human xylazine overdose: A comparative review with clonidine, phenothiazine and tricyclic antidepressants. *Clin. Toxicol.* 18, 663-678.

GARCIA-VILLAR, R., TOUTAIN, P.L., AND ALVINERIE, M. (1981). The pharmacokinetics of xylazine hydrochloride: an interspecific study. *J. Vet. Pharmacol. Therap.* 4, 87-92.

GREENE, S.A., AND THURMAN, J.C. (1988). Xylazine-a review of its pharmacology and use in veterinary medicine. *J. Vet. Pharmacol. Therap.* 11, 295-313.

GROSSO, M.A., BROWN, J.M., VIDERS, D.E., MULVIN, D.W., BANERJEE, A. VELASCO, S.E., REPINE, J.E. AND HARKEN, H. (1989). Xanthine-oxidase-derived oxygen radicals induce pulmonary edema via direct endothelial cell injury. *J. Surg. Res.* 46, 355-360.

HAGEN, T.M., BROWN, L.A. AND JONES, D.P. (1986). Protection against paraquat-induced injury by exogenous GSH in pulmonary alveolar type II cells. *Biochem. Pharmacol.* 35, 4537-4542.

KANNIAPPAN, M. AND RAMASWAMY, V.M. (1979). A study on the toxic effects of morphine, metamidazole and xylazine. *Cheiron* 8, 159-163.

KIMURA, R., TRABER, L.D., HERNDON, D.N., NEUHAUS, G.D. AND TRABER, D.L. (1988). Treatment of smoke-induced pulmonary injury with nebulized dimethylsulfoxide. *Circul. Shock* 25, 333-341.

KIRKPATRICK, R.M. (1978). Use of xylazine and ketamine as a combination anesthetic. *Canine Practice* 5, 53-57.

KITAMURA, Y., KAMISAKI, Y. AND ITOH, T. (1989). Hepatoprotective effects of cystathionine against acetaminophen-induced necrosis. *J. Pharmacol. Exp. Ther.* 250, 667-671.

KOMMONEN, B., KOSKIEN, L. (1984). Fluorescein angiography of the canine ocular fundus in ketamine-xylazine anesthesia. *Acta. Vet. Sacnd.* 25, 346-351.

LANGER, S.Z. (1980). Presynaptic regulation of release of catecholamine. *Phramacol. Rev.* 32, 337-364.

MARTIN, A., KORTHUIS, R.J., PERRY, M., TOWNSLEY, M.I. AND TAYLOR, A.E. (1986). Oxygen radical-mediated lung damage associated with α-naphthylthiourea. *Acta. Physiol. Scand.* Suppl. 548, 119-125.

MCCORD, J.M. AND ENGLISH, D. (1981). Superoxide dismutase: an anti-inflammatory drug. In *Enzymes as Drugs.* (J.S. Holcenberg and J. Roberts, Eds.), pp. 353-365. Wiley, New York.

MCINNES, G.T. AND BRODIE, M.J. (1988). Drug interactions that matter. *Drugs* 36, 83-110.

MINCHIN, R.F. AND BOYD M.R. (1983). Localization of metabolic activation and deactivation systems in the lung: significance to the pulmonary toxicity of xenobiotics. *Ann. Rev. Pharmacol. Toxicol.* 23, 217-238.

NATIONAL INSTITUTE OF HEALTH (1985). *Guide for the Care and Use of Laboratory Animals*. NIH, Bethesda, Maryland.

PARKS, D.A., BULKLEY, G.B., GANGER, D.N., HAMILTON, S.R. AND McCORD, J.M. (1982). Ischemia injury in the cat small intestine: Role of superoxide radicals. *Gastroenterol.* 82, 9-15.

POKLIS, A., MACKELL, M.A. AND CASE, E.S. (1985). Xylazine in human tissue and fluid in a case of fatal drug abuse. *J. Anal. Toxicol.* 9, 234-236.

REPINE, J.E. AND TATE, R.M. (1982). Neutrophils and lung edema. *chest* 81, 47S-50S.

SCHMIDTT, H., FOURNADJIEV, G. AND SCHMIDTT, H. (1970). Central and peripheral effects of 2-(2,6-dimethylphenyl-amino)-4-H-5, 6-dihydro-1, 3-thiazine (Bayer 1470) on the sympathetic system. *Eur. J. Pharmacol.* 10, 230-238.

SHASBY, M.D., FOX, R.B., HRADA, R.N. AND REPINE, J.E. (1982). Reduction of the edema of acute hyperoxic lung injury by granulocyte depletion. *J. Appl. Physiol.* 52, 1237-1244.

SIPES, I.G. AND GANDOLFI, A.J. (1986). Biotransformation of toxicants. In *Casarett and Doull's Toxicology* (C.D. Klaassen, M.O. Amdur and J. Doull, Eds.), pp. 64-98. Macmillan Publishing Company, New York.

STAUB, N.C. (1982). Pathophysiology of pulmonary edema. In *Edema* (N.C. Staub and A. E. Taylor, Eds.), pp. 719-746. Raven Press, New York.

TEPLITZ, C. (1979). Pulmonary cellular and interstitial edema. In *Pulmonary Edema* (A.P. Fishman and E.M. Renkin Eds.), pp. 97-111. American Physiological Society, Bethesda, Maryland.

UGGLA, A. AND LINDQVIST, A. (1983). Acute pulmonary oedema as an adverse reaction to the use of xylazine in sheep. *Veterinary Record* 111, 42.

VAN ZWIETEN, P.A. AND TIMMERMANS, P.B.M.W.M. (1983). Cardiovascular α_2-receptors. *J. Molec. Cell. Pharmacol.* 224, 501-507.

VARANI, J., FLIGIEL, S.E.G., TILL, G.O., KUNKEL, R.G., RYAN, U.S. AND WARD, P.A. (1985). Pulmonary endothelial cell killing by human neutrophils, possible involvement of hydroxyl radical. *Lab. Invest.* 53, 565-663.

VESSEL, E.S., PASSANANTI, G.T. AND GREEN, F.E. (1970). Impairment of drug metabolism in man by allopurinol and nortriptyline. *New Engl. J. Med.* 283, 1484-1488.

WHITE, P.F., WAY, W.L. AND TREVOR, A.J. (1982). Ketamine-Its pharmacology and therapeutic uses. *Anesthesiology* 56, 119-136.

CHAPTER IV

BIOCHEMICAL AND ULTRASTRUCTURAL ALTERATIONS IN XYLAZINE-INDUCED PULMONARY EDEMA

Introduction

Xylazine, an α_2-adrenergic agonist with analgesic, sedative and muscle-relaxant properties (Greene et al., 1988), is commonly used alone or in combination with ketamine in veterinary medicine. In a recent study, we have shown that xylazine caused pulmonary edema (PE) in rats which was characterized by rapid onset, cellular damage, extensive pleural effusion and protein-rich edema fluid (Amouzadeh et al., 1991). These are the hallmark of increased-permeability PE in contrast to increased-pressure PE which is characterized by lack of cellular damage, low protein edema fluid and benign course of development (Staub, 1984).

A number of drugs including analgesics, antiarrhythmic agents and opiates have been reported to cause pulmonary damage through non-cardiogenic factors such as direct toxicity or indirect augmentation of an inflammatory reaction (Cooper et al., 1986a and 1986b). In addition, toxicants such as α-naphthylthiourea, paraquat and 4-ipomeanol cause lung injury through reactive species generated by cytochrome P-450 (Boyd, 1980) which can cause endothelial injury and lead to PE. It was suggested that xylazine-induced PE could be classified as increase-permeability PE resulting from endothelial injury caused by xylazine or its metabolites (Amouzadeh et al., 1991). Also, oxygen radicals might be involved in the etiology of xylazine-induced PE and PLE (Amouzadeh et al., 1991).

Oxygen radicals, generated by xanthine oxidase (XO), neutrophils or drugs have been implicated in various experimental models of lung injury. Grosso et al. (1990), in a pulmonary hypoperfusion/ischemia-reperfusion model, have shown that xanthine oxidase-generated oxygen radicals are partially responsible for pulmonary capillary endothelial damage and PE. Neutrophil-generated oxygen radicals have been shown to cause acute lung injury and PE (Repine, 1982; Shasby et al., 1982; McDonald et al., 1987; Patterson et al., 1989). The pulmonary toxicity of paraquat and bleomycin has been proposed to be caused by oxygen radicals generated during their metabolism (Frank, 1985).

Cytokines such as IL-1 and TNF may also play a role in acute lung injury. IL-1 is produced primarily by monocytes/macrophages in response to infection and injury. Vascular endothelium, smooth muscle cells, astrocyte and microglial cells of the brain and B lymphocytes also produce IL-1 (Dinarello, 1988). Overall, IL-1 causes a number of cellular and biochemical effects which lead to congestion of vessels, formation of clot, infiltration of inflammatory cells and increased endothelial permeability (Dinarello, 1988). *In vitro*, IL-1 directly affects the vascular endothelium, causing increased adhesiveness for granulocytes (Bevilacqua et al, 1985; Cotran et al., 1986), increased synthesis of platelet-activating factor (Bussolino et al., 1986), and increased prostaglandin E_2, a vasodilator (Albrightson et al., 1985). IL-1 depresses both the levels and the activity of hepatic cytochrome P-450 in mice (Shedlofsky et al., 1987). The level of IL-1 is increased in bronchoalveolar fluid in patients with bacterial pulmonary infection (Wilmott, 1990), in alveolar macrophages from patients with Adult Respiratory Distress Syndrome (ARDS, Jacobs, 1989), and in ventricular fluid from patients with head injury (McClain et al., 1987). IL-1 and TNF levels are increased in patients with fulminant hepatic failure (Muto et al., 1988). Elevated levels of IL-1 have

also been reported in women after ovulation (Cannon and Dinarello, 1985). IL-1 bioactivity was increased in monocrotaline-treated rats (Gillespie et al., 1988). A monokine preparation containing IL-1 caused pulmonary edema and albumin leakage in rats (Gillespie et al., 1989). Goldblum et al. (1988) have shown that human IL-1 causes acute pulmonary vascular endothelial injury and lung edema in rabbits. It is suggested that the cytotoxic effects of IL-1 are caused indirectly through stimulating the release of oxygen radicals from endothelial cells (Matsubara and Ziff, 1986).

Tumor necrosis factor (TNF) is also produced mainly by monocytes/ macrophages (Fong and Lowry, 1990). TNF induces IL-1 production by the vascular endothelium (Dinarello, 1986; Nawroth et al., 1986), activates polymorphonuclear cell functions (Shalaby et al., 1985) and induces procoagulant activity of vascular endothelium (Bevilacqua et al., 1986; Nawroth and Stern, 1986). Although the production of TNF is associated with infection, elevated levels of TNF have been reported in non-infectious conditions such as cancer (Balkwill et al., 1987; Aderka, et al., 1985), thermal injury (Marano et al., 1990), renal allograft rejection (Maury and Teppo, 1987), head injury (Goodman et al., 1990), heart failure (Levine et al., 1990), hepatic failure (Muto et al., 1988) and ARDS (Roten et al., 1991). TNF production by peripheral blood mononuclear cells from anorexia nervosa patients (Schattner et al., 1990) and alveolar macrophages from patients with rheumatoid arthritis (Gosset et al., 1991) is increased. It appears that elevation of TNF is not exclusive to infectious diseases. Infusion of TNF into rats caused hypotension, metabolic acidosis, elevated hematocrit and potassium levels, and hyperglycemia leading to death from respiratory arrest (Tracy et al., 1986). At necropsy, prominent hemorrhagic lesions were found in the lung and gastrointestinal tract (Tracy et al., 1986). In mice, administration of TNF caused peripheral blood lymphopenia and neutrophilia accompanied by necrosis

in the small intestine due to endothelial injury (Remick et al., 1987). Systemic administration of TNF can cause pulmonary vascular endothelial injury and pulmonary edema in guinea pigs and rabbits (Stephens et al., 1988; Goldblum et al., 1989). This effect of TNF appears to be granulocyte-dependent, as granulocyte depletion prevents acute lung injury (Stephens et al., 1988). TNF caused generation of superoxide anion from neutrophils (Tsujimoto et al., 1986). TNF also increases endothelial cell permeability *in vitro* (Horvath et al., 1988; Henning et al., 1988; Brett et al., 1989; Shinjo et al., 1989; Royall et al., 1989; Goldblum and sun, 1990). This effect is thought to be independent of neutrophils (Horvath et al., 1988) and due endothelial cytoskeletal alteration involving G protein (Brett et al., 1989). Hocking et al. (1990) found that TNF caused neutrophil-dependent pulmonary edema in isolated, perfused guinea pig lung. The edema was attributed to increased pulmonary capillary pressure caused by thromboxanes, platelet activating factor (PAF), and increased capillary permeability mediated by PAF (Hocking, 1990). Results from studies involving endothelial cytotoxicity of TNF are contradictory and depend on the model used. TNF was not cytotoxic to human endothelium (Schuger et al., 1989, Pober and Cotran, 1990), except when TNF-pretreated human umbilical endothelial cells were transferred to a balanced salt solution (Schuger et al., 1989). It appears that TNF does not have direct endothelial cytotoxicity; rather, it increases the susceptibility of endothelium to injury. Pre-exposure of rat arterial endothelial cells to TNF increases the toxicity of activated neutrophils towards these cells (Varani et al., 1988). This is perhaps due to generation of cytotoxic superoxide anion from neutrophils stimulated by TNF (Tsujimoto et al., 1986). TNF-stimulated eosinophils are cytotoxic to human umbilical vein endothelium, possibly through peroxidase activity of eosinophils (Slungaard et al., 1990). Inhibition of protein synthesis in bovine pulmonary endothelial cells by

cycloheximide makes these cells susceptible to lysis by TNF (Nolop and Ryan, 1990).

Considering the multiple factors involved in the etiology of drug-induced acute lung injury, the purpose of this study was to elucidate the role of some of these factors and to characterize ultrastructural alterations in xylazine-induced PE.

