MORPHOLOGIC STUDY OF THE ADVANCING BORDER OF BOVINE PNEUMONIC PASTEURELLOSIS

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OF BOVINE PNEUMONIC PASTEURELLOSIS

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TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
II.	REVIEW OF THE LITERATURE: PNEUMONIC PASTEURELLOSIS	5
	Economic Importance	5
	Clinical Disease	6
	Causative Factors	7
	Predisposing Factors	8
	Infectious Agents	9
	Pasteurella haemolytica	11
	Pathogenesis of Pneumonic Pasteurellosis	12
	Vaccination	16
III.	PATHOLOGY OF BOVINE PNEUMONIC PASTEURELLOSIS AND BOVINE PULMONARY LYMPHATIC DRAINAGE	17
	Macroscopic Lesion	17
	Natural Disease	17
	Experimental Disease	19
	Microscopic Lesions	19
	Natural Disease	19
	Experimental Disease	22
	Bovine Pulmonary Lymphatic System	24
IV.	MATERIALS AND METHODS	27
	Natural Pneumonic Pasteurellosis	27
	Experimental Pneumonic Pasteurellosis	28
	Microorganism	28
	Experimental Animals	28
	Experimental Design	28
	Pathology	29
	Lymphatic Drainage From Intrapulmonic Injection Site	30
V.	RESULTS	32
	Gross Pathologic Findings	32
	Natural Disease	32
	Experimental Disease	33
	Histopathologic Findings	36
	Lymphatic Drainage From the Injection Site Into the Caudal Pulmonary Lobes	40
	Gross Findings	40

Chapter Page

Subgross Findings 40
Radiographic Findings 41

VI. DISCUSSION AND CONCLUSION 42

BIBLIOGRAPHY 49

APPENDIXES 57

APPENDIX A - TABLES 57

APPENDIX B - FIGURES. 61

LIST OF TABLES

Table | Page

I. Experiment A: Evaluation of Gross Pathology and Summary of Extending Lesions 58

II. Experiment B: Evaluation of Gross Pathology and Summary of Extending Lesions 60

LIST OF FIGURES

Figure		Page
1.	Natural Bovine Pneumonic Pasteurellosis: Bilateral Anteroventral Distribution of Pneumonic Area	62
2.	Natural Bovine Pneumonic Pasteurellosis: Anteroventral Distribution of Pneumonic Area	63
3.	Natural Bovine Pneumonic Pasteurellosis: Patchy Lobular Distribution of Pneumonic Area	63
4.	Experimental Bovine Pneumonic Pasteurellosis: Relatively Large and Ill-defined Broundaries of Non-resistant Lung Lesion	64
5.	Experimental Bovine Pneumonic Pasteurellosis: Well Circumscribed Area of Pneumonia From Resistant Lung.	64
6.	Radiographical Illustration of Distribution of the Radiopaque Substance From the Injection Site in the Caudal Lung Lobe	65
7.	Natural Disease: Area of Coagulative Necrosis	66
8.	Experimental Disease: Area of Coagulative Necrosis.	66
9.	Experimental Disease: Details of the Cellular Components at the Border of an Area of Coagulative Necrosis	67
10.	Experimental Disease: Vascular Thrombi in Areas of Coagulative Necrosis	67
11.	Experimental Disease: The Cellular Exudate in a Severely Inflamed Area.	68
12.	Experimental Disease: The Cellular Exudate in a Moderately Inflamed Area.	68
13.	Natural Disease: Fibrinous Pleuritis.	69
14.	Natural Disease: Alveolitis at the Margins of Lesion.	69

Figure Page

15. Experimental Disease: Mild Alveolitis Extending From Severely Inflamed Peribronchovascular Connective Tissue 70

16. Experimental Disease: Extensive Alveolitis Extending From Severely Inflamed Interlobular Septum 71

17. Experimental Disease: Peribronchovascular Lesion. 72

18. Experimental Disease: Peri-interlobular Septal Lesion 72

19. Natural Disease: Alveolitis at the Advancing Border of the Lesion 73

20. Experimental Disease: Alveolitis at the Advancing Border of the Lesion in a Non-resistant Lung. 73

21. Natural Disease: Numerous Bacteria Near the Center of Gross Lesion 74

22. Natural Disease: Sparsely Distributed Bacteria Near the Periphery of Pneumonic Lesion. 74

CHAPTER I

INTRODUCTION

Bovine respiratory disease is one of the most important infectious diseases of cattle; it causes great economic loss, particularly in the feedlot industry in the United States of America (46). The disease is also common in cattle in Canada, Europe, the United Kingdom (8), and in most countries where there is intensive beef or dairy cattle production. The movement of large numbers of cattle appears to encourage outbreaks of the disease.

The term "pneumonic pasteurellosis" has attained recognition as a distinct disease entity of the so-called "shipping fever complex" (79). It is appropriate to state here that "pneumonic pasteurellosis" in cattle as defined in this investigation is the acute pneumonic condition induced by Pasteurella haemolytica or Pasteurella multocida. The single term "pasteurellosis" will be avoided since it would be confused with haemorrhagic septicemia, mastitis, and other pasteurella infections unrelated to the respiratory tract or in which respiratory disease is a minor component.

The disease has been studied extensively under both field and experimental conditions. The voluminous amount of clinical and other experimental evidence has yet to be translated into effective preventive, control, or therapeutic measures. The nature of the disease, particularly its pathogenesis, is still inadequately defined.

Continuing research on the disease is needed to provide a better understanding of its nature and pathogenesis. Control of pneumonic pasteurellosis will be completely effective only when all contributing aspects of the disease, including pathogenesis, are elucidated.

The pathogenesis of bovine respiratory disease is complex. It is postulated that bacterial pathogenicity occurs when the host environment is suitably altered by other factors allowing the pasteurellae to express their pathogenic potential. Unfortunately, the definition of "other factors" is often vague and is frequently designated by the equally vague terms "stress" and "predisposing factors." The complexity of the etiology, pathogenesis, and manifestations of the syndrome warrent its designation as "bovine respiratory disease complex" or "shipping fever complex."

The salient lesion in pneumonic pasteurellosis is a fulminating fibrinous pneumonia. The tissue injury in pneumonia is "self-inflicted" through an excessive or poorly controlled response on the part of the host's inflammatory mechanism. Certainly microorganisms are usually initiative, but irrespective of specific etiologic considerations, what actually transpires in the injured lung is an intense inflammatory response characterized by massive exudation of leukocytes and fibrin, edema, and other defensive efforts which can eventually become threatening and perhaps ultimately more damaging than the initial insult provoking the inflammatory response (86). The study of the basic injury process, its manifestations in the lungs, and the critical interface between levels of injury and the levels of host response will lead to better understanding of the pathogenesis of this disease and eventually translate to means of

effective therapeutic intervention.

Morphologic lesions of both spontaneous and experimental bovine pneumonic pasteurellosis have been described by several authors (23, 26, 47, 51, 83). There are recent detailed reviews of the macroscopic and microscopic lesions of the natural disease (79, 102). The descriptions are of lesions of the terminal stages of the disease. One report by Friend et al. (23) described the bovine lung lesions following intratracheal inoculation with live P. haemolytica at 18, 72, and 168 hours. There apparently had been no previous study conducted on the appearance of the lesions in early stages of pneumonic pasteurellosis. An understanding of the chronology of the lesion is necessary for definition of the pathogenesis of the disease.

The lesion is hypothesized to extend from primary foci of catarrhal inflammation in the terminal bronchioles to rapidly involve the whole lobule and along the peribronchial, perivascular, and septal lymphatics to seed the lesion in other lobules. According to Panciera (75), there are often secondary lesions in accessory, and hilar regions of diaphragmatic and caudal intermediate lobes following transthoracic inoculation of P. haemolytica suspension into the middle region of the diaphragmatic lung lobe. This phenomenon is hypothesized to result from lymphatic spread of infection from the injection site toward the hilar region of the lung.

The lungs of cattle are abundantly supplied with lymphatics. Among the outstanding lesions in pneumonic pasteurellosis are enlarged, thrombosed lymphatic vessels and serofibrinous edema of interlobular, perivascular, peribronchial, and subpleural connective tissue. The role of lymphatic vessels and interstitial connective

tissue of the lung in the extension of pneumonic lesions warrants further investigation.

The objectives of this investigation are as follows:

1. To define the cellular events at the advancing border of the inflammatory process in experimental and spontaneous pneumonic pasteurellosis caused by P. haemolytica,

2. To define the role of interlobular septal, peribronchial and perivascular interstitial connective tissues in the spread of pneumonia in experimental and spontaneous diseases,

3. To estimate bacterial populations at the periphery versus the center of the lesion in experimental and spontaneous diseases,

4. To define the lymphatic drainage from the injection site of transthoracic injection in the diaphragmatic pulmonary lobe.

The findings of this study should provide additional insight into morphological patterns of pulmonary responses in pneumonic pasteurellosis, particularly in early stages of the disease, and elucidation of the dynamic nature of spreading of the lesion in the lung.

CHAPTER II

REVIEW OF THE LITERATURE: PNEUMONIC PASTEURELLOSIS

Acute pneumonic pasteurellosis is an acute aggressive fibrinous pleuropneumonia or fibrinous bronchopneumonia of complex etiology but whose final common etiology is Pasteurella haemolytica or Pasteurella multocida. Acute pneumonic pasteurellosis is included in the collective term "bovine respiratory disease (BRD)" and with few exceptions is the disease inferred by the term "shipping fever." Though the term "shipping fever" encompasses numerous etiologic factors, Jubb and Kennedy (51) used the term to refer specifically to the disease pneumonic pasteurellosis in their discussion of the fibrinous pneumonias of ruminants and swine. Haemophilus somnus (9, 74) and Mycoplasma mycoides (27) also cause fibrinous pneumonia in cattle.

Economic Importance

Modern methods of intensive cattle production particularly the gathering, mixing and transport of cattle to intensive feeding-fattening facilities incur numerous of the predisposing factors believed necessary for the development of acute pneumonic pasteurellosis. Although estimates of losses from bovine respiratory disease vary, the assessment of their importance does not. The Agricultural Research Service of the United States Department of Agriculture considers

respiratory disease the most economically devastating condition of the nation's cattle industry and estimates that $500 million is lost each year from the disease (86). Losses result from death, excessive shrinkage, cost of treatment, inefficient feed conversion, and delayed marketing. Medication costs and inefficiency in production of survivors of the disease probably represent expense far in excess to that of the death loss. The problem is of such significance that is has been the subject of numerous symposia in recent years (91, 92, 93) and has been assigned top priority for research support by the American National Cattlemen's Association for many years (40).

Clinical Disease

The disease usually develops in cattle within 10 to 14 days after they have been stressed (8). The first observable signs are slight depression and inappetence. Affected animals often stand alone with their heads down and ears drooping. However, sudden death without prodromal signs may be the first indication of an outbreak. In the early stages, there is no dyspnea. The respiratory rate may be elevated when the animal has a high fever, or when the ambient temperature is high. The muzzle may be dry and scabby in appearance. Nasal discharge may or may not be present, as it is more indicative of the severity of the predisposing upper respiratory viral disease than of the pneumonia. Though a soft cough is common, it is often difficult to identify the calves in an affected group that are coughing. The rectal temperature may range from slightly above normal to 42°C. Diarrhea is occasionally present.

In severe cases or those of several days duration with extensive

lung consolidation, there is marked depression, inappetence, and dyspnea with extended head and open-mouth breathing. The rectal temperature may be normal or subnormal. If the animal should respond to treatment in this stage, there is a marked tendency for relapse to occur after antimicrobial therapy is terminated. Those animals that do recover permanently may remain thin, grow slowly, and have a rough hair coat.

The course of the disease is usually short. If treated early, affected cattle recover in 24-48 hours, but peracute cases and those which have been ill for a few days before being treated, may die or become chronically affected in spite of intensive therapy (8). The morbidity rate may be as high as 100 percent and mortality can exceed 30 percent (38). Incidence of the disease is much higher during fall and winter than during spring and summer (47).

Causative Factors

The etiology of pneumonic pasteurellosis in cattle is currently believed to be an interaction of environment, host, and infectious agents. The precise cause or causes of the syndrome are not well identified. The long established generalization still holds true that predisposing factors, including a variety of environmental changes and primary viral, mycoplasmal, or chlamydial infections increase the susceptibility of cattle to the Pasteurella-induced pneumonia (28, 39, 47). Pasteurella infections extend the inflammatory process induced by the primary infection to the level of severe pneumonia and debilitating or fatal disease.

Predisposing Factors

Evidence that acute pneumonic pasteurellosis usually occurs following shipment and adverse weather conditions led to the adoption of the term "stress" to identify the physiologic consequences of animals subjected to such environmental changes (85). This term appears to be useful to designate predisposing factors in the etiology of bovine respiratory disease.

Stress has been defined recently by Stephens (89) as the homeostatic, physiological, and behavioral responses detectable in the animal resulting from its interactions with environmental stressors. Stressors denote any stimuli which deviate significantly from normal or are unusually prolonged or intense. Jensen et al. (47) emphasized the importance of stressing factors such as shipping, feedlot dust, and adaptation to diets in the etiology of shipping fever.

The exact actions of the various stressors are generally unknown. It is thought that they act non-specifically to lower the resistance of the calf to infection. Landi et al. (55) reported on the effects of shipping on immune functions in mice. Serum corticosterone values were markedly increased and immune function was significantly diminished in the mice at arrival and during the 48-hour period following shipment. Markedly increased plasma corticosteroid levels have been observed in cattle following shipment (76, 89). Glucocorticoids are generally considered to be anti-inflammatory and to suppress the immune system (81). The effects of elevated plasma cortisol concentrations in bovine were shown to depress lymphocyte blastogenesis in response to phytohemagglutinin (65, 80), concanavalin A, and pokeweed mitogen (80) and

to impair the myeloperoxidase-hydrogen peroxide-halide antibacterial system of the polymorphonuclear leukocytes (80). These effects may contribute to the increased susceptibility of cattle to pneumonic pasteurellosis following shipment.

