Pathologic and hematologic changes observed in swine inoculated with *Salmonella choleraesuis* var. *kunzendorf* and its endotoxin

by

Keith Conover Sherman

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major: Veterinary Pathology

Signatures have been redacted for privacy

Iowa State University
Ames, Iowa

1972

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INTRODUCTION

Salmonellosis, an enteric and/or septicemic disease of animals, is caused by many different species of the genus *Salmonella*. Closely related groups of animals appear to harbor specific species of salmonella. Many of the salmonellae are not species adapted and will produce disease in many different animals. Numerous species of salmonellae infect swine without producing apparent disease. The most common salmonella pathogen in swine in the United States is *Salmonella choleraesuis* var. *kunzendorf*.

Porcine salmonellosis and hog cholera have been closely associated through the years due to their similar clinical signs and lesions. The disease now known to be hog cholera was thought to be caused by *Salmonella choleraesuis* due to the absence of information and diagnostic capabilities for the isolation and identification of the virus. The virus of hog cholera was eventually identified and hog cholera and salmonellosis were separated into two distinct disease entities.

For many years hog cholera caused the most fatalities in swine in the United States. The incidence of hog cholera in this country is rapidly decreasing due to a cooperative state-federal eradication program and salmonellosis has become economically the most important disease problem of swine (Edwards and Galton, 1967). In view of the current relative

importance of salmonellosis, increased emphasis should be given to the development of better means for its diagnosis, prevention and treatment.

Many Gram-negative bacteria, including salmonellae, contain a potent endotoxin which is liberated when the cell wall disintegrates. Considerable research has been done on the effects of endotoxin in man and other animals. Most of the research has been done, however, with Gram-negative organisms other than salmonellae and in animals other than swine.

The gross and microscopic lesions of porcine salmonellosis have been well described for both the enteric and septicemic forms of the disease. The progressive hematologic changes which occur during the course of the disease and the role of endotoxin have not been well defined.

This experiment was designed to study the morphologic and hematologic changes in swine infected with virulent *S. choleraesuis* var. *kunzendorf* in the acute septicemic form and to compare these changes with those produced by the inoculation of extracted endotoxin from this species of *Salmonella*.

To accomplish this goal, 3 pigs each were inoculated intravenously with washed live cultures, washed killed cultures, and endotoxin of *S. choleraesuis* var. *kunzendorf* respectively. Hematologic and morphologic changes were studied and compared in each experimental group.

REVIEW OF LITERATURE

In 1886, Salmon and Smith isolated a bacterium from swine affected with hog cholera. They incorrectly postulated that this organism was the cause of hog cholera and proposed the name *Bacillus cholerae suis*. Fourteen years later, Lignieres suggested that this genus of bacteria be named *Salmonella* in honor of D. E. Salmon, the first Chief of the United States Bureau of Animal Industry (Merchant and Packer, 1967).

Following Salmon and Smith's first isolation numerous new species of salmonellae were isolated from many different members of the animal kingdom. It became apparent that host susceptibility and morphologic and biochemical characteristics were not sufficient criteria for species differentiation. Schuetze (1921) attempted to distinguish various groups of salmonellae through the use of absorbed serums. White (1925) formulated a system of classification based partially on the organism's antigenic composition. Kauffmann (1941) expanded and refined White's scheme and the present system of classification is based on morphologic, biochemical and serologic characteristics of each individual species.

Most species of salmonellae will infect a wide variety of animals, however, a few are host specific. All known species of salmonellae can be pathogenic for man or other animals but most animals will often harbor one or more species without exhibiting clinical disease (Breed *et al.*, 1957).

Dorset *et al*. (1905) reported that swine may have primary, secondary, or latent salmonellae infections. Murray *et al*. (1927) reproduced porcine salmonellosis by oral inoculation of swine with cultures of *Salmonella suipestifur* (*cholerae suis*). These experiments were continued by Biester *et al*. (1927) who described the sequential development of gross and microscopic lesions.

Salmonella choleraesuis var. *kunzendorf* has been the most common cause of clinical porcine salmonellosis in the United States (Edwards and Galton, 1967; Jubb and Kennedy, 1970). Numerous other salmonella serotypes have also been associated with disease in swine (Edwards *et al*., 1948; Moran, 1962; Edwards and Ewing, 1970; Harrington *et al*., 1971). Harrington *et al*. (1971) isolated salmonellae from 831 pigs of 2,774 suspected of being infected with hog cholera. Five hundred and nineteen or 62.5 percent of these 831 isolates were *Salmonella choleraesuis* var. *kunzendorf*. The remaining isolates were a conglomerate of 42 distinct serotypes.

Porcine salmonellosis can be divided into 2 clinical syndromes, an acute septicemic form and a chronic enteric form (Jubb and Kennedy, 1970). An animal that survives the acute syndrome usually develops the chronic enteric form. Acute salmonellosis usually affects young pigs, 2-4 months of age, and is only rarely seen before weaning (Jubb and Kennedy, 1970). Clinically the piglets are febrile, weak, dull and rapidly become moribund. Cyanosis of the snout,

ears, tail and distal portions of the extremities is often evident. Death usually occurs from 1-7 days after clinical signs appear. Mortality is high in pigs with clinical signs but herd morbidity is low (Sorensen, 1970). At necropsy there are widespread subserosal and submucosal hemorrhages that mimic those of hog cholera with regard to size and distribution. Renal cortical hemorrhages, serohemorrhagic lymphadenitis and splenomegaly are common (Sorensen, 1970; Jubb and Kennedy, 1970).

Microscopically the lesions are characterized by capillary thrombosis and focal necrosis. In the lungs there is subpleural hemorrhage, pulmonary edema, and congestion. The liver is usually congested and contains subcapsular petechial hemorrhages. Numerous small foci of necrosis, referred to as typhoid nodules, are scattered throughout the hepatic parenchyma (Robbins, 1962; Boyd, 1970; and Jubb and Kennedy, 1970). These small lesions vary in their development from simple coagulative necrosis to diffuse reactive histiocytic granulomas with Kupffer cell hyperplasia. Typically these lesions are not inflammatory as there is only minimal focal leukocytosis. Central necrosis occurs as the lesions progress. Very few fibroblasts are present surrounding the necrotic foci. A mild meningitis, characterized by perivascular cuffing with lymphocytes, plasma cells and a few macrophages, is sometimes present but an encephalitis is rare (Jubb and Kennedy, 1970). Infrequently there are small myocardial infarcts (Seghetti,

1946; Dade, 1966; and Sorensen, 1970).

The main clinical features of chronic enteric salmonellosis are diarrhea, dehydration and emaciation. Salmonellae produce the primary insult to the intestinal mucosa and subsequently many organisms along with salmonellae gain entry into the mesenteric lymphatic system and general circulation resulting in systemic reactions (Murray *et al*., 1927; Biester *et al*., 1927; Lawson and Dow, 1964; and Jubb and Kennedy, 1970). A deficiency of niacin can alter the intestinal mucosa so that it is more susceptible to invasion by salmonellae (Edwards and Galton, 1967). In the chronic form of the disease the host is usually able to eliminate the organism from the general circulation but the salmonellae often persist in the visceral lymph nodes and gall bladder. The main lesion seen at necropsy is a severe enteritis with necrosis and hemorrhages (Jubb and Kennedy, 1970; and Sorensen, 1970). Sorensen (1970) states that the necrotic intestinal mucosal lesion is often secondarily invaded by *Fusiformis necrophorus* or *Balantidium coli*. The systemic lesions when present are essentially the same as those described for the acute septicemic form although they may be less severe.

Very little has been done to establish hematologic changes produced by salmonella infection in swine. Sorensen *et al*. (1961) studied leukocyte counts in swine infected with virulent hog cholera virus (HCV) and modified HCV using *S. choleraesuis* as a stressing agent. He also studied a group

of pigs infected with _S_. _choleraesuis_ alone. In these animals he reported a decrease in thrombocytes and a leukocytosis. Seghetti (1946), studying a natural outbreak of salmonellosis caused by _S_. _choleraesuis_ var. _kunzendorf_, reported a moderate leukocytosis with an increase of neutrophils and a decrease in lymphocytes. Sorensen (1970) stated that hemograms of pigs with salmonellosis usually revealed a slight anemia and a marked leukocytosis with a regenerative shift to the left.

Studies of changes in the levels of serum enzyme activity have not been reported in porcine salmonellosis.

Most Gram-negative bacteria contain a complex protein-lipopolysaccharide endotoxin in their cell walls which is very toxic to animals (Nowotny, 1969). Endotoxin is released when the bacterium dies and the cell wall is lysed (Jawetz _et al_., 1970). The presence of a toxic substance in Gram-negative bacteria was suspected for many years prior to the discovery of endotoxin. In certain human diseases such as typhoid fever not all of the body responses could be attributed to the direct effect of the bacteria. Seibert (1923, 1925) reported a pyrogenic substance in Gram-negative bacteria and Boivin _et al_. (1933) extracted and identified the substance as endotoxin. Antigenically endotoxins appear to be distinct and are the antigens responsible for the O-antigenic specificities of Enterobacteriaciae of which the genus _Salmonella_ is a member (Work, 1971).

According to Braude (1964) and Work (1971) all endotoxins produce the same general signs in experimentally inoculated animals regardless of the Gram-negative bacterium from which they were derived. The known physiopathologic effects of endotoxin are more or less characteristic for this group of substances. Typically they are very pyrogenic (Work, 1971). After injection of an endotoxin there is a short latent period followed by a diphasic temperature rise. The interval between peaks in temperature is about one hour. A marked decrease in circulating neutrophilic leukocytes occurs shortly after the initial rise in temperature (Jawetz et al., 1970). Braude (1964) attributes the second rise in temperature to an endogenous pyrogen released from leukocytes damaged by the endotoxin. Braude (1964) also suggested that the temperature rise could be a direct effect of the endotoxin on the thermal control centers in the hypothalamus.

After repeated daily doses of endotoxin, a tolerance develops and there is a progressive diminution of febrile and other systemic responses (Beeson, 1947). Tolerance is thought to be due to the production of anti-endotoxin antibodies which enable the reticuloendothelial system to rapidly remove the endotoxin from the blood stream (Braude, 1964). If a blockage of the reticuloendothelial system is produced with particles of carbon, thorium dioxide or saccharated iron, tolerance can be abolished. The administration of adrenal cortical steroids also seem to diminish the effects of the

injected endotoxin (Braude, 1964; Jawetz *et al.*, 1970).

Zweifach (1964) studying the vascular effects of large doses of endotoxin produced a general circulatory collapse which resulted in a shock syndrome. A few minutes after inoculation with endotoxin the arterial blood pressure fell rapidly. The blood became sluggish and failed to adequately perfuse the brain, lungs and kidneys. This syndrome mimicked shock resulting from massive blood loss. Braude (1964) states that the collapse of the circulatory system is not due to a depletion of blood volume. He believes the collapse is the result of a slowing down of blood flow to the heart which in turn can not pump the normal volume of blood into the arterial circulation. Epinephrine greatly accentuates the toxic effects of endotoxin. Stetson (1955) and Thomas (1956) found epinephrine caused the blood vessels to overreact if they had been sensitized to endotoxin. The resultant impaired circulation caused extensive tissue damage. Brunson *et al.* (1955) and Zweifach (1964) stated that bacterial endotoxins mainly damage the microcirculation of the body.

Endotoxins can cause both local and generalized Schwartzman reactions. This phenomenon was first observed by Sanarelli (1924) but Schwartzman (1928) studied and described it. The local Schwartzman reaction is produced when an animal is injected intradermally with endotoxin and 24 hours later given an intravenous injection of the same substance. Severe inflammation and necrosis develop at the site of the first in-

jection. The generalized Schwartzman reaction is produced by two successive intravenous injections of endotoxin. After the second injection bilateral renal cortical necrosis develops as a result of the occlusion of glomerular capillaries by masses of fibrinoid material (Thomas and Good, 1952; Brunson et al., 1955). Lawson and Dow (1964) have produced this reaction in rabbits with S. choleraesuis endotoxin and Nordstoga (1970) has produced it in swine.

Nonspecific resistance can occur from the administration of small doses of endotoxin since it increases serum levels of properdin and stimulates phagocytic activity of leukocytes (Landy and Pillemer, 1956). Animals subjected to excessive amounts of ionizing radiation usually die of overwhelming Gram-negative bacterial infections. Increased resistance to the latter is produced by prior administration of small doses of endotoxin (Jawetz et al., 1970).

MATERIALS AND METHODS

Source of Animals

Fifteen, first generation, specific pathogen free (SPF), naturally farrowed Chesterwhite pigs were obtained from the herd at the National Animal Disease Laboratory (NADL), Ames, Iowa. There were 7 males and 8 females in the group. All pigs were approximately 3 months of age, weighed 30 to 40 pounds and had received no immunizations.

The pigs were raised on concrete and housed in isolation units away from other swine. Three pigs at a time were brought from the holding pens into the building where the experiment was conducted. The rooms in this building were all concrete and the temperature and relative humidity were kept at 70 F. and 45 percent respectively.

The pigs were fed a special pelleted feed diet,[1] which was nutritionally balanced and complete. The pigs were fed this diet from time of weaning until completion of the experiment. The feed contained no antibiotics or growth stimulants.

Rectal swabs were collected from each pig every third day until 3 specimens were obtained. The swabs were cultured immediately in selenite broth,[2] enriched with cystine,[3] as

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[1]NADL Pig Grower No. 539, Al Nuzum Supply Company, Baxter, Iowa.

[2]Difco Laboratories, Detroit, Michigan.

[3]Calbiochem Corporation, Los Angeles, California.

described by North and Bartram (1953). After incubation for 18 to 24 hours at 37 C. a loopful of this medium was streaked on brilliant green agar (BGS agar)[1] with Na sulfadiazene[1] added as an inhibitor for pseudomonas and proteus. The agar plates were incubated for 18 to 24 hours at 37 C. If salmonellae were present, there would be typical colony growth surrounded by a pink zone. If no growth was present on the plates at 24 hours they were incubated for another 24 hours to avoid missing slow growing organisms. All attempts to isolate salmonellae from the experimental pigs were negative.

Surgical Preparation of Experimental Animals

An indwelling arterial catheter was placed in the femoral artery of all pigs according to the method of Dougherty *et al.* (1965). The pigs were anesthetized with a halothane anesthetic,[2] and a polyvinyl catheter[3] (inside diameter 0.058" X outside diameter 0.08") was surgically inserted into the femoral artery in the midfemoral region. The catheter was inserted deep into the artery and passed anterior into the aorta to a point just caudal to the origin of the renal arteries. The catheter was anchored to muscle and fascia and the distal end passed subcutaneously to the lumbosacral region on the

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[1]Difco Laboratories, Detroit, Michigan.

[2]"Fluothane" - Ayerst Laboratories, Chicago, Illinois.

[3]Becton, Dickinson and Company, Rutherford, New Jersey.

back. A metal stopcock[1] (Figures 1 and 2) was attached to the exterior end of the catheter and firmly sutured to the skin.

A quaternary ammonia sanitizing agent,[2] diluted 1:1250 was applied daily to the suture wounds to prevent infection.

Each pig was placed in a separate pen to prevent mutilation of the surgical site.

Source of Inoculum

The strain of S. choleraesuis var. kunzendorf used was originally isolated from a natural outbreak and has been maintained as a type culture (70-13679) by the USDA, Animal and Plant Health Service, Diagnostic Services, NADL, Ames, Iowa. This isolate of S. choleraesuis var. kunzendorf belongs to the C_1 group of salmonellae, with somatic (O) antigens numbers 6, 7 and flagellar (H) antigens, phase 1, (C) and phase 2, 1, 5. This classification is in accordance with the Kauffmann-White schema as listed by Edwards and Ewing (1970).

Preparation of Inoculum

Experiment I

A 4 mm. loopful of the selected culture was inoculated into each of 6 milk dilution bottles which contained 30 ml.

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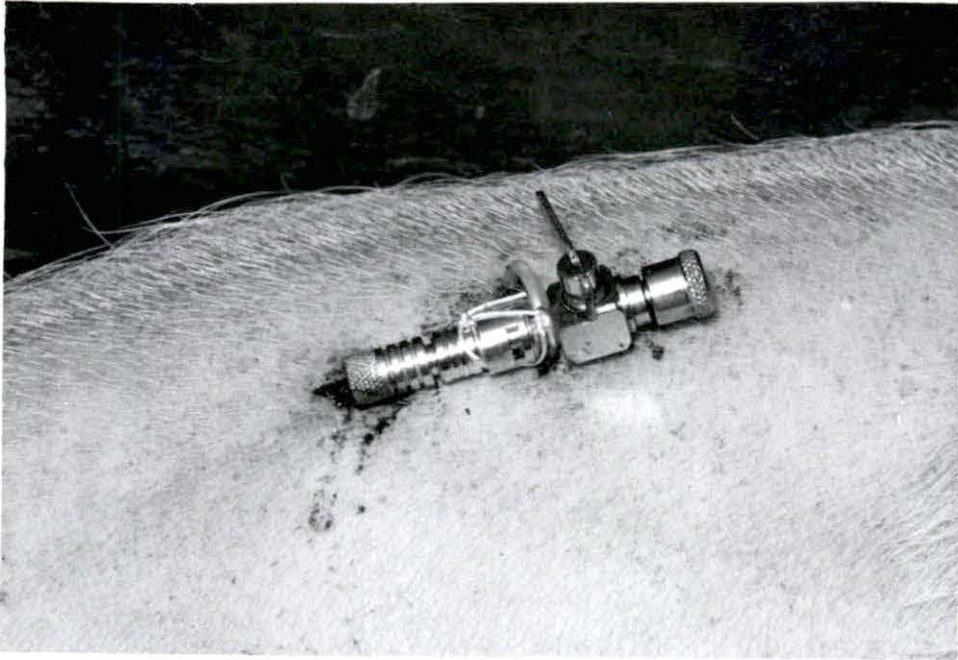
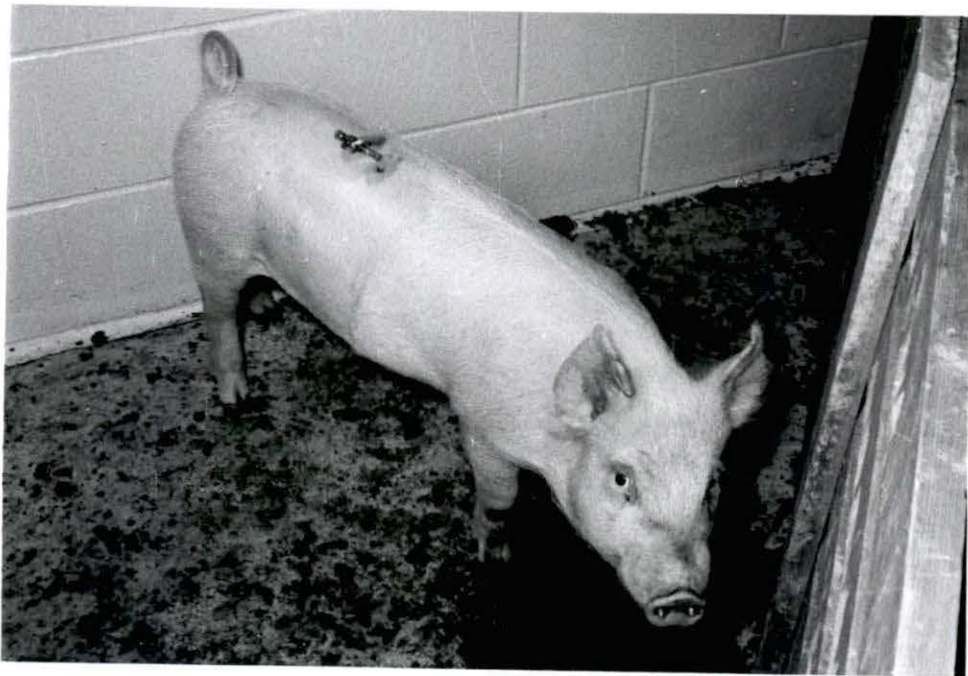
[1]Becton, Dickinson and Company, Rutherford, New Jersey.

[2]Roccal, Winthrope Laboratories, New York, New York.

Figure 1. Metal stopcock sutured to skin in the lumbar area

Figure 2. Pig with catheter in place. The catheter is protected from damage and filth in this location. There is minimal discomfort and no impairment of locomotion





of a liquid medium. The liquid medium was a mixture of 50 percent trypticase soy broth[1] and 50 percent tryptose broth[2] (TST media). The bottles were incubated at 37 C. for 18 hours. The medium was centrifuged for 30 minutes at 34,800 X G. The supernatant fluid was decanted and the pellet resuspended in sterile saline. This washing procedure was repeated 3 times. All saline solutions used in this experiment and subsequent experiments contained 0.85 Gm. of NaCl per 100 ml. of distilled water. The final pellet after centrifugation was resuspended in 20 ml. of sterile saline. This suspension was diluted to a McFarland nephelometer reading of 1.0 (McFarland, 1907).

Experiment II

Six milk dilution bottles containing 30 ml. of TST medium each were inoculated and incubated as in Experiment I. The contents of each bottle was emptied into a sterile metal centrifuge cup. The metal cups were placed in a boiling water bath for one hour. The cups were cooled and centrifuged at 34,800 X G for 30 minutes. The bacteria were washed and standardized as in Experiment I. A suspension equal to a McFarland nephelometer reading of 10 was prepared. A 4 mm. loopful

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[1]Baltimore Biologics, Division of Bioquest Laboratories, Baltimore, Md.

[2]Difco Laboratories, Detroit, Michigan.

of the bacterial suspension was streaked on BGS agar and incubated at 37 C. overnight. There was no bacterial growth in 24 hours incubation.

Experiment III

A milk dilution bottle containing 30 ml. of TST medium was inoculated as in Experiments I and II. The bottle was incubated at 37 C. for 6 hours. One ml. of the 6 hour culture was inoculated onto each of 56 large metal media pans. The large media pans were stainless steel cake pans 8 inches in diameter covered by 9 inch stainless steel cake pans.

The medium was prepared according to the following formula:

	Per liter
Trypticase soy broth	30 Gm.
Tryptose broth	20 Gm.
Yeast extract[1]	5 Gm.
Agar[1]	20 Gm.