Methods

Animals:

Specific virus antibody-free male Sprague-Dawley rats (Sasco Labs., Omaha, NE) of similar age and weight (225-250 g) were housed with temperature, humidity and air circulation maintained according to the Guide for the Care and Use of Laboratory Animals (NIH, 1985). They were allowed free access to food and water and were kept on a 12-hour light-dark cycle. One week after arrival, the rats were assigned to various treatment groups.

Drugs and Chemicals:

Xylazine (Gemini, Rockville center, NY), hypoxanthine, nitroblue tetrazolium, ethylene diamine tetra-acetic acid (EDTA), phenazine methosulfate (PMS) , Triton X-100, L(+)-lactic acid, 2-[4-iodophenyl]-3-[4-nitrophenyl]-5 phenyltetrazolium chloride (INT), ß-nicotinamide adenine dinucleotide (NAD), bovine serum albumin and 2-mercaptoethanol and actinomycin D (Sigma Chemical Co., St. Louis, MO), Tris and HEPES (Research Organics Inc., Cleveland, OH), analytical grade potassium phosphate dibasic and potassium phosphate monobasic, calcium chloride, sodium hydroxide and sodium bicarbonate (Fisher Scientific, Fair Lawn, NJ), paraformaldehyde (Polysciences Inc., Warrington, PA), cacodylic acid and glutaraldehyde (Electron Microscopy Science, Ft. Washington, PA), Click's medium (Irvine Scientific, Irvine, CA), fetal bovine serum

(Hyclone, Logan, UT), L-glutamine, gentamicin and Dubellco's Modified Eagles Medium (Gibco BRL, Grand Island, NY), ^{3}H-thymidine (ICN Radiochemicals, Irvine, CA) were purchased commercially. IL-1 was kindly provided by Dr. Peter Lomedico of Hoffmann La Roche, Inc., Nutley, NJ. TNF was a gift from Cetus Corporation, Emeryville, CA.

Assessment of Pulmonary Edema (PE):

PE was assessed by comparisons of the percent lung to body weight ratios (% LW/BW) of treated groups to that of control. The volume of pleural fluid, when present, was measured.

Experiment One: Determination of Protein, Lactate Dehydrogenase (LDH), Xanthine Oxidase (XO), Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF) in Bronchoalveolar Lavage Fluid (BALF)

Twenty four rats were given xylazine (42 mg/kg, im) and 3, 6 and 12 hr later they were killed by decapitation. Pleural fluid was collected and its volume was measured. Lungs and hearts were removed *en block*. The lungs were separated from the hearts, trimmed of fat and connective tissues, blot-dried with gauze and their wet-weight measured. Each lung was lavaged with 8.0 ml of phosphate buffered saline (PBS), pH 7.3, at room temperature using an infusion pump (Harvard Apparatus, Model 940) at 2.0 ml/min. A 2.0 min dwell time was allowed before withdrawal. The lavage fluid was centrifuged at 2000 x g for 15 min and the supernatant was collected and frozen.

Protein Assay. Protein concentration in the BALF was determined by bicinchoninic acid reagent (BCA, Pierce, Rockford, IL) with bovine serum albumin as standard.

LDH Assay. Lactate dehydrogenase was asssayed spectrophotometrically following the method of Korzeniewski and Callewaert (1986) using a 96-well microtiter plate assay. Briefly, 100 μl of BALF sample (triplicate determination) was placed in each well and 100 μl of assay mixture was added and the absorbance was measured at 490 nm for 2 min using a kinetic microplate reader (V_{max}, Molecular Devices, Menlo Park, CA). The assay mixture contained 5.4×10^{-2} M L(+) lactate, 6.6×10^{-4} INT, 2.4×10^{-4} PMS, 1.3×10^{-3} M NAD in 0.2 M Tris buffer (pH 8.2).

Xanthine Oxidase Assay. Xanthine oxidase was assayed by adaptation of the method of Fried (1966). The assay was done in 96-well microtiter plate. Each well contained 100 mM potassium phosphate buffer (pH 7.8), 10 mM EDTA, 0.3 % Triton X-100, 5.6 μg/ml phenazine methosulfate, 400 μg/ml nitro blue tetrazolium, 1 mM hypoxanthine and 80 μl of lavage fluid in a final volume of 200 μl. The absorbance was measured at 540 nm against a reference containing all components except hypoxanthine, using a kinetic microplate reader (V_{max}, Molecular Devices, Menlo Park, CA). All data (triplicate determination) were corrected for background rate of formazan formation in the absence of added substrate. The results are reported in U/L activity where one enzyme unit corresponds to the formation of one mole of formazan per minute.

IL-1 Assay. IL-1 was assayed following the method of Kaye et al (1984) using a murine interleukin-dependent helper T-cell clone (D10.G4.1, ATCC, TIB 244). The cells were grown in Click's medium containing 10% fetal bovine serum, 200 mM L-glutamine, 5×10^{-5} 2-mercaptoethanol and 50 μg/μl gentamicin supplemented to 5-10% with Con A-conditioned rat splenocyte supernate. The assay was performed in 96-well microtiter plates (triplicate determination) in a final volume of 200 μl. Each

well contained 2 x 10^4 D10.G4.1 cells in medium containing final concentration of 1 μg/ml Con A, 100 μl BALF (1:5 dilution). The cells were incubated 48 hr and labeled with ^{3}H-thymidine (specific activity, 34 Ci/mmole) at 1.0 μCi/well. After the labeling period, cells were harvested and processed for determination of ^{3}H-thymidine incorporation using a scintillation counter (Beckman LS 5000TD). IL-1 activity in BALF samples were determined from linear regression analysis of a standard curve generated from a titration of recombinant human IL-1α.

TNF Assay. TNF was assayed following the methods of Decker and Lohmann-Mathes (1988) using a TNF-sensitive murine fibrosarcoma cell line WEHI 164 (American Type Culture Collection CRL-1751). The cells were maintained in Dubellco's Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2-mercaptoethanol, 2 mM L-glutamine, 25 mM HEPES buffer, $NaHCo_3$ and 50 μg/ml gentamicin. The cells were passaged twice weekly by detachment with trypsin-EDTA and reconstituted with fresh medium to 1-3 x 10^5 cells/ml. The cells were then washed extensively with serum-free DMEM without phenol red to remove the LDH present in bovine serum. Three-fold dilution of BALF samples and TNF standard (rhTNF, 21-67 x 10^6 U/mg) were titrated in 96-well microtiter plates. To each well 1 x 10^4 WEHI cells in serum-free DMEM without phenol red was added. To enhance the sensitivity of cells to TNF, 10 μg/ml actinomycin D was also added and cells were incubated for 10 to 24 hr. Following incubation period, 0.1 ml of supernate was transferred to the corresponding wells of a flat bottomed microtiter plate and 0.1 ml LDH substrate mixture (5.4 x 10^{-2} M L(+) lactate, 6.6 x 10^{-4} INT, 2.4 x 10^{-4} PMS, 1.3 x 10^{-3} M NAD in 0.2 M Tris buffer, pH 8.2) was added to each well and absorbance was measured at 490 nm for 2 min (V_{max}, Molecular Devices, Melno Park, CA). TNF

activity in BALF samples was determined from regression analysis of a standard curve generated from a titration of rhTNF. A monoclonal antibody against rhTNF was used to verify the presence of TNF in BALF. The anti-human rTNF was added to BALF samples and the mixture was assayed for cytotoxic activity. The antibody reduced the cytotoxic activity of BALF considerably.

Experiment Two: Light and Electron Microscopy

Animals were given xylazine (42 mg/kg, im) and were killed by decapitation 0.5 (n=3) and 12 hr (n=4) later. The pleural fluid was removed and its volume was measured. Lungs and hearts were removed *en block*. The lungs were separated from the heart, trimmed of fat and connective tissues, blot-dried with gauze and their wet weight measured. The left lobe was fixed by tracheal instillation of Karnovsky's modified fixative (Karnovsky, 1965) at 20 cm H_2O to full lung expansion. The right lobes were fixed by immersion in the same fixative. After fixation, a section of the right lobe was routinely processed, sectioned at 6 μm and stained with H & E for histological examination. A section of left lobe was cut into 1 mm x 1 mm cubes and postfixed in osmium tetroxide and processed routinely for transmission electron microscopy.

Data Analysis:

The mean ± SE of percent lung to body weight ratio, pleural fluid volume, protein concentrations, LDH, XO, IL-1 and TNF levels were calculated. Data were analyzed by one-way analysis of variance using SAS General Linear Model procedure. The significance of treatment means as compared to control values was determined by Dunnett's test. A probability level of $P < 0.05$ was considered significant.

Results

Gross Pathological Findings:

Xylazine-treated rats had extensive serous pleural effusion. The fluid was typically straw-colored and clotted upon exposure to air. It also contained numerous intact red blood cells which settled from fluid upon centrifugation. The lungs were congested and edematous. Petechia and ecchymoses were commonly seen in the lung parenchyma of xylazine-treated animals. There were no gross pathological changes in other organs.

Pulmonary Edema

Percent LW/BW ratio was increased ($p < 0.05$) at 0.5, 3, 6 and 12 hr in xylazine-treated animals as compared to control. The volume of pleural effusion steadily increased throughout each period monitored (Figure 1).

Protein, LDH, XO, IL-1 and TNF

Protein concentration, LDH, XO and TNF levels were increased ($p < 0.05$) at 3, 6 and 12 hr in xylazine-treated animals as compared to control (Figures 2A, B, C, E). IL-1 level was unchanged at 3 and 6 hr, and was reduced ($p < 0.05$) at 12 hr (Figure 2D).

Light and Electron Microscopy

The lungs showed severe PE affecting the alveoli and perivascular stroma (Figure 3). Ultrastructurally, extensive endothelial damage such as thinning, detachment from basement membrane or bleb formation were observed (Figure 4). Both histological and ultrastructural changes were more pronounced at 12 hr after

administration of xylazine.

Discussion

The results of this study support our initial hypothesis that xylazine-induced PE is due to increased permeability of pulmonary endothelium. The increased level of LDH in the BALF is an indication of cellular injury which leads to leakage of protein into interstitial space, alveolar space and finally thoracic cavity. This protein leakage is reflected by the increased protein concentration in BALF from xylazine-treated rats as compared to control. Ultrastructural observations indicate that endothelial injury such as thinning, detachment from basement membrane or bleb formation are prominent features of xylazine-induced PE. Endothelial injury is evident as early as 0.5 hr after administration of xylazine and becomes more pronounced by 12 hr. These changes are similar to those caused by known edemagenic agents such as alloxan and α-naphthylthiourea which cause increased-permeability PE (Cottrel, et al., 1967; Cunningham and Hurley, 1972).

In a previous study (Amouzadeh et al., 1991), we found that DMSO was partially effective in preventing xylazine-induced PE and pleural effusion. This effect was attributed to oxygen radical scavenging activity of DMSO. Therefore, it was suggested that oxygen radicals are involved in etiology of xylazine-induced PE. The increased level of XO in BALF of xylazine-treated rats support this suggestion and indicates that xanthine oxidase-generated oxygen radicals may play a role in xylazine-induced PE. The XO could be activated by hypoxia/reperfusion during the initial hypertensive effect of xylazine. Alternatively, xylazine itself or one of its metabolites could damage pulmonary endothelium directly and lead to activation of XO. Such activation has been proposed by Jarasch et al. (1986) as a result of endothelial injury.

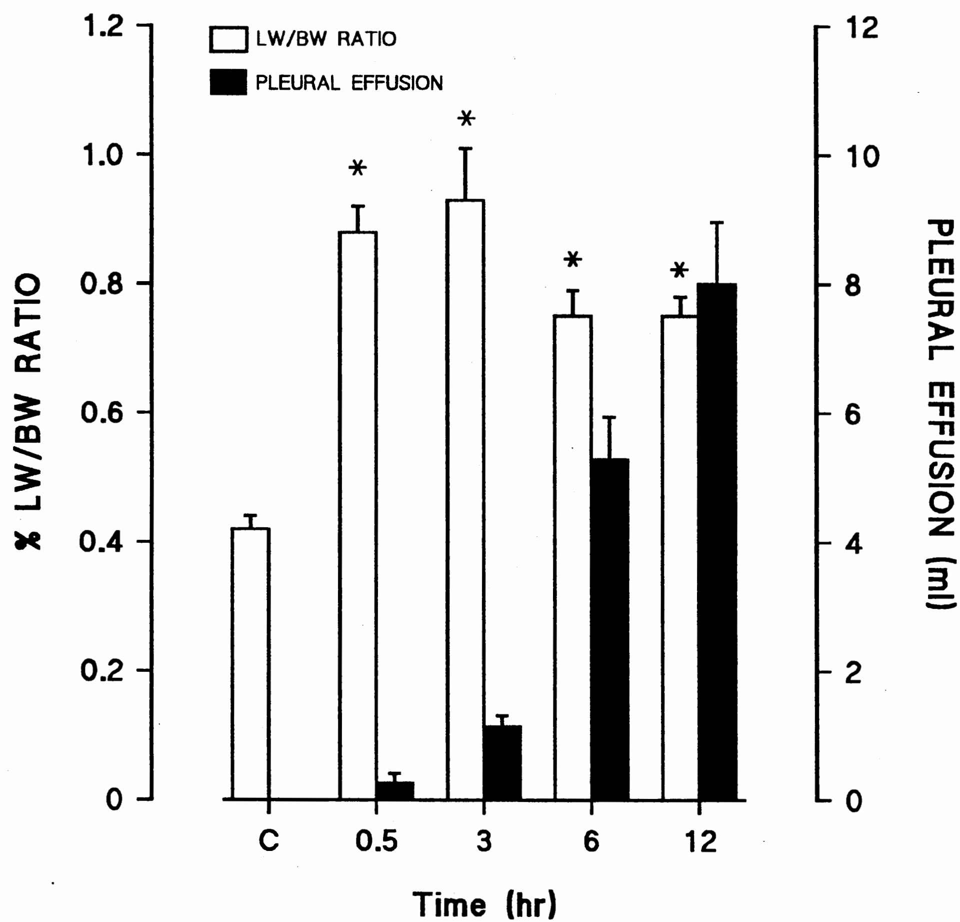


Figure 1. Percent lung to body weight (% LW/BW) ratio and pleural effusion volume in rats given 42 mg/kg xylazine (im, n=31). C = Control (Saline, im, n=13). (*) Significantly different from control ($p < 0.05$).

