

Experimental Hemorrhagic Septicemia: Gross and Microscopic Lesions Resulting from Acute Infections and from Endotoxin Administration

by Keith R. Rhoades, Kenneth L. Heddleston and Paul A. Rebers*

ABSTRACT

Gross and microscopic lesions observed in bovine and porcine hemorrhagic septicemia, and in bovine *Pasteurella multocida* endotoxemia are described. Widely distributed hemorrhages, edema, and general hyperemia were the most obvious tissue changes observed in the infected calves. Pneumonia was a constant lesion. Aerosol exposure produced a multiple focal fibrinosuppurative pneumonia, while intranasal and intramuscular inoculation resulted in generalized interstitial pneumonia. A slight lymphadenitis and degenerative changes in hepatic and renal parenchymal cells were also observed.

The predominant lesions observed in infected pigs were a diffuse, extensive, fibrinous pneumonia and fibrinous polyserositis. Edema and general hyperemia were observed, but the widespread hemorrhages which occurred in the calves were not present. Acute lymphadenitis and renal tubule vacuolar degeneration were observed. A slight amount of cloudy swelling and focal areas of necrosis were found in the liver.

Lesions in a calf that died following administration of *Past. multocida* endotoxin were widely distributed hemorrhages, edema, and general hyperemia. These lesions were especially evident in the lungs, and indicated widespread vascular alteration.

Hemorrhagic septicemia is a disease produced by a particular serotype of *Pasteurella multocida*. (5) This serotype is identified as type 2, (7) type B, (3) type I, (10) and type 6:B. (8) The disease is common in Asia, Africa and southern Europe, (2,4,6) and affects cattle and buffalo, as well as several other species.

Confirmed reports on infection with this

serotype in the United States have been very limited. It was isolated in 1922 (12) from an acutely infected American buffalo in Yellowstone National Park, and recently was again isolated from buffalo in Montana. (5)

During the course of experiments (5,9) which involved studies of *Past. multocida* (M-1404, serotype 2) isolated from buffalo in 1922, calves and pigs died from experimentally induced infections. The course of the disease was acute, with death occurring in less than 40 hours in the calves and less than 72 hours in the pigs. Since descriptions of the microscopic lesions produced by infections with this serotype are quite limited, gross changes were noted and selected tissues from these animals were examined microscopically.

Endotoxin extracted from the same isolate and administered intravenously resulted in the death of a calf in approximately 4 1/2 hours. (9) Tissues from this animal were also examined microscopically.

This report describes the gross and microscopic lesions observed in the aforementioned animals.

Materials and Methods

Three calves and 2 pigs were exposed to *Past. multocida*, utilizing intranasal inoculation, intramuscular inoculation, and aerosol techniques. One calf was exposed by each technique. The pigs were exposed by aerosol and intranasal inoculation. Descriptions of the animals, the organism used, exposure methods, dosages, and clinical signs of infection are included in another report. (5)

An additional calf included in this study received *Past. multocida* endotoxin intravenously. Details concerning this animal, extraction of the endotoxin, dosage, and clinical signs are also included in another report. (9).

* From the National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa.



Fig. 1. Subserosal splenic hemorrhages in septicemic calf.



Fig. 2. Omental hemorrhages in septicemic calf.

Tissues from the animals were fixed in 10% formalin, with the exception of brain tissue which was fixed in 20% formalin. Routine techniques for dehydrating, infiltrating, and embedding were used. Paraplast¹ was used as an infiltrating and embedding material. All tissue sections were stained with hematoxylin and eosin. (1) Giemsa stain (1) was utilized in some instances for identifying inflammatory cell types and bacteria.