The inoculum was smeared over the surface of the medium with a sterile L-shaped glass rod until the surface was evenly covered. The culture pans were incubated for 24 hours at 37 C.

The bacteria were harvested by adding 7 ml. of sterile saline to the surface of the media. The L-shaped glass rod was used to loosen the salmonellae from the medium. The

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[1]Difco Laboratories, Detroit, Michigan.

organism-saline suspension was pipetted into a sterile 100 ml. container. Another 7 or 8 ml. of sterile saline was added to the medium and the remaining salmonellae were recovered on the second washing.

The 56 large media pans yielded over 600 ml. of the bacteria-saline suspension. The volume was reduced to approximately 180 ml. by centrifugation and the organisms were washed 3 times in saline solution.

After the third washing the method of Roschka (1950) as outlined in Edwards and Ewing (1970) for the production of "O" or somatic antigen was used to kill and dry sufficient salmonellae for endotoxin extraction.

The endotoxin was extracted by the method of Westphal and Jann (1965) which utilized the hot water and phenol method for crude endotoxin extraction and ultracentrifugation for concentration and purification. The gelatinous endotoxin at the end of ultracentrifugation was resuspended in 25 ml. of sterile saline solution. This mixture was the final inoculum for Experiment III.

One ml. of the endotoxin mixture was drawn into a 10 ml. syringe. A syringe adapter (Swinny adapter)[1] with a millipore filter (Type AAWP - pore size 0.8 μ)[1] positioned inside was attached to the 10 ml. syringe. The millipore filter was prepared by drying in an oven at 110 C., then weighed.

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[1]Millipore Filter Corporation, Bedford, Massachusetts.

Pressure on the plunger forced the mixture through the filter. The filtrate was discarded. After filtration the filter was again dried in a 110 C. oven and weighed. The filter contained 300 mg. of solids. According to Westphal and Jann (1965) this residue is 97 percent lipopolysaccharide (endotoxin) and 3 percent bacterial nucleic acid.

Inoculation Procedures

Immediately following the collection of the pre-inoculation blood specimen through the catheter, the pigs were placed in a dorsal recumbent position in a wooden V-shaped trough. The inoculum was injected into the anterior vena cavae according to the following schedule.

Experiment I

Three pigs were each inoculated with 3 ml. of a suspension of live washed salmonellae at a concentration of 3 times a McFarland nephelometer 1.0 reading.

Experiment II

Three pigs were each inoculated with 3 ml. of a suspension of killed, washed salmonellae at a concentration of 10 times a McFarland nephelometer 1.0 reading.

Experiment III

Three pigs were each inoculated with 3 ml. of the final endotoxin extract from the salmonellae.

Controls

Three pigs were each inoculated with 3 ml. of sterile saline solution.

Hematologic Procedures

Blood samples were collected from each pig once a day for at least 4 days prior to inoculation and then immediately before inoculation to establish normal individual hematologic values.

Blood was collected at 15, 30, 45 and 60 minutes, and 2, 4, and 6 hours postinoculation (PI) for the first day. On subsequent days samples were collected at 9:00 a.m. and 3:00 p.m.

Approximately 10 ml. of blood was collected each time. Five ml. of blood was drawn into a "Vacutainer" (4727-3200 QS)[1] vial containing liquid anticoagulant, ethylenediamine-tetra-acetic acid trisodium salt (EDTA).[1] Another 5 ml. was drawn into a "Vacutainer" (4710-3200)[1] vial which contained no additives.

A drop of blood from the syringe was placed on a glass slide and a direct smear was made with another slide by the method of Schalm (1965). Two blood smears were made from each pig at each bleeding time for differential leukocyte counts.

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[1]Becton, Dickinson and Company, Rutherford, New Jersey.

The catheter system was emptied of heparinized saline by withdrawing a 3 ml. sample of blood which was discarded prior to collecting the desired sample. Following the specimen collection (Figure 3), the catheter system was refilled with sterile saline solution containing 1,000 International Units of heparin[1] per ml. and left until the next bleeding time.

The tests conducted on the unclotted blood samples were: erythrocyte sedimentation rates (ESR); packed cell volumes (PCV); total red blood cell counts (TRBC); and total white blood cell counts (TWBC).

The ESR was done using the modified Westergren method (Gambino _et al._, 1965). All ESR tests were started within 1 hour after blood was collected.

The PCV determinations were made by the micro-hematocrit tube method.

The erythrocytes were electronically counted by a Coulter, Model B, research counter.[2] Machine calibration and procedures used for enumerating swine erythrocytes was the method presented by Wisecup and Crouch (1963).

No TRBC counts were conducted on the 15, 30 and 45 minute PI bleedings. Total erythrocyte counts were done at 1, 2, 4 and 6 hours PI and on both bleedings on subsequent days.

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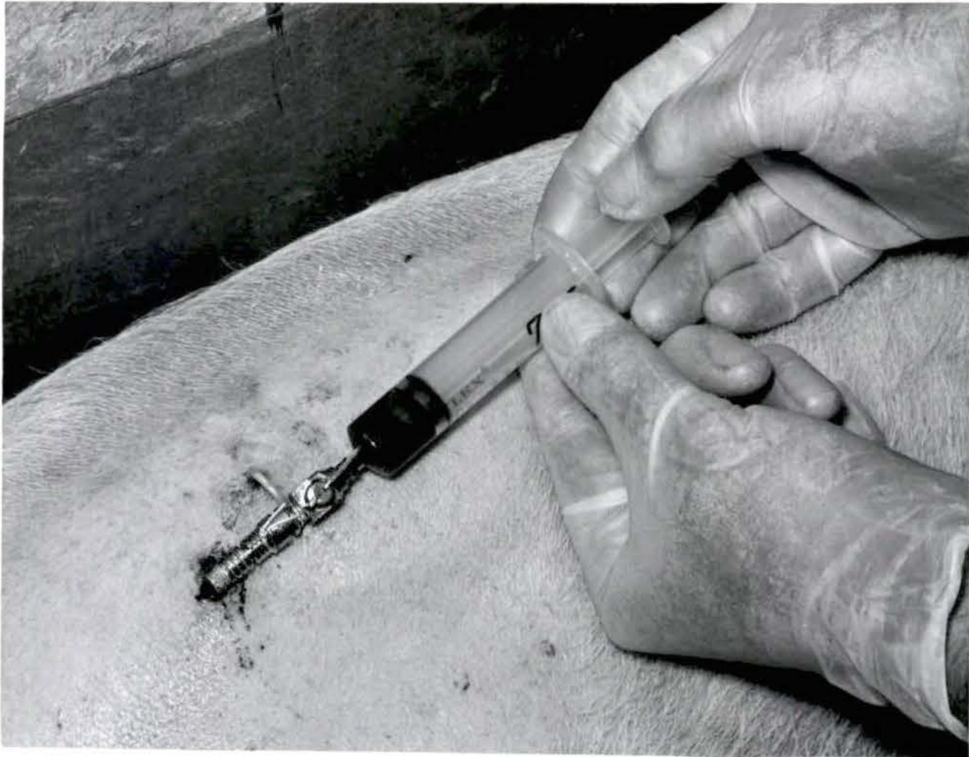
[1]Calbiochem Corporation, Los Angeles, California.

[2]Coulter Electronics, Inc., Hialeah, Florida.

Figure 3. Collection of a blood sample. Safety cap is removed and a syringe attached. Valve is turned to open the position and the blood drawn into the syringe. The catheter system is filled with heparinized saline except while blood is being collected



The TWBC counts were made by the same machine using different dilutions and calibrations, again following the methods described by Wisecup and Crouch (1963).

The blood with no additives was allowed to clot and then centrifuged at approximately 1,000 X G for 20 minutes to obtain the serum for the serum enzyme assays.

Serum glutamic oxaloacetic transaminase (SGOT) determinations were conducted using the colorimetric method of Reitman and Frankel (1957). Kits, commercially available from the Sigma Chemical Company, were used and the procedures followed were those outlined in Sigma Technical Bulletin No. 505.[1] Results were reported in Sigma-Frankel units which are defined in the same technical bulletin.

Serum lactic dehydrogenase (LDH) levels were determined by the colorimetric method of Cabaud _et al_. (1958). Reagent kits were used and procedures followed as described in Sigma Technical Bulletin No. 500.[1] Results of LDH activity were reported in Berger-Broida (B-B) units which are defined in Sigma Technical Bulletin No. 500.

The blood smears were stained with Wright's Blood Stain[2] and buffered by Wright's method for staining white blood cells. Differential leukocyte counts were performed by the cross section method of MacGregor, Richards and Loh (1940).

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[1] Sigma Chemical Company, St. Louis, Missouri.

[2] Wright's Blood Stain, Item No. 740, Hartmann-Leddon Company, Philadelphia, Pennsylvania.

Two hundred leukocytes were counted on all blood smears. The leukocytes were differentiated by their morphologic and characteristic staining qualities as described by Schalm (1965).

Necropsy Procedures

All pigs were killed 96 hours PI. Five ml. of a 10 percent solution of succinylcholine chloride[1] was injected into the arterial catheter after the last blood collection was made from each animal. Necropsies were performed according to the procedure of Jones and Gleiser (1954), with all body systems being examined. Gross lesions encountered during the necropsy were carefully recorded.

Representative tissue specimens were collected and placed in 10 percent buffered formalin solution for fixation. If lesions were present a specimen was also collected. The following is a list of tissues routinely collected from each animal:

Nervous system - intact brain and portion of cervical spinal cord.

Cardiovascular system - wall of right atrium and wall of right ventricle.

Respiratory system - diaphragmatic lobes of both lungs.

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[1]Sigma Chemical Company, St. Louis, Missouri.

Digestive system - liver, stomach, jejunum, ileum, caecum, colon and rectum.

Hemopoietic system - tonsil, spleen and lymph nodes. The lymph nodes were: mandibular, cervical, mediastinal, bronchial, gastric, mesenteric, colonic and internal iliac.

Urinary system - kidney.

Duplicate or representative tissue specimens of all the tissues listed for formalin fixation, with the exception of brain and spinal cord tissue, were collected in the fresh state. Each fresh tissue specimen was packaged in a small plastic envelope and identified as to tissue, location, and animal. These tissues were frozen in a -70 C. chest type freezer.

Histologic Procedures

Tissue specimens collected at necropsy were fixed in 10 percent formalin for 72 to 96 hours at room temperature. The fixed tissues were trimmed, dehydrated in graded concentrations of ethanol, cleared in clearing agent[1] and infiltrated with paraffin,[2] in the routine manner in an Autotechnicon.[1] Following infiltration with paraffin, the tissues

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[1]The Technicon Company, Chauncey, New York.

[2]Paraplast - Aloe Scientific, St. Louis, Missouri.

were then embedded in paraffin and cut into 6 micron thick sections which were mounted on glass slides for staining. All sections were stained with Harris' hematoxylin and eosin Y (H & E).[1] Special stains were also employed on selected tissues. MacCallum-Goodpasture's Gram stain,[1] and May-Grunwald Giemsa's stain[1] were used for bacteria. Periodic acid - Schiff (PAS) stain[1] was used to demonstrate fibrin accumulations. The techniques for all histologic and staining procedures follow those found in a manual published by the Armed Forces Institute of Pathology (1968).

Cultural and Fluorescent Antibody Procedures

Studies on HCV infected pigs were being conducted concurrently in the same isolation building, therefore, accidental contamination was a possibility. Tissues from every animal used in the project were examined for the presence of HC viral antigen.

Frozen tonsil, spleen and mandibular lymph node were used for the fluorescent antibody tests for hog cholera. Two tests were used, the fluorescent antibody tissue section technique (FATST), Bedell *et al*. (1969), and the fluorescent antibody tissue culture technique (FATCT), Turner *et al*. (1969).

Frozen liver, kidney, spleen and mandibular lymph node were used for fluorescent antibody (FA) detection of salmo-

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[1]Allied Chemicals, National Biological Stains and Reagents Department, Morristown, New Jersey.

nellae by the method of Ellis and Harrington (1968). The FA test was conducted on tissues from all animals used in the project.

Portions of frozen liver, kidney, spleen and mandibular lymph nodes from all pigs were also cultured for salmonellae. This was accomplished by mincing bits of tissue into tetrathionate broth[1] and incubating overnight at 37 C. A loopful of broth was streaked on brilliant green agar (BGS agar) with sulfadiazine and incubated overnight at 37 C. If present, salmonella colonies were selected and transferred into a tube of triple sugar iron agar (TSI agar)[1] and cultured overnight at 37 C. Hydrogen sulfide (H_2S) production, as evidenced by blackening of the TSI agar was indicative of _S. choleraesuis_ var. _kunzendorf_.

The FA conjugates for both the HC and salmonella diagnostic tests were produced by the Animal Health Division, Diagnostic Services, NADL, Ames, Iowa.

All FA test results were read on Leitz Orthoplan microscopes[2] equipped with a xenon ultraviolet light source and using a BG 38 heat absorbing filter, a BG 12 blue exciting filter and a K530 barrier filter.

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[1]Difco Laboratories, Detroit, Michigan.

[2]E. Leitz, Inc., New York, New York.

RESULTS

Experiment I

Clinical observations

Following inoculation of the pigs with live salmonellae there was no apparent reaction for 4 hours PI. All pigs ate within 1 hour PI. About 4 hours PI, the pigs lay down and did not resist when blood was collected. They would get up and move around when stimulated.

Twenty-four hours PI, the pigs were vomiting, depressed and reluctant to move. They had stopped eating. The feces were firm, mucus covered and in pellet form (Figure 4). The pigs were still able to get up and walk short distances when prodded.

From 24 to 48 hours PI, the pigs were very reluctant to move, but continued to respond when stimulated. They were still not eating. The feces were firm and mucus covered. Vomiting ceased during this period. Slight bleeding of the catheter surgery wounds was observed between 48 and 72 hours PI (Figure 5).

At 72 hours PI, the pigs appeared more alert but were still recumbent. They opened their eyes and watched while the caretakers cleaned their pens and put in fresh feed. They were not eating or drinking. A few small pellets of feces were scattered about the pen indicating the pigs had moved around during the night. They still offered no

Figure 4. Experiment I, 24 hours PI. The pig is severely depressed. Firm, dry feces and untouched feed are present in the pen

Figure 5. Experiment I, 48 hours PI. The pig is moribund and serosanguinous fluid from the surgical incision stains the back and sides

resistance when their temperatures were taken and the blood collected.

At 96 hours PI, the clinical appearance of all 3 pigs had improved. They were still recumbent and anorexic, but appeared more alert and showed some interest in feed. Body temperatures of all 3 pigs are recorded in Table 1. The temperatures became elevated rapidly. At 6 hours PI a 2 to 4 F. increase was disclosed. The temperatures remained elevated until 96 hours PI, at which time the pigs were killed and the experiment concluded.

Hematology

Erythrocyte sedimentation rate The ESR results are listed in Table 2. After 2 hours PI, the ESR began to increase rapidly. Pig 525 had an extremely fast ESR. The ESRs were markedly elevated in all 3 pigs at 24 hours PI and remained high until the pigs were killed at 96 hours PI.

Packed cell volume The PCV results are listed in Table 3. The greatest PCV values occurred within the first 2 hours PI. After 2 hours PI, a definite decreasing trend occurred which continued through 72 hours PI. Slight rises in the PCVs were noted at 96 hours PI.

Total red blood cells The TRBC counts are listed in Table 4. The highest TRBC counts were recorded prior to inoculation. A very gradual decrease in numbers of erythrocytes occurred throughout the course of the experiment. No sudden

Table 1. Body temperatures[a] of swine inoculated with virulent *S. choleraesuis* var. *kunzendorf*. Experiment I

Hours PI	Pig 524	Pig 525	Pig 550
-96	103.2	102.6	103.0
-72	101.8	103.0	102.6
-48	101.6	102.7	102.1
-24	102.0	104.4	103.4
0	102.2	104.8	104.4
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	...	...	...
2	...	...	...
4	...	...	...
6	106.6	106.4	106.0
24	106.8	105.0	106.4
30	107.8	107.0	106.6
48	107.2	107.4	106.6
54	107.4	107.2	107.2
72	106.4	106.6	107.0
78	107.0	106.6	106.8
96	106.6	106.8	106.2

[a]Temperatures recorded in degrees Fahrenheit.

[b]No temperatures taken.

Table 2. Erythrocyte sedimentation rates[a] of blood from swine inoculated with virulent S. choleraesuis var. kunzendorf. Experiment I

Hours PI	Pig 524	Pig 525	Pig 550
-96	7.0	22.0	21.0
-72	6.0	27.5	17.0
-48	6.5	41.0	28.0
-24	10.0	94.5	15.0
0	4.0	92.0	26.0
15 min.	4.5	120.0	21.5
30 min.	2.5	76.0	18.0
45 min.	4.0	66.0	15.0
1	3.0	47.0	11.0
2	2.5	96.0	15.0
4	15.0	144.0	39.5
6	20.0	131.0	55.5
24	42.0	128.0	89.0
30	49.5	147.0	102.5
48	45.0	146.0	92.0
54	48.0	151.0	98.0
72	51.0	101.0	59.0
78	55.0	99.5	54.0
96	46.0	112.0	60.0

[a]Packed cell volume reported as percentage.

Table 3. Packed cell volume[a] of blood of swine inoculated with virulent S. *choleraesuis* var. *kunzendorf*. Experiment I

Hours PI	Pig 524	Pig 525	Pig 550
-96	31.0	27.0	28.0
-72	32.5	27.5	28.0
-48	33.0	26.5	29.5
-24	34.5	28.5	30.5
0	32.0	28.0	30.5
15 min.	35.5	27.5	32.5
30 min.	34.0	26.5	34.0
45 min.	33.0	27.0	31.0
1	32.5	26.0	31.0
2	30.0	29.5	31.0
4	30.0	24.0	28.5
6	31.5	26.5	27.5
24	28.0	27.0	27.0
30	27.5	25.5	25.5
48	28.5	25.0	24.0
54	27.0	23.5	22.0
72	27.0	30.5	26.5
78	27.0	23.5	24.5
96	29.0	25.0	25.5

[a]Packed cell volume reported as percentage.

Table 4. Total red blood cell counts[a] from blood of swine inoculated with virulent S. choleraesuis var. kunzendorf. Experiment I

Hours PI	Pig 524	Pig 525	Pig 550
-96	515	446	469
-72	548	460	475
-48	620	487	548
-24	645	512	559
0	570	476	552
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	581	438	541
2	564	488	546
4	496	379	470
6	563	480	474
24	484	457	470
30	466	454	429
48	507	419	424
54	507	399	380
72	478	527	460
78	464	384	416
96	492	422	430

[a]Red blood cells reported as number of cells X 10^4/cu. mm.

[b]Counts not conducted on these bleedings.

or drastic changes were detected to have occurred at any time during the experiment.

Total white blood cells The TWBC counts are listed in Table 5. The counts slowly elevated from the time of inoculation to about 6 hours PI at which time they started to decline. The gradual decline continued from 24 to 48 hours PI. At 72 hours PI, a leukopenia (less than 10,000 WBCs) was found in 2 of the pigs (524 and 550). The TWBC counts continued to decrease in these pigs until 78 hours PI. A slight rise in TWBC counts occurred at 96 hours PI. Pig 525 maintained a high TWBC count throughout the experiment.

Serum glutamic oxaloacetic transaminase The levels of SGOT activity are listed in Table 6. Elevated levels were noted 24 to 48 hours PI. The values doubled every 24 hours PI until the pigs were killed at 96 hours PI. The highest levels of SGOT activity were present at 96 hours PI.

Lactic dehydrogenase The levels of LDH activity are listed in Table 7. The results of this serum enzyme determination were very erratic. During the course of the experiment the levels doubled with the greatest increases occurring 48 to 96 hours PI.

Differential leukocyte counts The results of the differential leukocyte counts on pig 524 are listed in Table 8. Lymphocytes were the predominant cells in the postsurgical and pre-inoculation blood smears. Segmented neutrophils decreased in total numbers during recovery from surgery.

Table 5. Total white blood cell counts[a] from blood of swine inoculated with virulent S. choleraesuis var. kunzendorf. Experiment I

Hours PI	Pig 524	Pig 525	Pig 550
-96	14,580	16,800	13,100
-72	14,580	20,800	12,600
-48	14,700	19,100	11,300
-24	15,000	23,100	12,600
0	16,300	22,700	15,200
15 min.	15,600	20,600	16,400
30 min.	12,700	19,700	16,700
45 min.	17,600	25,000	18,800
1	20,900	24,100	20,000
2	20,100	29,100	28,900
4	18,600	19,900	24,000
6	23,500	21,100	24,300
24	17,000	19,100	19,700
30	13,900	16,500	15,600
48	12,800	17,000	13,000
54	12,200	18,100	9,940
72	9,157	24,200	8,067
78	8,978	19,000	7,443
96	16,400	17,800	9,984

[a]White blood cell counts reported in number of cells/cmm.

Table 6. Serum glutamic oxaloacetic transaminase[a] levels of activity in serum of swine inoculated with virulent S. choleraesuis var. kunzendorf. Experiment I

Hours PI	Pig 524	Pig 525	Pig 550
-96	36	27	25
-72	21	21	23
-48	30	25	42
-24	25	20	25
0	27	20	44
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	32	22	38
2	32	25	34
4	30	22	32
6	36	25	32
24	38	42	48
30	34	24	48
48	100	25	120
54	104	25	174
72	275	62	275
78	395	57	315
96	600	70	680

[a]Glutamic oxaloacetic transaminase reported in Sigma-Frankel units.

[b]No determinations made.

Table 7. Lactic dehydrogenase[a] levels of activity in serum of swine inoculated with _S. choleraesuis_ var. _kunzendorf_. Experiment I

Hours PI	Pig 524	Pig 525	Pig 550
-96	1140	480	895
-72	1060	835	1060
-48	965	910	1075
-24	1040	785	975
0	925	720	1280
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	1005	835	1055
2	880	735	990
4	910	685	925
6	805	545	820
24	990	1315	975
30	700	735	965
48	785	625	1140
54	1200	770	1250
72	1150	965	1710
78	1435	925	2000
96	1910	1325	1825

[a]Lactic dehydrogenase reported in Berger-Broida (B-B) units.