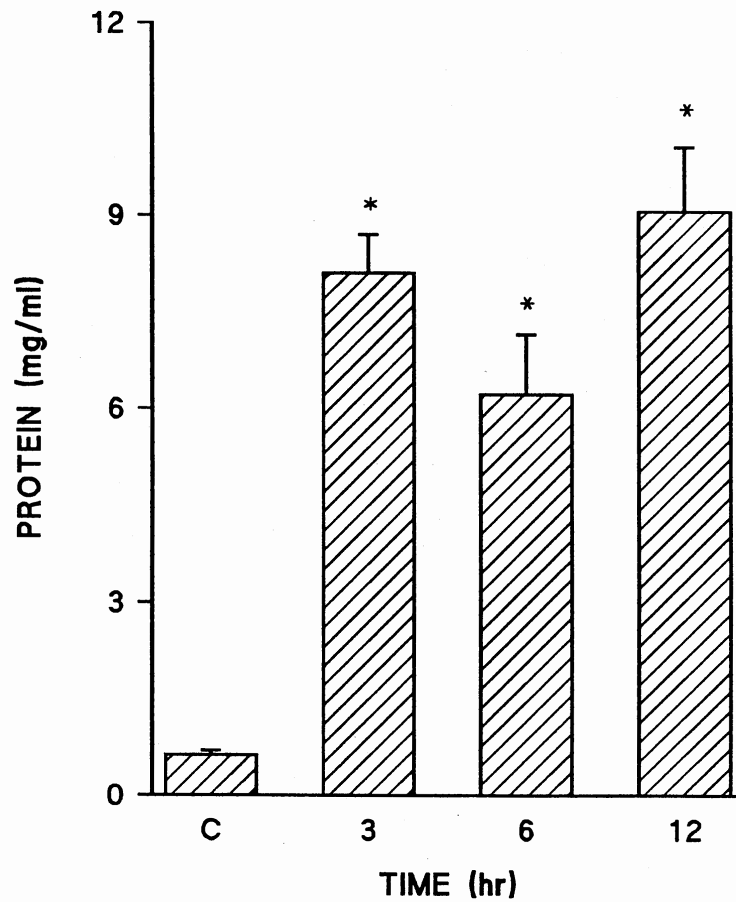


Figure 2A. Protein concentration in BALF from rats given 42 mg/kg xylazine (im, n=24). C = Control (saline, im, n=9). (*) Significantly different from control ($p < 0.05$).

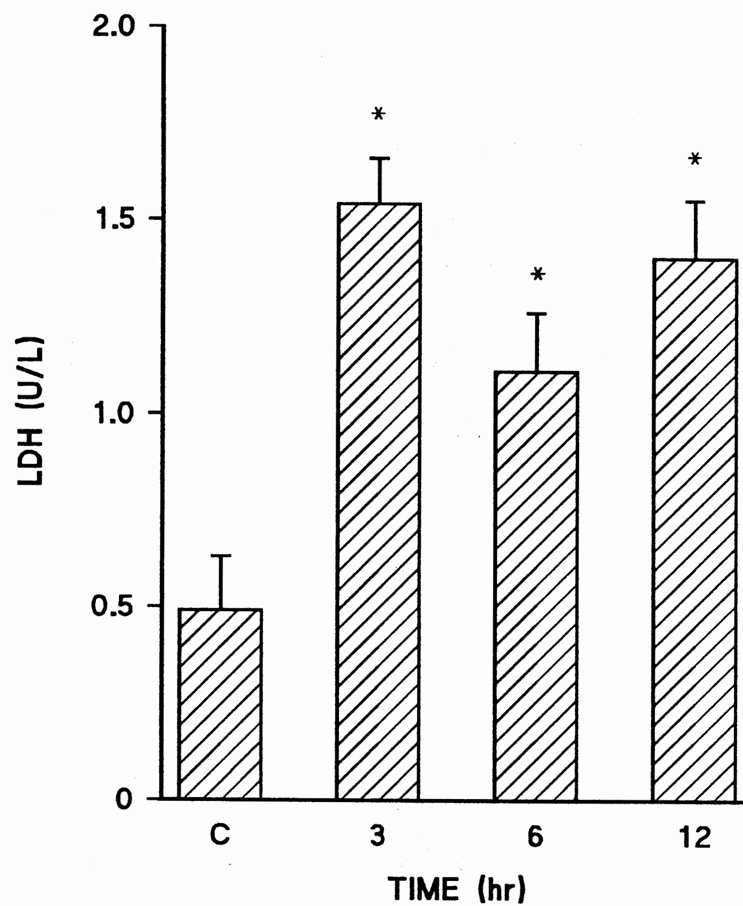


Figure 2B. Lactate dehydrogenase levels (LDH) in BALF from rats given 42 mg/kg xylazine (im, n=24). C = Control (saline, im, n=9). (*) Significantly different from control ($p < 0.05$).

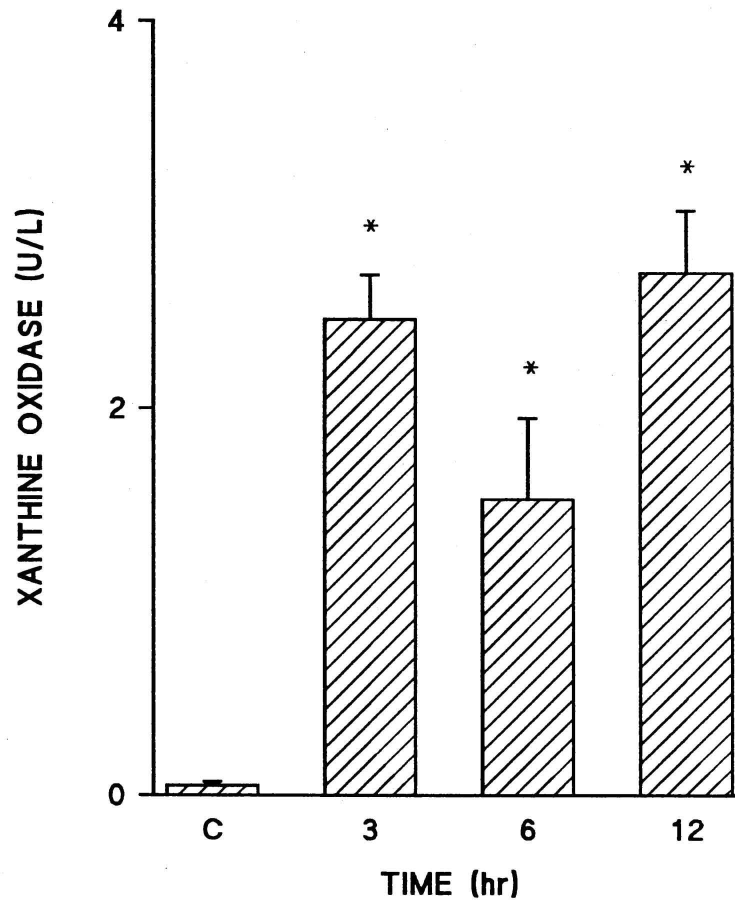


Figure 2C. Xanthine oxidase levels in BALF from rats given 42 mg/kg xylazine (im, n=24). C = Control (saline, im, n=9). (*) Significantly different from control ($p < 0.05$).

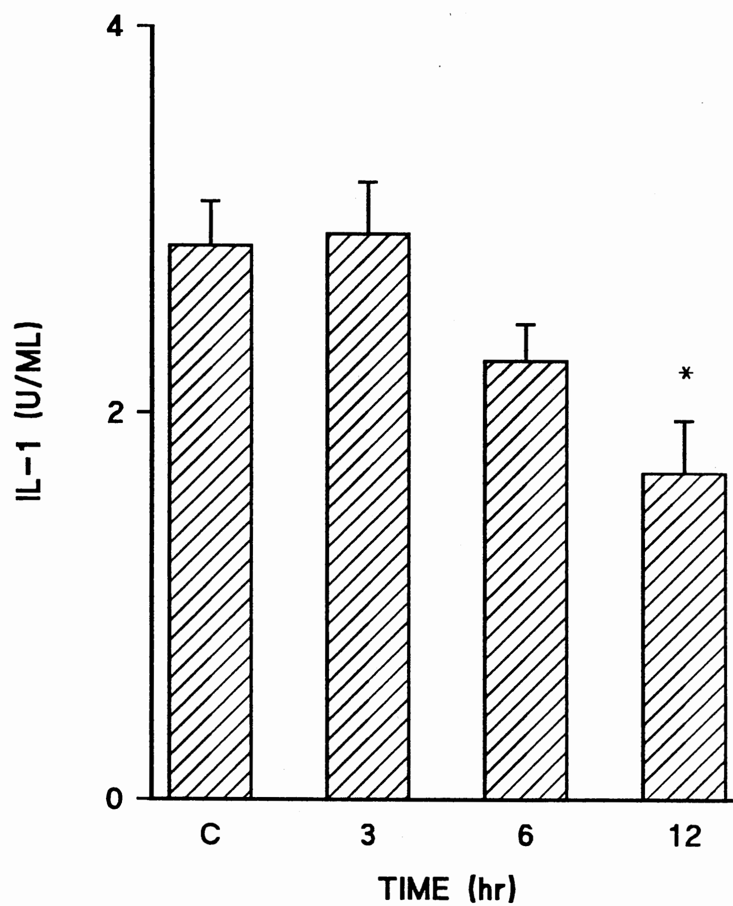


Figure 2D. Interleukin-1 (IL-1) activity in BALF from rats given 42 mg/kg xylazine (im, n=24). C = Control (saline, im, n=9). (*) Significantly different from control ($p < 0.05$).

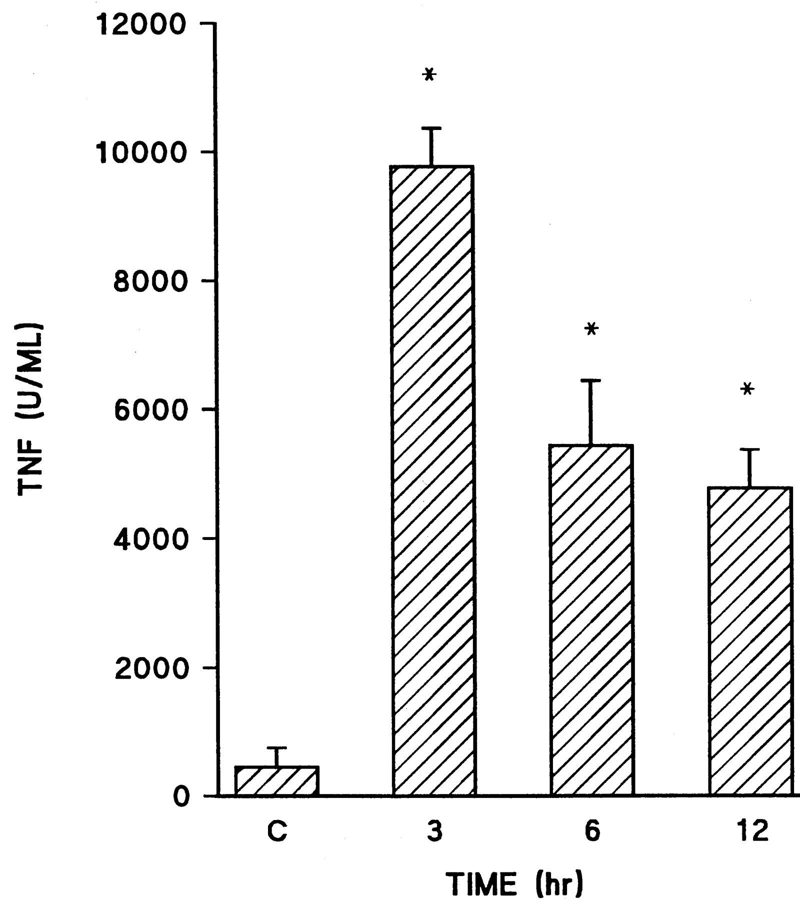


Figure 2E. Tumor necrosis factor (TNF) activity in BALF from rats given 42 mg/kg xylazine (im, n=24). C = Control (saline, im, n=9). (*) Significantly different from control ($p < 0.05$).