Results

GROSS LESIONS

Septicemic Calves. Widely distributed areas of hemorrhage were the most prominent lesions observed in the calves. Multiple, subcutaneous, petechial hemorrhages were found in the area of the thoracic inlet of the calves exposed by aerosol and intranasal inoculation. The hemorrhages of this area extended into the thymus and peritracheal adventitia, and were accompanied by edema. The calf exposed by intramuscular inoculation had little or no hemorrhage in the thoracic inlet area, but had hemorrhages in the thoracic portion of the thymus. The site of inoculation, the right midportion of the neck, was markedly hyperemic and an area of edema approxi-

mately 1/4 inch thick was observed subcutaneously. Edema was also observed in the subcutaneous tissue at the anterior portion of the sternum of the calf exposed by aerosol. The only female calf, exposed by intranasal inoculation, had diffuse hemorrhages in the mammary gland. Petechial and ecchymotic hemorrhages were found in the pericardium and under the parietal pleura, epicardium and endocardium of all calves.

Increased peritoneal fluid and subperitoneal hemorrhages involving the forestomachs, spleen (Fig. 1), mesentery, and omentum were found in all calves. These hemorrhages were petechial and ecchymotic in size, except that those in the omentum of the calf exposed intranasally, were diffuse (Fig. 2).

Lesions of the lower respiratory tract and related structures varied with the type of exposure. Hyperemia, which was most pronounced in the anteroventral portion of the lungs, was observed in all calves. The calf exposed by aerosol had, in addition, a well-developed interlobular edema accompanied by markedly increased fluid and fibrin in the pleural cavity.

Lymph nodes were often congested, but little, if any, enlargement was observed. All calves had a general passive hyperemia indicated by general venous dilatation. Ede-

¹Aloe Scientific, 3501 Raleigh Ave., So., Minneapolis, Minn.

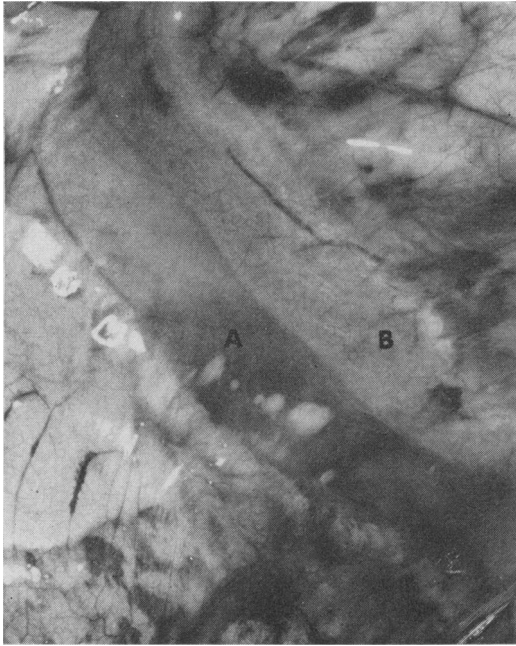


Fig. 3. Edema (A) surrounding duodenum (B) of septicemic calf.



Fig. 4. Fibrinous pleural adhesions in septicemic pig.

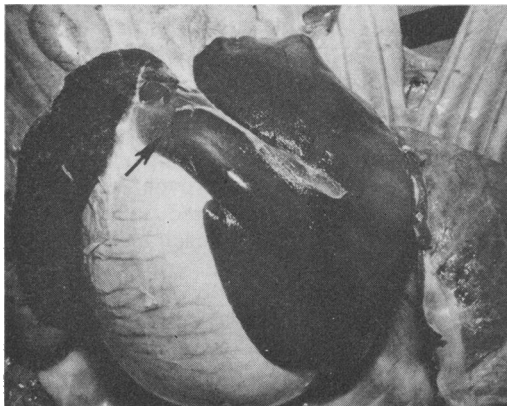


Fig. 5. Swollen spleen and fibrinous peritonitis (arrow) in septicemic pig.