[b]No determinations made.

Table 8. Differential leukocyte counts[a] on blood smears prepared from swine inoculated with virulent *S. choleraesuis* var. *kunzendorf*. Experiment I

			Pig 524					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	122	8	61	5	0	0	3	1
-72	130	8	54	3	1	0	4	0
-48	131	2	57	4	1	0	3	2
-24	158	4	31	2	0	0	4	1
0	137	4	47	5	0	0	7	0
15 min.	143	2	45	5	0	0	5	0
30 min.	149	1	45	0	0	0	2	3
45 min.	131	4	49	8	1	0	3	4
1	103	2	76	15	1	0	2	1
2	62	3	98	34	2	0	0	1
4	39	1	119	40	1	0	0	0
6	31	3	122	40	4	0	0	0
24	51	4	122	21	2	0	0	0
30	55	3	102	38	1	0	1	0
48	59	3	87	46	5	0	0	0
54	50	2	93	49	4	0	0	2
72	61	7	47	68	13	4	0	0
78	87	2	42	58	9	2	0	0
96	64	5	54	67	7	0	3	0

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

Forty-five minutes PI, the number of lymphocytes began to decrease and neutrophils increased. By 6 hours PI the number of neutrophils outnumbered the lymphocytes. A marked degenerative shift to the left, or an increase in the numbers of immature leukocytes (Schilling's index), was demonstrated. The TWBC counts increased only slightly during the first 6 hours PI. From 24 to 78 hours PI a degenerative shift to the left was slowly progressive. At 96 hours PI, a regenerative shift to the left was obvious. Immature neutrophils were the predominant cell types, but an increase in lymphocytes and segmented neutrophils was apparent.

The differential leukocyte counts for pig 525 are given in Table 9. The counts were similar to pig 524 but the shift to the left was not as drastic as in pig 524. The differential leukocyte counts on pig 550 are listed in Table 10. The results from this pig were similar to those of the other 2 pigs (524 and 525). Pig 550 had a marked shift to the left which from 48 to 72 hours PI was a degenerative shift to the left but by 96 hours PI it was regenerative. Numbers of eosinophils and monocytes decreased following inoculation in all 3 pigs.

The absolute differential leukocyte counts are listed in Tables 11, 12 and 13 on pigs 524, 525 and 550 respectively. The absolute counts did not disclose any significant differences from the relative counts.

Table 9. Differential leukocyte counts[a] on blood smears prepared from swine inoculated with virulent S. choleraesuis var. kunzendorf. Experiment I

			Pig 525					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	126	7	55	2	1	0	8	1
-72	101	4	81	3	2	0	5	4
-48	106	1	78	10	3	0	0	2
-24	78	3	101	7	8	0	1	2
0	88	4	88	7	5	0	7	1
15 min.	101	5	76	10	3	1	3	1
30 min.	63	0	124	7	2	0	3	1
45 min.	68	2	115	11	3	1	0	0
1	79	2	102	11	5	1	0	0
2	60	4	116	16	2	1	0	1
4	30	0	144	17	7	2	0	0
6	46	4	128	14	7	0	1	0
24	81	6	97	10	4	0	2	0
30	59	6	119	12	3	0	1	0
48	59	4	105	28	3	0	0	1
54	54	4	89	45	6	2	0	0
72	67	2	93	30	3	1	4	0
78	71	3	89	33	1	1	2	0
96	64	2	99	26	6	0	3	0

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

Table 10. Differential leukocyte counts[a] on blood smears prepared from swine inoculated with virulent S. choleraesuis var. kunzendorf. Experiment I

			Pig 550					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	64	10	101	14	1	0	4	6
-72	72	5	100	10	5	0	5	3
-48	92	2	91	10	3	0	1	1
-24	94	2	82	14	2	0	3	3
0	55	3	123	13	2	0	4	0
15 min.	64	1	122	7	1	0	4	1
30 min.	77	5	103	12	1	0	2	0
45 min.	58	0	127	14	0	0	0	1
1	60	2	123	12	3	0	0	0
2	41	1	135	20	2	0	1	0
4	57	2	122	14	3	1	1	0
6	21	0	164	13	2	0	0	0
24	36	2	145	13	3	0	1	0
30	36	1	145	13	1	0	4	0
48	38	0	77	69	8	2	6	0
54	49	7	76	53	12	1	2	0
72	65	6	45	61	14	7	2	0
78	75	4	16	52	38	13	2	0
96	84	2	67	34	10	2	1	0

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

Table 11. Absolute differential leukocyte counts[a] on blood smears prepared from swine inoculated with virulent _S. choleraesuis_ var. _kunzendorf_. Experiment I

			Pig 524					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	8894	583	4447	365	0	0	219	73
-72	9477	583	3937	218	73	0	292	0
-48	9629	147	4190	294	73	0	220	147
-24	11850	300	2325	150	0	0	300	75
0	11166	326	3831	407	0	0	570	0
15 min.	11154	156	3510	390	0	0	390	0
30 min.	9461	63	2858	0	0	0	127	191
45 min.	11528	352	4312	704	88	0	264	352
1	10763	209	7942	1568	104	0	209	105
2	6231	302	9849	3417	201	0	0	101
4	3627	93	11062	3720	93	0	0	0
6	3443	352	14335	4700	470	0	0	0
24	4335	340	10370	1785	170	0	0	0
30	3823	208	7089	2641	69	0	70	0
48	3776	192	5568	2944	320	0	0	0
54	3050	122	5673	2989	244	0	0	122
72	2793	320	2152	3113	595	184	0	0
78	3905	90	1885	2604	404	90	0	0
96	5248	410	4428	5494	594	0	246	0

[a]Absolute differential leukocyte counts expressed in numbers per cubic mm. of blood.

Table 12. Absolute differential leukocyte counts[a] on blood smears prepared from swine inoculated with virulent S. choleraesuis var. kunzendorf. Experiment I

			Pig 525					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	10584	588	4620	168	84	0	672	84
-72	10504	416	8424	312	208	0	520	416
-48	10123	95	7449	955	287	0	0	191
-24	9009	346	11667	808	924	0	115	231
0	9988	454	9988	794	568	0	794	114
15 min.	10403	515	7828	1030	309	103	309	103
30 min.	6205	0	12214	690	197	0	295	99
45 min.	8500	250	14375	1375	375	125	0	0
1	9520	241	12291	1325	603	120	0	0
2	8730	582	16878	2328	291	146	0	145
4	2985	0	14328	1692	696	199	0	0
6	4853	422	13504	1477	739	0	105	0
24	7736	573	9263	955	382	0	191	0
30	4868	495	9817	990	248	0	82	0
48	5015	340	8925	2380	255	0	0	85
54	4887	362	8055	4072	543	181	0	0
72	8107	242	11253	3630	363	121	484	0
78	6745	285	8455	3135	95	95	190	0
96	5696	178	8811	2314	534	0	0	0

[a]Absolute differential leukocyte counts expressed in numbers per cubic mm. of blood.

Table 13. Absolute differential leukocyte counts[a] on blood smears prepared from swine inoculated with virulent *S. choleraesuis* var. *kunzendorf*. Experiment I

			Pig 550					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	4192	655	6616	917	65	0	262	393
-72	4536	315	6300	630	315	0	315	189
-48	5198	113	5142	565	164	0	57	56
-24	5922	126	5166	882	126	0	189	189
0	4180	228	9348	988	152	0	304	0
15 min.	5248	82	10004	574	82	0	328	82
30 min.	6430	417	8601	1002	83	0	167	0
45 min.	5452	0	11938	1316	0	0	0	94
1	6000	200	12300	1200	300	0	0	0
2	5925	144	19508	2890	289	0	144	0
4	6840	240	14640	1680	360	120	120	0
6	2552	0	19926	1579	243	0	0	0
24	3546	197	14283	1280	296	0	98	0
30	2808	78	11310	1014	78	0	312	0
48	2470	0	5005	4485	520	130	390	0
54	2435	348	3777	2634	596	50	100	0
72	2622	242	1815	2460	565	282	81	0
78	2791	149	595	1935	1414	484	75	0
96	4193	100	3315	1697	499	100	50	0

[a]Absolute differential leukocyte counts expressed in numbers per cubic mm. of blood.

Necropsy findings

Gross lesions   Dehydration and loss of condition were quite apparent at the time of necropsy. There were no discolorations or other changes present in the skin. The natural body openings were normal as were the conjunctiva and mucous membranes. There were no abnormal swellings on the body and the joints were normal. Suture wounds at the site of the external catheter valve were not visibly inflamed.

Petechial hemorrhages were present in the visceral peritoneum and visceral pleura. The lymph nodes throughout the body were edematous and enlarged approximately 2 times. There was marked bilateral perirenal edema. Petechial hemorrhages were present in the renal cortex (Figure 6). Numerous renal infarcts that extended from the pelvis to the capsule were present (Figure 7). The livers were mottled and numerous small petechial hemorrhages were present (Figure 8). The gall bladders had submucosal petechial hemorrhages and the walls were consistently edematous (Figure 9). The stomach of pig 524 had a focal area of submucosal gelatinous edema along the greater curvature that measured approximately 6 cm. in diameter and 1 cm. thick.

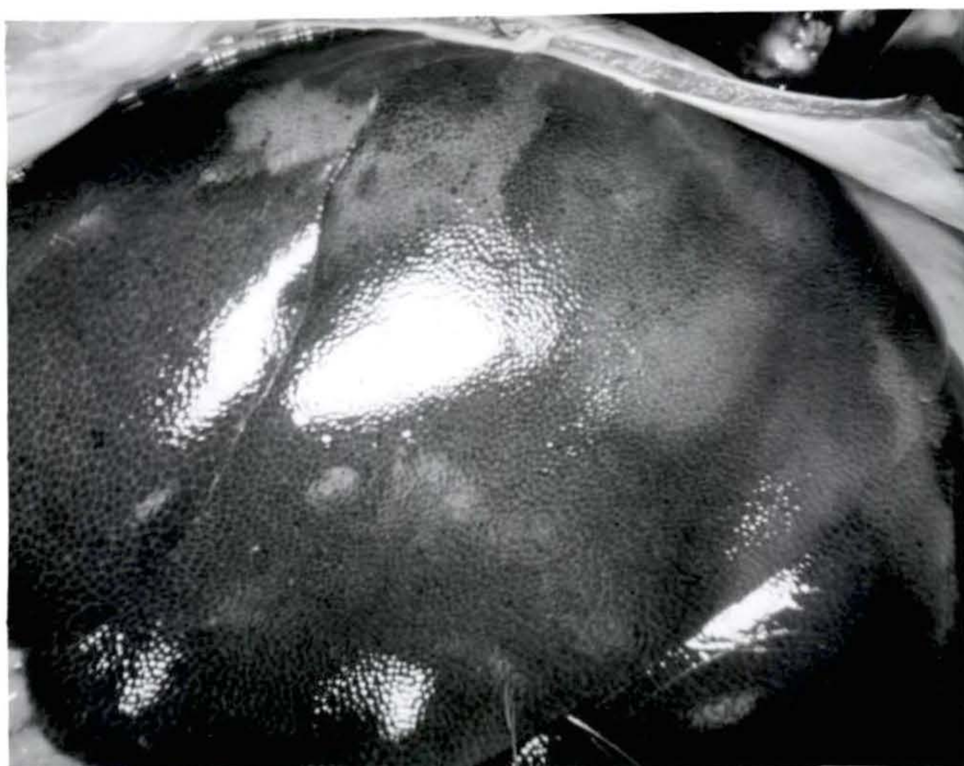
Microscopic lesions   The lesions observed on histopathologic examination were similar in all 3 pigs. The respiratory system was free of lesions except in the lungs where there were congestion and scattered interstitial accumulations of lymphocytes and plasma cells in the alveolar walls

Figure 6. Experiment I, 4 days PI. Multiple petechial hemorrhages are present in the renal cortex

Figure 7. Experiment I, 4 days PI. Renal infarcts at various stages of development are present in the cortex

Figure 8. Experiment I, 4 days PI. Multiple lobular hemorrhages and large pale foci are present on the surface of the liver

Figure 9. Experiment I, 4 days PI. Marked edema is present in the wall of the gall bladder



(Figure 10). Many small alveolar capillaries were plugged with neutrophils and lymphocytes (Figure 11). The capillary endothelial cells were swollen and reduced the diameter of the lumen nearly to the point of occlusion (Figure 12). Erythrocytes were found in the lumina of the alveoli and small bronchi with no apparent break in the capillary walls. There was lymphoid hyperplasia in areas surrounding the bronchi and larger bronchioles. The lung changes were not extensive.

Hepatic lesions were pronounced with multiple, small, focal areas of coagulative necrosis scattered throughout the liver tissue (Figure 13). These necrotic areas did not follow a definite pattern of distribution within the lobule (Figure 14). Some of these necrotic foci were near the central vein, others near the periphery of the lobule, but most were located in the midlobular region. The lesions appeared to arise between the liver cord cells in the sinusoids. There were numerous neutrophils in the sinusoids but they did not appear to be part of an inflammatory response from the lesions (Figure 15). The necrotic foci contained lymphocytes and macrophages but few neutrophils (Figure 16). Marked hyperplasia of the Kupffer cells was present throughout the liver. There was mild congestion of the liver. The cord cells were shrunken and the sinusoids were slightly dilated. The mucosa of the gall bladder was normal, but the vessels in the submucosa were congested. The submucosa and muscularis were

Figure 10. Experiment I, 4 days PI. Lung. Foci of alveolar hemorrhage surround vessel (a) and erythrocytes (arrows). H&E stain; 250 X

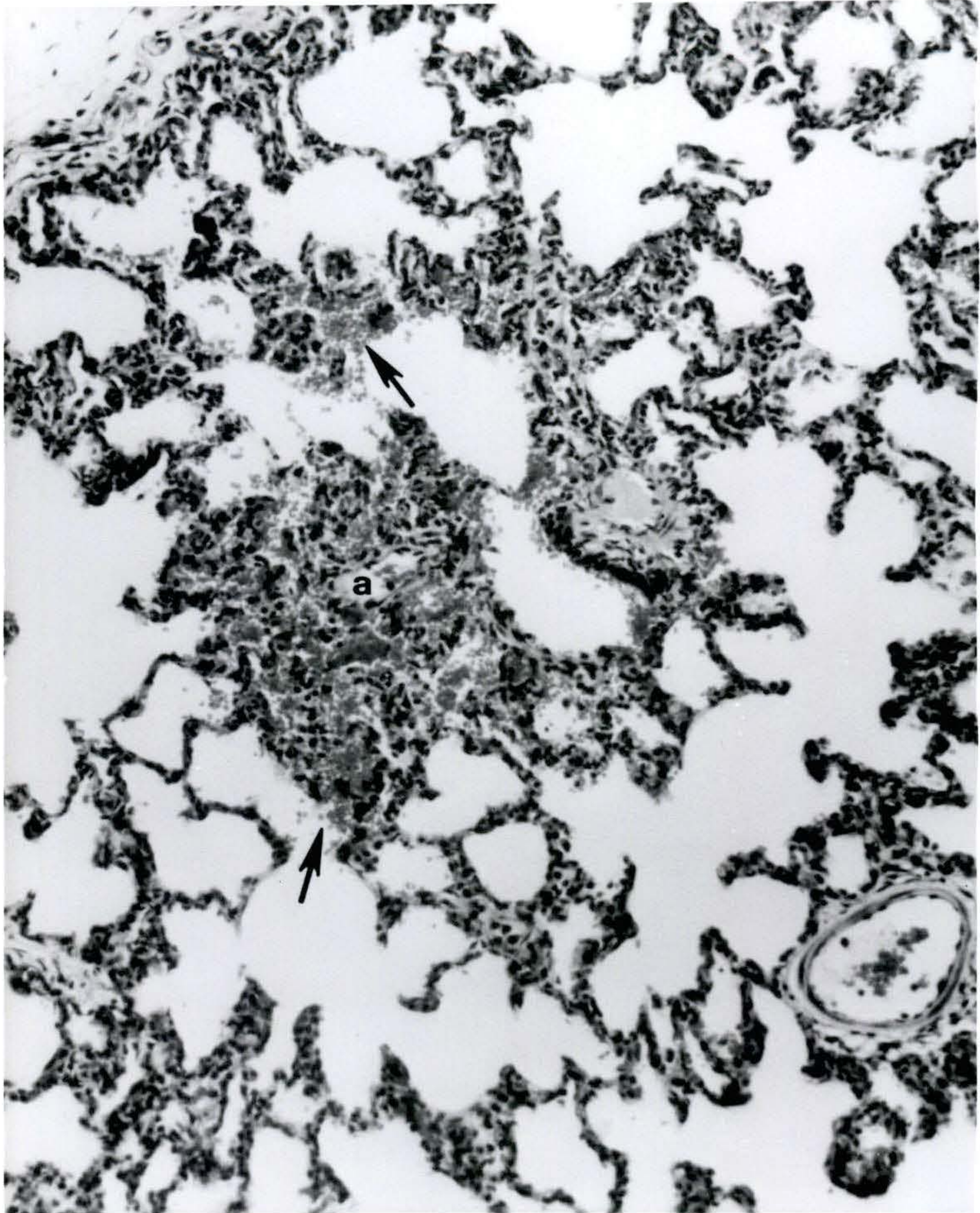
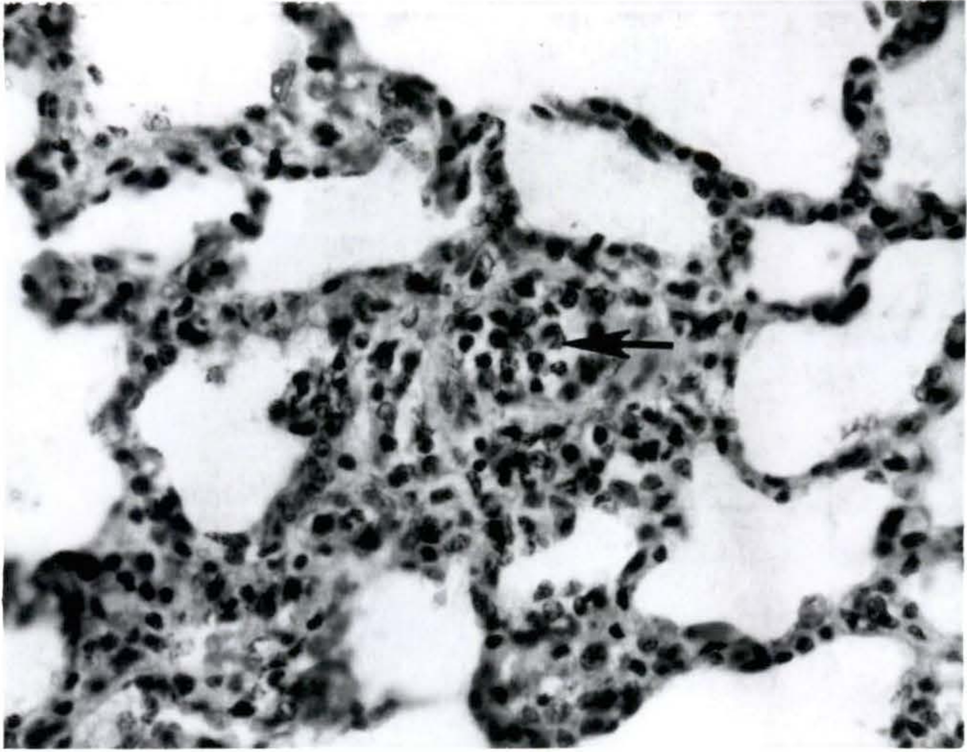

a

Figure 11. Experiment I, 4 days PI. Lung. Pulmonary capillary is occluded with neutrophils. H&E stain; 250 X

Figure 12. Experiment I, 4 days PI. Lung. The capillary is occluded by swollen endothelial cells and mononuclear leukocytes. H&E stain; 430 X