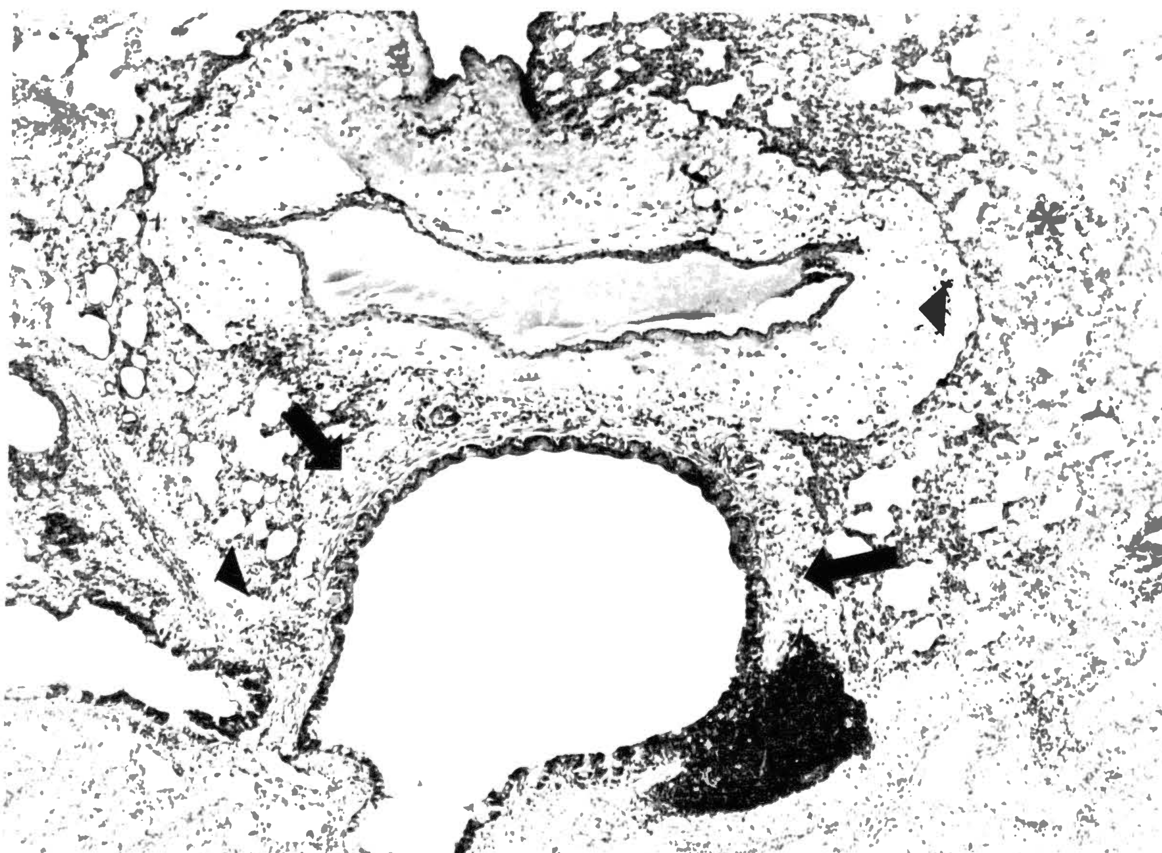


Figure 3. Light micrograph of the lung from a rat given 42 mg/kg xylazine (im, n=4) and sacrificed 12 hr later. Note perivascular (arrowheads), peribronchiolar (arrows) and alveolar edema (*). H&E x37.

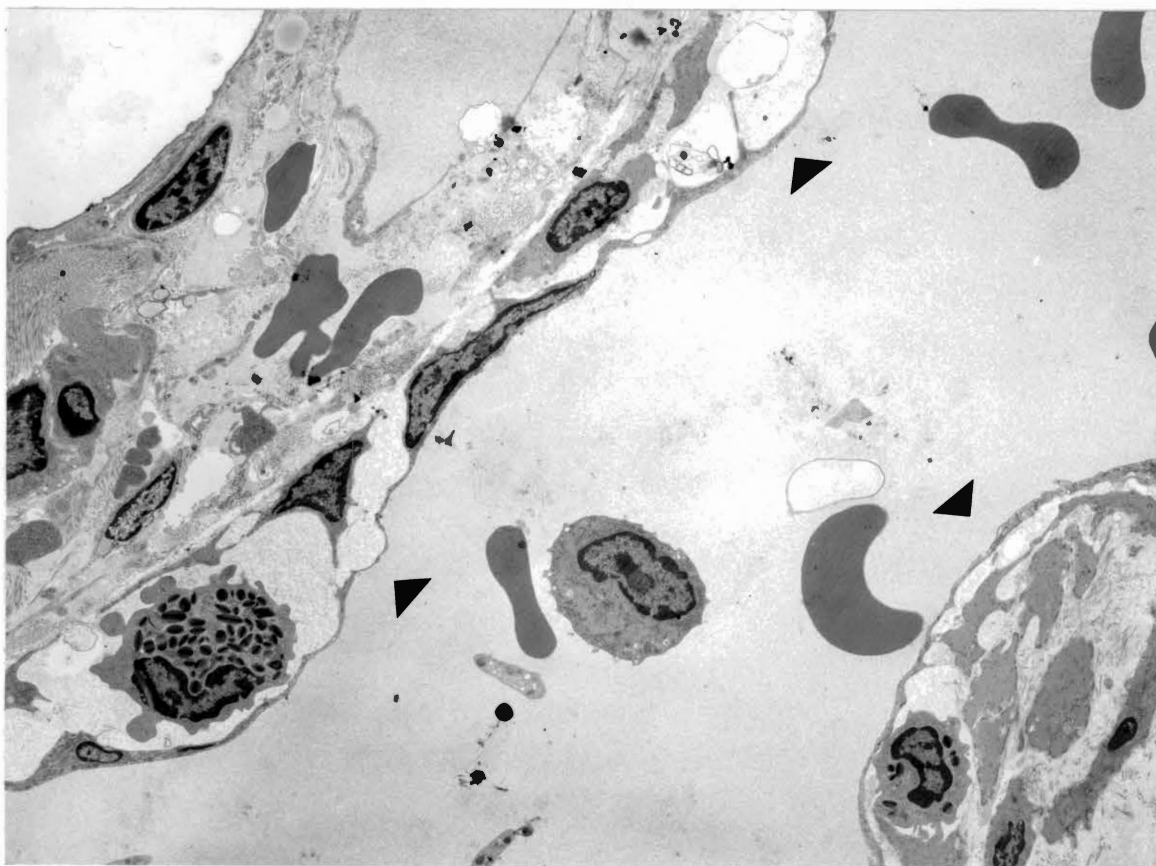


Figure 4. Electron micrograph of the lung of a rat given 42 mg/kg xylazine (im, n=4) and sacrificed 12 hr later. Note endothelial thinning, detachment from basement membrane or blabbing (arrowheads). Uranyl acetate and lead citrate. x9000.

The role of cytokines in etiology of xylazine-induced PE remains unclear. In this study, IL-1 activity remained unchanged and was reduced at 12 hr after xylazine administration. This reduction in activity is perhaps due to the displacement of a large volume of the plasma into the thoracic cavity as this will reduce the total IL-1 plasma level. Murine IL-1 caused granulocytopenia followed by granulocytosis and sustained pulmonary leukostasis in rabbits (Goldblum et al., 1987). Human recombinant IL-1 has been shown to cause pulmonary vascular endothelial injury accompanied with pulmonary leukostasis (Goldblum, 1988). It appears that lung injury caused by IL-1 is leukocyte-dependent. We have shown that leukocyte count is elevated only 12 hr after administration of xylazine. This is an indication that xylazine-induced PE is perhaps independent of leukocytes. Therefore, this could be the reason for the lack of elevated IL-1 levels as IL-1 lung injury appears to be leukocytes-dependent.

TNF levels were increased substantially throughout all time periods. This increase was highest at 1 hr indicating that TNF somehow plays a causative role in xylazine-induced PE. TNF-induced pulmonary endothelial injury in rabbits is independent of leukocytes as indicated by the lack of significant change in pulmonary leukostasis and myeloperoxidase activity (Goldblum et al., 1989). In addition, TNF increased transendothelial albumin movement in the absence of leukocytes in vitro (Goldblum et al., 1989). In contrast, granulocyte depletion prevented TNF-induced acute lung injury in guinea pigs (Stephens et al., 1988). The role of leukocytes in the pulmonary endothelial injury caused by IL-1 and TNF is not clear. Besides having many common characteristics with IL-1, TNF causes morphological changes in endothelium *in vitro* such as cellular elongation and overlapping (Stolpen et al., 1986), actin filament reorganization and loss of fibronection network as well as cytostatic effects

(Sato et al., 1986). The role of TNF in xylazine-induced pulmonary edema appears to be due to its effect on endothelial permeability or its cytotoxic effect.

In summary, these results indicate that xylazine causes increased-permeability PE through endothelial injury. Whereas oxygen radicals and TNF appear to be involved, IL-1 may not play a role in the etiology of xylazine-induced pulmonary edema.

References

ADERKA, D., FISHER, S., LEVO, Y., HOLTMAN, HAHN, T. AND WALLACE, D. (1985). Cachectin/tumor-necrosis-factor production by cancer patients. *Lancet* 2, 1190.

ALBRIGHTSON, C.R., BAENZIGER, N.L. AND NEEDLEMAN, P. (1985). Exaggerated human vascular cell prostaglandin biosynthesis mediated by monocytes: role of monokines and interleukin-1. *J. Immunol.* 135, 1872-1877.

AMOUZADEH, H.R., SANGIAH, S. AND QUALLS, C.W. (1989). Effects of some hepatic microsomal enzyme inducers and inhibitors on xylazine-ketamine anesthesia. *Vet. Hum. Toxicol.* 31, 532-534.

AMOUZADEH, H.R., SANGIAH, S. AND QUALLS JR., C.W., COWELL, R.L. AND MAUROMOUSTAKOS, A. (1991). Xylazine-induced pulmonary edema in rats. *Toxicol. Appl. Pharmacol.* 108, 417-427.

BALKWILL, F., OSBORNE, R., BURKE, F., NAYLOR, S., TALBOT, D., DURBIN, H., AVERNIER, J. AND FRIES, W. (1987). Evidence for tumor necrosis factor/ cachectin production in cancer. *Lancet* 1, 1229-1230.

BAKER, J.R. AND GATESMAN, T.J. (1985). Use of carfentanil and ketamine-xylazine mixture to immobilize wild grey seals (*Halichoerus grypus*). *Vet. Rec.* 116, 208-210.

BEVILACQUA, M.P., POBER, J.S., MAJEAU, G.R., COTRAN, R.S. AND GIMBRONE, M.A. (1986). Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the action of interleukin 1. *Proc. Natl. Acad. Sci.* 83, 4533-4537, 1986.

BEVILACQUA, M.P., POBER, J.S., WHEELER, M.E., COTRAN, R.S. AND GIMBRONE, M.A. (1985). Interleukin-1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. *J. Clin. Invest.* 76, 2003-2011.

BOYD, M.R. (1980). Biochemical mechanisms in chemical-induced lung injury: role of metabolic activation. *CRC Critical Reviews in Toxicology.* 7, 103-176.

BRETT, J., GERLACH, H., NAWROTH, P., STEINBERG, S., GODMAN, G. AND STERN, D. (1989). Tumor necrosis factor/cachectin increases permeability of endothelial cell monolayers by a mechanism involving regulatory G protein. *J. Exp. Med.* 169, 1977-1991.

BUSSOLINO, F., BREVIARIO, F., TETTA, C., MASSIMO, A., MANTOVANI, A. AND DEJANA, E. (1986). Interleukin-1 stimulates platelet activating factor production in cultured human endothelial cells. *J. Clin. Invest.* 77, 2027-2033.

CANNON, J.G. AND DINARELLO, C.A. (1985). Increased plasma interleukin-1 activity in women after ovulation. *Science* 227, 1247-1248.

COOPER, J.A.D., WHITE, D. AND MATTHAY, R.A. (1986a). Drug-induced pulmonary disease, Part 1: Cytotoxic drugs. *Am. Rev. Resp. Dis.* 133, 321-340.

COOPER, J.A.D., WHITE, D. AND MATTHAY, R.A. (1986b). Drug-induced pulmonary disease, Part 2: Noncytotoxic drugs. *Am. Rev. Resp. Dis.* 133, 488-505.

COTRAN, R.S., GIMBRONE, M.A., BEVILACQUA, M.P., MENDRICK, D.L. AND POBER, J.S. (1986). Induction and detection of a human endothelial activation antigen in vivo. *J. Exp. Med.* 164, 661-666.

COTTRELL, T.S., LEVINE, O.R., SENIOR, R.M., WEINER, J., SPIRO, D., FISHMAN, A.P. (1967). Electron microscopic alterations at the alveolar level in pulmonary edema. *Circ. Res.* 21, 783-797.

CUNNINGHAM, A.L., HURLEY J.V. (1971). Alpha-naphthyl-thiourea-induced pulmonary oedema in the rat: A topographical and electron microscope study. *J. Path.* 106, 25-35.

DEEB G.M., GRUM, C.M., LYNCH, M.J., GUYNN, T.P., GALLAGHER, K.P., LJUNGMAN, A.G., BOLLING, S.F. AND MORGANROTH, M.L. (1990). Neutrophils are not necessary for induction of ischemia-reperfusion lung injury. *J. Appl. Physiol.* 68, 374-381.

DINARELLO, C.A., CANNON, J.G., WOLFF, S.M., BRENHEIM, H.A., BEUTLER, B., CERAMI, A., FIGARI, I.S., PALLADINO, M.A. AND O'CONNER, J.V. (1985). Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J. Exp. Med.* 163, 1433-1450.

DINARELLO, C.A. (1988). Biology of interleukin 1. *FASEB J.* 2, 108-115.

FRANK, L. (1985). Oxygen toxicity in eukaryotes. In *Superoxide Dismutase VIII*, (L.W. Oberley, Ed.), pp. 1-43. CRC press, Boca Raton, FL.

FRIED, R. (1966). Colorimetric determination of xanthine dehydrogenase by tetrazolium reduction. *Anal. Biochem.* 16, 427-432.

GILLESPIE, M.N., GOLDBLUM, COHEN, D.A., AND MCCLAIN, C.J. (1988). Interleukin 1 bioactivity in the lung of rats with monocrotaline-induced pulmonary hypertension. *Proc. Soc. Exp. Biol. Med.* 187, 26-32.

GILLESPIE, M.N., OLSON, J.W., HENNING, B., COHEN, D.A., MCCLAIN, C.J. AND GOLDBLUM, S.E. (1989). Monokine-induced lung injury in rats: Similarities to monocrotaline-induced pneumotoxicity. *Toxicol. Appl. Pharmacol.* 98, 134-143.

GOLDBLUM, S.E., COHEN, D.A., GILLESPIE, M.N. AND MCCLAIN, C.J. (1988). Interleukin-1-induced granulocytopenia and pulmonary leukostasis in rabbits. *Am. J. Physiol.* 62, 122-128.