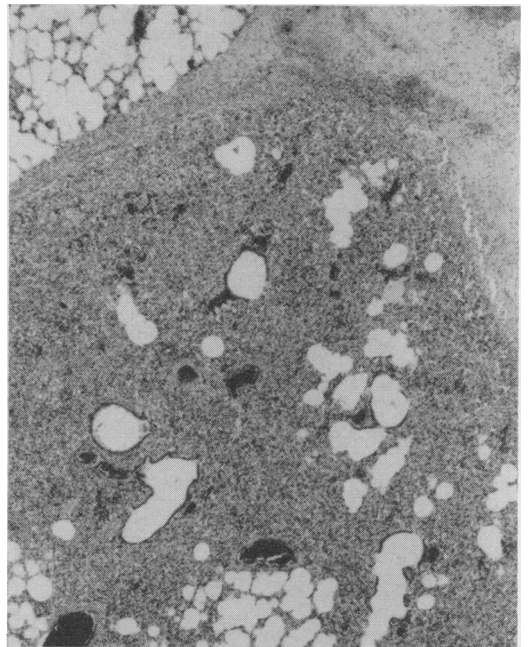


Fig. 6. Focal suppurative pneumonic area in septicemic calf exposed by aerosol. x40.

ma was observed around the small intestine of the calf exposed intranasally (Fig. 3). It extended approximately 10 inches along the intestine in the area of the bile duct entrance. Edema was also observed around the right kidney of the calf exposed intramuscularly. Hyperemia, hemorrhages, and edema were the only lesions observed in the abdominal viscera of the calves.

Endotoxemic Calf. The most severe lesions in the calf that died following endotoxin administration involved the respiratory system. The trachea and bronchi were completely filled with a stable yellowish froth. Extensive pulmonary edema involved both the interlobular septa and the parenchymateous tissue. There was an increased amount of pleural fluid and a few small subpleural hemorrhages. The small intestine, near the site of the bile duct entrance, was surrounded by an area of edema which was approximately $\frac{1}{2}$ inch thick and extended along the small intestine about 6 inches.

Lymph nodes were swollen and congested. No other lesions were observed.

Septicemic Pigs. Extensive lung lesions were constantly found in the pigs. Marked edema and congestion were diffuse in distribution, with consolidation occurring in the anteroventral portions. These pulmona-

ry lesions were accompanied by increased fluid and fibrin in the pleural and pericardial cavities and associated fibrinous serosal adhesions (Fig. 4).

Lymph nodes were, in general, congest-

ed and swollen. The spleens were usually enlarged (Fig. 5) and livers slightly swollen, but, with the exception of generalized hyperemia, the other visceral organs appeared normal. The peritoneal cavities of both pigs contained fibrin (Fig. 5) and increased amounts of fluid. Joints and skeletal musculature appeared normal. The skin of the extremities and ventral abdominal and thoracic areas was cyanotic.

MICROSCOPIC LESIONS

Septicemic Calves. Microscopic examination of the tracheas from the infected calves revealed small hemorrhages in the peritracheal adventitia and in the submucosa in the angle formed by the ends of the cartilagenous rings.

Pulmonary histologic differences were associated with the different exposure methods. Aerosol exposure resulted in multiple focal suppurative pneumonic areas (Fig. 6), edema, and hyperemia. The edema of the interlobular septa observed grossly was found to involve also the subpleural connective tissue (Fig. 7), and to a lesser degree, the peribronchial and perivascular connective tissue. Leukocytes, primarily neutrophils, and fibrin were found in these edematous areas. Bacteria were numerous and were often in lymph vessels. The pneumonic areas were characterized by leuko-