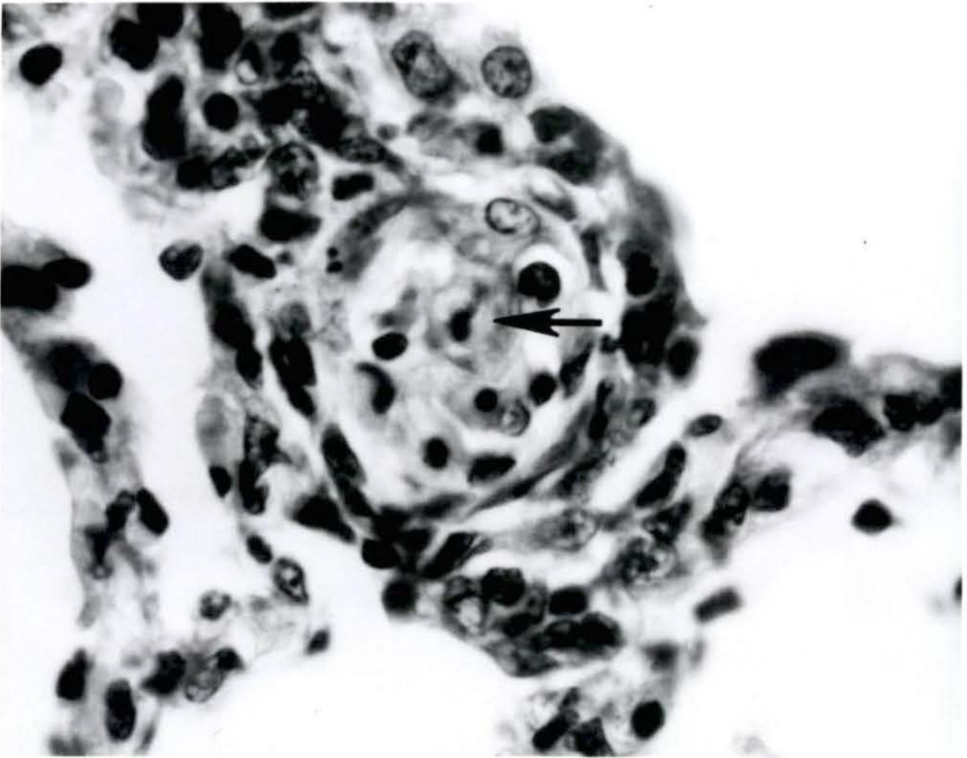
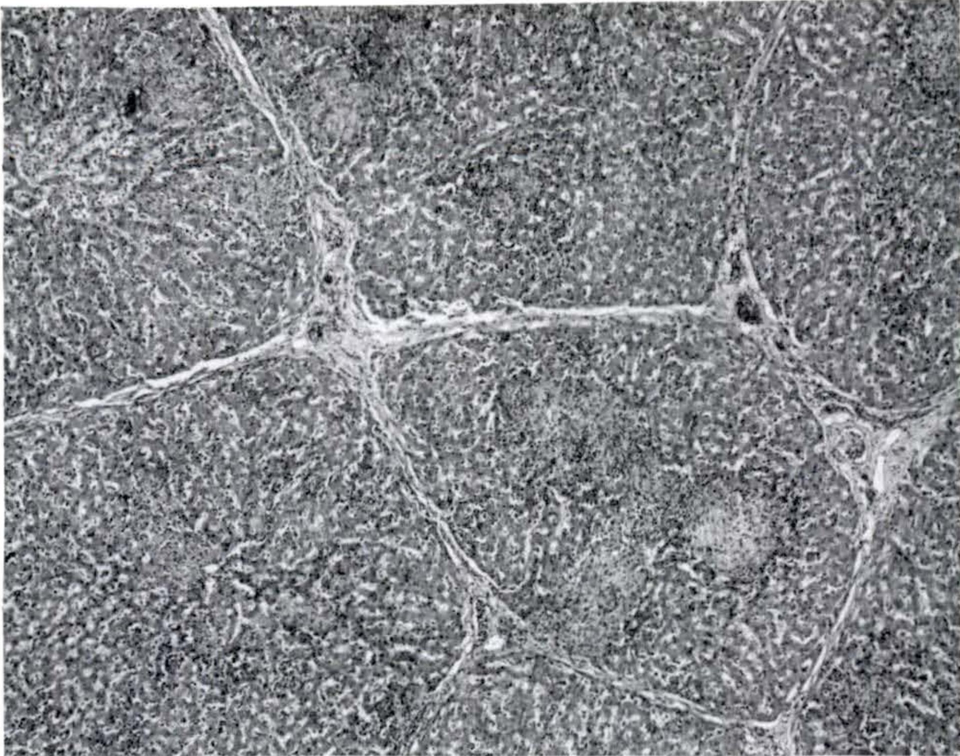


Figure 13. Experiment I, 4 days PI. Liver. Individual hepatic lobules contain multiple foci of coagulation necrosis. H&E stain; 80 X

Figure 14. Experiment I, 4 days PI. Liver. Randomly distributed foci of necrosis are present within one hepatic lobule. H&E stain; 200 X



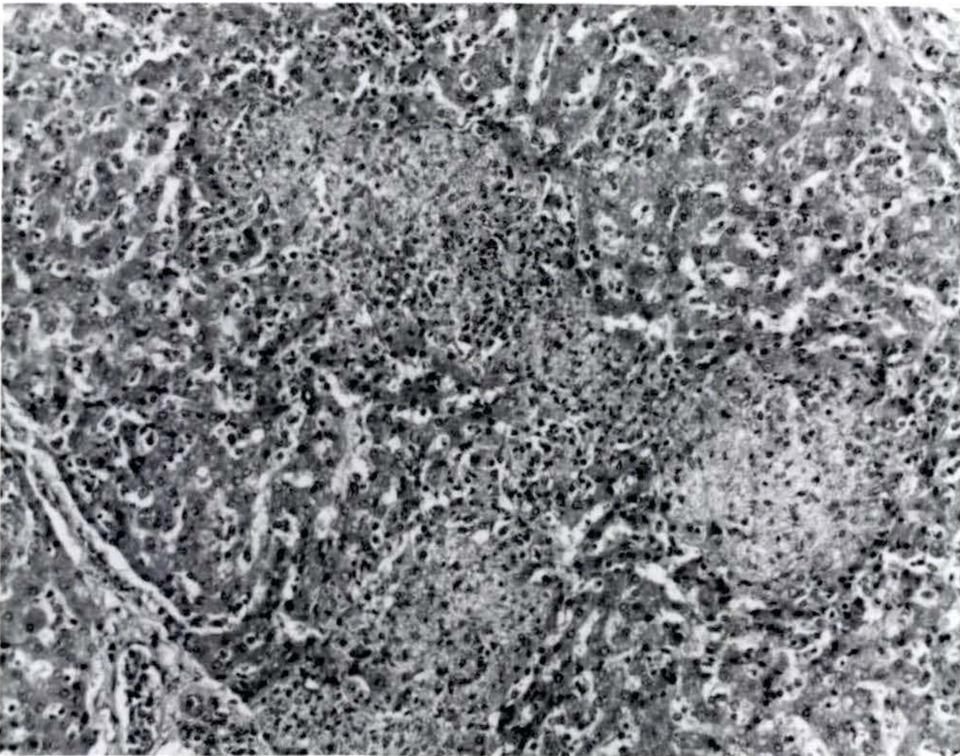
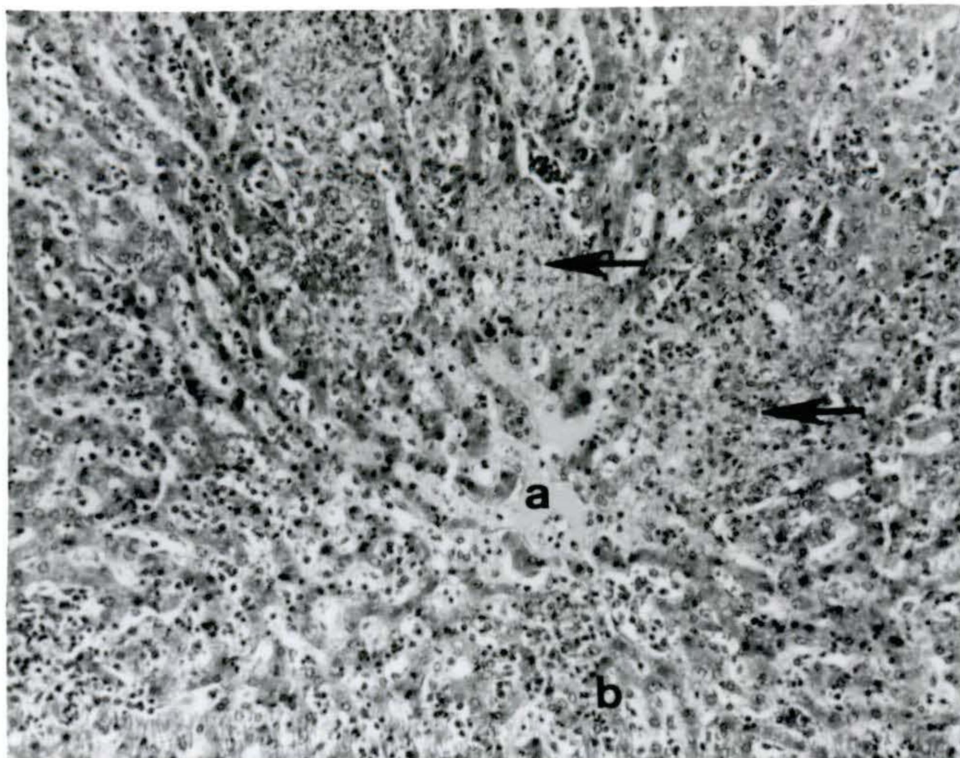


Figure 15. Experiment I, 4 days PI. Liver. Early stage in the formation of the focal areas of necrosis (arrows). A proteinaceous eosinophilic staining fluid (a) is present near the central vein and in dilated sinusoids. Foci of neutrophils trapped in sinusoids (b) appear to precede focal necrosis (arrows). H&E stain; 200 X

Figure 16. Experiment I, 4 days PI. Liver. Focus of hepatic necrosis with central hemorrhage (arrow). Neutrophils surround the necrotic focus (a) and fill the adjacent hepatic vein (b). H&E stain; 200 X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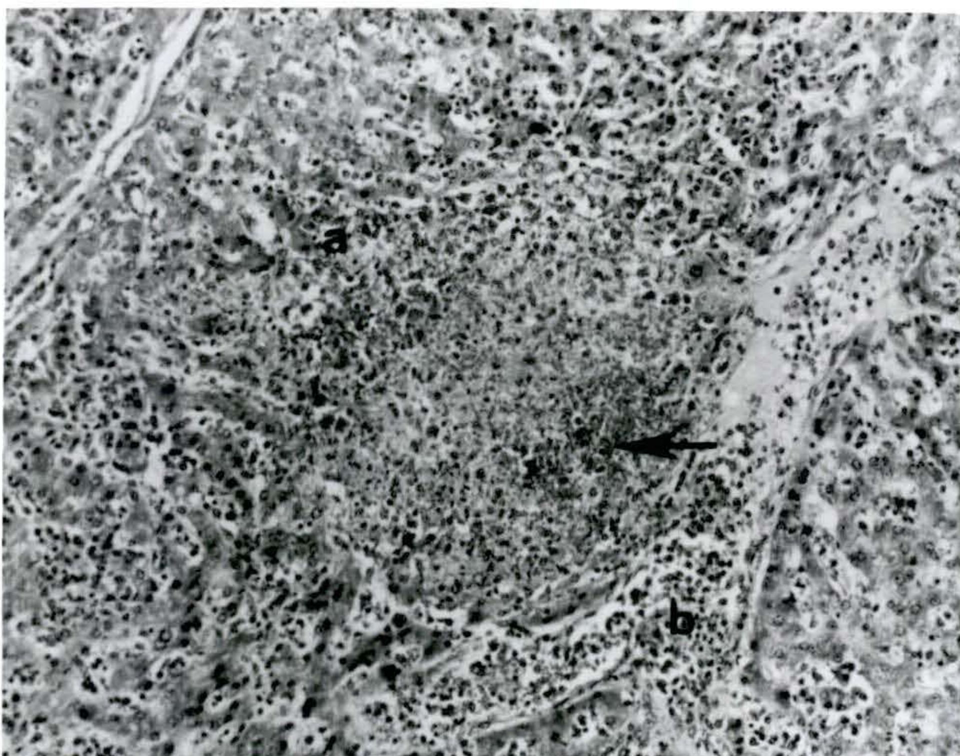

a
b

thickened due to the presence of edema (Figure 17).

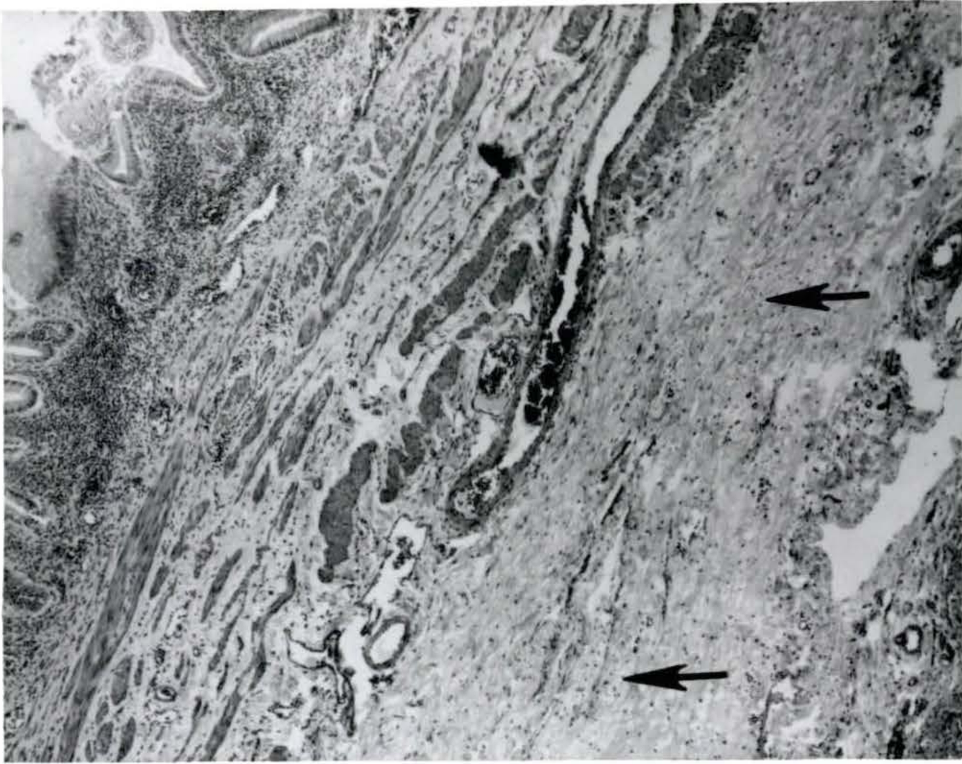
The spleen was congested and a diffuse reticuloendothelial cellular hyperplasia was prominent. No abscesses were present.

The renal infarcts seen grossly were clearly delineated microscopically by a zone of inflammation at the edge of the infarcted areas (Figure 18). Both red and white infarcts were present. The red infarcts contained many erythrocytes and there was coagulation necrosis of the tubules and glomeruli. The white infarcts were characterized by the lack of erythrocytes. Fibrous connective tissue had replaced the parenchymatous elements. Pathologic changes in the kidneys were minimal except in the infarcted areas. Some glomerular capillary tufts were congested and appeared to be hypercellular. In some glomeruli, Bowman's space appeared to be distended. One kidney, from pig 525, had numerous hyaline casts in the lumina of the distal tubules and in the collecting ducts. These casts were associated with the infarcted areas. No abscesses were present in any of the kidneys.

The lymph nodes were edematous, congested and some contained a few microabscesses (Figure 19). The microabscesses were characterized by a central zone of necrosis with much nuclear debris surrounded by an accumulation of neutrophils (Figure 20). The abscesses were not composed entirely of neutrophils, but contained other cells such as lymphocytes, plasma cells and mononuclear macrophages. These lesions

Figure 17. Experiment I, 4 days PI. Gall bladder. A wide zone of edema (arrows) is present in the muscularis and submucosa. H&E stain; 80 X

Figure 18. Experiment I, 4 days PI. Kidney. The edge of a renal infarct (a) is separated from normal parenchyma (b) by a zone of inflammation (c). H&E stain; 80 X



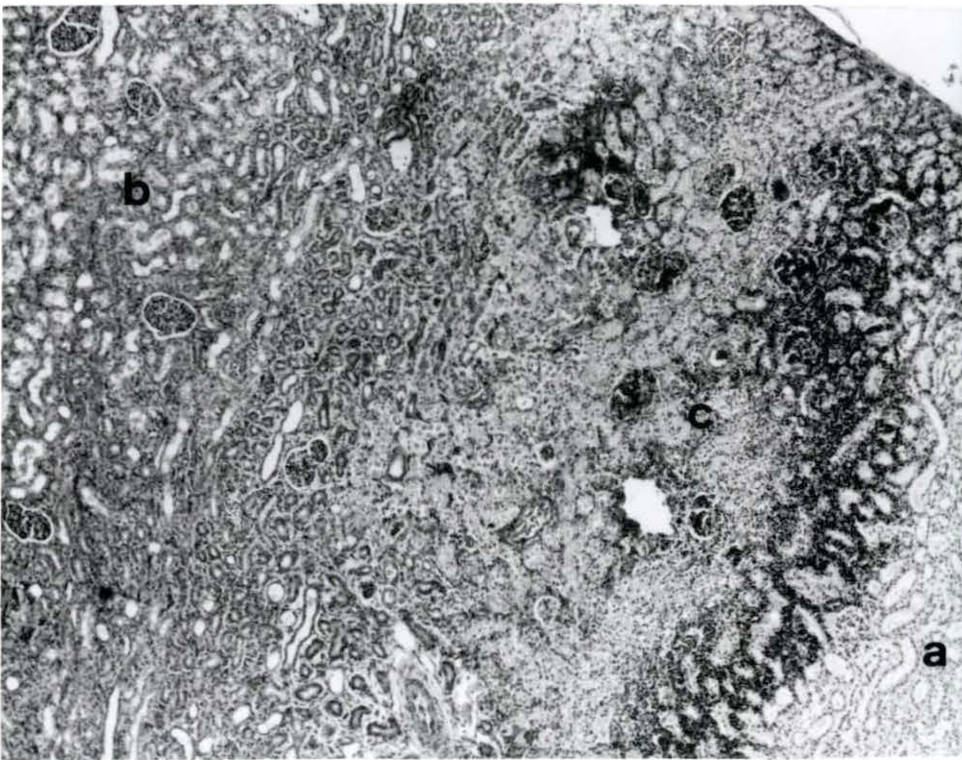
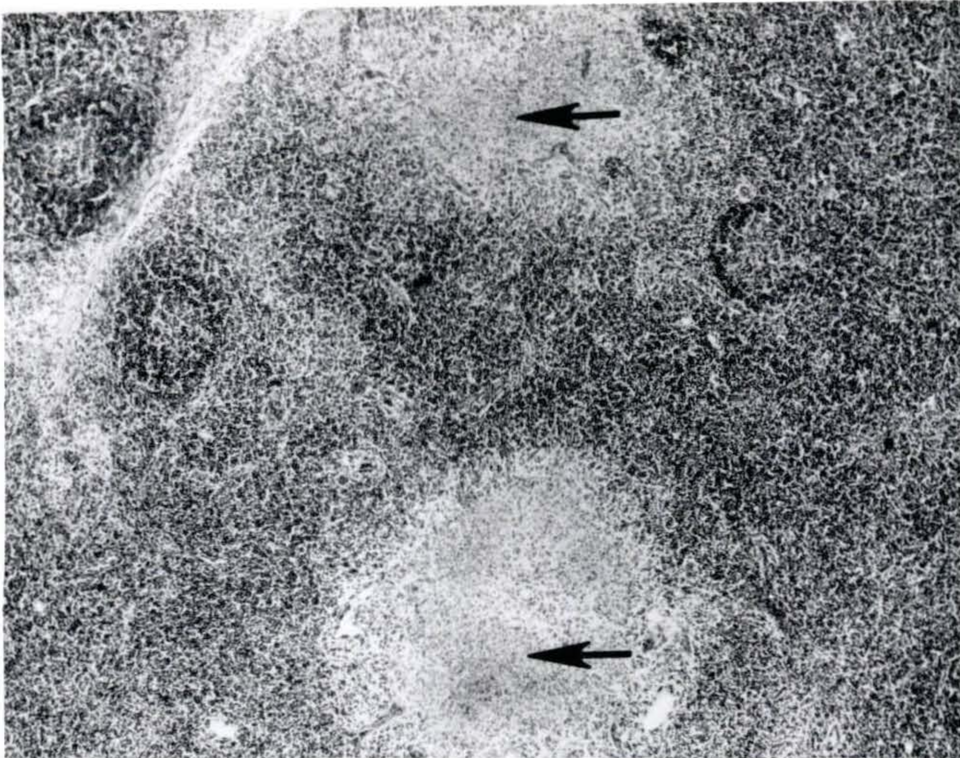
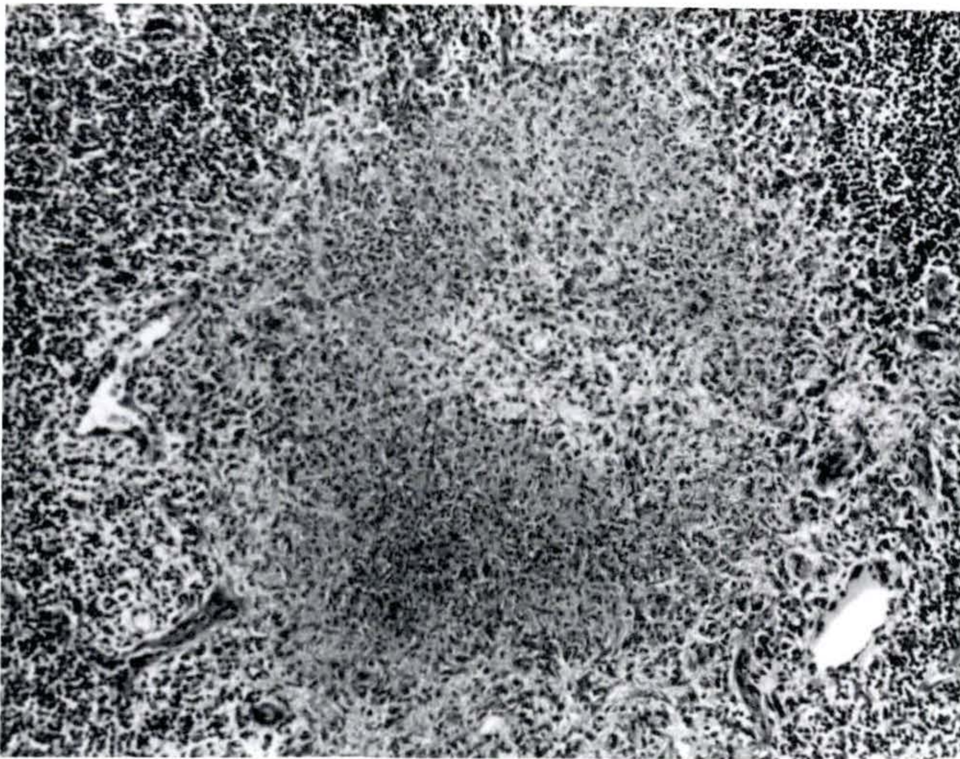

b
c
a

Figure 19. Experiment I, 4 days PI. Lymph node. Multiple microabscesses (arrows) with necrotic centers. H&E stain; 80 X

Figure 20. Experiment I, 4 days PI. Lymph node. The necrotic focus contains nuclear debris and many neutrophils. H&E stain; 200 X





developed in the stroma at the periphery of the germinal centers near the peritrabecular lymph spaces and in the cell poor area at the periphery of the nodes. The internal iliac lymph nodes, that received lymph from the leg that was catheterized, contained many erythrocytes and neutrophils in the subcapsular and peritrabecular sinuses. These lymph nodes were similarly involved in all 3 pigs.

No lesions were observed in the central nervous system.