GOLDBLUM, S.E., YONEDA, K., COHEN, D.A. AND MCCLAIN, C.J. (1988). Provocation of pulmonary vascular endothelial injury in rabbits by human recombinant interleukin-1ß. *Infec. Immun.* 56, 2255-2263.

GOLDBLUM, S.E., HENNING, B., JAY, M., YONEDA, K. AND MCCLAIN, C.J. (1989). Tumor necrosis factor α-induced pulmonary vascular endothelial injury. *Infec. Immun.* 57, 1218-1226.

GOLDBLUM, S.E. AND SUN, W.L. (1990). Tumor necrosis factor-α augments pulmonary arterial transendothelial albumin flux in vitro. *Am. J. Physiol.* 258, L57-L67.

GOODMAN, J.C., ROBERTSON, C.S., GROSSMAN, R.G. AND NARAYAN, R.K. (1990). Elevation of tumor necrosis factor in head injury. *J. Neuroimmunol.* 30, 213-217.

GOSSET, P., PEREZ, T., LASSALLE, P., DUQUESNOY, B., FARRE, J.M., TONNEL, A.B. AND CAPRON, A. (1991). Increased TNF-α secretion by alveolar macrophages from patients with rheumatoid arthritis. *Am. Rev. Respir. Dis.* 143, 593-597.

GREENE, S.A., AND THURMON, J.C. (1988). Xylazine-a review of its pharmacology and use in veterinary medicine. *J. Vet. Pharmacol. Therap.* 11, 295-313.

GROSSO, M.A., BROWN, J.M., VIDERS, D.E., MULVIN, D.W., BANERJEE, A. VELASCO, S.E., REPINE, J.E. AND HARKEN, H. (1989). Xanthine-oxidase-derived oxygen radicals induce pulmonary edema via direct endothelial cell injury. *J. Surg. Res.* 46, 355-360.

HENNING, B., HONCHEL, R., GOLDBLUM, S.E. AND MCCLAIN, C.J. (1988). Tumor necrosis factor-mediated hypoalbuminemia in rabbits. *J. Nutr.* 118, 1586-1590.

HOCKING, D.C., PHILLIPS, P.G. FERRO, T.J. AND JOHNSON, A. (1990). Mechanism of pulmonary edema induced by tumor necrosis factor-α. *Cir. Res.* 67, 68-77.

HORVATH, C. J., FERRO, T.J., JESMOK, G. AND MALIK, A.B. (1988). Recombinant tumor necrosis factor increases pulmonary vascular permeability independent of neutrophils. *Proc. Natl. Acad. Sci.* 85, 9219-9223.

JARASCH, E.D., BRUDER, G. AND HEID, H.W. (1986). Significance of xanthine oxidase in capillary endothelial cells. *Acta Physiol. Scand.* Suppl. 548, 49-46.

KARNOVSKY, M.J. (1965). A formaldehyde-gluteraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell. Biol.* 25, 137A-138A.

KAYE, J., GILLIS, S., MIZEL, S.B., SHEVACH, E.M., MALEK, T.R., DINARELLO, C.A., LACHMAN, L.B. AND JANEWAY, C.A. (1984). Growth of cloned helper T cell line induced by a monoclonal antibody specific for the antigen receptor: interleukin-1 is required for the expression of receptors for interleukin-2. *J. Immunol.* 133, 1339-1345.

KORZENIEWSKI, C. AND CALLWAERT, D.M. (1983). An Enzyme-release assay for natural cytotoxicity. *J. Immunol. Meth.* 64, 313-320.

LEVINE, B., KALMAN, J., MAYER, L., FILLIT, H.M. AND PACKER, M. (1990). Elevated circulating levels of tumor necrosis factor in sever chronic heart failure. *New Eng. J. Med.* 323, 236-241.

MARANO, M.A., FONG, Y., MOLDAWER, L.L., WEI, H., CALVANO, S.E., TRACEY, K.J., BARIE, P.S., MANOGUE, K., CERAMI, A., SHIRES, G.T. AND LOWRY, S.F. (1990). Serum cachectin/tumor necrosis factor in critically ill patients with burns correlates with infection and mortality. *Surg. Obst. Gynecol.* 170, 32-38.

MATSUBARA, T. AND ZIFF, M. (1986). Increased superoxide anion release from human endothelial cells in response to cytokines. *J. Immunol.* 137, 3295-3298.

MAURY, C.P.J. AND TEPPO, A.M. (1987). Raised serum levels of cachectin/tumor necrosis factor α in renal allograft rejection. *J. Exp. Med.* 166, 1132-1137.

MCCLAIN, C.J., COHEN, D., OTT, L., DINARELLO, C.D. AND YOUNG, B. (1987). Ventricular fluid interleukin-1 activity in patient with head injury. *J. Lab. Clin. Med.* 110, 48-54.

MCDONALD, R.J., BERGER, E.M. AND REPINE, J.E. (1987). Neutrophil-derived oxygen metabolites stimulate thromboxane release, pulmonary artery pressure increases, and weight gain in isolated perfused rat lungs. *Am. Rev. Respir. Dis.* 135, 957-959.

MUTO, Y., NOURI-ARIA, K.T., MEAGER, A., ALEXANDER, G.J., EDDELSTON, A.L.W.F. AND WILLIAMS, R. (1988). Enhanced tumor necrosis factor and interleukin-1 in fulminant hepatic failure. *Lancet* 2, 72-74.

NATIONAL INSTITUTE OF HEALTH (1985). *Guide for the Care and Use of Laboratory Animals*. NIH, Bethesda, Maryland.

NAWROTH, P.P. AND STERN, D.M. (1986). Modulation of endothelial cell hemostatic properties by tumor necrosis factor. *J. Exp. Med.* 163, 740-745.

NAWROTH P.P., BANK, I., HANDLEY, D., CASSIMERIS, J., CHESS, J. AND STERN D. (1986). Tumor necrosis factor/cachectin interacts with endothelial receptors to induce release of interleukin 1. *J. Exp. Med.* 163, 1363-1375.

NOLPO, K.B. AND RYAN, U.S. (1990). Enhancement of tumor necrosis factor-induced endothelial cell injury by cycloheximide. *Am. J. Physiol.* 259, L123-L129.

PALMORE, W.P. (1990). A fatal response to xylazine and ketamine in group of rabbits. *Vet. Res. Comm.* 14, 91-98.

PATTERSON, C.E., BARNARD, J.W., LAFUZE, J.E., HULL, M.T., BALDWIN, S.J. AND RHODES, R.A. (1989). The role of activation of neutrophils and microvascular pressure in acute pulmonary edema. *Am. Rev. Resp. Dis.* 140, 1052-1062.

POBER, J.S. AND COTRAN, R.S. (1990). Cytokines and endothelial cell biology. *Physiol. Rev.* 70, 427-451.

REMICK, D.G., KUNKEL, R.G., LARRICK, J.W. AND KUNKEL, S.L. (1987). Acute *in vivo* effects of human recombinant tumor necrosis factor. *Lab. Invest.* 56, 583-590.

REPINE, J.E., BOWMAN, M. AND TATE, R.M. (1982). Neutrophils and lung edema. *chest* 81, 47S-50S.

ROTEN, R., MARKERT, M., FEIHL, F., SCHALLER, M., TAGAN, M. AND PERRET, C. (1991). Plasma levels of tumor necrosis factor in adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* 143, 590-592.

ROYALL, J.A., BERKOW, R.L., BECKMAN, J.S., CUNNINGHAM, M.K., MATALON, S. AND FREEMAN, B. (1989). Tumor necrosis factor and interleukin 1α increase vascular endothelial permeability. *Am. J. Physiol.* 257, L399-L410.

SATO, N., GOTO, T., HARANAKA, K. SATOMI, N., NARIUCHI, H., MANO-HIRANO, Y. AND SAWASAKI, Y. (1986). Action of tumor necrosis factor on cultured vascular endothelial cells: Morphologic modulation, growth inhibition and cytotoxicity. *J. Natl. Canc. Inst.* 76, 1113-1121.

SCHATTNER, A., STEINBOCK, M., TEPPER, R., SCHONFELD, A., VAISMAN, N. AND HAHN, T. (1990). Tumor necrosis factor production and cell-mediated immunity in anorexia nervosa. *Clin. Exp. Immunol.* 79, 62-66.

SCHUGER, L., VARANI, J., MARKS, R.M., KUNKEL, S.L., JOHNSON, K.J. AND WARD, P.A. (1989). Cytotoxicity of tumor necrosis factor-α for human umbilical vein endothelial cells. *Lab. Invest.* 61, 62-68.

SHALABY, M.R., AGGARWAL, B.B., RINDERKNECHT, E., SVEDERSKY, L.P., FINKLE, B.S. AND PALLADINO, M.R. (1985). Activation of human polymorphonuclear neutrophil functions by interferon- and tumor necrosis factor. *J Immunol* 135, 2069-2073.

SHASBY, M.D., FOX, R.B., HRADA, R.N. AND REPINE, J.E. (1982a). Reduction of the edema of acute hyperoxic lung injury by granulocyte depletion. *J. Appl. Physiol.* 52, 1237-1244.

SHASBY, D.M., VANBENTHUYSEN, K.M., TATE, R.M., SHASBY, S.S., MCMURTURY, I. AND REPINE, J.E. (1982b). Granulocyte mediate acute edematous lung injury in rabbit and isolated rabbit lung perfused with phorbol myristate acetate: role of oxygen radicals. *Am. Rev. Resp. Dis.* 125, 443-447.

SHINJO, K., TSUDA, S., HAYAMI, T., ASAHI, T. AND KAWAHARADA, H. (1989). increase in permeability of human endothelial cell monolayer by recombinant lymphotoxin. *Biochem. Biophys. Res. Commun.* 162, 1431-1437.

SLUNGAARD, A., VERCELLOTTI, G.M., WALKER, G., NELSON, R.D. AND JACOBS, H.S. (1990). Tumor necrosis factor *α*/cachectin stimulates eosinophil oxidant production and toxicity towards human endothelium. *J. Exp. Med.* 171, 2025-2041.

STAUB, N.C. (1984). Pathophysiology of pulmonary edema. In *Edema* (N.C. Staub and A.E. Taylor, Eds.), pp. 719-746. Raven Press, New York.

STEPHENS, K. E., ISHIZAKA, A., Wu, Z., LARRICK, J.W. AND RAFFIN T.A. (1988). Granulocyte depletion prevents tumor necrosis factor-mediated acute lung injury in guinea pigs. *Am. Rev. Resp. Dis.*, 138, 1300-1307.

STEPHENS, K. E., ISHIZAKA, A., LARRICK, J.W. AND RAFFIN T.A. (1988). Tumor necrosis factor causes increased pulmonary permeability and edema. *Am. Rev. Resp. Dis.*, 137, 1364-1370.

TRACY, K.J., BEUTLER, B., LOWRY, S.F., MERRYWEATHER, J., WOLPE, S., MILSARK, I.W., HARIRI, R.J., FAHEY, T.J., ZENTELIA, A., ALBERT, J.D., SHIRES, G.T. AND CERAMI, A. (1986). Shock and tissue injury induced by recombinant human cachectin. *Science* 234, 470-473.

TSUJIMOTO, M., YOKOTA, S., VILCEK, J. AND WEISSMANN, G. (1986). Tumor necrosis factor provokes superoxide anion generation from neutrophils. *Biochem. Biophys. Res. Commun.* 137, 1094-1100.

VARANI, J., BENDELOW, M.J., SEALY, D.E., KUNKEL, S.L., GANNON, D.E., RYAN, U.S. AND WARD, P.A. (1988). Tumor necrosis factor enhances susceptibility of vascular endothelial cells to neutrophil-mediated killing. *Lab. Invest.* 59, 292-295.

CHAPTER V

EFFECTS OF XYLAZINE ON BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS IN CULTURE

Introduction

In a recent study, we reported that xylazine, a veterinary analgesic, sedative and muscle relaxant, caused pulmonary edema (PE) in rats characterized by rapid onset, cellular damage, extensive pleural effusion and protein-rich edema fluid (Amouzadeh et al., 1991). These characteristics are the hallmark of increased-permeability pulmonary edema (Staub, 1986). Subsequently, we showed that xylazine-induced PE is due to endothelial injury as indicated by the elevated protein and lactate dehydrogenase levels in bronchoalveolar lavage and ultrastructural changes such as endothelial thinning, detachment from basement membrane or bleb formation (Amouzadeh et al., 1991). The endothelial injury is likely caused by oxygen radicals since xanthine oxidase levels in bronchoalveolar lavage fluid was increased in xylazine-treated rats (Amouzadeh et al., 1991). Since inhibitors of cytochrome P-450 such as SK&F 525-A augmented the pulmonary toxicity of xylazine (Amouzadeh et al., 1991), it is thought that endothelial injury in xylazine-treated rats is due to direct effects of xylazine or its metabolites on the pulmonary vascular endothelium. Many classes of drugs such as antibacterial agents, nitrosoureas, alkylating agents, antimetabolites, analgesics, anticonvulsants, antiarrhythmic agents, diuretics, opiates and antirheumatic drugs have been associated with pulmonary diseases (Cooper et al., 1986a and

1986b). These drugs could be classified according to their mechanism of toxicity into two categories: cytotoxic and non-cytotoxic. Most of cytotoxic drugs are used in chemotherapy whereas non-cytotoxic drugs include a wider range of agents. The mechanism of the toxicity of many cytotoxic drugs is not well-understood and in many cases it might be the result of a combination of effects. In addition to drugs, toxicants such as paraquat, α-naphthylthiourea (ANTU), 4-ipomeanol and pyrrolizidine alkaloids also cause lung injury. The pulmonary toxicity of paraquat, 4-ipomeanol, ANTU and monocrotaline is caused by the reactive metabolites of these toxicants generated by cytochrome P-450 (Boyd, 1980). In the case of paraquat, these reactive metabolites are oxygen radicals (Boyd, 1980). Regardless of the source and the mechanism of injury, often pulmonary endothelium is the primary target of these agents. Bleomycin, cyclophosphamide, and nitrofurantoin generate oxygen radicals which are thought to be responsible for pulmonary toxicity of these agents (Cooper, 1986a; Frank, 1985). Endothelial injury is one of the common features of increased-permeability PE. Using endothelial cells isolated from porcine thoracic aorta, Ody and Junod (1985) have shown that paraquat is directly toxic to endothelial cells. Martin and Howard (1985) have also reported similar findings for amiodarone, an antiarrhythmic drug, using bovine pulmonary artery endothelial cells.