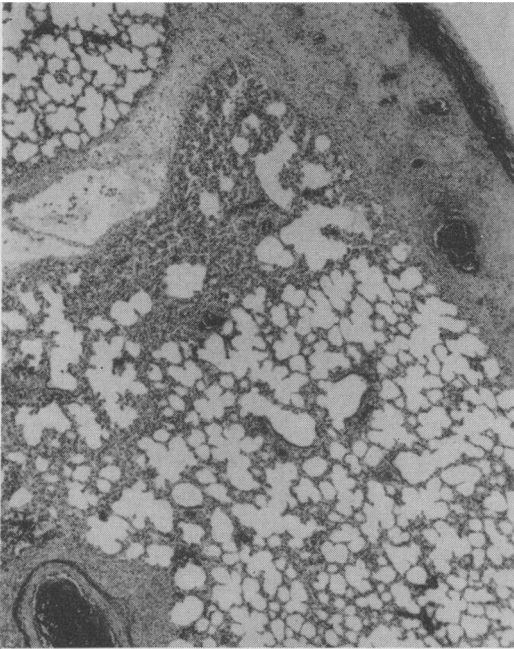


Fig. 7. Interlobular and subpleural pulmonary edema in septicemic calf exposed by aerosol. x40.

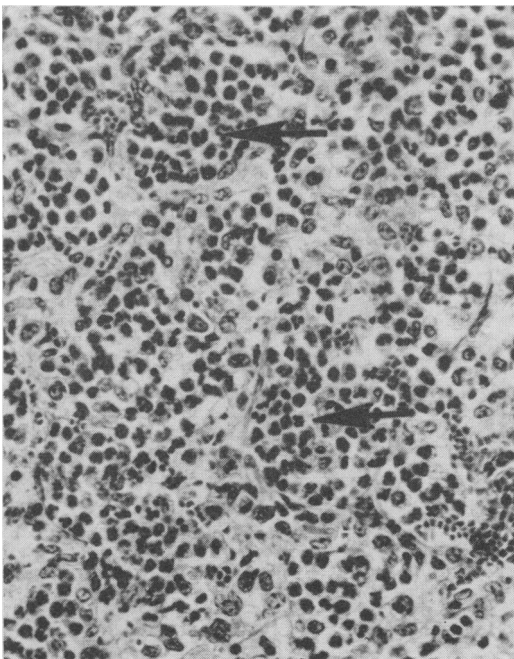


Fig. 8. Neutrophils in alveoli (arrows) of pneumonic area of septicemic calf exposed by aerosol. x325.

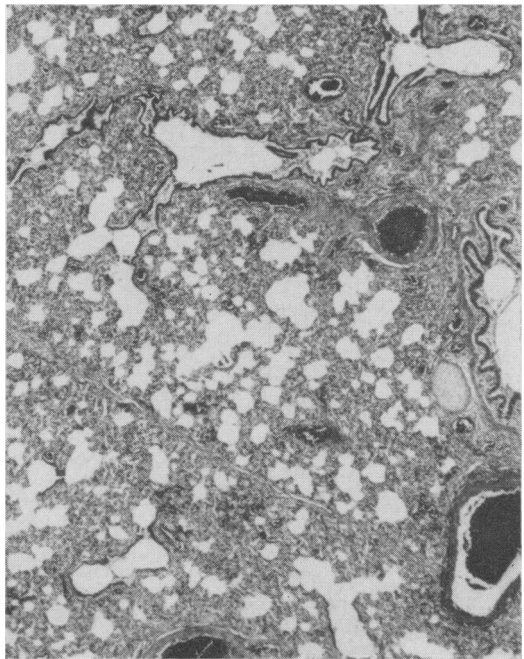


Fig. 9. Interstitial pneumonia involving apical lobe of lung of septicemic calf. x40.

cytic infiltration, hyperemia, and edema. Most of the leukocytes observed were neutrophils, but numerous macrophages and other cell types were also seen. Alveoli in affected areas contained large numbers of neutrophils (Fig. 8). The pneumonic areas varied in size, often affecting only a small portion of a lobule, but sometimes involving the entire lobule.