Cultural and FA results

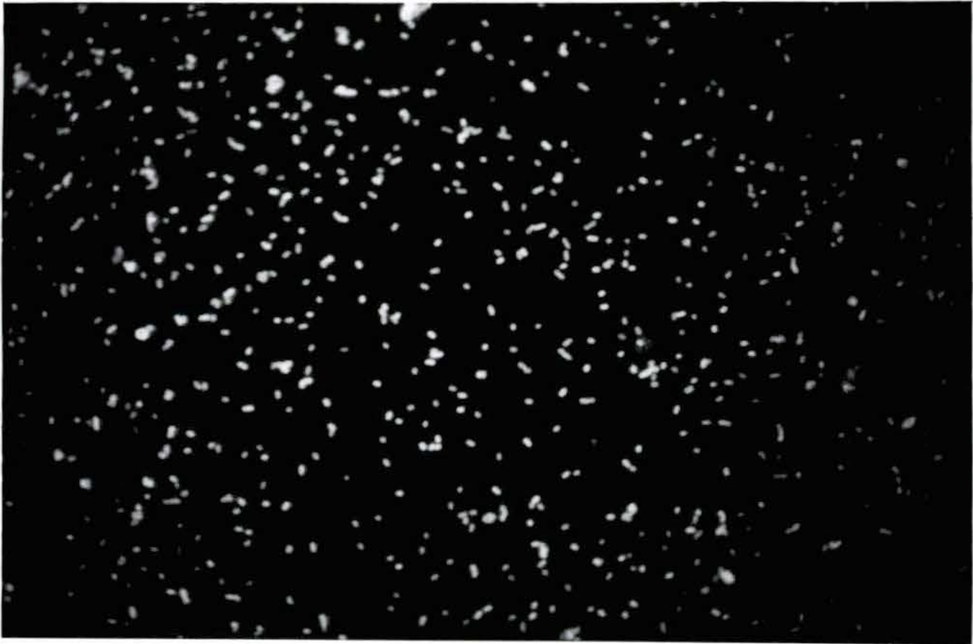
All tests were negative for hog cholera viral antigen. The results of the FA tests were positive for salmonellae on all specimens examined (Figure 21). *S. choleraesuis* var. *kunzendorf* was re-isolated from all the parenchymatous tissues in pure culture from all 3 pigs.

Experiment II

Clinical observations

Pigs exposed to a killed salmonellae cell suspension were not affected until approximately 1 hour PI when they began to look uncomfortable and 1 pig vomited. All 3 pigs were prostrate in 1 to 2 hours PI and did not resist when temperatures and blood specimens were taken. At 24 hours PI, the pigs were recumbent. They were not eating or drinking. No vomitus or feces were noted in the pens. The pigs still

Figure 21. Experiment I. Numerous bacilli isolated from parenchymatous organs are stained specifically with the fluorescein conjugated anti-salmonella antibody. 750 X



did not get up when the temperatures were taken or when the blood was collected. They would stand if prodded but would lie down immediately when left alone. They were rotated every few hours to prevent the development of pneumonia. By 48 hours PI, the pigs appeared to be markedly improved. Although the pigs were lying down when the caretakers entered the room they did stand and walk short distances. When fresh feed was offered they ate while the temperatures were taken and the blood collected. They remained recumbent for the rest of the day and were even more improved by 54 hours PI. Feces of normal consistency were present in 2 of the pens, but pig 564 passed feces that were firm and mucus covered. By 72 hours PI, all 3 pigs were eating. At 96 hours PI, when the experiment was concluded, the pigs appeared normal and had eaten all the feed left from the previous afternoon feeding. Normal feces were present in all 3 pens.

The body temperatures of the pigs are recorded in Table 14. The temperatures were elevated 4 to 5 F. by 1 hour PI and slowly increased another degree by 2 hours PI. At 4 hours PI, the temperatures began to decrease in 2 pigs (564 and 566) while in the third pig (565) the temperature continued to rise and peaked at 6 hours PI. At 24 hours PI, the temperatures of all 3 pigs were back within normal ranges. With minor fluctuations, the temperatures remained normal for the duration of the experiment which was concluded at 96 hours PI.

Table 14. Body temperatures[a] of swine inoculated with killed, washed suspension of _S. choleraesuis_ var. _kunzendorf_. Experiment II

Hours PI	Pig 564	Pig 565	Pig 566
-96	102.6	102.6	102.0
-72	103.2	103.0	103.4
-48	102.7	102.2	101.1
-24	101.6	101.4	101.4
0	100.8	101.4	100.8
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	105.6	105.0	105.4
2	106.6	105.4	105.2
4	104.2	105.8	105.4
6	104.2	106.0	103.4
24	102.4	102.8	102.6
30	101.8	102.4	102.6
48	103.0	102.4	102.2
54	103.0	102.2	102.2
72	102.4	102.6	102.0
78	103.0	102.8	102.0
96	102.0	101.8	101.8

[a]Temperatures recorded in degrees Fahrenheit.

[b]No temperatures taken.

Hematology

Erythrocyte sedimentation rate The ESR results of the pigs in this experiment are listed in Table 15. The rates were somewhat elevated at the time of inoculation. The ESRs decreased during the first hour PI and the lowest values recorded were at approximately 1 hour PI. A slight rise was noted at 2 hours PI, but the ESRs were definitely rising in all 3 pigs by 4 hours PI. A sharp rise was present from 4 to 6 hours PI. At 24 hours PI the highest ESRs were recorded. These ESR values were 5 times those recorded at the time of inoculation in 2 of the pigs (564 and 566). By 30 hours PI, a sharp drop in the ESRs was evident. The ESRs erratically decreased from 48 to 96 hours PI. At 78 hours PI the ESRs were near the pre-inoculation values.

Packed cell volume The changes in the PCV of blood from pigs in this experiment are listed in Table 16. The PCVs were somewhat elevated in the pre-inoculation hematologic studies and the greatest values for PCVs were at this time. Following inoculation a slight decrease occurred in all 3 pigs within 1 hour PI. The PCVs decreased from 2 hours through 72 hours PI. The lowest values were approximately 20 percent less than those at the time of inoculation. At 96 hours PI a slight increase in PCV was recorded in 1 pig (565) and a slight decrease in the other pigs (564 and 566).

Table 15. Erythrocyte sedimentation rates[a] of blood from swine inoculated with killed, washed suspension of *S. choleraesuis* var. *kunzendorf*. Experiment II

Hours PI	Pig 564	Pig 565	Pig 566
-96	7.0	14.0	7.0
-72	9.0	27.0	7.0
-48	10.0	29.5	8.0
-24	8.5	32.0	9.0
0	6.0	28.0	9.0
15 min.	8.0	20.0	7.0
30 min.	6.0	17.0	12.0
45 min.	5.0	13.0	7.5
1	6.0	10.0	5.5
2	6.0	13.0	9.5
4	9.0	23.0	19.0
6	26.0	44.0	20.0
24	30.0	63.0	49.0
30	17.0	32.5	35.0
48	19.5	47.5	24.5
54	15.5	30.0	17.5
72	17.5	31.5	23.0
78	8.0	24.0	11.0
96	11.5	27.0	15.0

[a]Erythrocyte sedimentation rate results in mm./hr.

Table 16. Packed cell volumes[a] of blood from swine inoculated with a killed, washed suspension of *S. choleraesuis* var. *kunzendorf*. Experiment II

Hours PI	Pig 564	Pig 565	Pig 566
-96	32.0	28.5	29.5
-72	40.0	32.0	35.0
-48	36.0	30.0	31.5
-24	32.5	31.0	35.0
0	35.0	30.0	31.5
15 min.	33.0	30.0	31.0
30 min.	31.0	29.0	31.0
45 min.	32.0	29.5	30.5
1	29.0	31.0	31.0
2	31.5	28.5	29.0
4	31.0	28.5	29.0
6	28.5	28.5	31.0
24	27.0	26.0	26.0
30	28.0	24.5	25.5
48	28.0	24.5	26.0
54	28.0	26.0	26.5
72	27.5	26.5	25.5
78	30.5	26.5	28.0
96	30.0	28.0	27.5

[a]Packed cell volume reported as percentage.

Total red blood cell count The TRBC counts on blood from these pigs are listed in Table 17. The counts closely paralleled the PCV values as the greatest numbers were recorded in the pre-inoculation studies. There did not appear to be any important changes immediately after inoculation. The lowest counts were at 24 to 48 hours PI and no sudden or drastic changes were detected at any specific time during the course of the experiment, which was concluded at 96 hours PI.

Total white blood cell count The TWBC counts are listed in Table 18 for the pigs in this experiment. The TWBC numbers were within normal range when the pigs were inoculated. During the first 6 hours PI there were increased numbers of WBCs in pigs 565 and 566, but no significant change in the TWBCs of pig 564. The TWBCs doubled in numbers in pig 565 from pre-inoculation to 6 hours PI. An erratic increase of about 25 percent was recorded in the same period of time in pig 566. At 24 hours PI, the TWBCs had decreased in all 3 pigs to near pre-inoculation values. From 48 to 96 hours PI, the TWBC numbers seemed to stabilize within normal range in 2 of the pigs (564 and 566). The lowest TWBC count in pig 565 occurred at 48 hours PI, and was approaching values of a leukopenia. A leukopenia is generally considered a TWBC count of less than 10,000 WBCs per/ml. of blood. From 48 to 96 hours PI the TWBCs remained low and did not return to pre-inoculation levels by the time the experiment was concluded

Table 17. Total red blood cell counts[a] from blood of swine inoculated with killed, washed suspension of _S. choleraesuis_ var. _kunzendorf_. Experiment II

Hours PI	Pig 564	Pig 565	Pig 566
-96	603	492	515
-72	607	537	589
-48	678	547	587
-24	634	582	660
0	628	540	563
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	524	534	559
2	542	487	514
4	566	509	513
6	527	514	565
24	482	448	448
30	521	433	448
48	501	432	450
54	516	471	496
72	482	432	450
78	558	460	496
96	523	475	471

[a]Red blood cells reported as number of cells X 10^4/cu. mm.

[b]TRBC counts not conducted on these bleedings.

Table 18. Total white blood cell counts[a] from blood of swine inoculated with killed, washed suspension of *S. choleraesuis* var. *kunzendorf*. Experiment II

Hours PI	Pig 564	Pig 565	Pig 566
-96	16,100	14,800	16,800
-72	24,800	17,700	22,500
-48	16,100	14,000	19,400
-24	17,600	13,400	15,300
0	22,700	14,300	16,900
15 min.	21,200	18,800	17,800
30 min.	19,500	15,500	16,700
45 min.	19,100	20,000	17,600
1	18,000	21,300	18,400
2	18,100	27,600	21,100
4	19,300	29,300	17,500
6	20,200	31,900	22,600
24	17,000	21,300	15,800
30	16,900	16,700	17,000
48	15,700	10,900	16,800
54	17,100	12,300	16,100
72	14,600	12,600	14,300
78	17,300	13,300	16,000
96	15,600	11,900	14,700

[a]White blood cell counts reported in number of cells/cmm.

at 96 hours PI.

Serum glutamic oxaloacetic transaminase The levels of SGOT activity are listed in Table 19 for the 3 pigs in this experiment. The highest levels were recorded in the pre-inoculation determinations in 2 of the pigs (564 and 565). The highest levels of SGOT activity occurred between 2 and 6 hours PI in all 3 pigs. At 24 hours PI, a significant decrease was recorded in SGOT activity. From 48 through 96 hours PI, the SGOT levels became quite erratic with no apparent trends.

Lactic dehydrogenase Serum LDH levels are listed in Table 20 for the pigs in this experiment. The highest levels were recorded in the pre-inoculation hematologic studies in 2 of the pigs (564 and 565). These values did not change appreciably following inoculation. Pig 566 did not reveal any significant changes in LDH from time of inoculation until 72 hours PI, when the LDH levels of activity started to elevate slightly. At 96 hours PI the highest values occurred and were higher than pre-inoculation values. The other 2 pigs (564 and 565) remained nearly the same throughout the experiment and no definite trends could be established.

Differential leukocyte count The differential leukocyte counts on pig 564 are listed in Table 21. Lymphocytes were the predominant cell type in the pre-inoculation studies. Within 1 hour PI, segmented neutrophils were beginning to

Table 19. Serum glutamic oxaloacetic transaminase[a] levels of activity from swine inoculated with killed, washed suspension of *S. choleraesuis* var. *kunzendorf*. Experiment II

Hours PI	Pig 564	Pig 565	Pig 566
-96	28	30	27
-72	75	41	41
-48	51	30	36
-24	32	28	27
0	32	22	38
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	27	22	34
2	34	23	43
4	32	25	52
6	27	25	45
24	25	20	28
30	23	18	20
48	20	25	19
54	36	19	30
72	30	19	34
78	27	20	32
96	22	27	23

[a]Glutamic oxaloacetic transaminase reported in Sigma-Frankel units.

[b]No determinations made.

Table 20. Lactic dehydrogenase[a] levels of activity in serum of swine inoculated with killed, washed suspension of *S. choleraesuis* var. *kunzendorf*. Experiment II

Hours PI	Pig 564	Pig 565	Pig 566
-96	665	895	850
-72	1085	1100	935
-48	1140	1005	835
-24	1375	935	895
0	950	850	1005
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	1005	895	950
2	965	805	1005
4	935	755	975
6	820	785	990
24	910	770	920
30	975	805	990
48	865	785	820
54	910	740	865
72	895	805	935
78	990	665	1015
96	965	945	1140

[a]Lactic dehydrogenase reported in Berger-Broida (B-B) units.

[b]No determinations made.

Table 21. Differential leukocyte counts[a] on blood smears from swine inoculated with killed, washed suspension of *S. choleraesuis* var. *kunzendorf*. Experiment II

			Pig 564					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	108	4	80	4	0	0	2	2
-72	100	8	90	2	0	0	0	0
-48	104	6	88	0	0	0	2	0
-24	105	4	84	4	0	0	3	0
0	112	2	83	1	0	0	0	2
15 min.	110	2	84	0	0	0	2	2
30 min.	116	6	77	1	0	0	0	0
45 min.	108	6	82	2	0	0	1	1
1	106	3	79	6	0	0	3	3
2	81	2	95	18	0	0	0	4
4	48	4	124	22	1	0	1	0
6	46	4	128	20	0	0	1	1
24	102	4	82	4	0	0	2	6
30	114	5	72	4	0	0	3	2
48	113	2	75	5	0	0	4	1
54	112	8	76	0	0	0	3	1
72	102	3	84	2	0	0	6	3
78	112	2	78	0	0	0	6	2
96	130	4	60	0	0	0	2	4

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

increase and the lymphocytes were decreasing. Immature forms of the neutrophils were also increasing in numbers, indicating a shift to the left (Schilling's index). The segmented and immature forms of neutrophils continued to rise throughout the first 6 hours PI while the lymphocytes decreased. The numbers of monocytes, eosinophils and basophils did not change appreciably, but a slight increase in eosinophils was present at 72 to 96 hours PI. After 24 hours PI, the lymphocytes increased and the segmented and immature forms of neutrophils decreased until pre-inoculation values had returned by 48 hours PI.

The differential leukocyte counts on pig 565 are listed in Table 22. The number of lymphocytes began to decrease as early as 30 minutes PI and the neutrophil became the predominant cell type. Immature forms of neutrophils began to appear by 1 hour PI and steadily increased in numbers through 6 hours PI. At 24 hours PI, the segmented and immature neutrophils still predominated as the most numerous cell types but by 30 hours PI the lymphocytes again outnumbered the neutrophils. From 30 to 96 hours PI, a return to pre-inoculation normals occurred.

The differential leukocyte counts on pig 566 are listed in Table 23. The counts on this pig are very similar to pigs 564 and 565. Neutrophils began to increase and lymphocytes to decrease after 1 hour PI. A definite shift to the left occurred through 6 hours PI. At 24 hours PI, the differential

Table 22. Differential leukocyte counts[a] on blood smears from swine inoculated with killed, washed suspension of S. choleraesuis var. kunzendorf. Experiment II

			Pig 565					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	80	3	110	2	0	0	3	2
-72	75	6	109	0	0	0	10	0
-48	123	2	73	2	0	0	0	0
-24	121	1	72	2	0	0	4	0
0	106	2	86	1	0	0	3	2
15 min.	118	2	75	0	0	0	2	3
30 min.	87	0	111	2	0	0	0	0
45 min.	94	2	98	0	0	0	6	0
1	65	3	123	4	0	0	4	1
2	52	2	134	12	0	0	0	0
4	32	5	136	26	0	0	1	0
6	43	1	124	30	2	0	0	0
24	76	4	105	10	0	0	5	0
30	100	2	82	7	0	0	7	2
48	108	3	84	3	0	0	1	1
54	102	2	90	0	0	0	6	0
72	101	4	90	2	0	0	1	2
78	95	4	96	3	0	0	2	0
96	122	3	68	1	0	0	4	2

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

Table 23. Differential leukocyte counts[a] on blood smears from swine inoculated with killed, washed suspension of *S. choleraesuis* var. *kunzendorf*. Experiment II

			Pig 566					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	112	2	82	1	0	0	3	0
-72	127	1	69	0	0	0	2	1
-48	120	4	74	0	0	0	0	2
-24	133	3	60	1	0	0	3	0
0	137	0	58	0	0	0	3	2
15 min.	124	3	70	1	0	0	2	0
30 min.	110	4	84	0	0	0	2	0
45 min.	126	1	72	0	0	0	1	0
1	132	5	61	2	0	0	0	0
2	69	2	109	18	0	0	0	2
4	56	0	134	9	1	0	0	0
6	51	2	129	18	0	0	0	0
24	106	4	78	5	3	0	4	0
30	141	3	48	4	1	0	3	0
48	132	0	59	0	0	0	7	2
54	131	1	62	2	0	0	3	1
72	114	2	83	1	0	0	0	0
78	112	3	82	0	0	0	2	1
96	120	1	76	0	0	0	2	1

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

leukocyte counts were back to the pre-inoculation values.

The absolute differential leukocyte counts are listed in Tables 24, 25 and 26 for pigs 564, 565 and 566 respectively. The absolute counts did not disclose any significant differences from the relative counts.

Necropsy findings

Gross lesions   The necropsy findings in all 3 pigs in this experiment were essentially the same. No gross external lesions were observed and the surgical wounds associated with catheterization of the femoral artery were healed. Lesions were not visible in the viscera with the exception of multiple bilateral renal infarcts in all 3 pigs.

Microscopic lesions   The alveolar walls of the lungs were moderately thickened due to infiltrations of macrophages along with lymphocytes and plasma cells. The plugged capillaries were present as in Experiment I. There was an obvious hyperplasia of the Kupffer cells of the liver (Figure 22). The hepatocytes were foamy in appearance and not shrunken as in Experiment I (Figure 23). The spleen exhibited mild, diffuse reticuloendothelial hyperplasia. The renal infarcts were the same as described in Experiment I. In the lymph nodes there were slight edema and a depletion of the small lymphocytes at the periphery of the germinal centers (Figure 24). No other microscopic lesions were observed.

Table 24. Absolute differential leukocyte counts[a] on blood smears prepared from swine inoculated with killed, washed suspension of S. choleraesuis var. kunzendorf. Experiment II

			Pig 564					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	8694	322	6440	322	0	0	161	161
-72	12400	992	11160	248	0	0	0	0
-48	8372	483	7084	0	0	0	161	0
-24	9240	352	7392	352	0	0	264	0
0	12712	227	9421	113	0	0	0	227
15 min.	11660	212	8904	0	0	0	212	212
30 min.	11310	585	7508	97	0	0	0	0
45 min.	10314	573	7831	191	0	0	96	95
1	9540	270	7110	540	0	0	270	270
2	7331	181	8597	1629	0	0	0	362
4	4632	386	11966	2123	96	0	97	0
6	4646	404	12928	2020	0	0	101	101
24	8670	340	6970	340	0	0	170	510
30	9633	423	6084	338	0	0	253	169
48	8871	157	5887	393	0	0	314	78
54	9576	684	6498	0	0	0	257	85
72	7446	219	6132	146	0	0	438	219
78	9688	173	6747	0	0	0	519	173
96	10140	312	4680	0	0	0	156	312

[a]Absolute differential leukocyte counts expressed in numbers per cubic mm. of blood.

Table 25. Absolute differential leukocyte counts[a] on blood smears prepared from swine inoculated with killed, washed suspension of S. choleraesuis var. kunzendorf. Experiment II

			Pig 565					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	5920	222	8140	148	0	0	222	148
-72	6638	531	9646	0	0	0	885	0
-48	8610	140	5110	140	0	0	0	0
-24	8107	67	4824	134	0	0	268	0
0	7579	143	6149	72	0	0	214	143
15 min.	11092	188	7050	0	0	0	188	282
30 min.	6743	0	8602	155	0	0	0	0
45 min.	9400	200	9800	0	0	0	600	0
1	6923	319	13100	426	0	0	426	106
2	7176	276	18492	1656	0	0	0	0
4	4688	733	19924	3809	0	0	146	0
6	6859	159	19778	4785	319	0	0	0
24	8094	426	11183	1065	0	0	532	0
30	8350	167	6847	585	0	0	584	167
48	5886	164	4578	163	0	0	54	55
54	6273	123	5535	0	0	0	369	0
72	6363	252	5670	126	0	0	63	126
78	6318	266	6384	199	0	0	133	0
96	7259	179	4046	59	0	0	238	119

[a]Absolute differential leukocyte counts expressed in numbers per cubic mm. of blood.