There have been few studies on the relation of breed, sex, age, and color on the susceptibility of cattle to acute pneumonic pasteurellosis. The disease may occur in any animal: recently weaned beef calves, feeder cattle which have recently arrived in a feedlot, or mature cows (8). A higher incidence of pneumonia has been noted in young calves than in older cattle (85). Inbred cattle appear to be more severely affected following shipment than outbred cattle (17).

Infectious Agents

Disruption of inherent pulmonary defense mechanisms may be the major predisposing factor leading to outbreaks of severe acute pneumonic pasteurellosis. In addition to environmental and host factors previously mentioned, a number of viral and other infective agents appear to occur as primary infections and predispose the bovine respiratory tract to invasion by *Pasteurella* spp. They include bovine herpesvirus type 1 [infectious bovine rhinotracheitis (IBR) virus], bovine herpesvirus type 3 [malignant catarrhal fever (MCF) virus], bovine herpesvirus type 4 (Movar 33/63, DN 599 virus), bovine adenovirus types 1 to 10, bovine parainfluenza 3 virus (PI-3), bovine respiratory syncytial virus (BRS), bovine viral diarrhea (BVD) virus, reovirus types 1 and 2, and bovine enterovirus types 1 to 7 (64, 78).

Each of the viral agents mentioned are capable of inducing respiratory disease alone or in concert with other viruses. Most bovine

respiratory virus infections without secondary bacterial complications are mild and self-limiting. Serologic studies indicate that serum antibodies to IBR, BVD and PI-3 viruses are widespread in non-vaccinated and clinically healthy cattle (24). Other serologic surveys also suggested widespread infection of BRS virus, adenoviruses, rhinoviruses, and reoviruses in cattle in the U.S.A.; DN-599 virus was not found to have such a widespread distribution or prevalence (78).

Certain viral infections of the respiratory tract suppress pulmonary antibacterial defenses. Susceptibility of the respiratory tract to bacterial attachment and colonization is increased, mucociliary clearance and surfactant levels are diminished, and alveolar-macrophage functions are impaired (44, 97). Jakab (44) stated that the magnitude of the virus-induced alterations was an important factor since not all viral pneumonias predispose to bacterial superinfections. Studies in cattle have shown that infection with either PI-3 (50, 58) or IBR viruses predisposes them to pulmonary infection with P. haemolytica (49).

Although Mycoplasma mycoides causes contagious bovine pleuropneumonia, a severe and primary pleuropneumonia of cattle, the role of other mycoplasmas in bovine respiratory disease is inconclusive. It is suggested that mycoplasmas may cause a subclinical pneumonia and may suppress humoral and cell-mediated immune responses thus reducing the ability of the pulmonary system to resist Pasteurella spp. and other bacterial infection (88). Chlamydial infection may also render the respiratory tract susceptible to invasion by other bacteria (90).

The microbial flora of the normal bovine respiratory tract is controversial (1, 91). According to Carter (12), few, if any, bacteria or

fungi reside in the trachea, bronchi or lungs. However, he listed several species of bacteria among which were Pasteurella haemolytica and P. multocida as the normal flora in the upper respiratory tract. The role of other species of bacteria presently appears to be less important than Pasteurella (11, 13, 14, 95). The fact that Pasteurella spp. were isolated from upper respiratory tracts of healthy cattle (14, 61) and from non-pneumonic bovine lung tissue (1) has caused some doubt about the pathogenicity of these bacteria. However, there is considerable evidence of the role of P. haemolytica and P. multocida in acute pneumonic pasteurellosis (13, 28, 57, 95). Although P. multocida is more prevalent in the upper respiratory mucosa, P. haemolytica is more often isolated from cases of bovine pneumonic pasteurellosis (48, 83). P. haemolytica induces more severe lesions associated with fibrinous pleuropneumonia, whereas P. multocida induces somewhat less aggressive lesions associated with fibrinous bronchopneumonia (83).

Pasteurella haemolytica. Pasteurella haemolytica is a gram-negative coccobacillus. It is identical to those referred to as Bacillus bovisepticus Group 1 (79). The name P. haemolytica was given by Newsom and Cross in 1932 and has been widely accepted (10). It has the ability to hemolyze equine and bovine erythrocytes and does not produce indol. Two biotypes of P. haemolytica, A and T, have been identified (87). Biotype A ferments arabinose within 7 days and biotype T ferments trehalose within 2 days. Both somatic and capsular antigens are described (7). Capsular antigens are more stain-specific and more useful in classification. There are twelve known serotypes, designated 1 to 12. When the 12 serotypes were grouped according to

biotype, serotypes 3, 4 and 10 were biotype T; all others were biotype A (21). There is some disagreement on the prevalence of the various serotypes and biotypes of P. haemolytica (102). According to Frank (21, 22), serotype 1 is the predominant serotype isolated from cattle; however, serotype 2 and untypeable isolates are frequently isolated. P. haemolytica, serotype 1, is most frequently isolated from calves with respiratory disease and is the serotype most frequently found in pneumonic lungs (101).

Pathogenesis of Pneumonic Pasteurellosis

The pathogenesis of pneumonic pasteurellosis is complex and a subject of continuing investigation. A major problem in defining its pathogenesis is the difficulty in experimentally reproducing the disease. Although there are several methods for the production of pneumonia utilizing Pasteurella, all involve exposure of the airways or lung to massive numbers of bacteria.

Under normal conditions, the defense mechanisms of the bovine respiratory tract are highly effective in the clearance of microorganisms from the lower respiratory tracts and the pulmonary parenchyma (44, 56, 94). The efficient clearance of invasive infectious agents from the respiratory tract depends on adequate and rapid function of the host's defense mechanisms, which in turn depends on the level of nutrition, hydration, stress, and immunity of the host prior to and during respiratory infection (20). The defense mechanisms of the respiratory tract include mechanical factors such as sedimentation of large particles (10 to 20 microns in diameter) in the nasopharyngeal region, the cough reflex, tracheobronchial mucous secretion, and mucociliary escalator

clearance from the lower respiratory tract (15, 32, 67). Specific immunity on mucosal surfaces of the upper or lower respiratory tract contributes to resistance to infections (15, 32, 53, 67, 100). Non-specific soluble factors such as α-1-antitrypsin (antiproteolytic enzyme), lysozyme, and complement also contribute to the pulmonary defense mechanism (67). Small particles (1.6 to 3.2 microns in diameter) are deposited in the terminal bronchioles and alveoli (15) and are cleared from the lung parenchyma by phagocytic cells. Phagocytosed debris is subsequently cleared from the pulmonary region by mucociliary movement. The lymphatic drainage of the lung is also important to pulmonary defense mechanisms. Macklin (60) described areas he called "sumps" at terminal bronchioles through which particles that had not been phagocytosed might gain entrance directly to the lung-lymphatic system.

Both alveolar macrophages and polymorphonuclear leukocytes have been shown to be important in the defense of the lung against pathogenic bacteria (29, 32). The microbicidal effects of both phagocytes are partially mediated by the intracellular release of proteolytic enzymes into phagolysosomes. Release of these enzymes into the extracellular milieu has also been documented to occur during phagocytosis, allowing for the possibility of proteolytic lung damage (70, 98). Because important differences exist between the proteolytic enzyme systems of alveolar macrophages and polymorphonuclear leukocytes (45), the phagocytic cell response to bacteria in the lung has important implications regarding its potential for causing lung injury. In a murine model, it was demonstrated that there were species-specific differences of the phagocytic cells in responding to bacterial instillation of the lung (71, 72).

Alveolar macrophages are the primary phagocytic cell for protection of the respiratory membrane of the lung from infections (15). Particles on the walls of alveoli are usually engulfed by these cells (18). Whenever the alveolar macrophages fail to ingest and destroy the microbial agents, the infection can establish. Once a pulmonary infection is established, an inflammatory response occurs which mobilizes the host defenses of the blood stream. Granulocytes and monocytes (macrophages) are attracted to the site by bacteria, dead tissue components, fibrin and collagen split products and fractions of the complement system. It has been documented in guinea pigs, rhesus monkeys, cattle, and humans that the alveolar macrophage plays a role in modulating the migration of neutrophils to the lung. Following stimulation by phagocytosis or merely by attachment to a glass surface, alveolar macrophages secrete a low molecular weight (<5,000 daltons) chemotactic factor that preferentially attracts polymorphonuclear leukocytes (41, 42, 54).

Several authors have recently summarized the hypothetical steps in the initiation of acute pneumonic pasteurellosis as follows: P. haemolytica is commonly carried in undetectable numbers in the nasopharyx of healthy cattle (21, 61, 95). Stress or concurrent disease(s) allows a marked increase in the number of P. haemolytica in the nasopharynx which are then inhaled to the alveolar level via aerosol droplets. Lillie (57) listed four possible modes of extension of the infection from the upper to the lower respiratory tract. They included gravitational drainage, inhalation of droplet nuclei, lymphatic drainage, and hematogenous dissemination. The first two modes were considered more likely because P. haemolytica was isolated from the tracheal air of cattle with colonized nasal passages (33). These modes of

extension correlated well with the distribution of lesions at the level of respiratory bronchiole (51).

Normally, alveolar macrophages dispose of inhaled bacteria even if inhaled in large numbers. It was documented that 75% of experimentally inhaled P. haemolytica were cleared in two hours by the calf lungs, 90% were cleared in four hours and 92% were cleared in eight hours (56). Three major factors appear to be responsible for failures of this mechanism in bovine pneumonic pasteurellosis. Initially, P. haemolytica is highly toxic to alveolar macrophages and many macrophages may be killed in trying to phagocytose these organisms (5, 52, 62, 63, 84). Secondly, a concurrent respiratory viral infection may interfere with normal pulmonary bacterial clearance (44, 97). Parainfluenza-3 virus has been demonstrated to interfere with the clearance of P. haemolytica (58); however, there was no significant effect of either bovine viral diarrhea virus or Mycoplasma bovis on the bovine pulmonary clearance rate of P. haemolytica (59). Lastly, stress conditions may inhibit normal pulmonary defense mechanisms (47).

The cytotoxic factors of P. haemolytica are currently under active investigation. Berggren et al. (6) reported that cytotoxic factors impaired the phagocytic capabilities of bovine neutrophils; others have noted a similar effect on alveolar macrophages (5, 62, 63). Himmel et al. (37) identified cytotoxic factor as a highly immunogenic protein with a molecular weight of approximately 150,000 daltons; however, Baluyut et al. (3), described cytotoxin as a heat labile protein, susceptible to extremes of pH, with a molecular weight of 300,000 daltons or more. The biochemical nature of cytotoxic factor awaits further classification and description.

Tissue damage due to Pasteurella infections is hypothesized to occur by two mechanisms. Cytotoxic effects of the bacteria create pneumonic lesions in combination with necrosis caused by endotoxin-induced lymphatic and vascular clotting and thrombosis (47). Secondly, the excessive or poorly controlled inflammatory response initiated by the P. haemolytica infection results in widespread tissue destruction (86). It is likely that both of these mechanisms account for the lesions of pneumonic pasteurellosis.

Vaccination

The role of the immune system in preventing pulmonary disease has been the subject of intense study for several years. Current research is in large part directed toward development of an effective immunizing agent, but various difficulties with developing and testing Pasteurellae vaccines have been encountered (99). Two major approaches have been taken in this research: Vaccination against the viruses which appear to predispose the animal to the development of pasteurellosis and vaccination with agents directed against the bacteria or their products. Neither method, however, has yielded satisfactory results.

CHAPTER III

PATHOLOGY OF BOVINE PNEUMONIC PASTEURELLOSIS AND BOVINE PULMONARY LYMPHATIC DRAINAGE

Even though bovine pneumonic pasteurellosis has been extensively studied, reports of the pathology of the disease are few. Descriptions are of the well developed lesions observed in advanced stages or fatal cases of disease (47, 51, 83). There apparently is no literature dealing with sequential morphological changes in the natural disease under field conditions. The lesions in spontaneous and experimental disease were recently reviewed by Rehmtulla and Thomson (79), and Yates (102).

Macroscopic Lesion

Natural Disease

The pathologic basis of bovine pneumonic pasteurellosis is an acute fulminating fibrinous pneumonia (51). Inflammatory lesions involve the anteroventral portion of one-third or more of each lung (79, 96). The pneumonic areas are enlarged, heavy, firm and flesh-like. On cross section, both lobular and stromal changes exist. Affected lobules are consolidated, they are either dark-red or gray-white, and bordered by thick yellow fibrinous or clear edematous interlobular septa (83). A cut surface is moist and exudes serous fluid. The pneumonic process is patchy in distribution with some normal appearing lobules in the

affected area. The junction between normal and pneumonic tissue is edematous (47).

In more chronic cases, gray circular dry areas representing coagulation necrosis or small cavities containing yellowish cheesy pus can be seen amidst the solid tissue (79). Jubb and Kennedy (51) state that necrosis of major portions or entire lobules is common. There is often difficulty in determining how much of the necrosis to attribute to direct microbial action on the parenchyma and how much to vascular injury. Jensen et al. (47) described hemorrhagic infarcts and areas of coagulative necrosis as the complications resulting from vascular thrombosis.

Acute fibrinous or serofibrinous pleuritis is present in most cases of pneumonic pasteurellosis (51, 83). In addition to fibrinous pleuritis, serofibrinous pericarditis is frequently present (69). Bronchial and mediastinal lymph nodes are swollen, edematous and congested. Palotay and Newhall (73) described the presence of abundant yellowish pleural fluid which coagulated rapidly on exposure to air. However, most descriptions made no mention of the presence or absence of pleural fluid.