Intranasal and intramuscular exposure resulted in generalized interstitial pneumonia. Hyperemia, edema, and cellular response were most extensive in the anteroventral portion of the lung (Figs. 9, 10), with less involvement of the diaphragmatic lobes (Fig. 11). An increased number of lymphocytes and macrophages were found in the thickened alveolar septa (Figs. 10, 11), along with a few neutrophils. The lesions were no more pronounced in peribronchial areas than in other areas, and the interlobular septa were unaffected except for a slight hyperemia.

Lymph nodes were hyperemic and the medullary areas contained an increased number of macrophages. A moderate number of plasma cells and neutrophils were also observed. Large numbers of neutrophils were infrequently observed in some areas of the marginal and intermediate sinuses of thoracic lymph nodes.

Examination of spleens from all calves

revealed subserosal hemorrhages, hyperemia, and a slight increase in the number of red pulp macrophages. Bacteria were infrequently observed in microcolonies, apparently in lymph vessels.

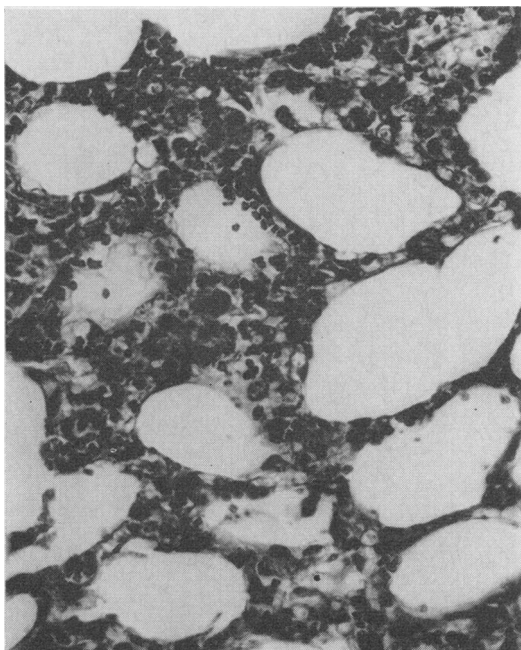


Fig. 11. Interstitial pneumonia involving diaphragmatic lobe of lung of septicemic calf. x325.

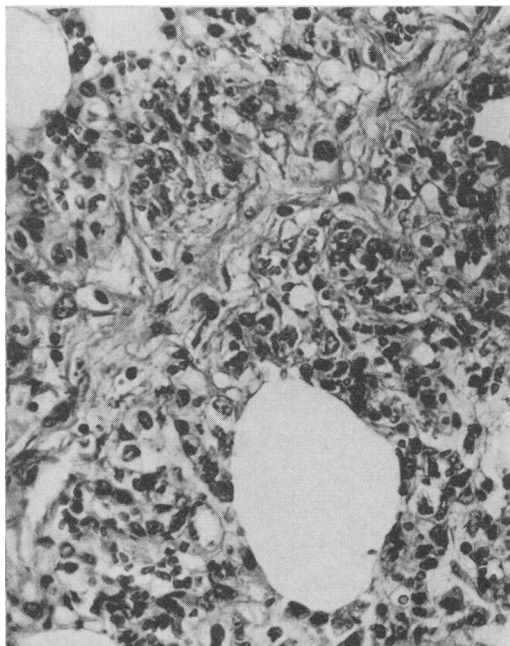


Fig. 10. More highly magnified portion of tissue represented in Fig. 9. x325.