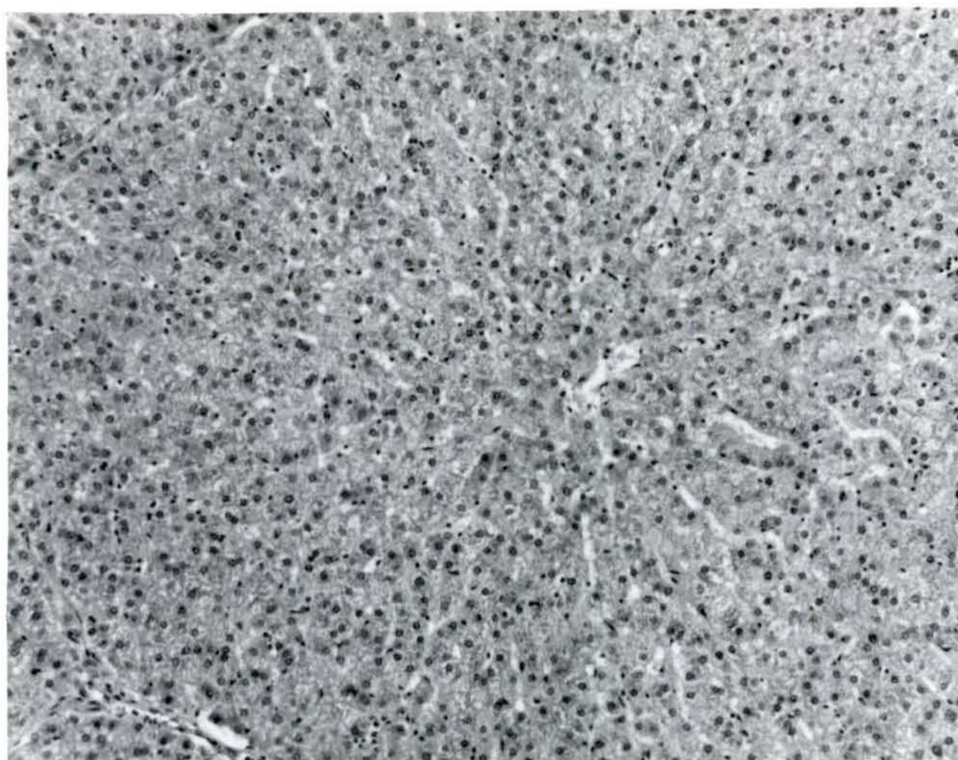
Table 26. Absolute differential leukocyte counts[a] on blood smears prepared from swine inoculated with killed, washed suspension of S. choleraesuis var. kunzendorf. Experiment II

			Pig 566					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	9408	168	6888	84	0	0	252	0
-72	14288	113	7762	0	0	0	225	112
-48	11640	388	7178	0	0	0	0	194
-24	10175	229	4590	77	0	0	229	0
0	11577	0	4901	0	0	0	253	169
15 min.	11036	267	6230	89	0	0	178	0
30 min.	9185	334	7014	0	0	0	167	0
45 min.	11088	88	6336	0	0	0	88	0
1	12144	460	5612	184	0	0	0	0
2	7280	211	11499	1899	0	0	0	211
4	4900	0	11725	788	87	0	0	0
6	5763	226	14577	2034	0	0	0	0
24	8374	316	6162	395	237	0	316	0
30	11985	255	4080	340	85	0	255	0
48	11088	0	4956	0	0	0	588	168
54	10546	80	4991	161	0	0	242	80
72	8151	143	5935	71	0	0	0	0
78	8960	240	6560	0	0	0	160	80
96	8820	73	5586	0	0	0	147	74

[a]Absolute differential leukocyte counts expressed in numbers per cubic mm. of blood.

Figure 22. Experiment II, 96 hours PI. Liver. Hyperplasia of the Kupffer cells is present throughout the liver tissue. H&E stain; 200 X

Figure 23. Experiment II, 96 hours PI. Liver. The hepatocytes appear to be foamy and distinct cords or sinusoids not easily seen. H&E stain; 200 X



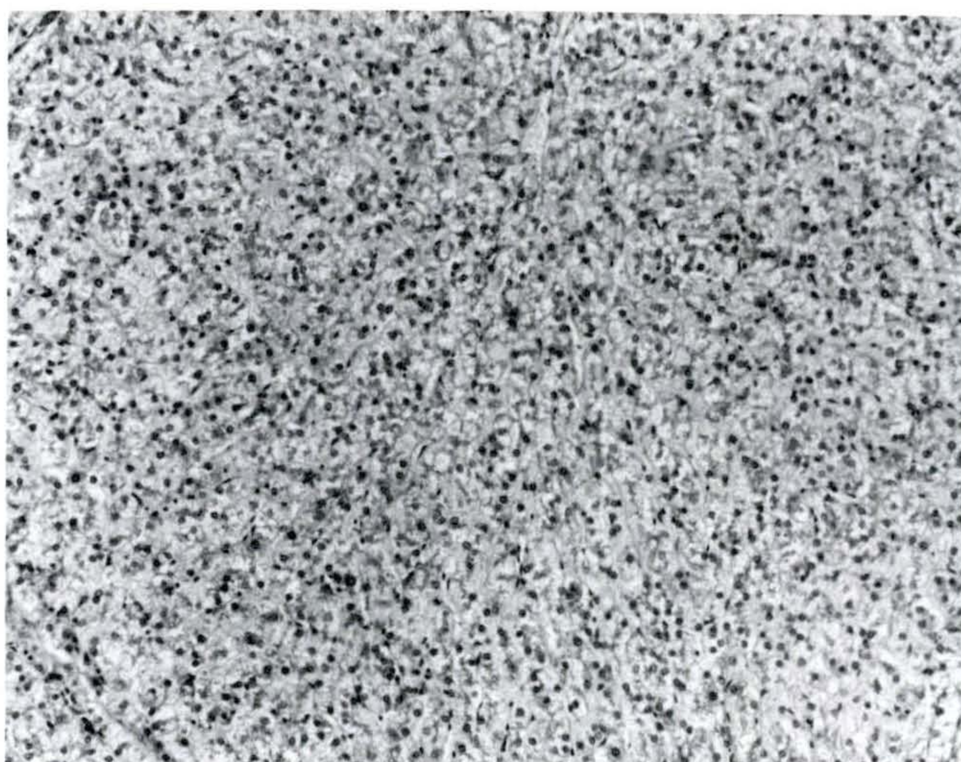
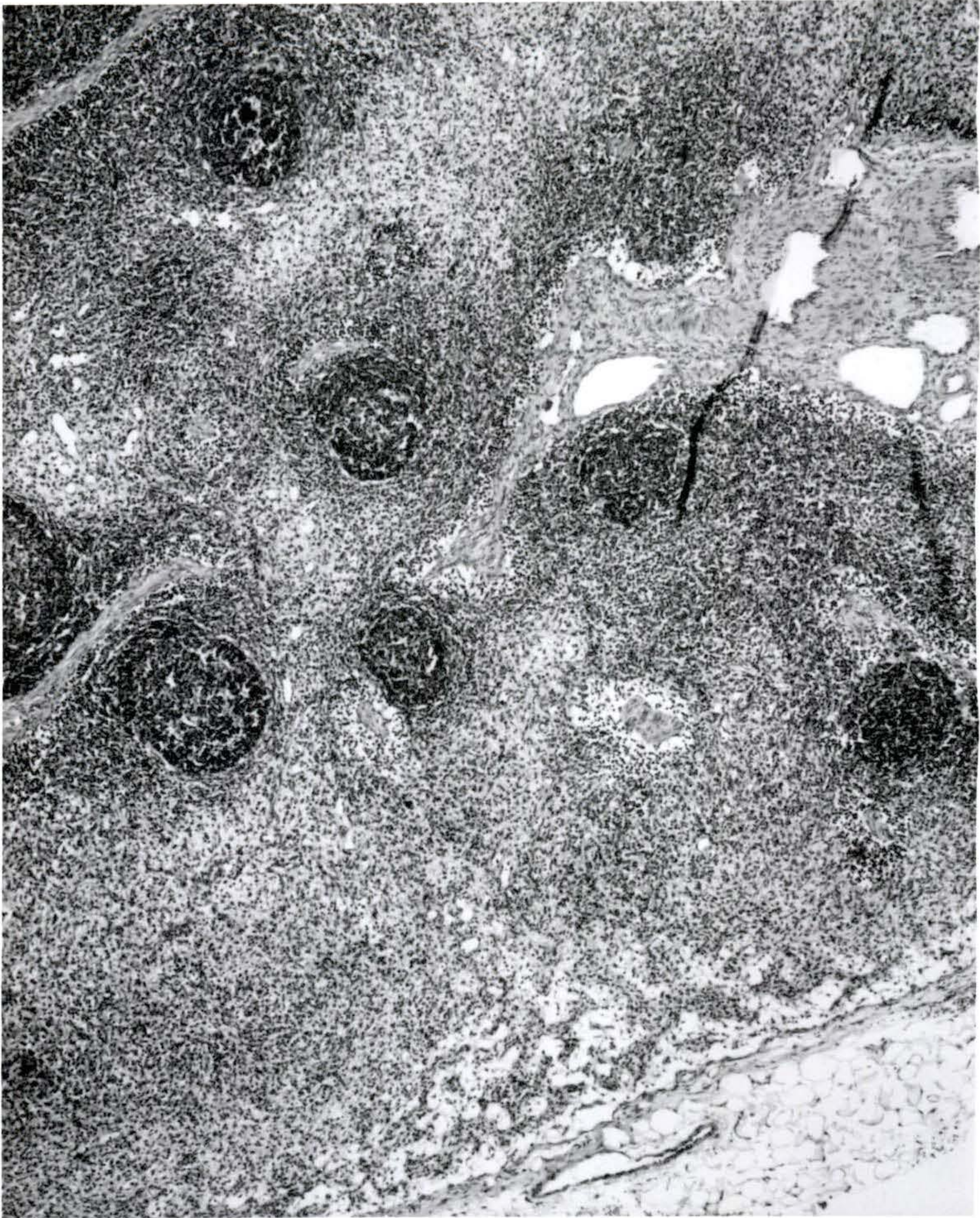


Figure 24. Experiment II, 96 hours PI. Lymph node. Slight edema and cellular depletion of small lymphocytes are evident. H&E stain; 120 X



Cultural and FA results

All fluorescent antibody tests for HC viral antigen and salmonellae were negative. All attempts to culture salmonellae from the tissues were also negative.

Experiment III

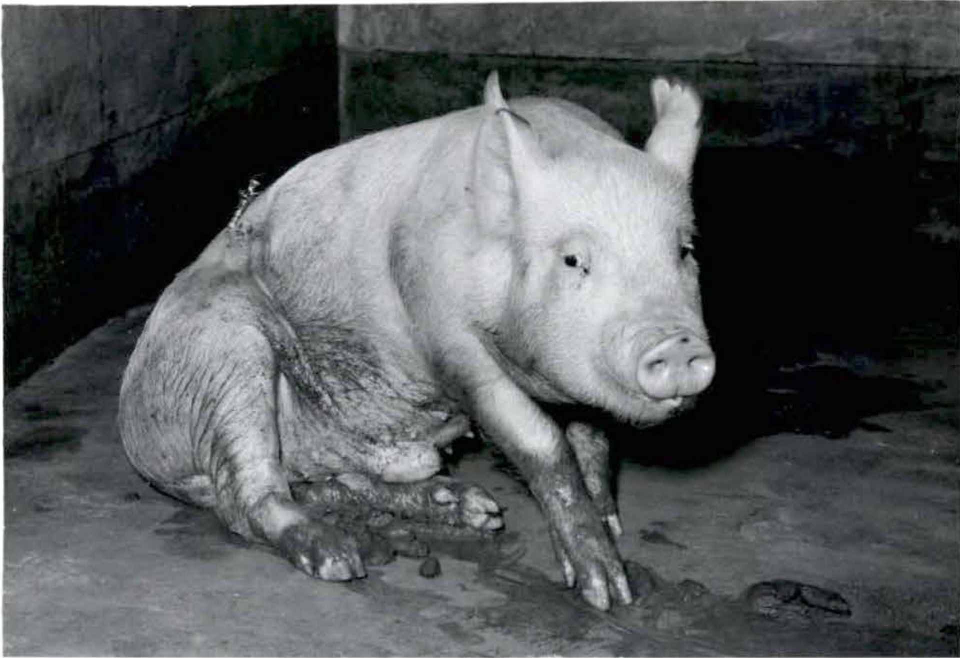
Clinical observations

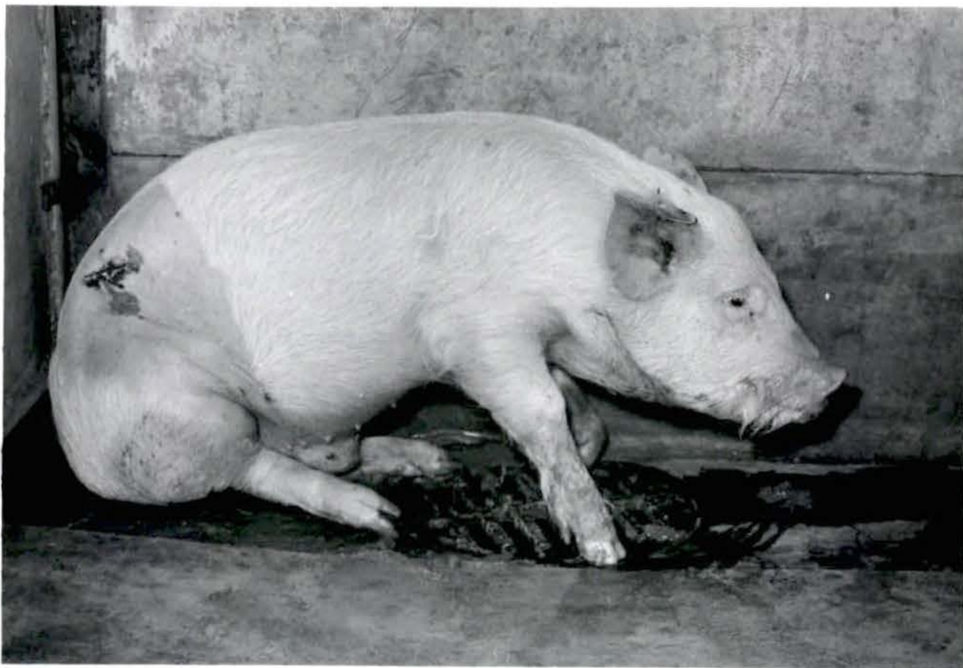
About 5 minutes after inoculation with endotoxin, pig 706 went into shock. It started shaking and lay down. Respiration was rapid and labored, and the skin became cyanotic. The pig vomited and had a bowel movement. The pig appeared to be in danger of dying, but after 15 to 20 minutes PI it began to recover. Respiration became easier and cyanosis of the skin disappeared. The vomiting continued, however, and the feces became fluid. A pronounced diarrhea followed.

The other 2 pigs (705 and 707) did not go through the shock stage but after 10 minutes PI, vomiting and large bowel movements occurred. Each pig vomited about 5 times in the next 15 minutes. The pigs exhibited slight incoordination and posterior weakness (Figure 25). The feces were at first firm in consistency but a definite diarrhea was present in all 3 pigs by 1 hour PI. For the remainder of the first hour PI, the pigs were restless and would lie down, then get up, walk around and lie down again. After the first hour PI,

Figure 25. Experiment III, 30 minutes PI. As a result of posterior weakness and incoordination the pig is unable to stand

Figure 26. Experiment III, 2 hours PI. Posterior weakness persists. Salivation is present following repeated vomiting and defecation





the pigs were prostrate and would not stand to vomit or defecate. They did not resist when temperatures and blood samples were taken.

Vomiting and diarrhea continued 2 hours PI with frequent regularity. Between 1 and 2 hours PI, attempts to vomit were eventually no longer productive and retching was severe (Figure 26). Profuse salivation was obvious by 2 hours PI (Figure 27). The salivation was so copious it became frothy and would drip from the mouth (Figure 28). Vomiting and diarrhea began to subside 4 hours PI, and the salivation lasted approximately another 2 hours.

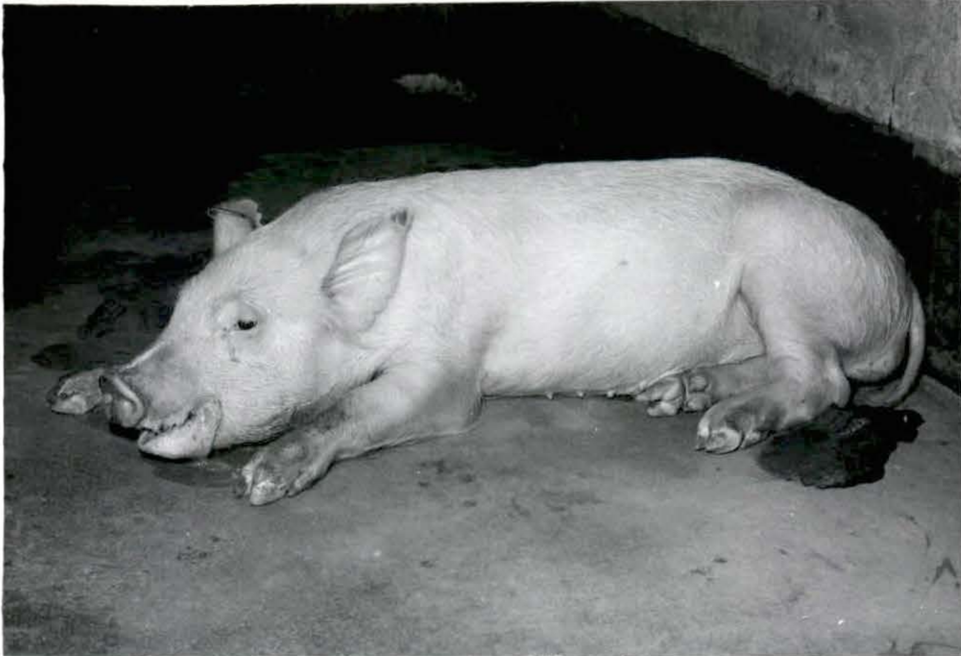
Six hours PI, the pigs were recumbent and almost comatose, but would respond if prodded. The salivation had diminished and vomiting and diarrhea had ceased. When the thermometer was removed from the rectum streaks of fresh blood in the fecal material adhered to it.

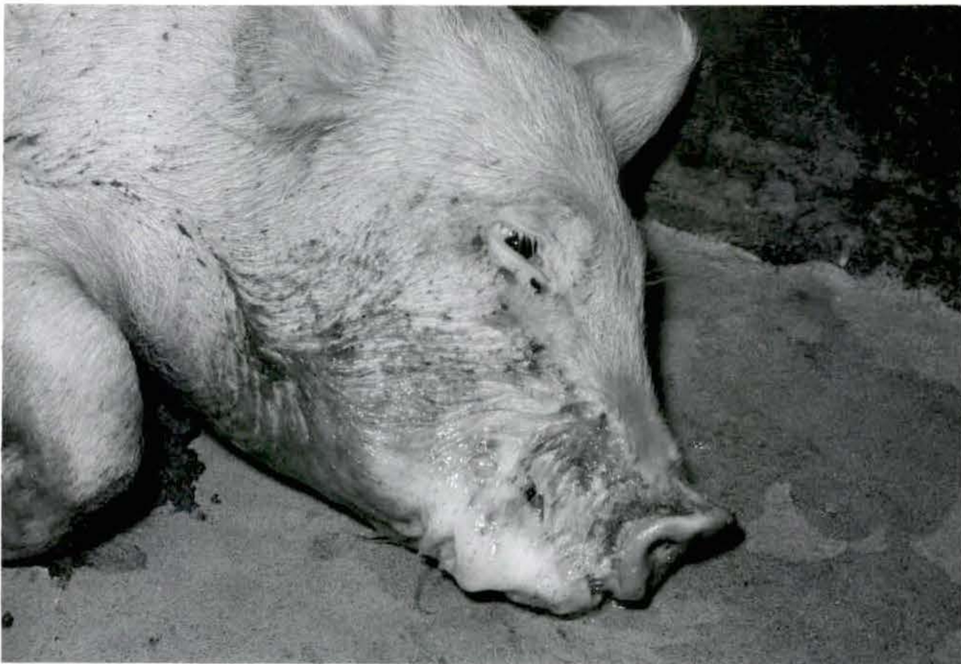
Eight hours PI, the pigs had not moved and were nearly comatose. Fresh blood oozed from the anal orifice. The external surgical wounds from the catheter surgery bled slightly in all 3 pigs. Due to the seriousness of the clinical signs and to the bleeding the pigs were observed continuously for the first 12 hours PI. The pigs had not moved since about 6 hours PI so each was turned to the opposite side. Bleeding was still evident but apparently not increasing in intensity.

Twenty-four hours PI, the condition of the pigs was

Figure 27. Experiment III, 2 hours PI. Pig is recumbent. Note feces and vomitus. Open mouth breathing and profuse salivation are present

Figure 28. Experiment III, 3 hours PI. Note extensive frothy salivation. Pig is bright eyed but too weak to rise





practically unchanged from the 12 hours PI observations. External bleeding had stopped. The pigs continued to lie as if comatose but would rise if severely prodded. Little, if any, change was noted in their overall condition from 24 to 48 hours PI. Feed and water had not been touched. No vomitus or feces were present in the pens.

Forty-eight hours PI, dehydration and loss of condition were apparent. The pigs were awake and watched the animal caretakers move about their pens, but did not get up or eat. In the afternoon, 30 hours PI, 2 of the pigs (705 and 707) were on their feet and were observed to eat small amounts of feed. Firm, mucus covered feces were present in the pens. The feces were dark colored but no fresh blood was noted. The third pig (706) was showing some improvement but made no attempt to stand.

Seventy-two hours PI, all 3 pigs were on their feet and ate some feed while the temperatures were taken and the blood collected. The pigs were quite weak and stood for only short periods. They lay down as soon as the animal handlers left the pens. The afternoon observations were practically the same as in the morning but the pigs appeared to be stronger, more alert and definitely recovering from the effects of the endotoxin.

At 96 hours PI, the pigs were on their feet and hungry. Loss of condition and weakness were the only changes from their pre-inoculation physical state.

The body temperatures are recorded in Table 27. All pigs were within the normal temperature range when inoculation occurred. The temperatures elevated 2 to 3 F. during the first hour PI. At 2 hours PI, 2 of the pigs (705 and 707) had marked decreases in temperatures, but pig 706 went up another degree. At 4 hours PI, all 3 pigs had temperatures near or less than their pre-inoculation readings. At 6 hours PI, pig 705 had a 3.0 F. rise in temperature. The other 2 pigs (706 and 707) remained in the normal temperature range. At 24 hours PI, all 3 pigs had elevated temperatures. From 48 to 96 hours PI the temperatures were within the normal temperature range in all 3 pigs.