There is remarkable consistency in the observation relating to interlobular septal changes. The interlobular tissue is very markedly distended due chiefly to the presence of coagulated exudate. Thickening of the interlobular septa with fibrin and infiltration with serum, which in some instances extends out under the visceral pleura, are common findings (47, 79, 83). Bronchi are hyperemic and partly filled with blood, pus, and mucus (83).

Experimental Disease

Descriptions of macroscopic lesions in experimental pneumonic pasteurellosis are few. This may reflect an inability to reproduce the shipping fever complex consistently (2, 34, 36). Evaluations of the degree and nature of lung lesions produced in successful trials were either not given or appeared to be inconsistent with those of pneumonic pasteurellosis (79).

Macroscopic lesions produced by intratracheal inoculation of a large dose of P. haemolytica by Friend et al. (23) were characteristic of fibrinous pneumonia as described by Jubb and Kennedy (51). The gross lesions were graded according to the severity and extent of lesions at 18 hours, 3 days and 7 days. The most severe gross lesions were present at three days postinoculation and were described as an extensive dark red consolidation of apical, cardiac and diaphragmatic lobes, with separation of interlobular septa by fibrin and fibrinous pleuritis. The milder lesions, in calves 18 hours and 7 days post-inoculation, were also distributed in the anteroventral pulmonary lobes and varied from collapsed flaccid red lobules to dark red, very firm lobules with a mild fibrinous pleuritis.

Microscopic Lesions

Natural Disease

The histological lesions of bovine pneumonic pasteurellosis are distinctive and characterically those of an acute fibrinous pneumonia and pleuritis (51, 83). The histological descriptions of the pleura are seldom mentioned, presumably because pleural lesions do not differ

from the gross lesions of fibrinous exudation. The descriptions of the lung lesions are slightly variable depending on the stage when examined. The common features are predominantly fibrinocellular exudate composed of mononuclear cells, neutrophils and edematous fluid; bronchiolitis; characteristic swirly dark macrophages in alveoli and terminal bronchioles, and extreme dilatation of interstitial septa by thrombosed lymph vessels. Multifocal areas of coagulation necrosis are present in the parenchyma of the severely affected areas (47, 51, 79, 83, 96). Graham (30) indicated the most consistent finding to be interlobular septal changes characterized by edema, fibrin, leukocyte infiltration and distended lymphatics which were frequently thrombosed. Other findings are occasionally emphysema and rarely fibrosis.

Lumens of bronchi and bronchioles contain exudate composed of mucus, bacteria, neutrophils, macrophages, and erythrocytes (47). The epithelial lining of bronchi and bronchioles are well preserved. Jubb and Kennedy (51) suggest this feature indicates the drift of exudate from the alveoli up bronchioles and bronchi, which could result in secondary inflammation of the mucosa of bronchioles and bronchi. They further described an early accumulation of mononuclear inflammatory cells and subsequently an enormous distension of the peribronchial lymphatics and connective tissue spaces as in the septa.

The alveoli in affected lobules contain edema, fibrin, and occasionally hemorrhage in variable proportions. Inflammatory cells within alveoli may be neutrophils and/or macrophages. Early changes in alveolar walls are alveolar capillary congestion, with occasional hemorrhage into the alveoli (79). There is exudation of serous fluid and later desquamation of alveolar epithelial cells into alveoli. The

outstanding and characteristic lesions are multifocal areas of coagulation necrosis and the presence of degenerate "oat-shaped" macrophages. The necrotic areas are variable in size and shape and often involve whole or confluent lobules, but not whole lobes. The necrotic areas, the margins of which are distinctly marked by a line of intense leukocytic infiltration, expand out from a primary focus at the terminal bronchiole (51). Scheifer et al. (83) recognized two types of macrophages in the lesions; first, large round cells having pale eosinophilic cytoplasm, and second, tightly packed fusiform cells (oat-shaped cells). The dark fusiform macrophages are also present at the edge of necrotic areas and along thrombosed lymph vessels in the septa (83).

The presence of bacteria in histological lesions was described by some, and not mentioned by other authors. Jubb and Kennedy (51) described them as numerous in sections and considered them to be an important criterion to differentiate pneumonic pasteurellosis from contagious bovine pleuropneumonia. Schiefer et al. (83) found them in cases associated with P. multocida, but not P. haemolytica. Jensen et al. (47) described bacteria admixed with fibrin, serum, erythrocytes and cellular exudate in alveolar spaces in many lobules. Phacocytosis of bacteria was sometimes evident. Greater numbers of bacteria and the heaviest accumulations of exudate were in alveoli at the periphery of the lobules.

Reference to changes in the blood vessels are few. Jensen et al. (47) and Schiefer et al. (83) stated many blood vessels, both arterial and venous, as well as septal capillaries, contained homogenous blood clots. Thrombosed veins were dilated and often associated with hemorrhagic infarcts. Jubb and Kennedy (51) indicate that inflammatory

injury and necrosis of arteries are prominent changes in severe cases but occlusive thrombi are difficult to demonstrate. They further state the periarteriolar lymphatics and connective tissue spaces are distended and implied the spread of the inflammation along these structures to involve successive lobules as well as along the peribronchial lymphatics and connective tissue spaces. Most lymphatic vessles of pneumonic tissue are dilated and some are occluded with fibrin clots. A fibrinous mesh entraps erythrocytes and leukocytes. Subpleural, interlobular, perivascular, and peribronchial lymphatics are equally affected (47).

In chronic cases, the fluids and exudates are organized. Fibrocytes grow into fibrinous exudate of alveolar lumens and pleural surfaces, fibrinous thrombi of lymphatics, and into interlobular stroma. Connective tissue forms a capsule around foci of necrosis (47).

Experimental Disease

Gilka et al. (26) observed bronchiolitis 4 hours subsequent to aerosolized inoculation with P. haemolytica. Polymorphonuclear leukocytes were consistently present in the peribronchiolar and peripheral alveolar septa. Peribronchiolar and peripheral alveoli were common sites of deposition of P. haemolytica and sites of reaction of the pulmonary tissue to bacteria. Alveolar macrophages were the major phagocytic cells for P. haemolytica in lungs. A small proportion of neutrophils participated in phagocytosis of P. haemolytica (26). There were areas of peribronchiolar and peripheral atelectasis. Later, these areas were associated with widely dilated lymphatics. They postulated that edema fluid might have removed the alveolar surfactant material into these adjacent dilated lymphatic vessels resulting in subsequent

collapse of the alveoli.

Friend et al. (23) reported the major findings in affected areas at 18 hours post intratracheal inoculation of P. haemolytica to be varying degrees of atelectasis, infiltration of neutrophils into alveolar spaces and bronchioles as well as accumulation of macrophages and fibrin in alveoli. Exudate was more prominent in and around bronchioles. At three days postinoculation the lesions were characterized by the presence of marked generalized distension of lymphatic vessels with fibrin and neutrophils and frequently, lymphatic thrombosis. Neutrophils were prominent in the epithelium and lamina propria of bronchioles and purulent exudate was in bronchiolar lumens. Alveolar spaces contained large numbers of neutrophils, large pale eosinophilic macrophages and occasionally syncytial giant cells. Homogenous eosinophilic fibrin clots filled many alveoli. There were scattered areas of coagulative necrosis bordered by fusiform elongate dark basophilic macrophages and mononuclear cells. This mantle of macrophages was invariably associated with necrosis of the enclosed lung tissue but they also occurred in groups of alveoli in whorl-like patterns in a random distribution unassociated with necrotic tissue. Seven days postinoculation the lesions in general were an aftermath of those observed at three days. Lymphoid hyperplasia around bronchi and bronchioles were extensive and organizing obliterative bronchiolitis was observed. Neutrophilic and mononuclear infiltration of the walls of bronchioles were pronounced. Small areas of necrosis were replaced by granulation tissue. The granulation tissue was prominent but loosely arranged at the edges of the large necrotic areas. Organization of fibrinous exudate in lymphatic vessels and on the pleura as well as in some alveolar spaces was present.

Irregularly distributed areas of thickened alveolar walls and epithelialization of the lining cells were prominent around bronchioles.

Bovine Pulmonary Lymphatic System

The classical lesions of bovine pneumonic pasteurellosis include extensive involvement of the lymphatic system (47, 51, 79, 83). The lymphatic system of the lung is, in principle, the same as that of other organs. It is composed of three major parts, the lymphoid tissue, the lymphatic vessels and the extravascular fluid pathway system (66). The system consists of cells and organs that are designed to protect the internal environment from invasion and damage by foreign substances. The defensive aspects of the lymphatic system are manifested in various ways, e.g., production of defensive cells, transport of materials via lymphatic vessels, filtration of lymph and blood through constituent organs, phagocytosis and production of immunoglobulins (4). The character of the lymphatic system is complicated by the fact that, although it participates in the defense mechanisms of the organ, it also takes part in the development of the disease process (66). Comprehension of the normal morphology of pulmonary lymph vessels is needed to interpret their role in the defense mechanism of the lung.

Most inhaled particles are cleared via the bronchial mucociliary escalator, but a small proportion of these particles accumulate in the lymphatic system of the lung (31). The first site of accumulation seems to be in the lymphoid tissue at the terminal bronchiole near Macklin's sump, where centrilobular (periarterial) lymphatic vessels begin (18). These vessels then follow the branches of the pulmonary artery along the bronchial tree to the hilus of the lung. Enroute,

they are interrupted by larger and larger lymph nodes, which filter out many of the particles in the lymph. Perilobular (perivenous or paraseptal) lymphatic vessels begin at the venous side of the capillaries and then accompany the veins that course along the septa (18). The lymph vessels of the lung form two main sets of interconnected vessels. They are the superficial (pleural) plexus and the deep (peribronchovascular and septal) plexus. The deepest branches are the juxta-alveolar lymph vessels in the connective tissue adjacent to peripheral alveolar walls. Lymph vessels are not present within the interalveolar septa (18, 25). Interstitial fluid in alveolar walls flows during respiration centripetally and centrifugally to the centrilobular and perilobular lymphatics, respectively.

According to Nagaishi (66), the lymph flow in the interlobular lymph vessel (deep lymphatic plexus) is directed either to the hilar lymph nodes or pass through the subpleural connective tissue (superficial lymphatic plexus) to the hilar lymph nodes. He stated that the subpleural nets of the lymph capillaries drained through either collecting lymph vessels in the interlobular septa or subpleural connective tissue to the hilar lymph nodes. The peribronchial lymph capillaries drained through collecting lymph vessels in the interlobular septa and then through the subpleural connective tissue to the hilar lymph nodes. Galina (25) stated that, based on morphology of the lymphatic valves in normal bovine lung, there is a unidirectional flow of lymph from septal lymph vessels to pleural lymph vessels. However, there was no mention of the possibility of lymphatic drainage from septal lymph vessels directly to the hilar lymph nodes.

The lymph nodes associated with the bovine lung formed two

lymphocenters; first, the mediastinal lymphocenter consisted of cranial, middle, and caudal mediastinal lymph nodes; second, the bronchial lymphocenter consisted of cranial, right, middle and left tracheobronchial lymph nodes and the pulmonary lymph nodes (25). The drainage of lymph from the different lobes of the lungs to the lymphocenters was described by Galina (25) as follows: "The drainage of the cranial lobe was first to the cranial tracheobronchial lymph node and then to the cranial mediastinal lymph nodes. The drainage of the middle and caudal segments of the cranial lobes courses first to the middle then to the right and then to the left tracheobronchial lymph nodes at the division of the trachea into the primary bronchi. From the tracheobronchial lymph nodes, the drainage was to the middle mediastinal lymph node and then to the thoracic duct, except for the lymph drained from the caudal pulmonary lobes which was collected in the caudal mediastinal lymph node and then drained into the thoracic duct."

CHAPTER IV

MATERIALS AND METHODS

Natural Pneumonic Pasteurellosis

Lungs were selected for study from five cattle with spontaneous pneumonic pasteurellosis confirmed by isolation of Pasteurella haemolytica. The lungs were carefully examined and the pneumonia characterized according to severity, distribution, and extent. Lesions of the pleura, pericardial sac, tracheobronchial and mediastinal lymph nodes, trachea, and major bronchi were examined as well. Color, consistency, and lobular and stromal appearances of the lateral, medial and cut surfaces were noted in the subgross study. Well demarcated areas of pneumonic lung and grossly normal appearing lung were collected and fixed in 10% neutral buffered formalin solution for histopathologic study. After routine processing and paraffin embedding, sections of tissue were cut at 6 microns and stained with hematoxylin and eosin. Selected tissue sections were stained with Mallory's phosphotungstic acid hematoxylin (PTAH) and modified Phloxine methylene blue azure II (PMBA II) for demonstration of intravascular thrombi and bacteria, respectively.

Airways, vascular structures, alveoli, and interstitial connective tissues (interlobular septal, peribronchial, perivascular, and subpleural) were examined microscopically with special attention on the

cellular reactions at the margins of the lesions. Relative bacterial populations at the periphery and the center of the lesions were estimated from selected PMBA II stained sections.

Experimental Pneumonic Pasteurellosis

Microorganism

Cultures of *P. haemolytica* Biotype A, Serotype 1, originally isolated from feedlot steers were used in these studies (68). The organism was grown on brain heart infusion agar with 5% citrated bovine blood, 1% horse serum, and 1% yeast hydrolysate at 37°C in candle jars. The bacterial suspension used for vaccination and challenge exposure was prepared as previously described (16). Organisms were suspended at an approximate concentration of 10^9 colony-forming units (CFU)/ml as measured by a modified plate count method (68).

Experimental Animals

Twenty-seven, 6-8 month old Hereford, Angus, and Hereford-Angus cross-bred male and female calves (160 to 225 kg) were used. All calves were obtained from a closed herd with a low prevalence of respiratory disease. These calves were used for morphologic study of pneumonic pasteurellosis. Two mature sheep and one one-day-old calf were used for study of pulmonary lymphatic drainage from the injection site.