The purpose of this study was to determine whether xylazine-induced PE is caused by the direct effect of xylazine on pulmonary endothelium using bovine pulmonary endothelial cells as an *in vitro* model.

Materials and Methods

Endothelial Cells. Bovine pulmonary artery endothelial cells (BPAEC) were purchased from American Type Culture Collection at passage 16 (ATCC No. CCl 209). The cells

were cultured in minimum essential medium supplemented with 20 % fetal calf serum (complete medium) at 37 °C in 95% O_2 and 5% CO_2 and used at passage 19.

Chemicals and Cell Culture Medium. Minimum essential media, fetal calf serum, trypsin-EDTA (Gibco-BRL Grand Island, NY), xylazine hydrochloride, trypan blue and Hank's balanced salt solution (Sigma Chemical Co., St. Louis, MO) were purchased commercially.

Experimental Procedure. Cells were plated in 24-well plates at a density of 3.5×10^4 cells per well and allowed to grow to confluency. Upon confluency, the medium was removed from each well and the monolayer was rinsed with 0.5 ml complete medium. The xylazine was dissolved in distilled water and filtered through 0.2 μ filter. Various concentrations of xylazine (0.3 - 300 μg/ml) in a volume of 10 μl was mixed with 990 μl of complete medium and was placed in wells. The control well contained 10 μl of filtered distilled water (quadruplicate determinations). The plates were incubated for either 0.5 or 3 hr at 37 °C in 95% O_2 and 5% CO_2. After incubation, the monolayers were photographed using a phase-contrast microscope (Olympus, CK1) and the medium was removed and frozen. The monolayer was removed with trypsin-EDTA and the number of live and dead cells were determined by dye exclusion assay using 0.4% trypan blue. Cell viability was calculated as percentage of the ratio of live to the total number of cells. Morphological changes were assessed by comparison of the phase-contrast micrographs of treated monolayer to that of control.

Data Analysis. The mean ± S.E. of percentage viability for control and each dose was calculated and analyzed by one way analysis of variance using SAS general Linear

model procedure. The significance of treatment means as compared to control value was determined by Dunnett's test. A probability level of $P < 0.05$ was considered significant.

Results

Phase-contrast micrographs did not show any change up to 30 μg/ml xylazine (Figure 1A, B, D & C). At 300 μg/ml xylazine, cells appeared elongated and retracted from growth surface (Figure 1E). The viability of the BPAEC was not affected by xylazine at any dose or time period (Figure 2).

Discussion

The result of this study indicate that xylazine may not have any direct toxic effects on BPAEC. Since a dose-dependent response could not be demonstrated and absence of any change in cell viability, the morphological changes caused by 300 μg/ml of xylazine could be attributed to non-specific effects. This dose could also be an in vitro toxic threshold, however, it will not have any in vivo toxicological consequences. The pharmacokinetic data of xylazine in rats are not known at present, however, extrapolation of the pharmacokinetics parameters of xylazine from dogs and horses (Garcia-Villar et al., 1981) suggests that the concentration of xylazine in plasma after administration of 42 mg/kg xylazine should not exceed 42 μg/kg. Therefore, xylazine-induced pulmonary edema could not be the result of the direct toxicity of xylazine. Since we used bovine pulmonary artery endothelial cells, the possibility of a species-dependent response could not be ruled out. However, considering similar morphological (cobble-stone appearance) and biochemical (presence of angiotensin converting enzyme, prostaglandin metabolism) characteristics of endothelial cells

derived from different sources (bovine, porcine, and human) it is unlikely that species variation is an important factor. It is also possible that pulmonary toxicity of xylazine is caused by its metabolites, some of which could be reactive. Pulmonary toxicity of paraquat, ANTU and monocrotaline is thought to be due the reactive metabolites, generated by cytochrome P-450 either in liver or lung (Boyd, 1980). The role of metabolites of xylazine in its pulmonary toxicity needs further investigation.

In summary, xylazine does not appear to have any toxicity toward BPAEC in vitro indicating that xylazine-induced pulmonary edema is mediated through mechanisms other than direct toxicity.

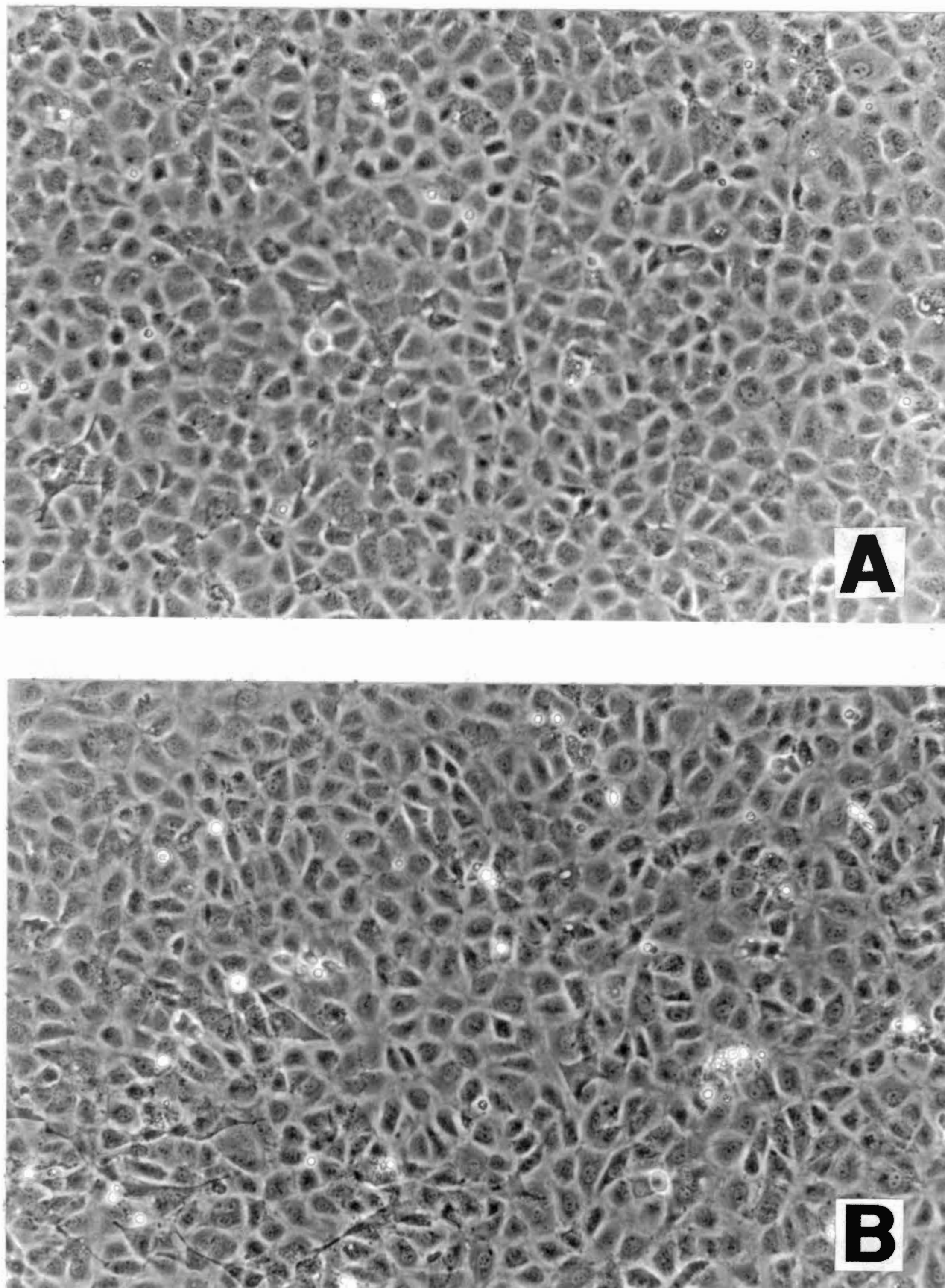
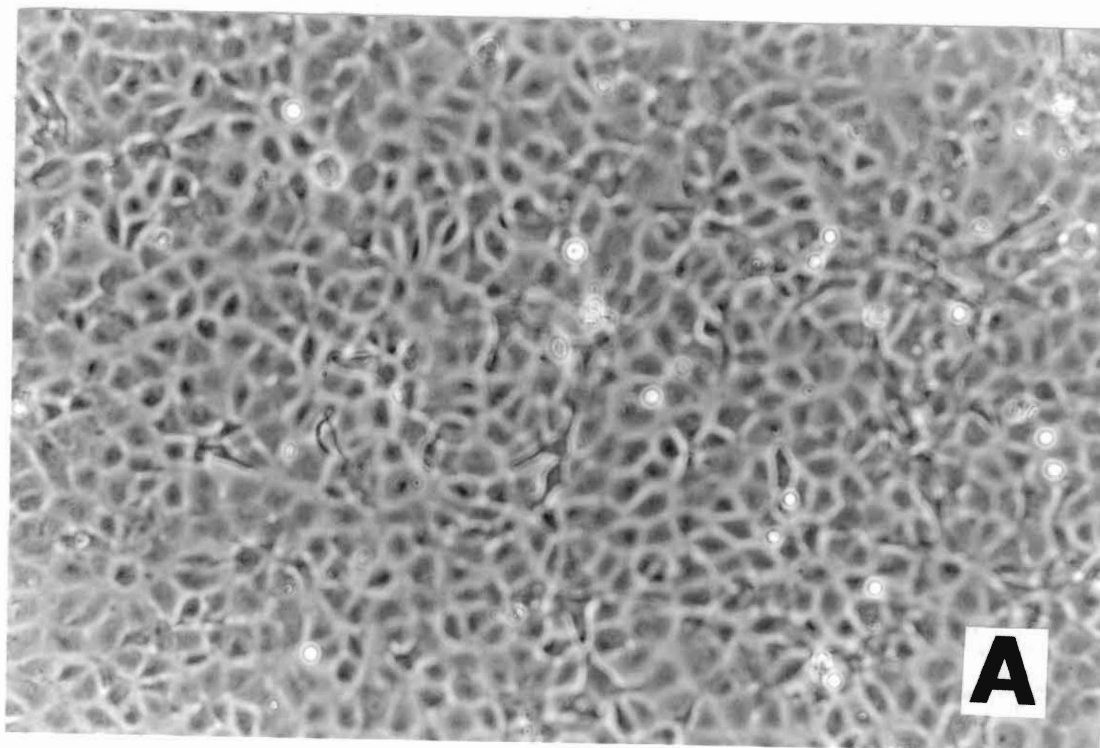


Figure 1A. Phase-contrast micrograph of the morphology of the BPAEC monolayer 0.5 hr (A) and 3.0 hr (B) after treatment with distilled water (control).



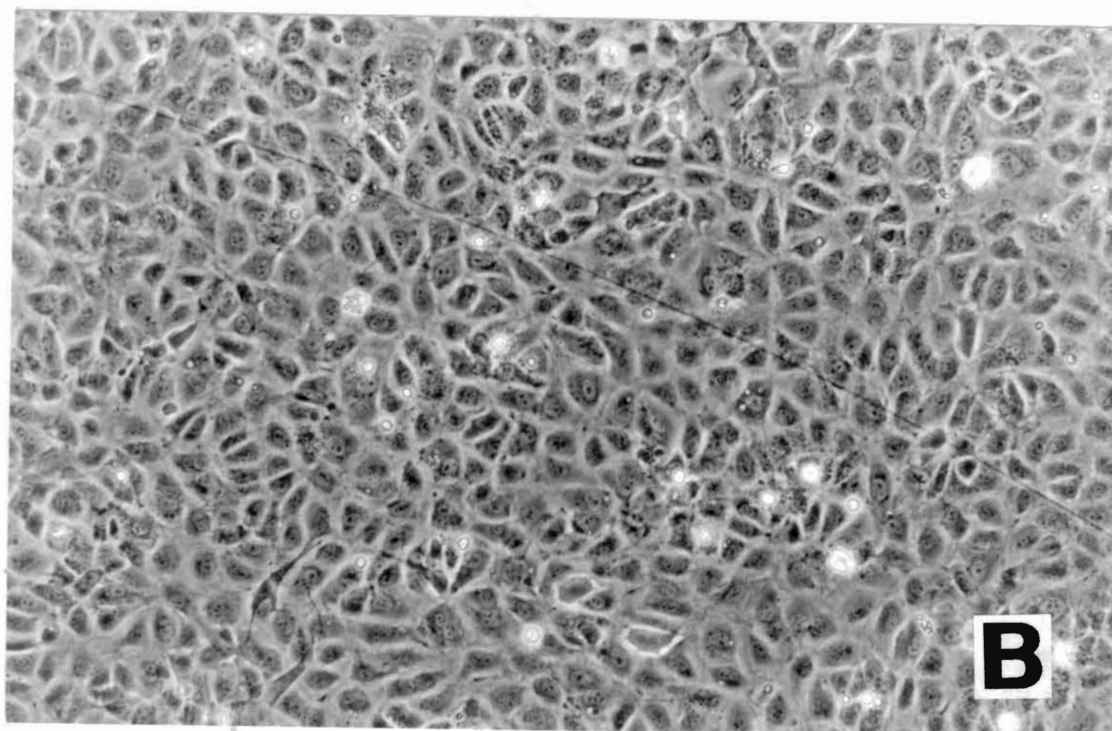
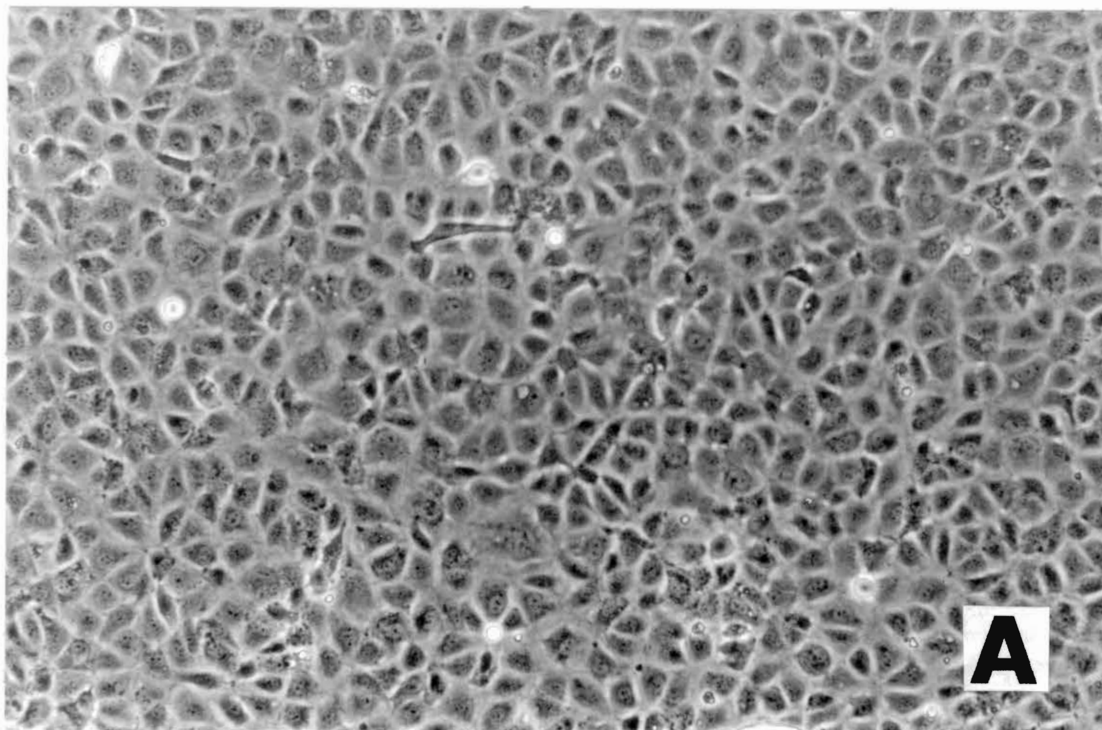