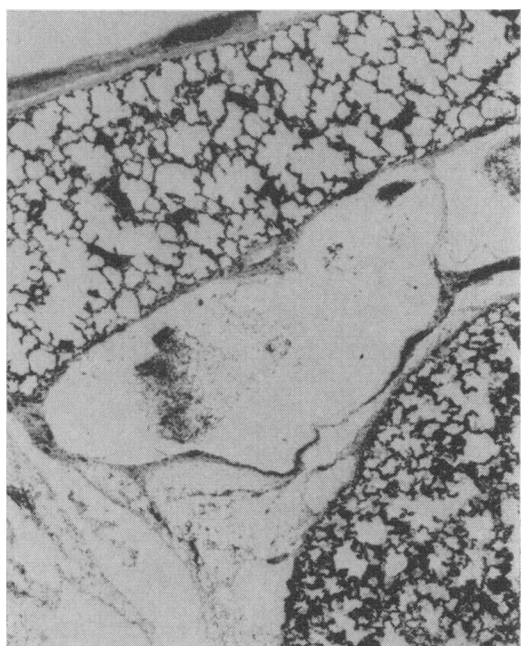


Fig. 12. Hyperemia, hemorrhage, and edema of lung of endotoxemic calf. x40.

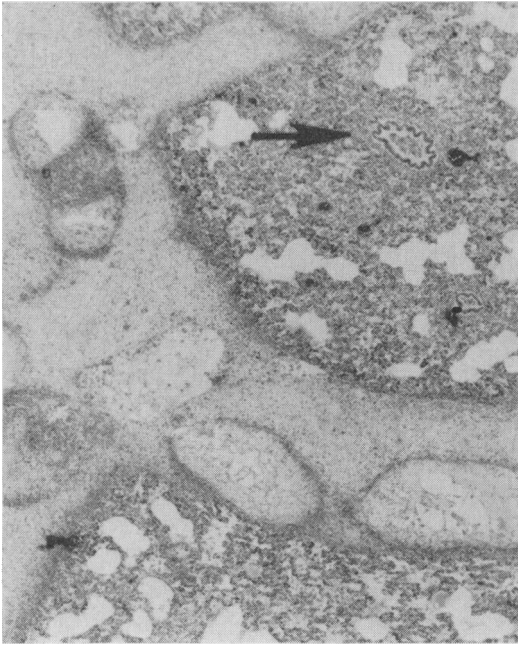


Fig. 13. Interlobular edema and pneumonia in septicemic pig. x40.

Microscopic examination of the gastrointestinal tracts revealed hyperemia and subserosal hemorrhages. The liver was hyperemic and, in some areas, cloudy swelling and fatty degeneration were observed in the parenchymal cells. These degenerative changes were not extensive, and were predominantly perilobular in distribution.

Examination of the kidneys revealed cloudy swelling and pyknosis of tubular epithelial cells in some areas and diffuse hyperemia. No urinary bladder lesions were observed.

Microscopic examination of brain tissue was limited to one calf, exposed intranasally. Hyperemia was observed in the brain and meninges, along with a slight amount of hemorrhage in perivascular spaces of the brain. These hemorrhagic areas were not confined to any particular portion of the brain and did not involve the meninges.

Examination of heart sections revealed marked hyperemia along with subepicardial and subendocardial hemorrhages. The adrenals were slightly hyperemic, and in some areas small cortical hemorrhages were found. The thymuses were hyperemic, had cortical and subcapsular hemorrhages, and varying degrees of interlobular edema. Lymphatics of the cortical area of the thymus were evident because of their distention. The mammary gland of the female

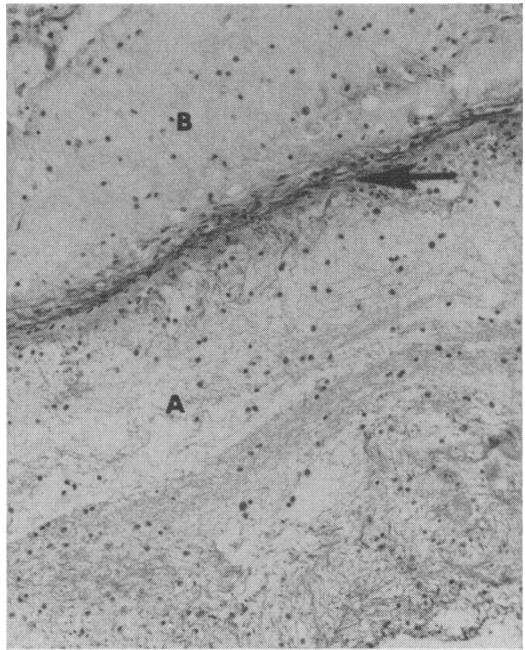


Fig. 14. Fibrinous pleuritis (A) and subpleural edema (B) in septicemic pig. Arrow indicates location of pleura. x125.

calf infected was hyperemic and markedly hemorrhagic.