Experimental Design

The twenty-seven calves were divided into two experimental groups, group A containing 15 animals (Table I) and group B containing 12

animals (Table II). Twelve of the group A calves were vaccinated twice on days 0 and 7 using live P. haemolytica bacteria and a commercial bacterin[1] by different routes (Table I). The remaining 3 animals in group A were sham vaccinated on the same days using phosphate buffer saline solution (PBSS). Details of the vaccination procedures were described previously (68). Two weeks after the second vaccination (day 21), the calves were inoculated with 5 ml of suspensions of live P. haemolytica by direct transthoracic intrapulmonic injection as previously described (68).

Group B consisted of twelve non-vaccinated calves. The calves were intrapulmonically inoculated with 5 ml of suspensions of live P. haemolytica as has been described in group A.

The animals which died after challenge exposure were necropsied and samples were collected for pathologic examination.

Pathology

Surviving calves were slaughtered four days following the challenge inoculation and the respiratory tracts were collected and examined grossly. Lesions were described according to size and location, distribution and character of pleural inflammation, the size and shape of the central intensely inflamed focus at the inoculation site, the size and character of the zone of interlobular edema peripheral to the focus of intense inflammation, and other parameters that implied spread of the inflammatory process from the focus of inoculation. The identify and

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[1] Pasteurella haemolytica-multocida bacterin, Bovine Isolates, Aluminumhydroxide adjuvanted (Ultrabac-PR; Beecham Laboratories, Bristol, Tennessee 37620, U.S.A.

orientation of each artificially induced lesion was maintained throughout the examination.

The cases with gross evidence of extension of the pneumonic lesions from the injection site toward the pulmonary hilar region were selected for histopathologic study. These tissues were fixed in 10% neutral buffered formalin solution. In order to relate histologic observations to the gross lesion, the fixed tissues were dissected and then photographed or diagrammed. After routine processing and paraffin embedding, sections of tissue were cut at 6 microns and stained with hematoxylin and eosin. Selected sections were stained also with PTAH and PMBA II.

In group B, the gross and histopathologic lung changes of all calves were examined and evaluated in the same manner as described for group A.

Lymphatic Drainage from Intrapulmonic Injection Site

Lymphatic drainage from the intrapulmonic injection site in the caudal lobes was traced by the injection of traceable substances followed by slaughter and examination of the lungs and mediastinal structures. The injected material was a mixture of 8 ml of radiopaque contrast medium (Conray)[2] and 2 ml of 5% aqueous solution of Brilliant Blue FCF.[3]

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[2]Conray^{R}-400 (Iothalamate sodium injection U.S.P. 66.8%) Mallinckrodt, Inc., St. Louis, Missouri 63134, U.S.A.

[3]Brilliant Blue FCF, CI No. 4290-CARC, Pfaltz & Bauer, Inc., Stamford, Connecticut 06902, U.S.A.

The pilot studies were conducted to obtain the optimal dosage of the tracers and prenecropsy time in two sheep. At one and one-half hours after intrapulmonic injection of the traceable media into the left and right lungs, the calf was killed by injection of an overdose of euthanasia solution[4] and necropsied immediately. The lungs were inflated with air to usual size and radiographically examined. The lung was frozen subsequently at -15°C and carefully dissected to determine drainage of the dye from the injection site.

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[4]Euthanasia solution[R], Veterinary Laboratories, Inc. Lanexa, Kansas 66215, U.S.A.

CHAPTER V

RESULTS

Gross Pathologic Findings

Natural Disease

The lungs had typical fibrinous pleuropneumonia. The pneumonia was bilateral but unequal in the left and right lungs, generally involved the cranial ventral portion of each lung, and was usually well demarcated from the remaining pale, often overinflated, dorsocaudal portion of the lung (Figures 1 and 2). Occasionally, the demarcation was poorly defined. The accessory lobe was frequently, entirely or partly, involved. Affected areas were heavy, dark red, and consolidated. On cut surface, the lobules were dark red, red, or gray (Figure 3). The interlobular septa were widened due to infiltration of yellow, dark red, or clear edematous material. Dry gray areas of necrosis involving portions or entire lobules were common.

Visceral and parietal pleurae overlying inflamed areas of lung were usually dull and coated with variable amounts of fibrin. There were occasionally 2-3 liters of serosanguineous fluid in the pleural cavity. The outer surface of the pericardial sac was sometimes dull and cloudy with small amounts of fibrin. Ecchymotic hemorrhages were occasionally observed on the epicardium and endocardium. The tracheobronchial and mediastinal lymph nodes were usually dark red and

enlarged. Tracheal and bronchial mucosa were diffusely reddened. The lower bronchioles, particularly those that supplied severely affected pulmonary lobes, contained mild-to-moderate amount of bloody and frothy exudate or occasionally mucopurulent exudate.

In more chronic cases, the lesions were generally similar to those described above. In addition, fibrinous adhesions were observed between visceral pleura of interpulmonary lobes, between visceral and parietal pleurae, and/or between visceral pleura and the pericardial sac. Necrotic areas were circumscribed by a prominent connective tissue capsule. Bronchioles, bronchi, and occasionally the trachea were filled with variable amounts of mucopurulent exudate.

Experimental Disease

Lesions at the site of intrapulmonic injection in animals killed 96 hours post challenge were consistently present though variable in appearance. The injection site lesions were expanding foci of pneumonia, qualitatively typical of spontaneous acute pneumonic pasteurellosis: The lung surrounding the injection site was remarkably swollen and moderately firm. This resulted in a broad, smoothly swollen area visible on both the lateral and medial surfaces of the lung. The visceral pleura was thickened and edematous and underlying interlobular septa were distended. The visceral (and occasionally the opposed parietal) pleura associated with the injection sites were often covered with granular, shaggy, or sheet-like deposits of adherent fibrin. On cut surface, the intense focus of pneumonia was usually irregularly spherical or ovoid, sometimes angular, densely firm, and deep red or gray mottled. Usually the area was partially bordered and often

intersected by translucent yellow-gray, widely distended interlobular septa. There were focal or diffuse serofibrinous pleuritis, and regional (thoracic) lymphadenitis.

Lesions were graded according to the size, severity, and extent of invasion of the inflammatory process to the surrounding lung tissue and spreading to remote hilar regions. Lesions were classified as either susceptible, intermediate, or resistant. Susceptible lung lesions were characterized by relatively large size and ill-defined boundaries (Figure 4). Swelling on the medial and lateral surfaces of injection site lesions was prominent and edema of interlobular septa in inoculated lobes was extensive. On cut surface, the inflammatory process appeared highly invasive extending from the locus of inoculation into the surrounding lung tissue and tended to spread to the remote lobules in the hilar regions. Exudate and edema within interlobular septa often extended several centimeters beyond the borders of the focus of intense inflammation. The septal exudate was yellow and translucent within and immediately adjacent to the intense focus but became clear further from the intensely inflamed focus. The invasive nature of the inflammatory process was characterized by broad zones of reddened and moderately firm lung parenchyma adjacent to affected septa, yet outside the intense focus. Evidence of pneumonic cord-like areas extending from the intense focus cranially along peribronchovascular connective tissue toward the hilar region of the lungs was occasionally present. However, the secondary lesions in the hilar region of the diaphragmatic, intermediate, accessory, and cranial lobes were frequently present without gross evidence of a continuous pneumonic area from the primary induced lesion. The hilar lesions were smaller than the primary intense

lesions, but their cut surface appearance was similar. Lesion migration from the primary focus in right and left lungs was compared in ten experimental cases. Evidence of cranial migration of the lesion was found in eight right lungs, whereas only two left lungs had this cranial spreading phenomenon (data not shown). Tracheobronchial lymph nodes were edematous, enlarged, and occasionally uni-or-multifocally necrotic.

In resistant lungs, lesions were generally smaller, less swollen and more circumscribed and usually lacked extensions of the inflammatory process into interlobular septa and lobular parenchyma of adjacent lung (Figure 5). Pleuritis and pleural edema were considerably limited or absent. There was apparently no evidence of cranial extension of the induced lesion to hilar regions.

Gross necropsy lesions in animals that died within 24-72 hours post inoculation (calf number 1, 2, and 3 in experiment B; Table II) included severe, diffuse congestion and edema of the lung parenchyma, interstitium, and visceral and parietal pleurae. Intensely congested, edematous, and firm areas surrounded the injection site lesions for extensive distances. The visceral pleura, and to a less extent, the parietal pleura, was often diffusely dull, granular, and petechiate. Extensive congestion, edema and occasionally necrosis were present in thoracic lymph nodes. In addition, evidence of fibrinous polyarthritis and generalized petechiation on serosal surfaces was consistently found. Occasionally, panophthalmitis and meningitis were present. P. haemolytica was isolated from bone marrow, spleen, and liver of these calves.

Histopathologic Findings

General features of both spontaneous and experimental lesions are similar. Vascular congestion, exudation of protein-rich edema fluid, fibrin and varying numbers of inflammatory cells constituted the major inflammatory changes in affected areas. The well advanced lesion was characterized by areas of coagulative necrosis which were irregular in size and shape (Figures 7 and 8) and commonly occupied a portion or entire pulmonary lobules. These areas were bordered by dark zones of leukocytic infiltration composed of eosinophilic rounded or darkened fusiform macrophages, and degenerate and/or intact neutrophils (Figure 9). Less severely affected areas were massively infiltrated with mononuclear cells and neutrophils (Figure 11). The presence of whorling and streaming patterns of darkened fusiform nuclei macrophages in alveoli and terminal bronchioles were a common finding. In experimental lesions, multinucleated giant cells were occasionally present with the other cellular components in alveoli. Bronchi and bronchioles were filled with variable amounts of serous, mucous, and fibrinous exudate, mixed inflammatory cells, and cellular debris. The mucosal epithelium of bronchi and bronchioles was relatively intact, however, considerable numbers of mononuclear inflammatory cells and neutrophils were found in the peribronchovascular connective tissue. Pleuritis was characterized by marked edema and a moderately severe infiltration of neutrophils and macrophages, a prominence of fibroblasts, hemorrhage and congestion, lymphatic distension and thrombosis, mesothelial cell hypertrophy and hyperplasia, and fibrocellular exudation adherent to the pleural surfaces (Figure 13).

In experimental lesions, peribronchial lymph nodules were often hyperplastic. Lymphoplasmacytic cells and macrophages were infiltrated in the perilymphatic connective tissue of the septal and juxta-alveolar lymphatic vessels. This phenomenon was particularly prominent in lung lesions of calves which had been vaccinated. Alveolar hemorrhage was occasionally present. Blood vessels were generally congested. Vascular thrombosis was occasionally observed, particularly in necrotic areas (Figure 10). The endothelium was generally swollen and prominent. Lesions in the hilar region of experimental cases were generally similar in appearance to those described for the primary injection site; however, they were smaller, generally limited to one or several lobules and their borders sharply defined by inflamed interlobular septa.

The interlobular septal, peribronchial, perivascular and subpleural interstitial connective tissues were severely inflamed. Within and near areas of intense parenchymal damage of both natural and the nonresistant experimental lesions, interlobular septa were markedly distended with degenerating and necrotic inflammatory cell debris. Septal blood and lymphatic vessels were often thrombosed and their walls inflamed or necrotic. Septa somewhat removed from areas of intense inflammation were markedly distended by fibrillar and homogeneous proteinaceous material, numerous inflammatory cells, numerous large fusiform fibroblasts, and varying degrees of hemorrhage. Septal lymphatics were widely dilated with eosinophilic material, scattered inflammatory cells and erythrocytes, or were thrombosed. Septal lymphatic thrombi in experimental lung lesions of nonresistant cattle tended to contain many inflammatory cells and cellular debris and were bordered by dark rounded or fusiform macrophages. Large and small

arteries and veins were engorged and sometimes thrombosed. The inflammatory lesions within interlobular septa always extended beyond the area of parenchymal inflammation. This appearance suggests that spreading of the lesion was occurring through the interlobular septa (interlobular extension) and to a lesser extent acorss the septa to adjacent parenchymal lobules (translobular extension).

The experimental lesions of resistant lungs were rather well demarcated between the severe, moderate, and mild inflammatory parenchymal lobules by the interlobular septa. The inflammatory changes in the septa were mild, and occasionally fibroblast proliferation and neovascularization was present in the severely affected areas. The cellular components in the interlobular septa were mainly mononuclear cells (macrophages, and probably fibroblasts) and a few neutrophils.

The advancing border of both natural and experimental lesions showed variable degrees of exudative fibrinous pneumonia and alveolitis (Figures 14 and 15). Lesions were more severe in the peripheral and peribronchovascular regions of lobules adjacent to the grossly affected areas. Bands or irregular patches of the lesions extended from the region of greater severity into the center of the lobule. Occasionally, streaks or bands of alveolitis connecting the peripheral lobular lesions to peribronchovascular alveolitis in that lobule were present. Airways were relatively clear of exudate. This feature represents the extension of the inflammatory process from the interlobular septa into bordering interalveolar septa (Figure 18), into the adventitia of intralobular airways and vessels, and to or through the visceral pleura. The lesions were characterized by alveolar capillary engorgement with erythrocytes, hypercellularity of alveolar walls, and infiltration of serofibrinous

exudate. In severe areas, the hypercellularity and serofibrinous exudate was sufficiently extensive to obliterate alveolar spaces (Figure 11). The cellular components of thickened alveolar walls consisted of mononuclear cells (macrophages, pneumocytes, and mesenchymal cells) and polymorphonuclear neutrophils. The severity of inflammatory changes in interlobular septal and peribronchovascular connective tissues correlated with that of adjacent parenchymal lesions. In experimental cases, luminal exudate adjacent to severely diseased interlobular septa consisted largely of dense deposits of fibrin and few macrophages. More distant from the interlobular septa, alveolar content was less fibrillar but densely eosinophilic and more highly cellular (mononuclear cells and neutrophils) (Figure 16). In areas of mild inflammation, the alveolitis consisted of thickened interalveolar septa, congestion, edema, and mononuclear cell and neutrophil infiltration of the septal stroma. The majority of alveolar spaces were free of exudate. In moderately inflamed regions, which were located between the severe and mild areas of inflammation, alveolar lining cells were hypertrophied and minimal amounts of cellular or fluid exudate was present in alveolar lumens in addition to similar changes as described above for mildly inflamed areas (Figure 12). In natural lesions and the assigned experimental resistant lesions, mononuclear cells appeared to constitute the majority of the inflammatory cell infiltrate, whereas in the assigned experimental non-resistant lesions, there were approximately equal numbers of neutrophils and mononuclear inflammatory cells in these areas.