Figure 1B. Phase-contrast micrograph of the morphology of BPAEC monolayer 0.5 hr (A) and 3 hr (B) after treatment with 0.3 μg/ml xylazine.



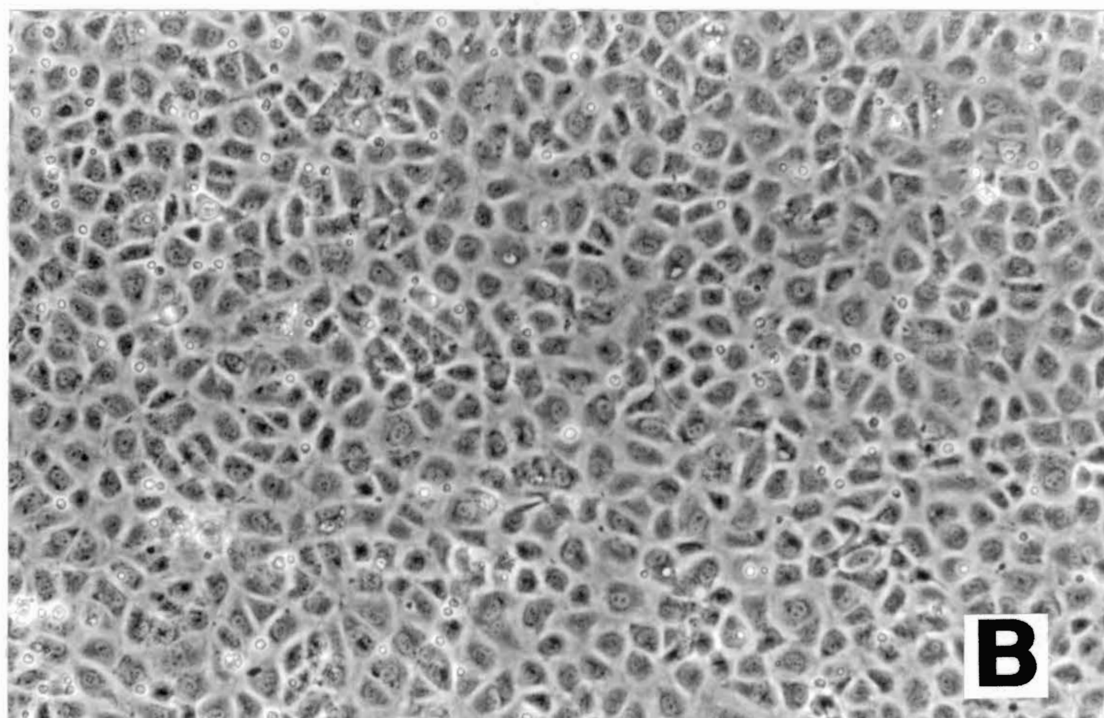
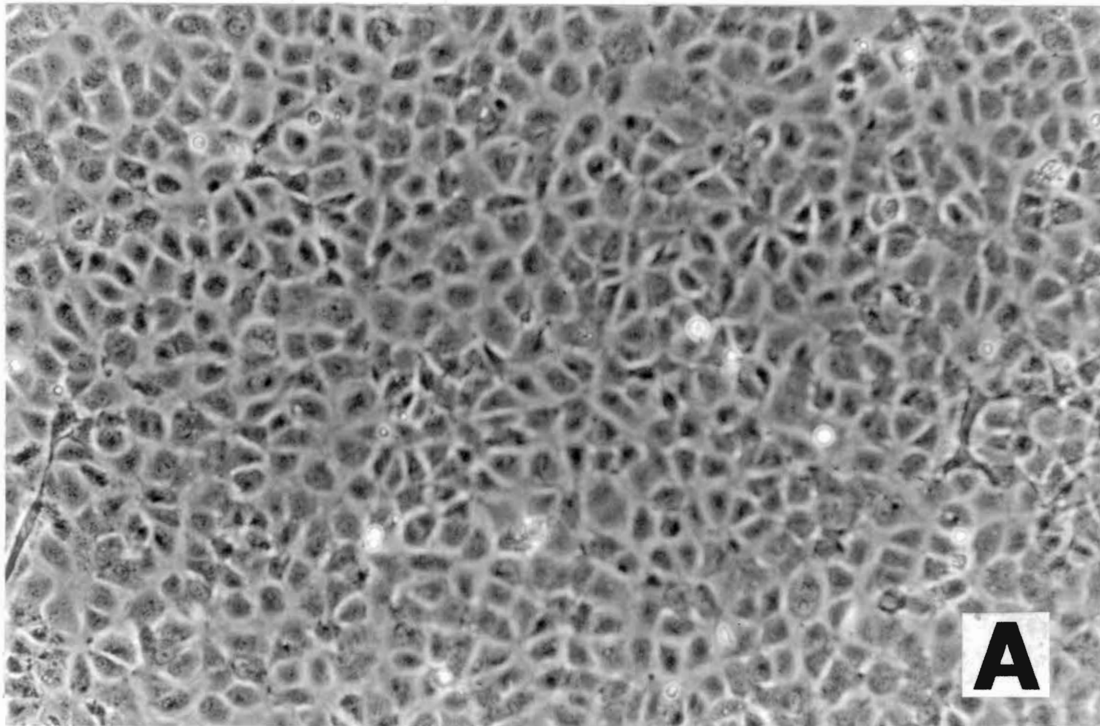


Figure 1C. Phase-contrast micrograph of the morphology of BPAEC monolayer 0.5 hr (A) and 3 hr (B) after treatment with 3 μg/ml xylazine.



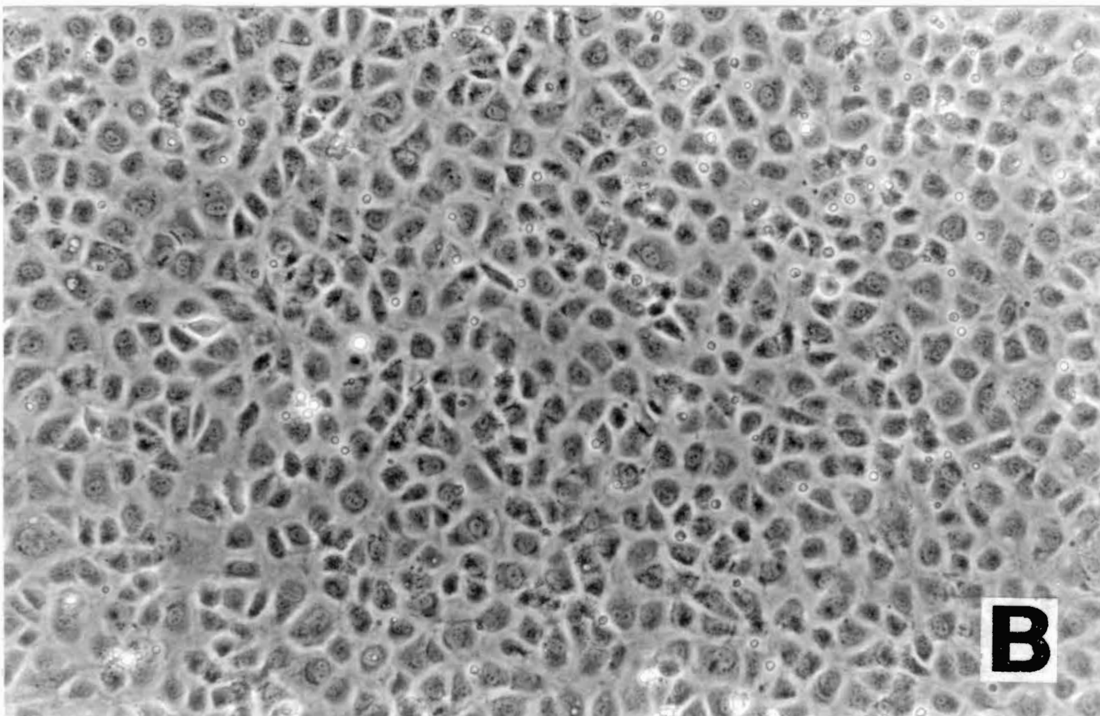


Figure 1D. Phase-contrast micrograph of the morphology of BPAEC monolayer 0.5 hr (A) and 3 hr (B) after treatment with 30 μg/ml xylazine.

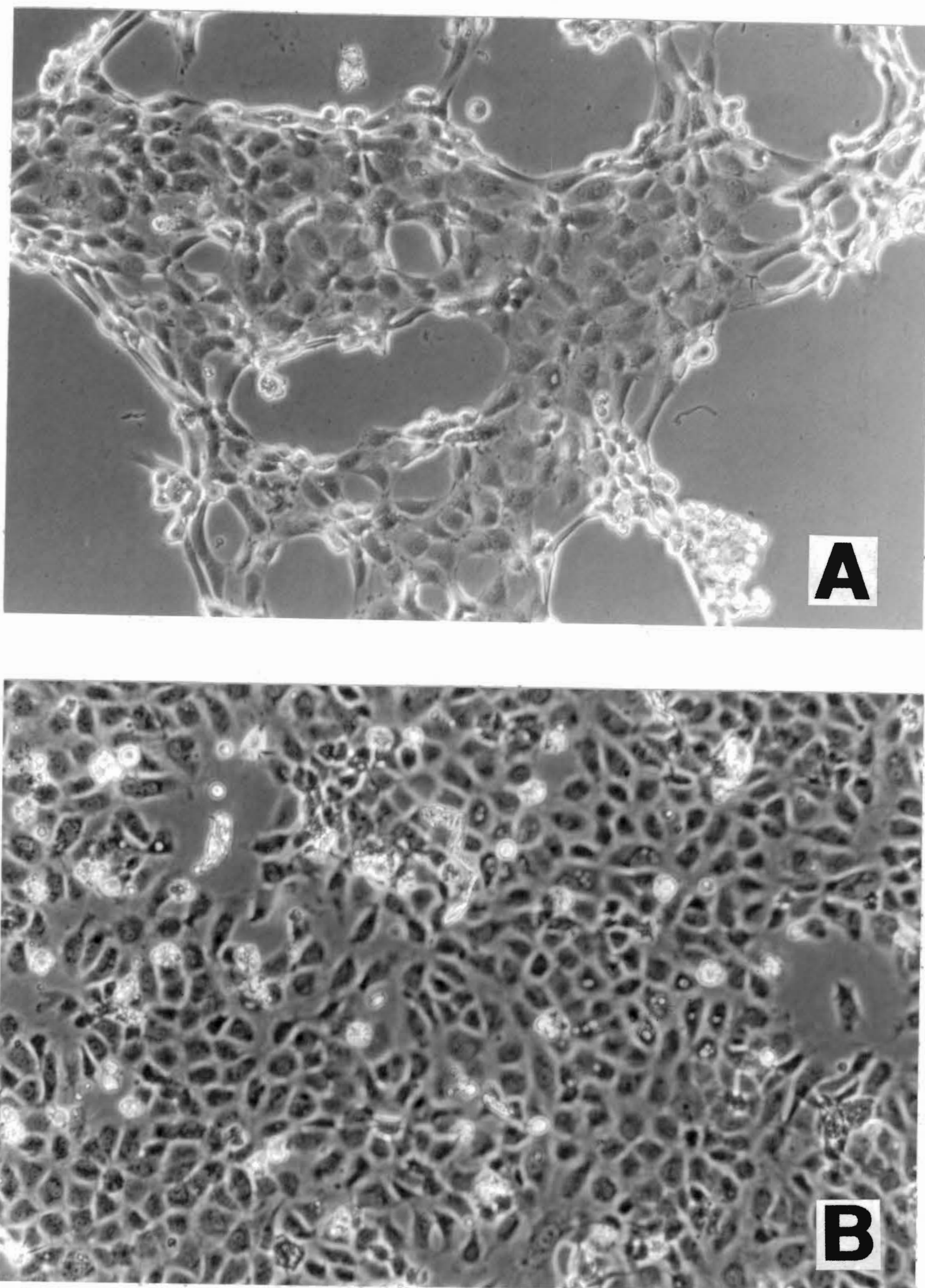


Figure 1E. Phase-contrast micrograph of the morphology of BPAEC monolayer 0.5 hr (A) and 3 hr (B) after treatment with 300 μg/ml xylazine. Note elongation and retraction of cells.