Hematology

Erythrocyte sedimentation rate The ESRs are listed in Table 28. The pre-inoculation values were elevated following surgery. These levels were decreasing at the time of inoculation and for 4 hours PI. Between 4 and 6 hours PI, the ESRs began to rise. At 24 hours PI, the ESRs of all pigs were approximately 4 times the level of the 6 hour PI bleeding. This was the maximum ESR for pig 706, but the other 2 pigs (705 and 707) did not reach their peak until 48 hours PI. The ESRs remained elevated in all 3 pigs until the experiment was concluded at 96 hours PI.

Packed cell volume The PCV results are listed in Table 29. The PCVs were slightly elevated during the

Table 27. Body temperatures[a] of swine inoculated with endotoxin of *S. choleraesuis* var. *kunzendorf*. Experiment III

Hours PI	Pig 705	Pig 706	Pig 707
-96	102.2	102.0	101.8
-72	102.0	102.6	102.6
-48	101.8	101.8	103.0
-24	102.1	102.0	102.8
0	102.4	101.6	102.0
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	104.0	104.0	104.8
2	102.8	105.0	102.4
4	102.0	102.4	100.6
6	105.0	101.6	101.6
24	104.0	104.2	104.4
30	104.4	104.2	103.6
48	102.6	101.8	102.2
54	101.4	101.8	101.0
72	101.8	101.4	102.6
78	103.0	101.2	102.8
96	102.0	102.2	102.0

[a]Temperatures recorded in degrees Fahrenheit.

[b]No temperatures taken.

Table 28. Erythrocyte sedimentation rate[a] of blood from swine inoculated with endotoxin of _S._ _choleraesuis_ var. _kunzendorf_. Experiment III

Hours PI	Pig 705	Pig 706	Pig 707
-96	3.0	4.5	3.0
-72	9.0	10.0	5.5
-48	19.0	20.0	8.0
-24	17.0	19.0	11.0
0	15.0	15.0	9.0
15 min.	3.5	1.5	3.0
30 min.	4.0	6.0	3.0
45 min.	3.0	3.5	2.5
1	3.5	4.5	3.0
2	2.0	2.5	2.0
4	5.0	3.5	1.5
6	16.5	22.5	7.5
24	67.0	94.5	32.0
30	77.0	69.5	31.0
48	89.0	60.0	36.5
54	91.5	58.0	38.0
72	75.5	85.5	36.0
78	36.5	73.0	11.0
96	64.0	72.5	28.0

[a]Erythrocyte sedimentation rate results in mm./hr.

Table 29. Packed cell volume[a] of blood from swine inoculated with the endotoxin of S. choleraesuis var. kunzendorf. Experiment III

Hours PI	Pig 705	Pig 706	Pig 707
-96	36.5	36.0	33.0
-72	33.0	37.0	35.5
-48	31.0	33.5	33.0
-24	30.5	30.0	31.0
0	29.5	29.5	28.5
15 min.	34.5	34.5	32.5
30 min.	35.0	34.5	34.5
45 min.	36.5	34.5	35.5
1	35.0	33.0	34.5
2	34.5	33.0	33.5
4	35.5	34.0	35.0
6	35.5	33.5	34.5
24	29.0	25.0	29.5
30	26.0	27.0	28.0
48	24.0	25.0	25.0
54	23.5	23.5	26.5
72	24.0	22.5	26.5
78	24.5	22.5	27.5
96	23.0	25.5	27.5

[a]Packed cell volume reported as percentage.

pre-inoculation bleedings but were decreasing with the lowest results recorded immediately before inoculation. The PCVs were all increased at 15 minutes PI and remained elevated for the next 6 hours PI. In 2 of the pigs (705 and 707) the highest readings occurred at 45 minutes PI. In pig 706, the PCVs did not change from 15 to 45 minutes PI. At 24 hours PI, all PCVs were considerably lower than at 6 hours PI. The PCV readings continued downward from 24 hours PI to 54 hours PI. In 2 of the pigs (705 and 706) the PCV results had reached the lowest point by 54 hours PI and were rising from 72 to 96 hours PI. The lowest PCV in blood from pig 707 was at 48 hours PI.

Total red blood cell counts The TRBC counts are listed in Table 30. The results are very similar to the PCV results. The pre-inoculation levels were decreasing when inoculation occurred. No TRBC counts were made during the first hour PI. At 1 hour PI, the counts were again elevated in all 3 pigs and remained slightly elevated through 6 hours PI. At 24 hours PI, the TRBC levels decreased considerably and continued to decrease through 72 hours PI. In all 3 pigs the TRBC values began to rise on the 78 and 96 hours PI bleedings.

Total white blood cell counts The TWBC counts are listed in Table 31. The values were within normal ranges in the pre-inoculation hematologic studies. Very little change was noted in the 15 minute PI blood samples, but a

Table 30. Total red blood cell counts[a] in blood of swine inoculated with the endotoxin of *S. choleraesuis* var. *kunzendorf*. Experiment III

Hours PI	Pig 705	Pig 706	Pig 707
-96	621	613	565
-72	572	634	628
-48	566	601	607
-24	549	544	578
0	534	518	524
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	620	589	619
2	618	589	628
4	601	577	644
6	642	584	630
24	486	424	527
30	428	476	487
48	380	396	456
54	415	401	448
72	406	386	448
78	422	395	488
96	382	439	483

[a]Red blood cells reported as number of cells X 10^4/cu. mm.

[b]TRBC not conducted on these bleedings.

Table 31. Total white blood cell counts[a] of blood from swine inoculated with the endotoxin of *S. choleraesuis* var. *kunzendorf*. Experiment III

Hours PI	Pig 705	Pig 706	Pig 707
-96	14,600	14,700	12,600
-72	12,700	15,400	15,000
-48	12,600	14,600	14,000
-24	11,100	13,600	13,600
0	12,500	13,900	10,500
15 min.	11,000	12,600	11,400
30 min.	4,677	7,758	4,880
45 min.	4,658	8,583	5,375
1	4,262	6,064	5,728
2	1,656	3,182	3,001
4	1,269	2,534	1,071
6	1,558	3,412	1,430
24	20,200	25,800	23,900
30	29,000	32,900	28,500
48	38,700	41,300	53,400
54	37,700	36,700	54,800
72	16,800	17,700	21,000
78	15,200	15,500	19,200
96	10,400	13,900	14,800

[a]White blood cell counts reported in number of cells/cmm.

50 percent decrease in WBCs was recorded at 30 minutes PI. The levels remained about the same for 1 hour PI and then again began to decrease. The leukopenia was most pronounced in all 3 pigs at 4 hours PI. By 24 hours PI, a leukocytosis had developed, and by 48 hours PI it was more evident. The TWBC values returned to near normal at 72 hours PI and remained in the normal range until the conclusion of the experiment at 96 hours PI.

Serum glutamic oxaloacetic transaminase The SGOT levels of activity are listed in Table 32. The postsurgical and pre-inoculation values decreased until the time of inoculation. At 1 hour PI, the SGOT levels of activity were increased, and they continued to rise through 6 hours PI. At 24 hours PI, the highest levels of SGOT activity were obtained in all 3 pigs. At 48 hours PI, the levels were almost reduced by half from the 24 hour PI values. The SGOT values decreased from 48 through 96 hours PI and were below the pre-inoculation levels at 96 hours PI.

Lactic dehydrogenase The LDH levels are listed in Table 33. Following surgery the LDH levels were elevated but were decreasing when inoculation occurred. One hour PI, the LDH levels were elevated slightly above the values at the time of inoculation. At 2 hours PI, the levels decreased in all 3 pigs to approximately the values at inoculation. At 4 hours PI, the LDH levels were again elevated and were about the same at 6 hours PI. The highest levels of LDH

Table 32. Serum glutamic oxaloacetic transaminase[a] levels of activity from swine inoculated with the endotoxin of _S. choleraesuis_ var. _kunzendorf_. Experiment III

Hours PI	Pig 705	Pig 706	Pig 707
-96	23	27	15
-72	27	40	18
-48	21	23	12
-24	21	21	9
0	21	22	13
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	30	34	27
2	34	37	22
4	61	48	42
6	75	57	57
24	120	79	89
30	104	70	79
48	45	48	45
54	36	40	42
72	23	22	17
78	---[c]	---	---
96	18	19	17

[a]Glutamic oxaloacetic transaminase reported in Sigma-Frankel units.

[b]No determinations made.

[c]Tubes broken in centrifuge.

Table 33. Lactic dehydrogenase levels[a] of activity from swine inoculated with the endotoxin of _S. choleraesuis_ var. _kunzendorf_. Experiment III

Hours PI	Pig 705	Pig 706	Pig 707
-96	1060	1060	935
-72	1175	1030	1015
-48	990	700	735
-24	1160	700	820
0	820	685	700
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	895	850	880
2	770	700	720
4	1115	850	895
6	1115	865	1015
24	1305	990	1150
30	1070	770	1225
48	1030	925	1150
54	1100	755	1140
72	925	735	895
78	---[c]	---	---
96	990	755	895

[a]Lactic dehydrogenase reported in Berger-Broida (B-B) units.

[b]No determinations made.

[c]Tubes broken in centrifuge.

activity occurred 24 hours PI in 2 pigs (705 and 706) and at 30 hours PI in pig 707. From 48 through 96 hours PI, the LDH levels slowly decreased, but did not return to the values obtained at the time of inoculation.

Differential leukocyte counts The differential leukocyte counts on blood smears from pig 705 are listed in Table 34. Pre-inoculation blood smears following surgery disclosed the neutrophils to be more numerous than the lymphocytes. Lymphocytes increased and the neutrophils decreased in numbers and were in the range of normal when inoculation occurred. Monocytes and basophils were within normal ranges, but the eosinophils were slightly above normal values. Little change was present at 15 minutes PI, but at 30 minutes PI there was a drastic change. A leukopenia was striking. Lymphocytes were about the only leukocytes present on the blood smears. Segmented neutrophils were practically gone from the circulating blood. The differential WBC count did not change much from 30 to 60 minutes PI, but by 2 hours PI a drastic shift to the left was evident. A few segmented neutrophils were also observed. From 2 hours PI through 6 hours PI, the shift to the left was spectacular with neutrophilic leukocytes being the predominant cell type. At 24 hours PI, the immature neutrophilic leukocytes were still the dominant cell types and a definite leukocytosis was present. The immature forms were primarily band cells and metamyelocytes, but also a few myelocytes were observed. By 48 hours PI, the segmented neutrophil

Table 34. Differential leukocyte counts[a] on blood smears from swine inoculated with the endotoxin of S. choleraesuis var. kunzendorf. Experiment III

Pig 705

Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	87	6	105	1	0	0	1	0
-72	97	8	87	0	0	0	8	0
-48	106	4	80	1	0	0	7	2
-24	133	4	59	1	0	0	2	1
0	120	5	61	3	0	0	11	0
15 min.	127	1	64	1	0	0	6	1
30 min.	193	1	3	0	0	0	2	1
45 min.	177	3	5	5	0	0	10	0
1	183	2	2	4	0	0	8	1
2	180	3	10	3	1	0	2	1
4	168	4	14	8	0	0	6	0
6	136	2	20	36	2	0	2	2
24	39	6	52	84	16	3	0	0
30	19	2	71	82	19	7	0	0
48	29	2	99	51	4	1	13	1
54	36	2	126	31	2	0	2	1
72	76	5	90	11	1	0	17	0
78	92	3	77	8	0	0	20	0
96	121	7	61	2	0	0	8	1

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

was the most numerous, but a few immature forms were present. A return toward normal was evident by 72 hours PI as the lymphocytes again outnumbered the neutrophilic leukocytes. At 96 hours PI, the differential WBC counts were back to pre-inoculation values.

The results of differential WBC counts on blood smears from pig 706 are listed in Table 35. The results were similar to those described for pig 705. The lymphocyte count did not go quite as high in pig 706 as in pig 705, but there were no neutrophilic leukocytes present at 30 minutes PI in pig 706. The leukopenia and shift to the left was every bit as drastic however between 1 and 6 hours PI as in pig 705. From 24 to 48 hours PI, a leukocytosis was present with mature and immature forms of neutrophils predominating. The differential WBC counts for pig 707 are listed in Table 36. The differential counts were similar to pigs 705 and 706. The counts were similar not only in numbers but also in times when the changes occurred.

The absolute differential leukocyte counts for pigs 705, 706 and 707 are listed in Tables 37, 38 and 39 respectively. The absolute counts did not reveal significant variations from the relative differential leukocyte counts.

Table 35. Differential leukocyte counts[a] on blood smears from swine inoculated with the endotoxin of *S. choleraesuis* var. *kunzendorf*. Experiment III

Pig 706

Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	76	5	114	5	0	0	0	0
-72	132	8	50	3	0	0	4	3
-48	138	6	40	6	0	0	9	1
-24	152	6	34	2	0	0	2	4
0	134	5	51	6	0	0	2	2
15 min.	130	4	63	0	0	0	1	2
30 min.	188	4	0	0	0	0	4	4
45 min.	176	2	11	5	0	0	4	2
1	178	4	6	5	0	0	4	3
2	165	2	14	10	0	0	7	2
4	143	0	28	25	1	0	2	1
6	99	0	46	48	3	1	1	2
24	39	1	55	87	13	4	1	0
30	40	1	81	63	7	3	4	1
48	29	4	131	24	2	2	6	2
54	41	1	141	13	1	0	3	0
72	103	4	77	6	2	0	5	3
78	127	1	60	5	0	0	6	1
96	126	7	58	1	0	0	7	1

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

Table 36. Differential leukocyte counts[a] on blood smears from swine inoculated with the endotoxin of S. choleraesuis var. kunzendorf. Experiment III

			Pig 707					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	111	4	81	4	0	0	0	0
-72	120	2	69	0	0	0	9	0
-48	116	2	74	0	0	0	8	0
-24	125	3	65	0	0	0	4	3
0	114	1	75	0	0	0	8	2
15 min.	121	1	74	0	0	0	3	1
30 min.	190	0	4	0	0	0	6	0
45 min.	187	0	5	2	0	0	1	5
1	177	2	15	0	0	0	5	1
2	194	0	2	2	0	0	0	2
4	181	0	7	0	0	0	6	6
6	154	2	30	9	0	0	0	5
24	25	1	34	113	22	5	0	0
30	33	1	49	94	20	3	0	0
48	19	3	104	64	7	1	2	0
54	30	5	113	48	2	0	2	0
72	57	7	112	16	1	0	7	0
78	86	6	94	5	0	0	8	1
96	123	5	55	4	0	0	11	2

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

Table 37. Absolute differential leukocyte counts[a] from blood smears from swine inoculated with the endotoxin of S. choleraesuis var. kunzendorf. Experiment III

			Pig 705					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	6351	438	7665	73	0	0	73	0
-72	6160	508	5524	0	0	0	508	0
-48	6678	252	5040	63	0	0	441	126
-24	7382	222	3274	56	0	0	111	55
0	7500	313	3812	188	0	0	687	0
15 min.	6985	55	3520	55	0	0	330	55
30 min.	4513	23	71	0	0	0	47	23
45 min.	4123	70	116	116	0	0	233	0
1	3900	43	44	84	0	0	170	21
2	1490	25	83	25	8	0	17	8
4	1066	25	89	51	0	0	38	0
6	1058	16	156	280	16	0	16	16
24	3939	606	5252	8484	1616	303	0	0
30	2755	290	10295	11890	2755	1015	0	0
48	5612	387	19156	9869	774	193	2516	193
54	6786	377	23751	5844	377	0	377	188
72	6384	420	7560	924	84	0	1428	0
78	6992	228	5852	608	0	0	1520	0
96	6292	364	3172	104	0	0	416	52

[a]Absolute differential leukocyte counts expressed in numbers per cubic mm. of blood.

Table 38. Absolute differential leukocyte counts[a] from blood smears from swine inoculated with the endotoxin of *S. choleraesuis* var. *kunzendorf*. Experiment III

Pig 706

Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	5586	368	8379	367	0	0	0	0
-72	10164	616	3850	231	0	0	308	231
-48	10074	438	2920	438	0	0	657	73
-24	10336	408	2312	136	0	0	136	272
0	9313	348	3544	417	0	0	139	139
15 min.	8190	252	3969	0	0	0	63	126
30 min.	7293	155	0	0	0	0	155	155
45 min.	7553	85	472	215	0	0	172	86
1	5397	121	182	152	0	0	121	91
2	2625	32	223	159	0	0	111	32
4	1812	0	355	317	13	0	25	12
6	1689	0	785	819	51	17	17	34
24	5031	129	7095	11223	1677	516	129	0
30	6580	165	13324	10364	1151	493	658	165
48	5989	826	27051	4956	413	413	1239	413
54	7524	183	25874	2385	183	0	551	0
72	9116	354	6814	531	177	0	443	265
78	9843	77	4650	388	0	0	465	77
96	8757	487	4031	69	0	0	487	69

[a]Absolute differential leukocyte counts expressed in numbers per cubic mm. of blood.

Table 39. Absolute differential leukocyte counts[a] from blood smears from swine inoculated with the endotoxin of S. choleraesuis var. kunzendorf. Experiment III

			Pig 707					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	6993	252	5103	252	0	0	0	0
-72	9000	150	5175	0	0	0	675	0
-48	8120	140	5180	0	0	0	560	0
-24	8500	204	4420	0	0	0	272	204
0	5985	53	3937	0	0	0	420	105
15 min.	6897	57	4218	0	0	0	171	57
30 min.	4636	0	98	0	0	0	146	0
45 min.	5026	0	134	54	0	0	27	134
1	5069	57	430	0	0	0	143	29
2	2911	0	30	30	0	0	0	30
4	970	0	37	0	0	0	32	32
6	1101	14	215	64	0	0	0	36
24	2988	119	4063	13504	2629	597	0	0
30	4703	142	6983	13395	2850	427	0	0
48	5073	801	27768	17088	1869	267	534	0
54	8220	1370	30962	13152	548	0	548	0
72	5985	735	11760	1680	105	0	735	0
78	8256	576	9024	480	0	0	768	96
96	9102	370	4070	296	0	0	814	148

[a]Absolute differential leukocyte counts expressed in numbers per cubic mm. of blood.

Necropsy findings

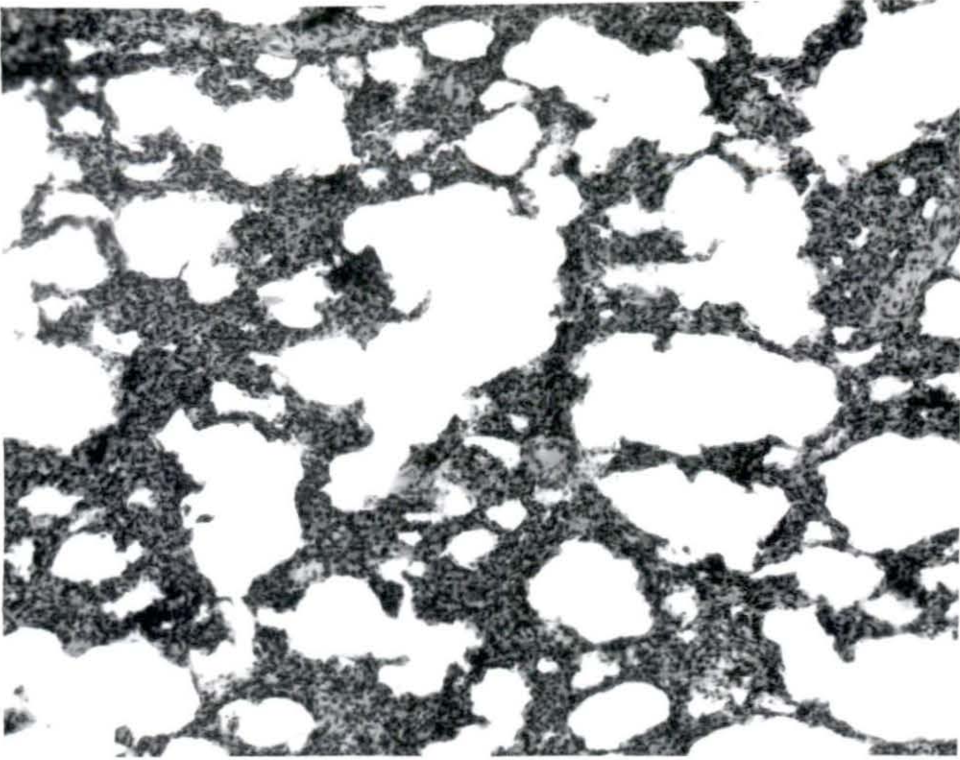
Gross lesions The only external changes observed in the pigs were dehydration and loss of condition. The internal lesions were minimal with a few subpleural petechial hemorrhages in the lungs. Large renal infarcts were again present in both kidneys of all 3 pigs as in Experiments I and II. The lymph nodes were soft and slightly edematous but otherwise normal.

Microscopic lesions The lungs contained small focal areas of hemorrhage (Figure 29). The alveolar walls were infiltrated with lymphocytes, plasma cells and macrophages. There was slight edema of the interstitial connective tissue (Figure 30). The cellular infiltrations in the alveolar walls compressed the alveolar lumina, many of which were filled with homogenous eosinophilic staining material (Figure 31). The livers had numerous focal collections of lymphocytes and macrophages (Figure 32) and necrotic lesions similar to those found in Experiment I (Figures 33 and 34). The liver lesions in this experiment were not as numerous as in Experiment I.

The heart of pig 707 had a mild endocarditis and a lymphadenitis was present in several of the lymph nodes. The subcapsular space and peritrabecular spaces contained many neutrophils. No definite abscesses were present. These lesions were present in only this pig (707) and not in pigs 705 or 706.