Considerable numbers of small coccobacilli, presumably *P. haemolytica*, were found free in the serofibrinous exudate, particularly in

alveoli at the periphery of the severely affected lobules (Figure 21). Occasionally, they were present in lymphatic vessels and interlobular septal matrix. The bacteria were readily found in the margins of the natural lesions and in the experimental septicemic lesions, whereas they were difficult to identify in the margins of other experimental lesions. The relative numbers of bacteria were higher at the center of the lesions than at the periphery in the natural disease and were higher in the center of experimental lesions than in the adjacent invasive pneumonic areas or the secondary hilar lesions in the experimental disease.

Lymphatic Drainage From the Injection Site Into the Caudal Pulmonary Lobes

Gross Findings

On the lateral surface of the lung, the brilliant blue FCF dye densely filled the subpleural space at the injection sites, and less densely in other lobar areas. On the medial surface, the dye was located mainly in the subpleural space at the hilar region of intermediate, caudal, and accessory lobes. Findings were similar in both left and right lungs.

Subgross Findings

Following freezing overnight, the lungs were serially sectioned for subgross examination. The most intensely stained areas were present along the peribronchovascular connective tissue spaces (presumably dilated lymph vessels) extending from the injection site toward the region of tracheal bifurcation. The pulmonary parenchyma surrounding

the injection sites and the subpleural space of the injection sites stained with lesser intensity. Findings were similar in both left and right lungs.

Radiographic Findings

Migration of the radiopaque substance (Conray^{R}) from the injection site in the caudal lobes toward hilar region and to surrounding areas of the injection site was determined radiographically. Findings were similar in both left and right lungs (Figure 6).

CHAPTER VI

DISCUSSION AND CONCLUSION

The gross and histopathological findings in natural pneumonic pasteurellosis in this study were similar to those previously described (47, 51, 79, 83, 102). In addition, gross and histopathologic findings in the experimental disease were reasonably similar to counterpart lesions of the natural disease suggesting that transthoracic intrapulmonic inoculation of P. haemolytica could serve as a method of practical and consistent production of experimental pneumonic pasteurellosis.

The experimental lesions could be considered artificial when compared to lesions resulting from aerogenous exposure in natural infection; however, the difference in route of exposure between natural and experimental disease appears insignificant. The method is more advantageous to other techniques which rely either on aerosol, intratracheal, or intrabronchial instillation of Pasteurella sp. suspension with or without prior viral infection or artificially induces stress (23, 26, 49, 50, 58). In the direct intrapulmonic injection technique, known numbers of bacteria are deposited into the caudal lung lobe and consistently produce unifocal expanding fibrinous pneumonia at the site of injection. These unifocal pneumonic lesions can be differentiated readily from naturally occurring pneumonic lesions and can be quantified into susceptible, intermediate, and resistant lung lesions by

objective evaluation of gross and histopathologic morphological changes. Whereas, in the aerosol, intratracheal, or intrabronchial inoculation technique, numbers of bacteria instilled is likely to vary depending on aerodynamic factors and the particle size of the generated aerosol. Furthermore, the latter techniques produce lesions in anteroventral lobes which may be difficult to differentiate from concurrent lesions resulting from natural exposure. Aerogenous experimental lesions have been quantified according to the number of lobes affected and/or the percentage of lung affected. These measurements are visual estimates and are therefore subjective.

Resistant lungs typically arrested the progress of the challenge lesion and maintained its unifocality rather than allowed the inflammatory process to extend to involve a large percentage of the lung. Susceptible lungs, however, frequently developed a locally extensive infiltrating pneumonia centering around the injection site. Thus, transthoracic intrapulmonic injection can serve as a challenge method to accurately assess lung resistance which is of critical value in the study of the pathogenesis of the disease and evaluation of the efficacy of biologic and/or therapeutic agents for control of the disease.

Gross and histopathologic appearance of the secondary hilar lesions were similar to that of the lesion at the injection site. The route of spread to the hilar region may be lymphatic drainage, venous drainage, or aerogenous retrograde movement by aspiration of the injected bacterial suspension from the locus of inoculation. Evidence of morphologic changes of the secondary hilar lesion which spread from the peripheral septa into the center of the parenchymal lobule, and lack of airways mucosal lesion, suggest that aerogenous spreading has

unlikely occurred. In addition, the finding of lymphatic drainage from the injection site supports the concept that lymphatic drainage plays an important role in spreading of the lesion toward the hilar region.

The pattern of bovine pulmonary lymphatic drainage was determined by the radiographic intensity of radiopaque medium (Conray^{R}) and upon distribution of dye revealed by subgross dissection of injected regions. Drainage from the intrapulmonic injection site occurred mainly via the collecting lymph vessels in the peribronchovascular connective tissue toward the hilar region. To a lesser extent, lymph drainage occurred via collecting lymph vessels in interlobular and subpleural connective tissues surrounding the injection site. This finding differs from the concept of a unidirectional flow of lymph in bovine lung from septal lymph vessels to subpleural lymph vessels as described by Galina (25). It is also in contrast to the statement of Saar and Getty (82) that lymph drains from the caudal pulmonary lobes, is collected in the caudal mediastinal lymph nodes, and then drains into the thoracic duct. The pattern of lymph flow determined by this investigation agrees well with that described by Nagaishi (66). He stated that lymph flows in the interlobular lymph vessels either to the hilar lymph nodes directly or through the subpleural connective tissue and then to the hilar lymph nodes.

The margins of the affected areas were histopathologically characterized by variable degree of alveolitis. The inflammation of interlobular septa in these areas frequently extended beyond the areas of parenchymal inflammation. Histologic examination of the margins of experimental lesions as well as the margins of spontaneous lesions revealed extension of the inflammation through interlobular septa into

the stroma of adjacent interalveolar septa and to peribronchial and perivascular connective tissue. The interstitial inflammation extended into alveolar epithelium and alveolar lumens of affected alveoli. The lack of early lesions in bronchi and bronchioles supports the concept of a centripetal spread of the lesions from the interlobular septa, through the alveoli towards the bronchus in the lobule as described by Schiefer et al. (83).

The inflammatory cell infiltrates at the margin of lesions was composed of both neutrophils and mononuclear inflammatory cells (Figures 19 and 20). The finding is in agreement with the concept that both macrophages and neutrophils are important as the phagocytic cell in the lung (29, 32, 71). This is in contrast to Jubb and Kennedy (51) who stated neutrophils are not a significant part of the alveolar reaction. Their observation was apparently based on the terminal stages of the disease in which mononuclear inflammatory cells represent the majority of the inflammatory cell infiltrate. According to Issekutz et al. (43), monocytes initially migrate into bacterial inflammatory sites simultaneously with neutrophils and histologically become the predominant cell type after 72 hours. This is due to the continued migration of monocytes into these lesions long after neutrophil migration has stopped. This differential migration is a probable explanation of the observation in the present study that the margins of lesions in lungs from calves that died of septicemia within 24-72 hours following inoculation contained a high proportion of neutrophils. The margins of experimental lesions which were classified as non-resistant (96-hour-old lesions) contained approximately equal numbers of neutrophils and mononuclear inflammatory cells (Figure 20). However, naturally

diseased lungs, and experimentally diseased ones classified as resistant, contained a high proportion of mononuclear inflammatory cells (Figure 19). Therefore, it is postulated that in septicemic lungs and non-resistant lung lesions, the neutrophils are still recruited into the margin of the lesion. The neutrophilic chemotactic factor that is secreted by alveolar macrophages (41, 42, 54) is probably also responsible for this feature. Thus, the border lesion of these lungs may represent an acutely progressive inflammatory response in which neutrophils are the predominant inflammatory cell, whereas the borders of lesions of natural disease and experimental disease classified as resistant represent a subacute inflammatory response. The milder inflammatory response and tissue injury in the border areas as compared to the intense inflammation at the center of a lesion may be the result of two factors: duration and intensity of the inflammatory stimuli. It is likely that both factors are contributing to the development of this feature.

In natural disease, aggregates of P. haemolytica were more readily identified in the peripheral areas of severely affected lobules (Figure 21). This finding is compatible with an observation of Jensen et al. (47). Schiefer et al. (83), however, mentioned the presence of bacteria in histological sections only in cases associated with P. multocida. In the experimental disease, it appeared that the injected P. haemolytica remained localized at the injection site. The bacteria were found mainly free in alveolar or septal exudate in the intense foci of inflammation. The relative population of bacteria in the border of the lesion is less than that in the center of the lesion in both experimental and natural diseases (Figures 21 and 22). In this study, small numbers of

bacteria were found in the margins of lesions of natural disease and experimentally susceptible lung sections stained with Phloxine methylene blue azure II (Figure 22). Bacteria were rarely demonstrated in the margins of experimentally resistant lesions. Further experiments using the fluorescent antibody technique as described by Newman et al. (68) are needed to substantiate this observation.

Animals dying of septicemia within 1-3 days following the challenge exposure had numerous bacteria in alveolar and interstitial exudates at the injection site and fewer at the periphery of the lesion. The mixture of bacteria and fusiform macrophages was particularly prominent in septal lymphatic vessels. In contrast to the findings of Friend et al. (23), who stated that the characteristic swirling dark fusiform cells accumulated in alveoli and alveolar ducts from three-day old lesions onward, these cells were identified in alveoli and vascular thrombi of one-day-old lesions of septicemic cases in this study.

The presence of numerous bacteria, fusiform macrophages, and coagulative necrosis implies that the bacteria or their toxic factor(s) were responsible for degeneration and necrosis of the macrophages and probably for the coagulative necrosis of lobular parenchyma. Even though microthrombi in alveolar capillaries were not clearly elucidated in PTAH-stained histologic sections, intravascular thrombi in coagulative necrotic areas were obvious. P. haemolytica endotoxin induced thrombosis of capillary plexuses, pulmonary and lymphatic vessels resulting in ischemic necrosis of the lobule as described by Pierson and Kainer (77) cannot be ruled out. Thus, it is likely that both the direct action of bacteria and vascular injury were responsible for the coagulation necrosis. Experiments using endotoxin or cytotoxic factor of

P. haemolytica should be undertaken to compare the morphological changes with this study.

A correlation between inflammatory changes in the parenchyma and interstitial tissue can be made. In severe pneumonic lesions, there were large numbers of neutrophils and mononuclear inflammatory cells in the interstitial tissue that accounted for features of peribronchitis and perivasculitis. The interlobular septa of lesions from resistant animals tended to contain a higher proportion of plump rounded or spindle-shaped mononuclear cells.

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APPENDIX A

TABLES

TABLE I

EXPERIMENT A: EVALUATION OF GROSS PATHOLOGY
AND SUMMARY OF EXTENDING LESIONS

Vaccination or treatment before challenge inoculation	Calf Number	Gross Lesion[a]	Extending lesions from the injected site							
			Left Lung			Accessory lobe	Right Lung			
			Apical lobe	Intermediate lobe	Diaphragmatic lobe[b]		Apical lobe	Cranial intermediate lobe	Caudal intermediate lobe	Diaphragmatic lobe[b]
PBSS[c], 15 min. aerosol exposure	1	I[d]	O	O	X[f]	O	O	O	O	Y[g]
6 hr. culture live P. haemolytica 15 min. aerosol exposure	2	I	O	O	O	X	O	O	X	X
6 hr. culture live P. haemolytica 15 min. aerosol exposure	3	R[h]	O	O	O	X	O	O	O	O
22 hr. culture live P. haemolytica 15 min. aerosol exposure	4	I	O	O	X	O	O	O	X	X
22 hr. culture live P. haemolytica 15 min. aerosol exposure	5	S[i]	O	O	X	X	O	O	O	Y
22 hr. culture live P. haemolytica 15 min. aerosol exposure	6	I	O	O	O	O	O	O	X	X
22 hr. culture live P. haemolytica 15 min. aerosol exposure	7	R	O	O	X	O	O	O	O	Y

TABLE I (Continued)

Vaccination or treatment before challenge inoculation	Calf Number	Gross Lesion[a]	Extending lesions from the injected site							
			Left Lung				Right Lung			
			Apical lobe	Intermediate lobe	Diaphragmatic lobe[b]	Accessory lobe	Apical lobe	Cranial intermediate lobe	Caudal intermediate lobe	Diaphragmatic lobe[b]
22 hr. culture live P. haemolytica 15 min. aerosol exposure	8	R	O	O	Y	X	O	O	X	O
Beecham bacterin subcutaneously	9	S	O	X	O	O	X	X	O	X
22 hr. culture live P. haemolytica direct intrapulmonic vaccination into the right lung	10	I	O	O	Y	O	O	O	O	O
22 hr. culture live P. haemolytica subcutaneously	11	I	O	O	Y	O	O	O	O	O
Beecham bacterin subcutaneously	12	S	O	O	Y	X	X	O	O	X
PBSS, 15 min. aerosol exposure	13	S	O	O	Y	X	O	O	X	Y
20 hr. culture live P. haemolytica 15 min. aerosol exposure	14	I	X	X	Y	O	O	O	O	Y
PBSS, 15 min. aerosol exposure	15	S	O	O	Y	X	O	O	X	X