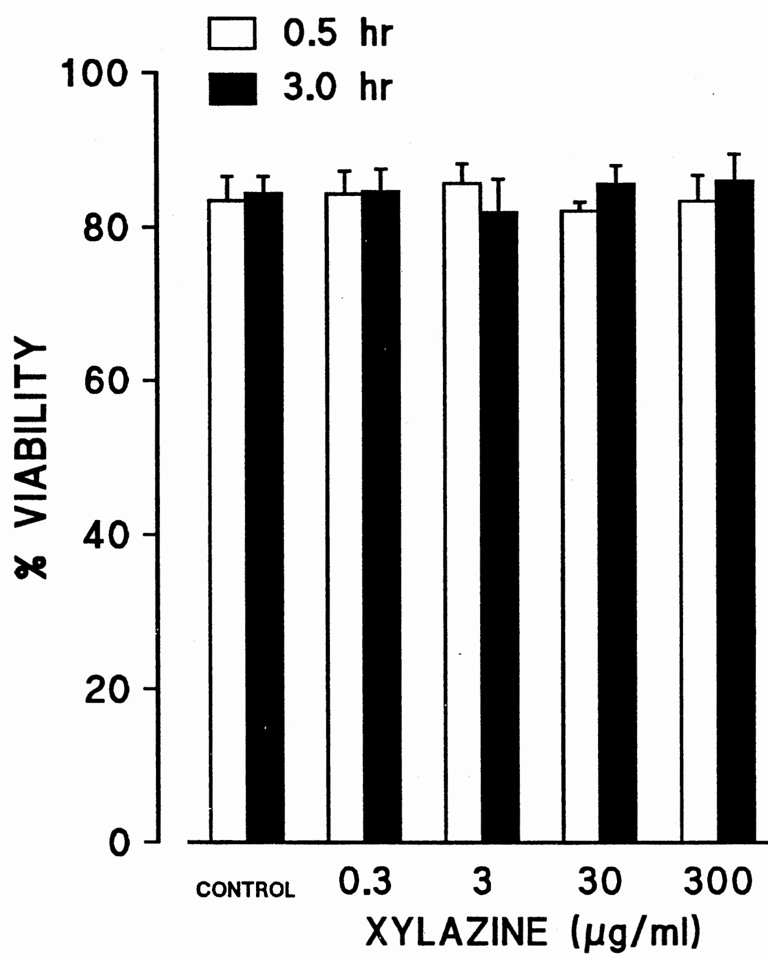


Figure 2. Effect of various concentration of xylazine (0.3 - 300 μg/ml) on viability of BPAEC after 0.5 and 3 hr incubation.

References

AMOUZADEH, H.R., SANGIAH, S. AND QUALLS, C.W. (1989). Effects of some hepatic microsomal enzyme inducers and inhibitors on xylazine-ketamine anesthesia. *Vet. Hum. Toxicol.* 31, 532-534.

AMOUZADEH, H.R., SANGIAH, S. AND QUALLS JR., C.W., COWELL, R.L. AND MAUROMOUSTAKOS, A. (1991). Xylazine-induced pulmonary edema in rats. *Toxicol. Appl. Pharmacol.* 108, 417-427.

BOYD, M.R. (1980). Biochemical mechanisms in chemical-induced lung injury: role of metabolic activation. *CRC Critical Reviews in Toxicology.* 7, 103-176.

COOPER, J.A.D., WHITE, D. AND MATTHAY, R.A. (1986b). Drug-induced pulmonary disease, Part 2: Noncytotoxic drugs. *Am. Rev. Resp. Dis.* 133, 488-505.

COOPER, J.A.D., WHITE, D. AND MATTHAY, R.A. (1986a). Drug-induced pulmonary disease, Part 1: Cytotoxic drugs. *Am. Rev. Resp. Dis.* 133, 321-340.

FRANK, L. (1985). Oxygen toxicity in eukaryotes. In *Superoxide Dismutase VIII*, (L.W. Oberley, Ed.), pp. 1-43. CRC press, Boca Raton, FL.

GARCIA-VILLAR, R., TOUTAIN, P.L., AND ALVINERIE, M. (1981). The pharmacokinetics of xylazine hydrochloride: an interspecific study. *J. Vet. Pharmacol. Therap.* 4, 87-92.

GREENE, S.A., AND THURMON, J.C. (1988). Xylazine-a review of its pharmacology and use in veterinary medicine. *J. Vet. Pharmacol. Therap.* 11, 295-313.

MARTIN, W.J. and HOWARD, D.M. (1985). Amiodarone-induced lung toxicity. In vitro evidence for the direct toxicity of the drug. *Am. J. Pathol.* 120, 344-450.

ODY, C. AND JUNOD, A.F. (1985). Direct toxic effects of paraquat and oxygen on cultured endothelial cells. *Lab. Invest.* 52, 77-84.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Various inducers and inhibitors of hepatic cytochrome P-450 microsomal enzymes were studied for their effects on xylazine-ketamine anesthesia. Pretreatment of Sprague-Dawley rats with chloramphenicol, cimetidine, ketoconazole, and SK&F 525-A significantly increased the duration of anesthesia in rats injected with ketamine and xylazine. Pretreatment with phenobarbital did not affect the duration of anesthesia significantly. The increase in duration of anesthesia in animals pretreated with SK&F 525-A and ketoconazole was accompanied by secondary respiratory distress about 6 hours after recovery from anesthesia, often leading to death within 24 hours. Lesions including extensive serous pleural effusion, alveolar edema rich in macrophages and extensive pulmonary hilar edema with hemorrhage were found at necropsy. These results indicated that acute lung injury could be a consequence of xylazine-ketamine anesthesia when the metabolism of these drugs is inhibited.

To determine the cause of PE, Sprague-Dawley rats were given a single anesthetic dose of xylazine alone or in combination with ketamine and/or SK&F 525-A and percent lung to body weight (% LW/BW) ratios (as an indicator of PE) were compared. The results indicated that xylazine caused PE which was independent of ketamine and was enhanced by SK&F 525-A. Subsequently, it was determined that 42 mg/kg xylazine, im is an optimal edemagenic dose. At this dose, xylazine increased the % LW/BW ratio as compared to control and pleural effusion (PLE) of various amounts was observed in 75 % of the animals. The pleural fluid to serum (PF/S)

protein ratio for xylazine was similar to that obtained for α-naphthylthiourea. Extensive serous PLE and alveolar edema with hemorrhage were found at necropsy in xylazine-treated rats. Pretreatment with yohimbine, prazosin, tolazoline, yohimbine plus prazosin, atropine, dimethyl sulfoxide, allopurinol, superoxide dismuatse , catalase, BW755C, ibuprofen, cystathionine plus taurine did not affect the % LW/BW ratio. PLE was increased by yohimbine, yohimbine plus prazosin, and allopurinol, reduced by DMSO, and not changed in other groups.

The increase in PF/S protein ratio to above 0.7 and the near unity of the ratio of albumin, ß-globulins and ϒ-globulins in pleural fluid to that in serum suggested that large protein molecules gain access to pleural space, most likely through injured pulmonary vascular endothelium. Failure of α-adrenergic antagonists (yohimbine, tolazoline, prazosin) and cholinergic antagonist (atropine) to prevent xylazine-induced PE and PLE was an indication that receptor-mediated hemodynamic changes are not the cause of PE. In addition, PE caused by hemodynamic factors are characterized by low protein edema fluid, lack of cellular damage and prolonged course of development. The lack of protection by inhibitors of cyclooxygenase and lipoxygenase pathways implied that arachidonic acid metabolites were not involved. Leukocytes were thought not to be the cause, since an increase in leukocyte count at a later period (12 hr) suggested that leukocytes were responding to an acute injury rather than being the cause themselves. Based on these results, xylazine-induced pulmonary edema can be classified as increased-permeability PE as a consequence of pulmonary endothelial injury and protein leakage. Hemodynamic factors, leukocytes and arachidonic acid metabolites were ruled out as primary factors. However, oxygen radicals may play an important role. In addition, the role of cytokines such as IL-1 and TNF needed investigation. The biochemical and ultrastructural alterations were also characterized

to further elucidate the mechanism of xylazine-induced PE. Sprague-Dawley rats were given xylazine and lungs were lavaged with phosphate-buffered saline 3, 6 and 12 hr later. Total protein, lactate dehydrogenase (LDH), xanthine oxidase (XO), interleukin-1 (IL-1) and tumor necrosis factor (TNF) were measured in lavage fluid. Protein concentration, LDH, XO and TNF levels were significantly increased in lavage fluid from xylazine-treated rats as compared to control. IL-1 level was unchanged at 3 and 6 hr, and was significantly reduced at 12 hr. Another group of rats were given 42 mg/kg xylazine intramuscularly and lungs were fixed 0.5 and 12 hr later for ultrastructural observations. Endothelial damage such as thinning, detachment from basement membrane or bleb formation were observed. The results provided direct evidence and supported the initial hypothesis that xylazine-induced PE is the result of increased pulmonary endothelial permeability. It is possible that xylazine or its metabolites are the cause. The initial findings after inhibition of the metabolism of xylazine-ketamine indicated that the acute lung injury is perhaps due to direct effect of xylazine on pulmonary endothelium.

Endothelial cell culture was used to clarify the role of xylazine. Xylazine, at various concentration (3 - 300 μg/ml), was incubated with confluent monolayers of bovine pulmonary artery endothelial cells (BPAEC) for 0.5 or 3 hr. Phase contrast microscopy and dye exclusion assay were used to assess the effects. Phase-contrast micrographs did not show any change up to 30 μg/ml xylazine. However, at 300 μg/ml xylazine, cells appeared elongated and retracted from growth surface. The viability of the BPAEC was not affected by xylazine at any dose or time period. Since a dose-dependent response could not be demonstrated, and absence of any change in cell viability, the morphological changes caused by 300 μg/ml of xylazine was attributed to non-specific effects. This dose could also be an in vitro toxic

threshold, however, it will not have any *in vivo* toxicological consequences. The pharmacokinetic profile of xylazine in rats is not known. Extrapolation of the pharmacokinetics parameters of xylazine from dogs and horses suggests that the concentration of xylazine in plasma after administration of 42 mg/kg xylazine should not exceed 42 μg/kg. Therefore, xylazine-induced PE could not be the result of the direct toxicity of xylazine on pulmonary artery endothelial cells.

Overall, the results of this study indicated that xylazine caused increased permeability PE and PLE as a result of pulmonary endothelial injury and leakage of protein. The adverse cardiovascular effects of xylazine do not appear to be the cause. Leukocytes and IL-1 are probably not involved and oxygen radicals, specifically those generated by xanthine oxidase, and tumor necrosis factor may play an important role. However, it has to be determined that whether oxygen radical are the cause or simply an effect resulting from the initial respiratory distress caused by xylazine or endothelial injury. The isolated perfused lung may provide a useful model for determination of the role of each of the proposed factors.

It is very likely that metabolite(s) of xylazine, perhaps reactive in nature, produced either in liver or lung causes endothelial injury. Studies on the metabolism of xylazine are basically non-existent with the exception of one study which indicated that metabolism of xylazine in rats yields 20 metabolites, resulting from the breakdown of thiazine ring, with dimethyl benzylamine being the major metabolite. Unfortunately, no further attempt has been made to determine the chemical nature of the remaining metabolites. It is hypothesized that inhibition of the metabolism of xylazine or increasing it dose could shift its metabolism to a toxifying pathway or saturate the detoxifying pathway and result in the formation of a reactive metabolite capable of damaging the pulmonary endothelium. It is also possible that conjugation of

metabolites results in the formation of reactive species. Both the shift to a toxifying pathway and the formation of reactive metabolites as a result of glutathione conjugation have been proposed as the mechanism of toxicity of drugs and toxicants. Elucidation of the exact mechanism of xylazine-induced PE can be useful in therapeutic management of xylazine toxicity in animals and man.

VITA

Hamid Reza Amouzadeh

Candidate for the Degree of

Doctor of Philosophy

Thesis: XYLAZINE-INDUCED PULMONARY EDEMA

Major Field: Physiological Sciences

Biographical:

Personal Data: Born in Firouzkouh, Iran, April 12, 1958, the son of Farajollah Amouzadeh and Fakhrozzaman Hoseini.

Education: Graduated from Dehkhoda High School, Semnan, Iran, in June, 1976; Received Bachelor of Science Degree in Arts and Sciences in Microbiology in December, 1982 from Oklahoma State University, and the degree of Master of Science in Physiological Sciences in July, 1986 from Oklahoma State University; completed requirements for the degree of Doctor of Philosophy at Oklahoma State University, July, 1991.

Professional Experiences: Data Analyst, Department of Agricultural Economics, Oklahoma State University, September, 1983 to November 1983; Laboratory Technician, Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, November, 1983 to January, 1984; Graduate Research Assistant, Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, September, 1984 to December, 1984; Graduate Research Assistant, Department of Plant Pathology, Oklahoma State University, November, 1984 to August, 1985; Graduate Teaching Associate, Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, July, 1985 to July, 1990. Graduate Research Assistant, Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, September, 1990 to June, 1991.

Professional Organizations: Member of Society of Toxicology and Sigma Xi.