Endotoxemic Calf. Examination of the lungs from the calf which died following intravenous administration of *Pasteurella* endotoxin revealed marked hyperemia (Fig. 12) and numerous areas of hemorrhage. Edema was extensive in the interlobular tissue (Fig. 12), and in the lung parenchyma. As a result of hemorrhage, erythrocytes were numerous in the interlobular lymphatics and surrounding edematous areas. Areas of a moderate degree of alveolar emphysema were also observed.

There was a slight hyperemia of the digestive tract, liver, spleen, and pancreas and moderate hyperemia of the kidneys. Other alterations observed were a slight degree of cloudy swelling in the liver, interlobular edema in the pancreas, increased amounts of blood pigments in macrophages of the spleen, and vacuolar degeneration in a large number of tubules in the kidney. This degenerative change in the kidney primarily involved the distal convoluted tubules.

Examination of the lymph nodes revealed hyperemia and edema. A moderate number of erythrocytes were observed in the medullary and intermediate sinuses, suggesting that they were transported from hem-

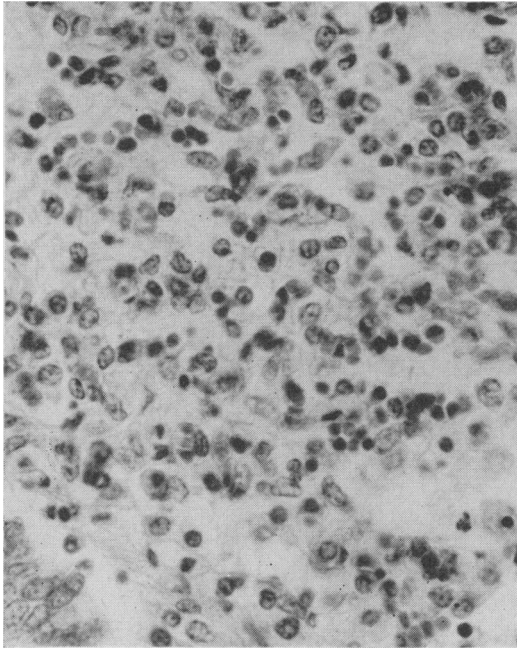


Fig. 15. Pneumonia with macrophage accumulation in septicemic pig; arrow in Fig. 13 locates low magnification view of same area. x525.

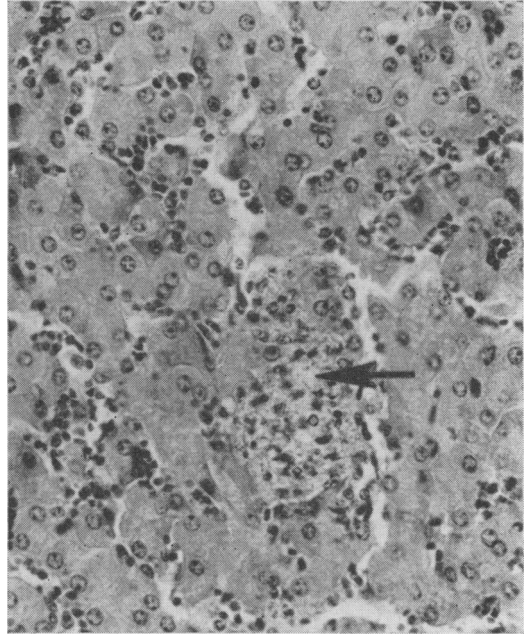


Fig. 16. Focal necrotic area (arrow) in liver of septicemic pig. x375.

orrhages in other tissues. Focal areas of extravascular erythrocytes were observed in primary follicles, indicating hemorrhage.