(a) = The criteria for evaluation were described in text.
(b) = Extending lesions from primary intense inflammation.
(c) = PBSS = Phosphate Buffered Saline Solution
(d) = I = Intermediate lesion
(e) = O = Absence of lesion
(f) = X = Presence of secondary focal lesion in the hilar region.
(g) = Y = cranial extension of lesion from the injected site toward the hilar region.
(h) = R = Resistant lesion
(i) = S = Susceptible lesion

TABLE II

EXPERIMENT B: EVALUATION OF GROSS PATHOLOGY
AND SUMMARY OF EXTENDING LESIONS

Calf Number	Gross[a] Lesion	Extending lesions from the injected site						Accessory Lobe	Extending lesions from the injected site			
		Note[b]	Left Lung						Right Lung			
			Mediastinal Lymph Node	Apical Lobe	Intermediate Lobe	Diaphragmatic Lobe[c]			Apical Lobe	Cranial Intermediate Lobe	Caudal Intermediate Lobe	Diaphragmatic Lobe[c]
1	**Septicemia**[d]	1 day	----------	Extensive Diffuse Lesion		---------		0	---- Extensive Diffuse Lesion ----------			
2	**Septicemia**	1 day	----------	Extensive Diffuse Lesion		---------		0	---- Extensive Diffuse Lesion ----------			
3	**Septicemia**	3 day	----------	Extensive Diffuse Lesion		---------		0	---- Extensive Diffuse Lesion ----------			
4	S[e]		X	O[f]	X[g]		X	X	O	O	X	X
5	S		O	O	O		Y[h]	X	O	O	X	X;Y
6	S		O	O	O		X;Y	X	O	O	X	X;Y
7	S		O	O	O		Y	O	---------- not inoculated ----------			
8	I[i]		O	O	O		O	X	O	O	X	Y
9	I		O	O	O		O	O	O	O	O	X
10	I		O	O	O		O	O	O	O	O	O
11	I		O	O	O		O	O	O	O	O	O
12	R[j]		O	O	O		O	O	---------- not inoculated ----------			

(a) The criteria for evaluation were described in text
(b) Period of spontaneous death following challenge inoculation.
(c) Extending lesions from primary intense inflammation.
(d) Details were described in text
(e) S = Susceptible lesion
(f) O = Absence of lesion
(g) X = Presence of secondary focal lesion in the hilar region.
(h) Y = Cranial extension of lesion from the injected site toward the hilar region.
(i) I = Intermediate lesion
(j) R = Resistant lesion

APPENDIX B

FIGURES

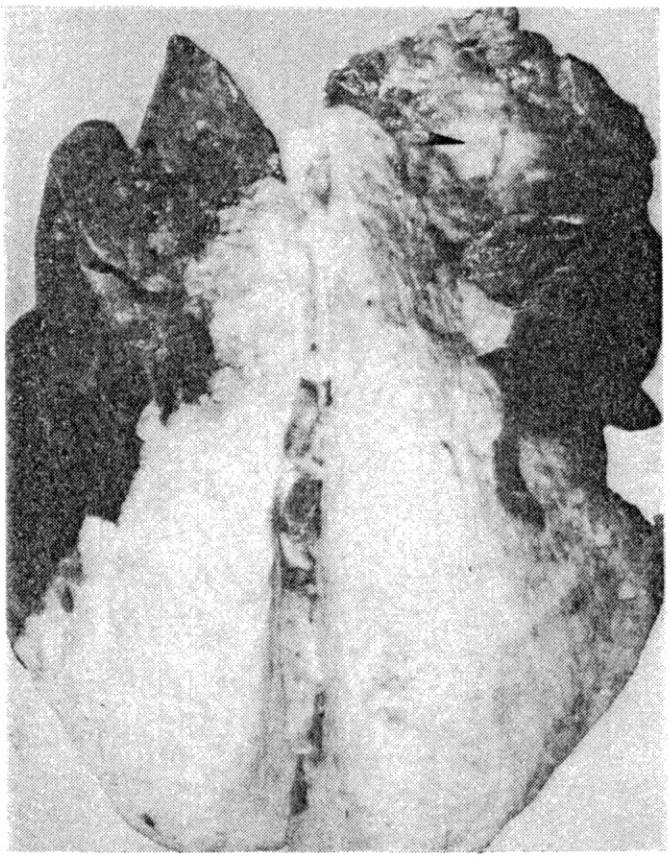

Figure 1. Natural bovine pneumonic pasteurellosis: Illustration of bilateral anteroventral pneumonic area, dorsoventral view. Note the white fibrinous exudate on the lateral surface of right apical lobe (arrow).

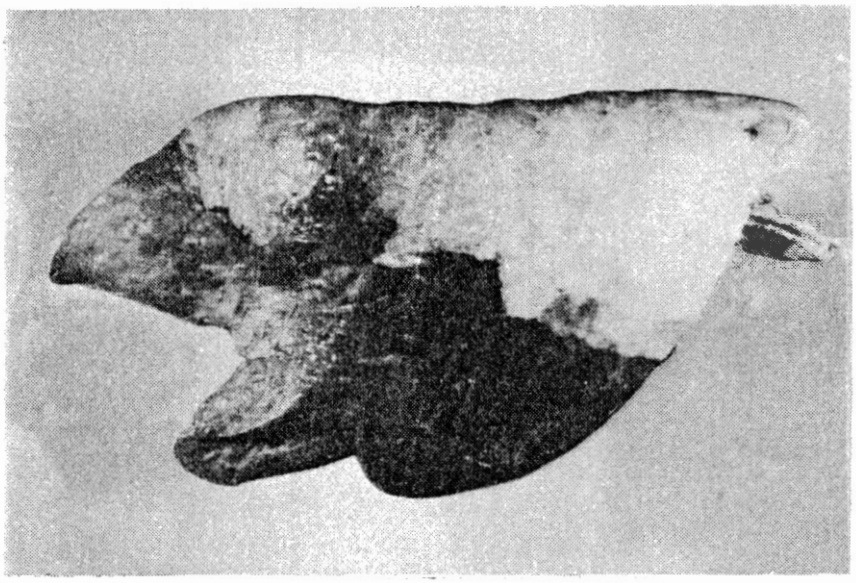

Figure 2. Natural bovine pneumonic pasteurellosis: Illustration of anteroventral distribution of pneumonic area, lateral view.

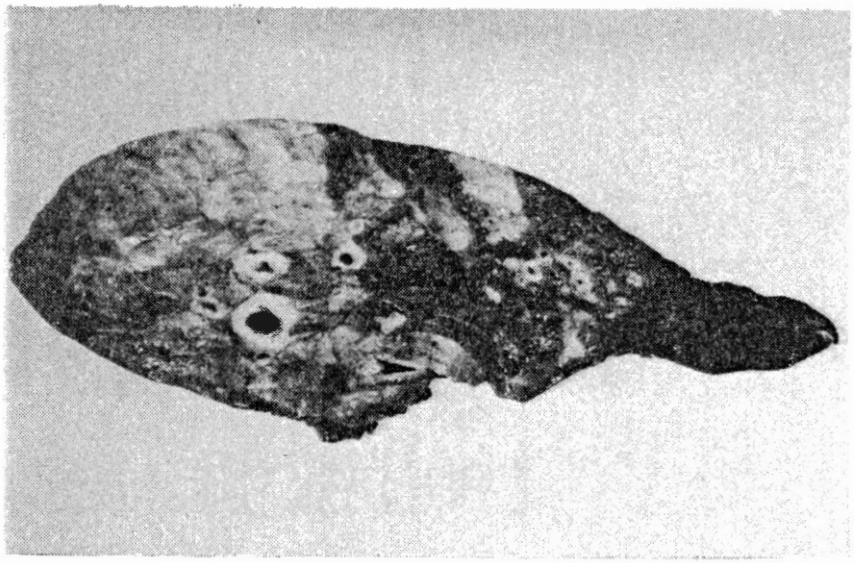

Figure 3. Natural bovine pneumonic pasteurellosis: Illustration of patchy lobular distribution of pneumonic areas, cut surface. Note subpleural and interlobular emphysematous space (arrow).

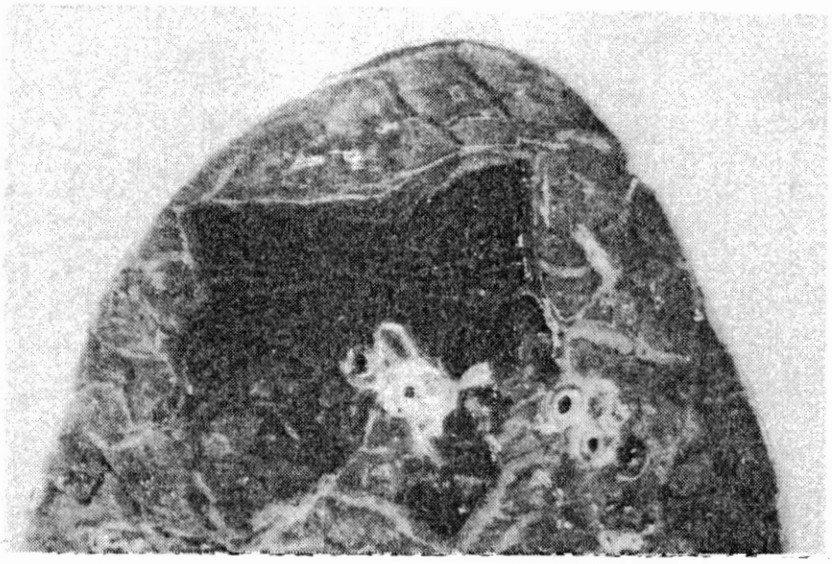

Figure 4. Experimental bovine pneumonic pasteurellosis: Relatively large and ill-defined boundaries of non-resistant lung lesion. (Courtesy of Dr. R. J. Panciera)

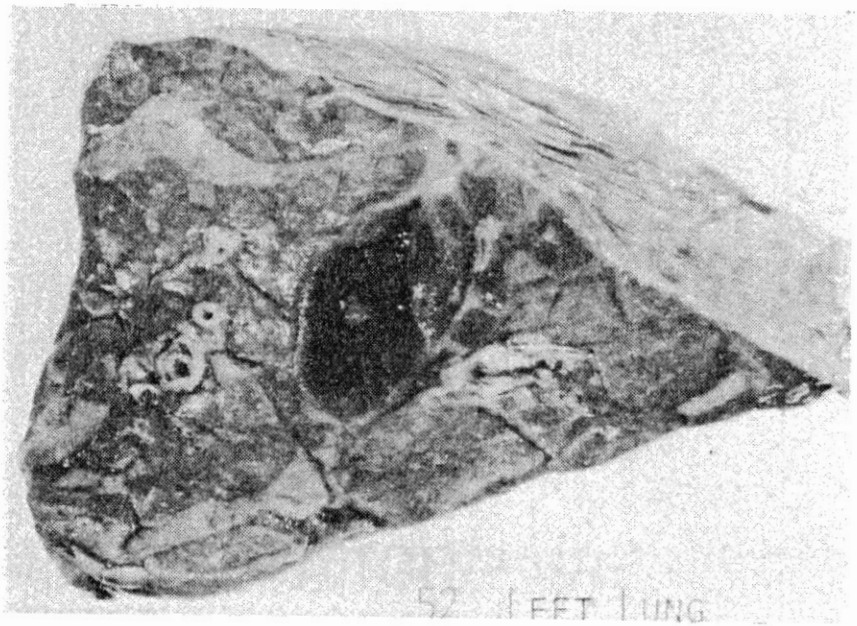

Figure 5. Experimental bovine pneumonic pasteurellosis: Well circumscribed area of pneumonia from resistant lung. (Courtesy of Dr. R. J. Panciera)

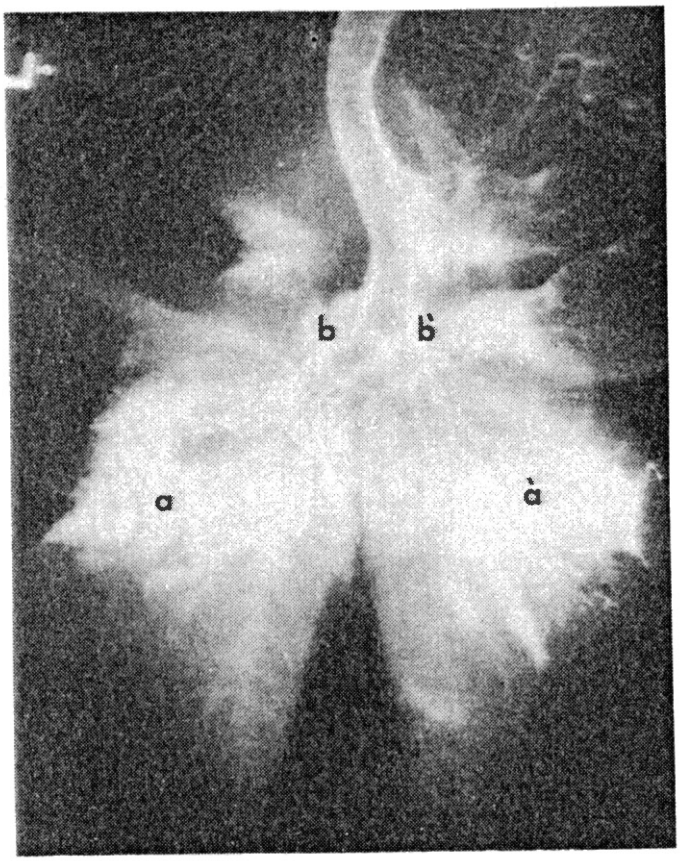

Figure 6. Radiographical illustration of distribution of the radiopaque substance (Conray^{R}) from the injection site in the caudal lung lobe (a,a'), predominantly toward the hilar region (b,b'), dorsoventral view.

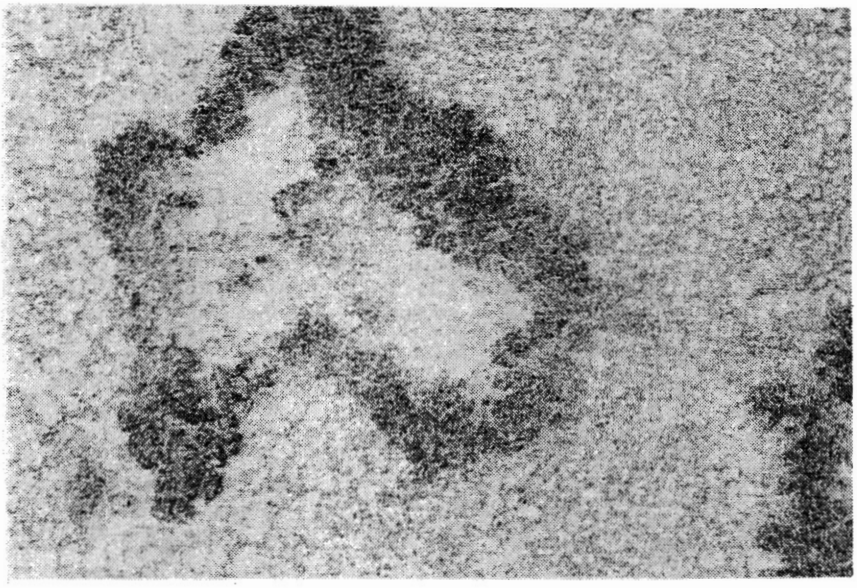

Figure 7. Natural disease: Area of coagulative necrosis bordered by zone of intense inflammatory cell infiltrate.

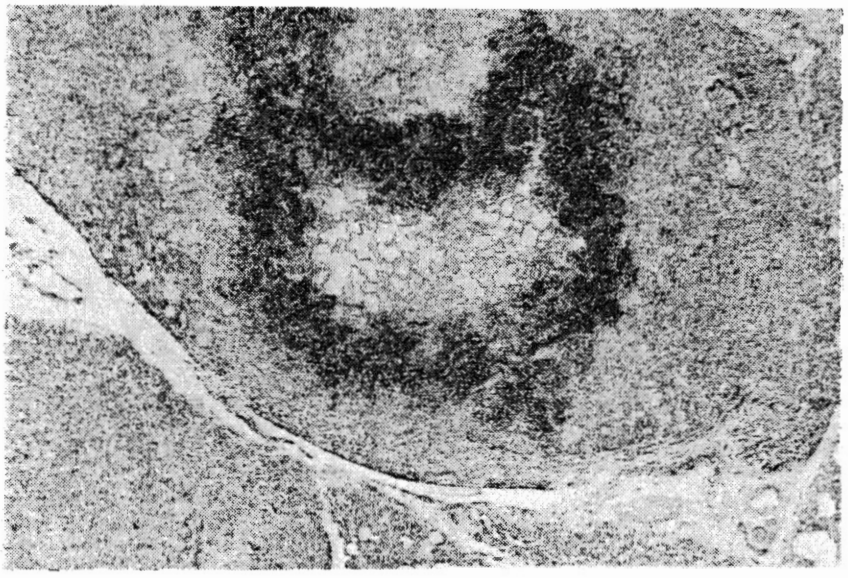

Figure 8. Experimental disease: Focal coagulative necrotic area. Note the similarity between natural and experimental lesions.

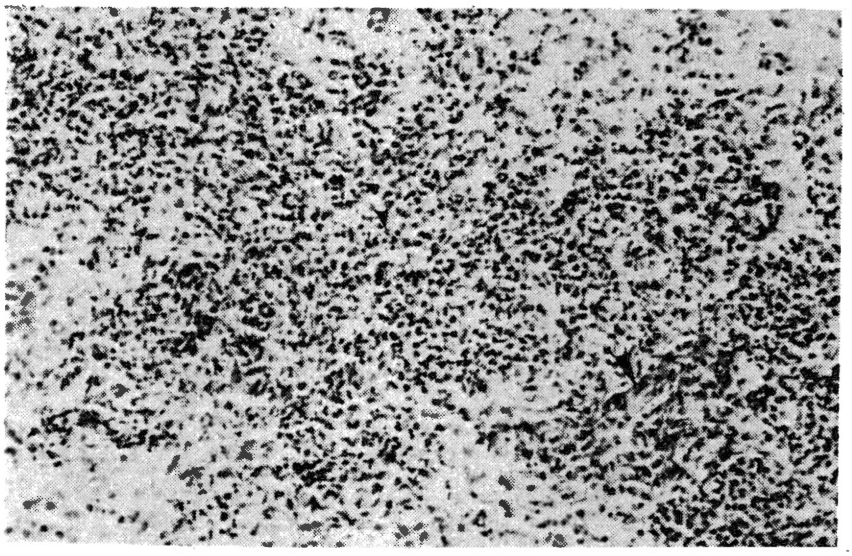

Figure 9. Experimental disease: Details of the cellular components at the border of an area of coagulative necrosis. They are composed of rounded or fusiform (oat-shaped) macrophages (large arrow), degenerate cells and some intact neutrophils (small arrow).

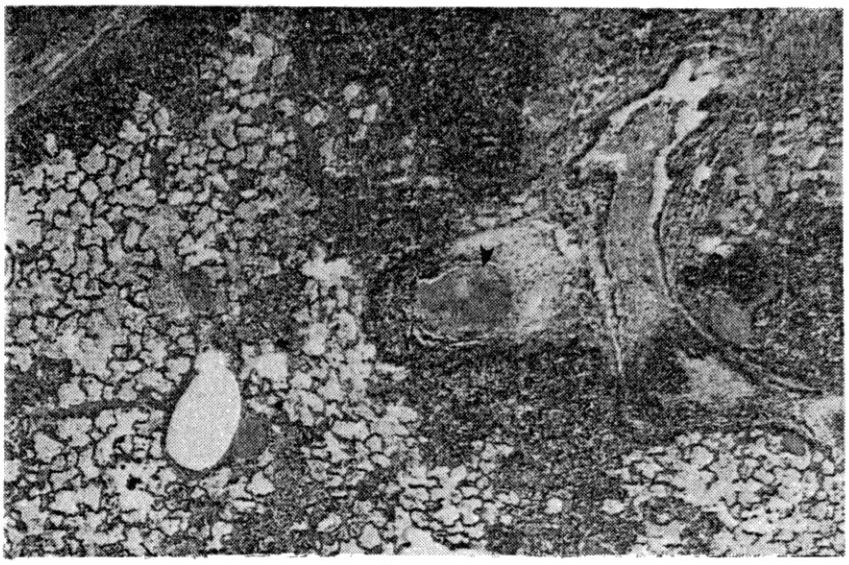

Figure 10. Experimental disease: Vascular thrombi (arrows) in areas of coagulative necrosis.

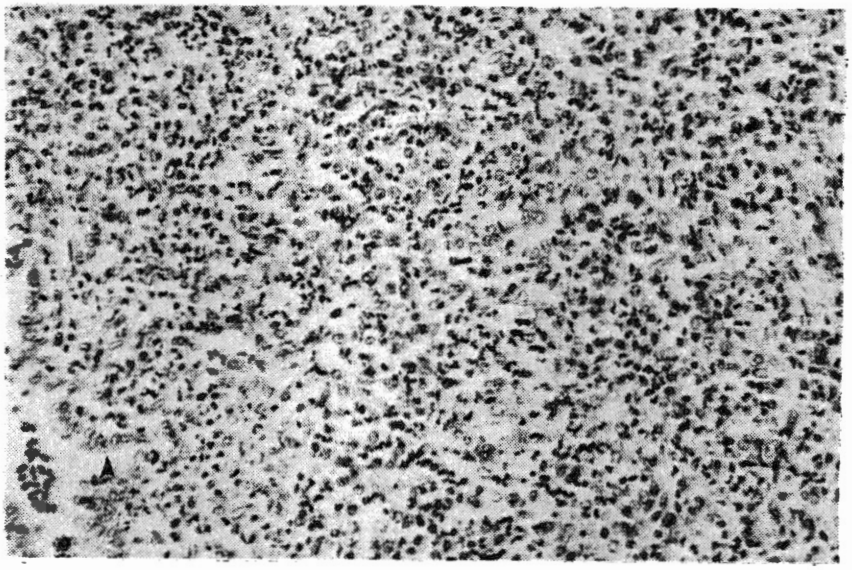

Figure 11. Experimental disease: The cellular exudate in a severely inflamed area is composed of mononuclear cells and neutrophils. The bronchiolar mucosa is relatively intact (arrow).

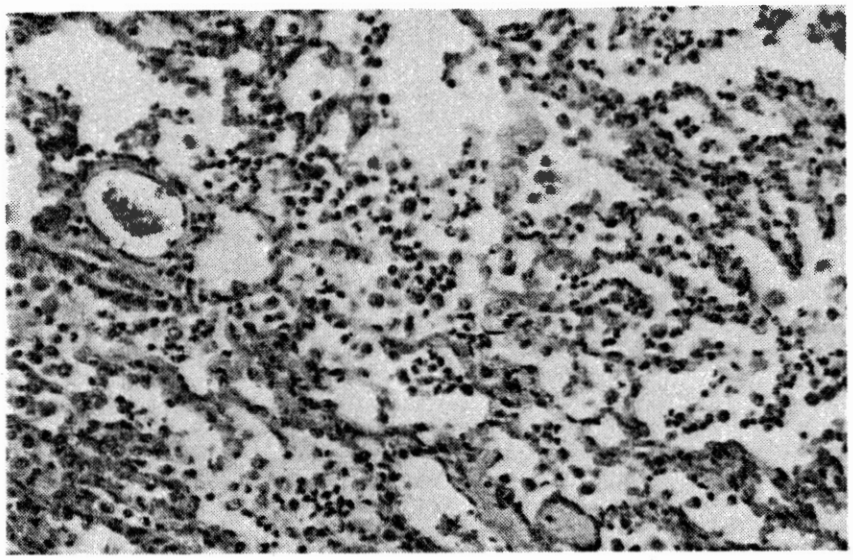

Figure 12. Experimental disease: A moderately inflamed region demonstrating hypertrophied alveolar lining cells with moderate amount of cellular exudate in alveolar lumens.

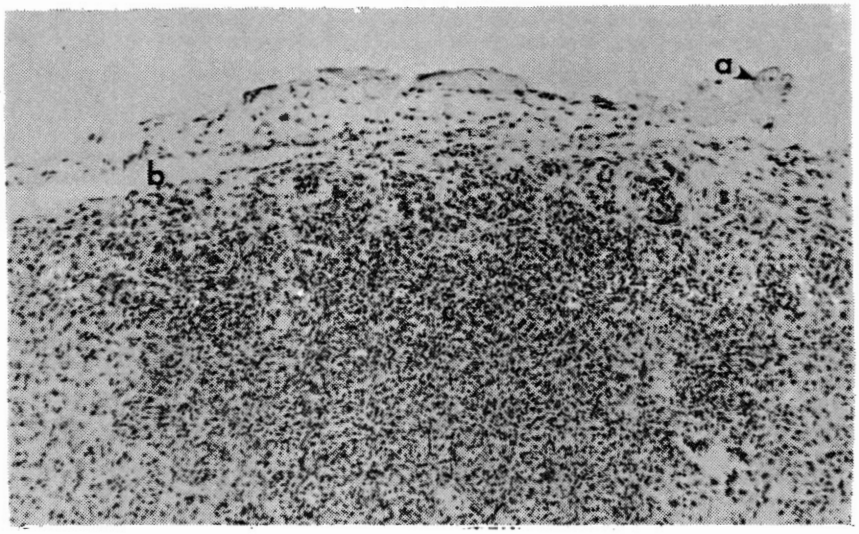

Figure 13. Natural disease: Fibrinous pleuritis, fibrinous exudate (a), dilated subpleural lymph vessel (b), an accumulation of degenerated leukocytes and oat-shaped macrophages (c). The lesion in accessory lobe.

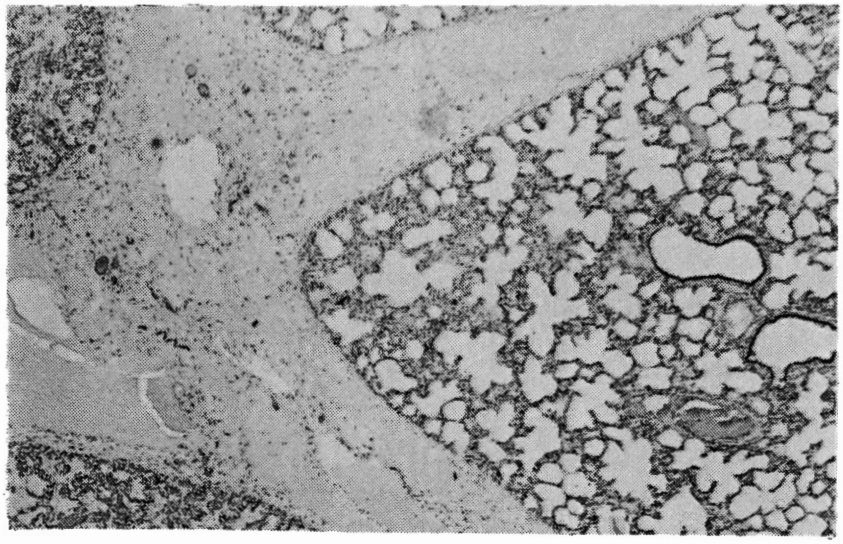

Figure 14. Natural disease: Alveolitis at the margins of lesion. Interlobular septae are distended by fibrillar and homogeneous proteinaceous material, inflammatory cells, and congestion.