Septicemic Pigs. A marked degree of hyperemia and edema, a fibrinous pleuritis, and a very extensive pneumonia were observed microscopically in the pig lung tissues. The hyperemia was general in distribution. The edema involved the interstitial tissue and alveoli, perivascular and peribronchial areas, and the interlobular and superpleural spaces (Fig. 13). Fibrin was observed in the edematous areas. The perivascular, interlobular, and subpleural edematous areas contained large numbers of neutrophils and macrophages, along with a few lymphocytes and plasma cells. Large numbers of inflammatory cells lined the endothelium of the interlobular and subpleural lymphatics (Fig. 13) and in some cases occluded these vessels. A large amount of fibrin was attached to the pleura (Fig. 14). Neutrophils and macrophages were the predominant cells in this exudate.

The lung parenchyma, in addition to the hyperemia and edema, had extensive accumulations of macrophages (Fig. 15). Neutrophils and other cell types were observed but were much less numerous than macrophages. Fibrin was distributed in the ede-

matous areas. These pneumonic lesions were diffuse in distribution. Exudate, containing both neutrophils and macrophages, was present in the bronchioles. Examination of the tracheas revealed only a slight hyperemia.

Examination of the cardiac and pericardial tissue revealed an extensive fibrinous pericarditis and epicarditis similar to the pleuritis previously described.

An acute lymphadenitis, indicated by marked hyperemia, extensive accumulations of macrophages in the sinuses, and edema, was observed microscopically. Neutrophils and eosinophils were also observed but were relatively few in number compared to the macrophages.

Examination of the liver sections revealed widespread cloudy swelling and multiple small focal areas of necrosis (Fig. 16) in the parenchyma. The necrotic areas were primarily midzonal and periportal in distribution and contained macrophages and neutrophils.

The kidneys were markedly hyperemic and some of the tubules had undergone vacuolar degeneration. These affected tubules, although not extremely numerous, were widely distributed. The distal convoluted tubules were primarily affected. No urinary bladder lesions were observed.

Gastrointestinal lesions observed microscopically were limited to a slight degree of passive hyperemia. Examination of the serosal surfaces of the abdominal viscera revealed a fibrinous peritonitis similar to the pericarditis and pleuritis described.

Hyperemia and a moderate macrophage increase were observed in the spleen sections. The only adrenal change was a slight hyperemia in the medullary portion. The skeletal muscle sections and brain sections examined were normal except for a slight degree of hyperemia.

Discussion

The lung lesions in the infected calves in this study varied with the exposure method. A focal suppurative pneumonia along with interlobular edema resulted from aerosol exposure, while an interstitial pneumonia resulted from intranasal and intramuscular exposure. Apparently aerosol exposure caused a primary respiratory infection and a concomitant septicemia, while the interstitial pneumonias observed were only part of a general reaction to septicemia. The pneumonic lesions in the calves were not severe, especially when compared to the lesions observed in the pigs.

The pneumonic lesions in the calves varied from the fibrinous pneumonias often seen in *Pasteurella* infections in cattle, (6, 11) probably because of the acute septicemic nature of the infection in this study.

All infected animals examined in this study developed a septicemia as determined by microbiological examination (5). When bacteria were noted in the descriptions of microscopic lesions they were found in large numbers, usually as microcolonies in lymph vessels. No effort was made to determine the distribution of bacteria in the tissues, since the septicemic nature of the infection had already been determined.

The widespread lesions of a vascular disturbance, hemorrhage, edema, and general hyperemia observed in the calf that died following endotoxin administration are compatible with changes associated with shock. In this case, the most extensive lesions were in the lungs.

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