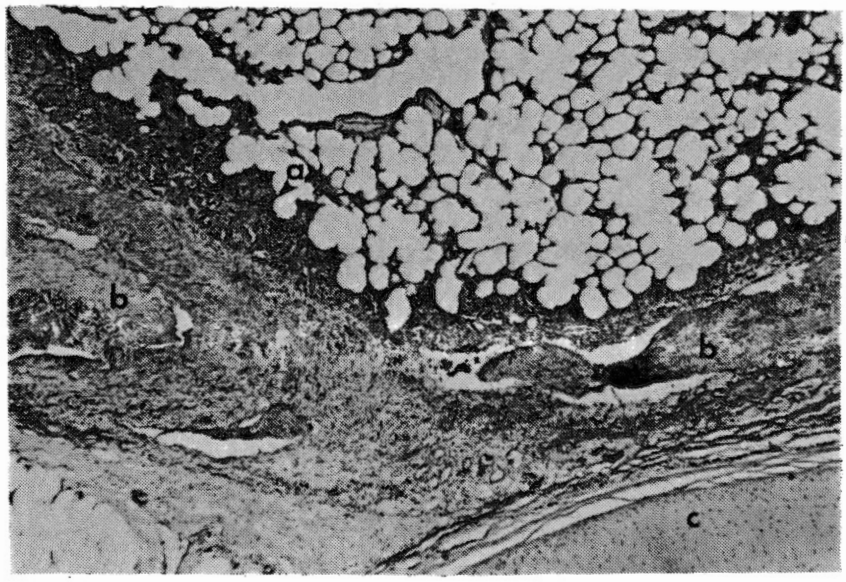

Figure 15. Experimental disease: Mild alveolitis (a) extending from severely inflamed peribronchovascular connective tissue, thrombi in peribronchial lymph vessels (b), bronchial cartilage (c).

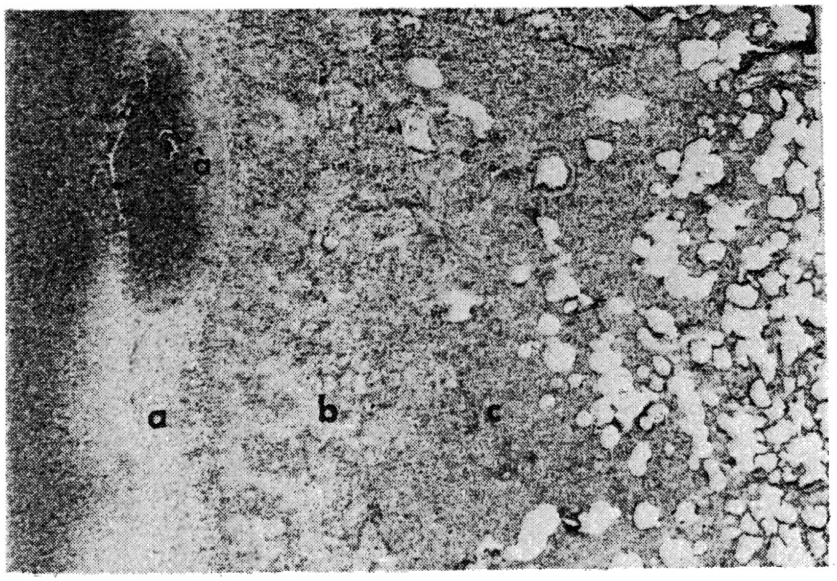

Figure 16. Experimental disease: Extensive alveolitis extending from severely inflamed interlobular septum (a). Note lymphatic thrombosis (a'); alveolar luminal exudate adjacent to the severely diseased interlobular septum consists largely of fibrin with few macrophages (b). More distant from the interlobular septum the alveolar content is less fibrillar, but densely eosinophilic and highly cellular (c).

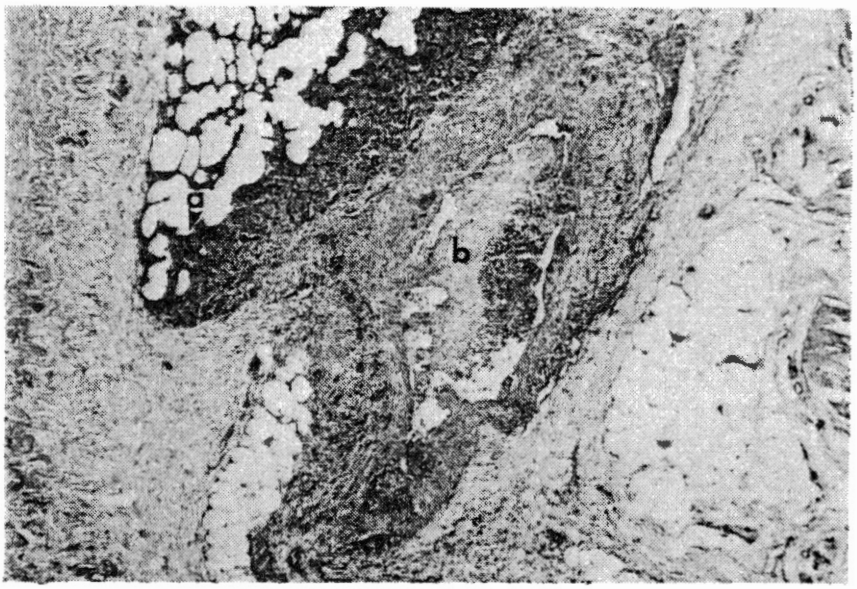

Figure 17. Experimental disease: Peribronchovascular spread of the lesion from the injection site. Alveolitis extends from the perivascular connective tissue (a), intralymphatic thrombus (b).

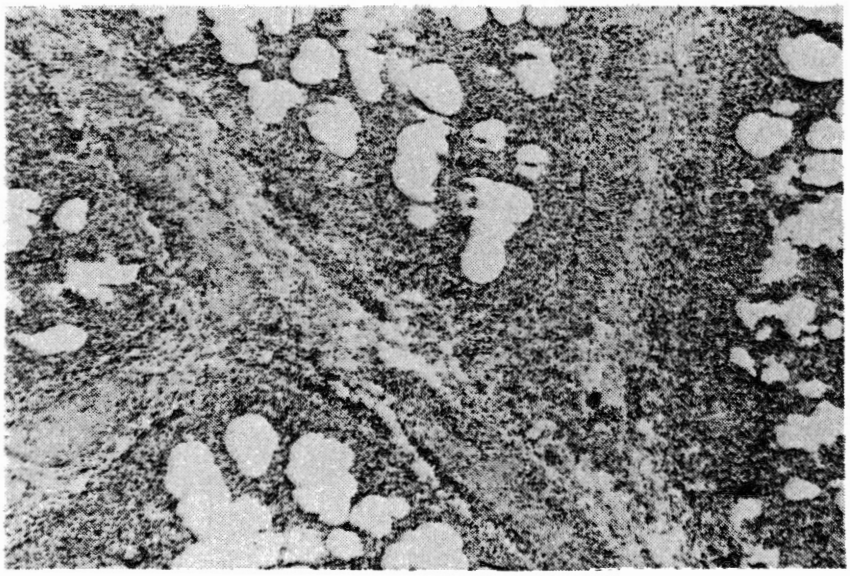

Figure 18. Experimental disease: Alveolitis extending from the interlobular septa.

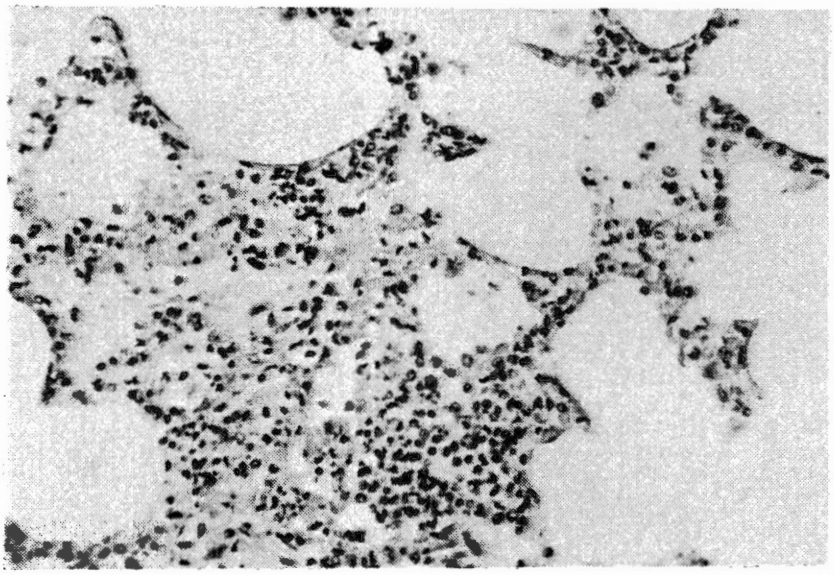

Figure 19. Natural disease: Alveolitis at the advancing border of the lesion. Note the relatively high porportion of mononuclear cells.

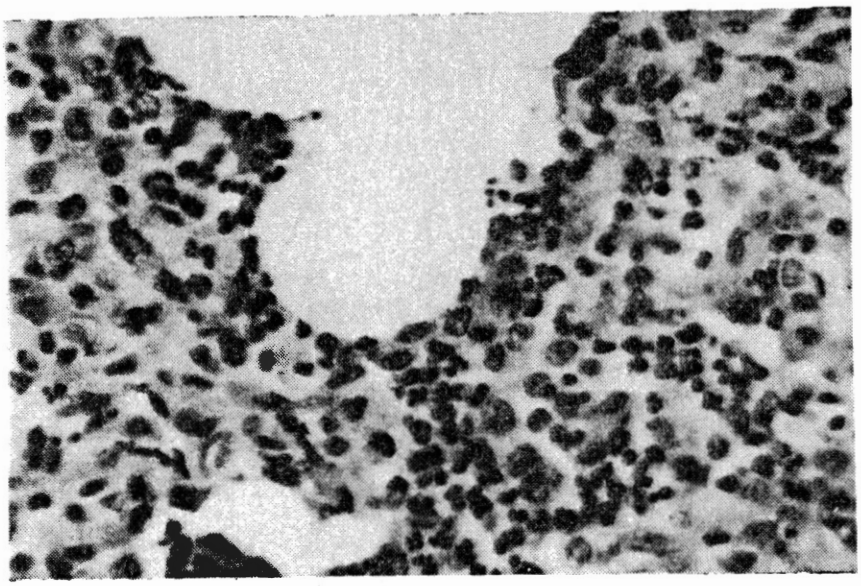

Figure 20. Experimental disease: Alveolitis at the advancing border of the lesion in a non-resistant lung. Note the approximately equal numbers of neutrophils and mononuclear cells.

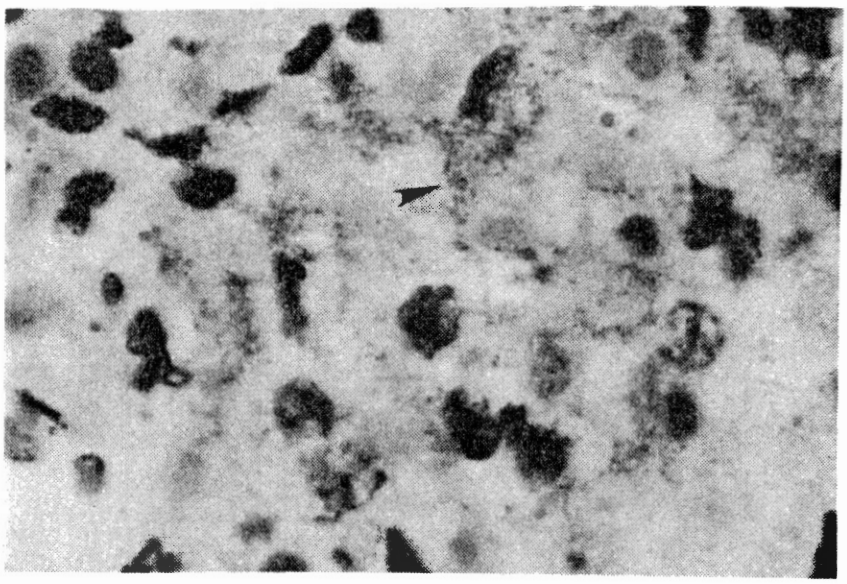

Figure 21. Natural disease: Numerous bacteria (arrow) near the center of gross lesion.

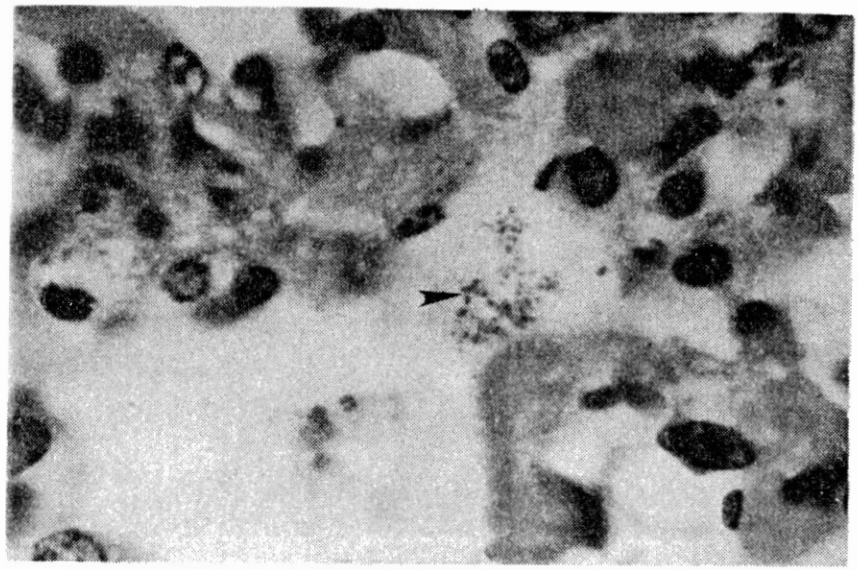

Figure 22. Natural disease: Sparsely distributed bacteria (arrow) near the periphery of pneumonic lesion.

VITA 2

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Master of Science

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