Figure 29. Experiment III, 4 days PI. Lung. The alveolar walls contain diffuse infiltrations of mononuclear leukocytes. H&E stain; 200 X

Figure 30. Experiment III, 96 hours PI. Lung. The alveolar lumina are greatly reduced in size and the walls are diffusely infiltrated with mononuclear leukocytes. The interlobular septum is slight edematous. H&E stain; 80 X



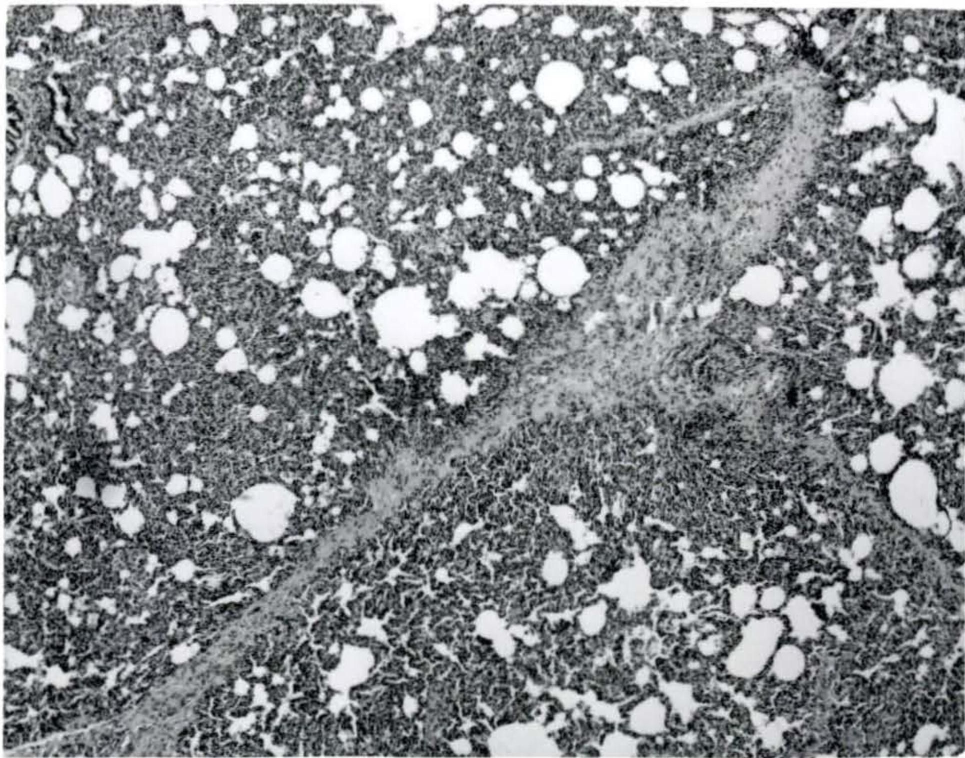
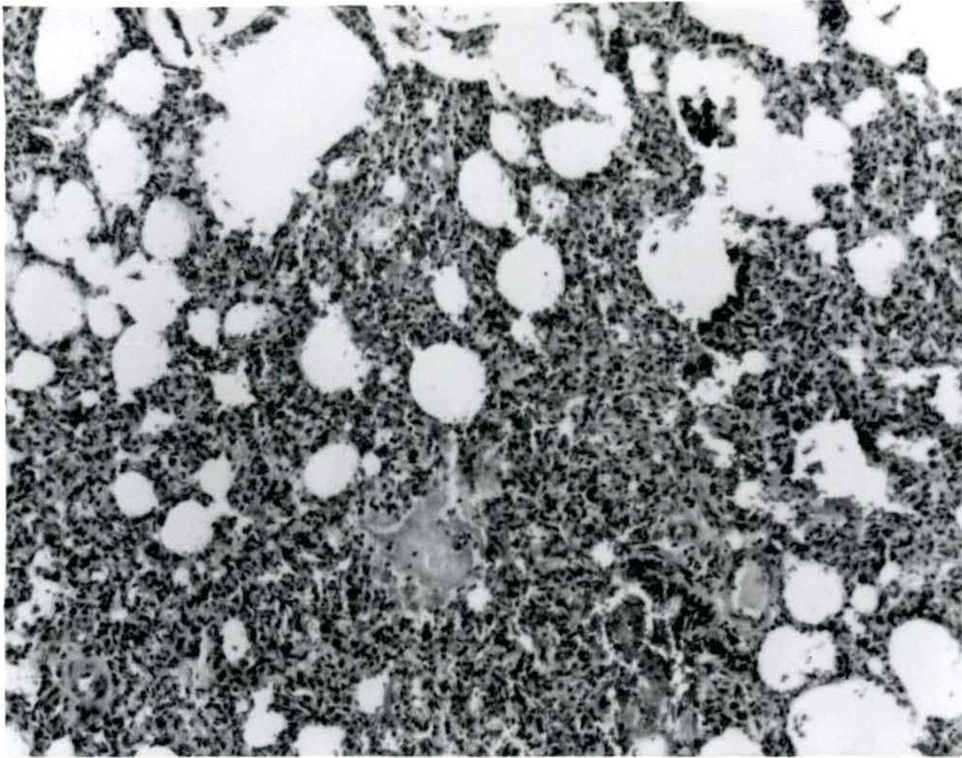


Figure 31. Experiment III, 96 hours PI. Lung. The alveolar walls are diffusely infiltrated with mononuclear leukocytes. Homogenous eosinophilic material and scattered erythrocytes are present in a few alveolar lumina. H&E stain; 200 X

Figure 32. Experiment III, 96 hours PI. Liver. Mononuclear leukocytes are present at the peripheral margins of a hepatic lobule. H&E stain; 200 X



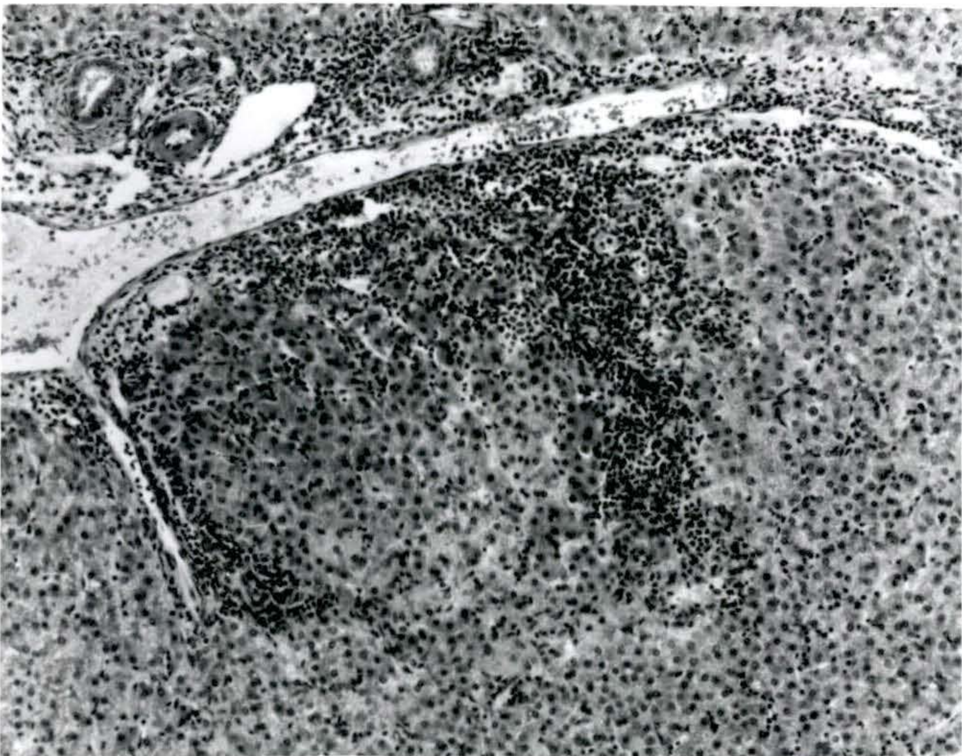
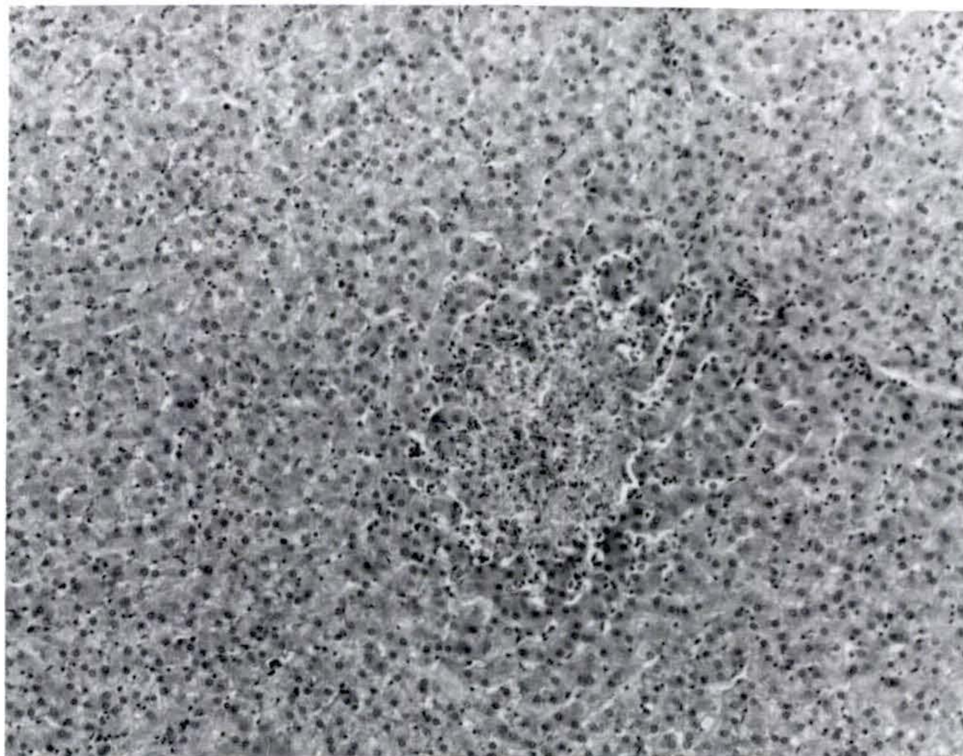


Figure 33. Experiment III, 96 hours PI. Liver. A focus of coagulation necrosis is present in the midzonal portion of the lobule. The necrotic area contains numerous neutrophils, macrophages and lymphocytes. H&E stain; 200 X

Figure 34. Experiment III, 96 hours PI. Liver. The focus of necrosis contains fewer leukocytes than Figure 33. Note the prominent Kupffer cells in the parenchyma surrounding the necrotic focus. H&E stain; 200 X



Cultural and FA results

The tissues were negative for hog cholera virus on both the FATST and the FATCT diagnostic tests.

The tissues were cultured for the presence of salmonellae and were negative. When the lesions were found in the heart and lymph nodes on histopathology, the heart, lymph nodes, spleen and liver were again cultured. Streptococcus sp. was isolated but no attempt was made to identify the species. Very few colonies were present on the culture plates.

Swine Controls

Clinical observations

Following inoculation with sterile saline, the pigs were eating within 1 hour PI. The incision in pig 577 became inflamed where the catheter emerged to attach to the catheter stopcock. Although a germicide was applied at the time of each bleeding, the inflammation extended deeper than could be reached by the germicide. The catheter acted as a wick as it moved slightly in and out as the pig walked. This movement occurred in other pigs also but inflammation did not occur. The inflammation was apparently accompanied by pruritus as the pig was observed to rub the area on any protruding object in the pen or against the wall. When blood was collected and the catheter valve handled, the pig tried to rub and it was difficult to hold the stopcock and collect the blood.

No clinical signs were observed during the course of the experiment which was concluded at 96 hours PI.

The body temperatures are listed in Table 40. Pig 577 had an elevated temperature which was attributed to infection in the external catheter wound.

Hematology

Erythrocyte sedimentation rate   The ESR values for the control pigs are listed in Table 41. All ESRs were within the range of normal when inoculation with saline occurred. Two of the pigs (575 and 576) remained in the normal range but pig 577 had a slightly elevated ESR.

Packed cell volume   The PCV results for the control pigs are listed in Table 42. The PCVs decreased slightly in all 3 pigs during the course of the experiment. No sudden or drastic changes were detected at any specific time following inoculation with saline.

Total red blood cell counts   The TRBC counts for the control pigs are given in Table 43. The values were relatively stable in all 3 pigs but there were minor fluctuations. The TRBC values decreased slightly during the experiment but not significantly.

Total white blood cell counts   The TWBC counts are listed in Table 44. Minor changes were noted but no significant or abrupt changes were demonstrated during the course of the experiment. Pig 577 developed a slight leukocytosis

Table 40. Body temperatures[a] of catheterized swine inoculated with saline. Controls

Hours PI	Pig 575	Pig 576	Pig 577
-120	103.4	102.6	103.4
-96	102.2	101.4	103.6
-72	101.0	103.6	102.0
-48	103.4	102.4	103.0
-24	103.2	103.0	101.8
0	102.4	101.4	102.0
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	101.4	103.0	103.8
2	101.7	102.5	104.0
4	103.4	103.0	103.5
6	103.5	103.0	103.4
24	102.0	101.6	103.4
30	102.8	102.6	104.0
48	101.8	102.6	103.0
54	102.8	102.6	103.2
72	102.4	101.4	104.6
78	102.8	102.4	106.0
96	101.8	101.6	102.8

[a]Temperatures recorded in degrees Fahrenheit.

[b]No temperatures taken.

Table 41. Erythrocyte sedimentation rates[a] of blood from catheterized swine inoculated with saline. Controls

Hours PI	Pig 575	Pig 576	Pig 577
-120	4.0	9.0	8.0
-96	11.0	6.0	6.0
-72	8.0	6.5	4.5
-48	12.0	14.0	7.0
-24	4.0	9.5	4.0
0	3.0	6.0	4.0
15 min.	2.0	6.0	3.5
30 min.	1.0	7.5	4.0
45 min.	1.5	3.0	3.0
1	2.0	5.5	4.0
2	1.0	3.0	2.0
4	2.0	5.5	9.0
6	3.0	5.0	11.0
24	5.5	6.0	7.0
30	3.5	5.5	5.0
48	6.0	5.0	4.0
54	2.5	4.5	4.0
72	4.5	3.0	9.0
78	4.0	6.0	15.0
96	7.0	6.0	11.5

[a]Erythrocyte sedimentation rate results in mm./hr.

Table 42. Packed cell volumes[a] of blood from catheterized swine inoculated with saline. Controls

Hours PI	Pig 575	Pig 576	Pig 577
-120	32.0	29.0	31.0
-96	30.0	32.0	34.5
-72	31.5	29.0	32.5
-48	29.0	30.0	32.0
-24	33.0	32.5	34.5
0	29.5	31.5	34.5
15 min.	30.0	30.0	34.0
30 min.	29.5	29.5	34.0
45 min.	30.5	32.0	35.0
1	30.0	31.5	33.0
2	31.0	30.0	32.0
4	29.5	28.0	29.5
6	29.5	29.0	32.0
24	27.5	31.0	29.5
30	28.0	31.0	32.0
48	28.5	33.0	32.5
54	28.5	31.5	31.5
72	27.5	29.0	27.0
78	28.0	28.0	28.0
96	26.5	29.5	28.0

[a]Packed cell volume reported as percentage.

Table 43. Total red blood cell counts[a] on blood from catheterized swine inoculated with saline. Controls

Hours PI	Pig 575	Pig 576	Pig 577
-120	590	526	580
-96	581	612	672
-72	606	561	622
-48	595	591	673
-24	639	646	733
0	570	602	654
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	552	600	631
2	576	556	584
4	548	491	550
6	548	542	620
24	499	577	531
30	537	559	596
48	529	603	631
54	548	615	591
72	454	501	481
78	491	523	527
96	501	550	515

[a]Red blood cells reported as number of cells X 10^4/cu. mm.
[b]TRBC counts not conducted on these bleedings.

Table 44. Total white blood cell counts[a] on blood from catheterized swine inoculated with saline. Controls

Hours PI	Pig 575	Pig 576	Pig 577
-120	18,700	10,400	12,000
-96	19,200	12,500	15,800
-72	16,700	13,000	16,400
-48	19,400	12,900	16,300
-24	17,900	10,900	14,700
0	26,300	16,200	17,500
15 min.	26,100	14,600	20,700
30 min.	25,900	15,000	19,900
45 min.	27,200	15,700	20,900
1	26,700	14,900	20,200
2	23,300	15,000	20,400
4	21,200	16,100	21,300
6	23,500	16,100	24,200
24	18,700	13,100	19,300
30	19,600	14,300	19,000
48	19,300	12,900	17,500
54	19,600	16,700	17,200
72	20,700	11,100	24,400
78	20,600	13,900	29,900
96	19,600	14,700	23,900

[a]White blood cell counts reported in number of cells/cmm.

between 72 and 96 hours PI.

Serum glutamic oxaloacetic transaminase The SGOT levels of activity from the control pigs are listed in Table 45. Pig 575 had a high postsurgery SGOT level but it dropped rapidly prior to inoculation with saline. The SGOT levels were essentially normal for the remainder of the experiment which was concluded at 96 hours PI.

Lactic dehydrogenase The LDH levels of activity in the serum of the control pigs are listed in Table 46. Pig 575 had a high LDH reading following surgery but it decreased slowly until inoculation with saline. The LDH values for the other 2 pigs (576 and 577) were relatively stable for the duration of the experiment.

Differential leukocyte counts The differential leukocyte counts for pigs 575, 576 and 577 are listed in Tables 47, 48 and 49 respectively. The numbers of neutrophils are increased the first 24 hours PI. Lymphocytes became the most numerous cell types in 2 of the pigs (575 and 576) from 24 to 96 hours PI, but the neutrophil count remained elevated in pig 577 through 96 hours PI when the experiment was concluded.

Absolute differential leukocyte counts are listed in Tables 50, 51 and 52 for control pigs 575, 576 and 577 respectively. The absolute values did not reveal significant differences from the relative differential counts.

Table 45. Serum glutamic oxaloacetic transaminase[a] levels of activity from catheterized swine inoculated with saline. Controls

Hours PI	Pig 575	Pig 576	Pig 577
-120	130	31	54
-96	65	25	53
-72	37	33	39
-48	22	41	34
-24	25	32	34
0	29	22	40
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	25	30	51
2	27	36	48
4	29	34	51
6	25	32	51
24	19	34	40
30	45	44	52
48	28	27	48
54	28	28	56
72	30	22	51
78	20	23	40
96	10	20	34

[a]Glutamic oxaloacetic transaminase reported in Sigma-Frankel units.

[b]No determinations made.

Table 46. Lactic dehydrogenase[a] levels of activity in serum of catheterized swine inoculated with saline. Controls

Hours PI	Pig 575	Pig 576	Pig 577
-120	1250	920	1245
-96	1425	1085	1455
-72	1055	1080	1280
-48	835	1100	1015
-24	910	1235	1085
0	865	1085	1060
15 min.	...[b]	...	...
30 min.	...	...	...
45 min.	...	...	...
1	820	1005	1185
2	755	1055	1100
4	805	1085	1175
6	880	1015	1200
24	735	1100	1030
30	685	950	1035
48	720	1040	1085
54	655	1100	1045
72	710	990	1100
78	605	1015	865
96	665	1570	895

[a]Lactic dehydrogenase reported in Berger-Broida (B-B) units.

[b]No determinations made.

Table 47. Differential leukocyte counts[a] on blood smears prepared from catheterized swine inoculated with saline. Controls

			Pig 575					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	115	5	78	0	0	0	2	0
-72	146	4	44	1	0	0	5	0
-48	119	5	70	0	0	0	4	2
-24	123	4	68	2	0	0	2	1
0	120	6	68	1	0	0	4	1
15 min.	115	2	75	0	0	0	6	2
30 min.	118	3	72	2	0	0	4	1
45 min.	104	4	89	3	0	0	0	0
1	106	4	80	4	0	0	4	2
2	91	4	102	2	0	0	1	0
4	96	5	86	9	0	0	3	1
6	94	3	97	3	0	0	3	0
24	113	4	79	2	0	0	0	2
30	114	6	74	0	0	0	4	2
48	120	7	73	0	0	0	0	0
54	136	2	56	2	0	0	2	2
72	113	2	77	2	0	0	4	2
78	109	3	78	0	0	0	6	4
96	126	4	65	1	0	0	2	2

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

Table 48. Differential leukocyte counts[a] on blood smears prepared from catheterized swine inoculated with saline. Controls

Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
			Pig 576					
-96	144	5	48	0	0	0	3	0
-72	133	6	55	2	0	0	4	0
-48	136	3	57	2	0	0	2	0
-24	136	2	58	0	0	0	2	2
0	134	4	55	0	0	0	4	3
15 min.	133	2	63	1	0	0	0	1
30 min.	110	7	78	1	0	0	3	1
45 min.	124	1	71	2	0	0	2	0
1	103	4	93	0	0	0	0	0
2	94	4	100	1	0	0	0	1
4	74	3	117	2	0	0	2	2
6	109	3	84	1	0	0	3	0
24	116	6	69	0	0	0	9	0
30	111	5	72	0	0	0	10	2
48	123	2	68	0	0	0	4	3
54	132	2	63	0	0	0	3	0
72	140	2	52	0	0	0	5	1
78	124	1	70	0	0	0	3	2
96	127	6	63	0	0	0	0	4

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

Table 49. Differential leukocyte counts[a] on blood smears prepared from catheterized swine inoculated with saline. Controls

			Pig 577					
Hours PI	Lymph	Mono	Segs	Bands	Meta	Myl	Eosino	Baso
-96	117	3	75	2	0	0	3	0
-72	128	4	61	0	0	0	5	2
-48	122	6	57	3	0	0	9	3
-24	127	4	57	2	0	0	10	0
0	116	7	67	0	0	0	8	2
15 min.	123	3	68	1	0	0	4	1
30 min.	116	4	72	2	0	0	3	3
45 min.	92	5	95	3	0	0	4	1
1	109	2	88	1	0	0	0	0
2	122	2	76	0	0	0	0	0
4	123	0	75	0	0	0	0	2
6	68	4	124	3	0	0	1	0
24	91	3	100	1	0	0	3	2
30	97	6	91	0	0	0	4	2
48	110	2	84	0	0	0	3	1
54	92	5	91	1	0	0	8	3
72	70	4	120	4	0	0	0	2
78	57	4	129	3	0	0	5	2
96	74	3	114	0	0	0	7	2

[a]Differential leukocyte counts expressed in percentage of 100 cells; 200 cells counted, therefore, $\frac{\text{number of cells}}{2}$ = percentage.

Table 50. Absolute differential leukocyte counts[a] on blood smears prepared from catheterized swine inoculated with saline. Controls

		Pig 575				
Hours PI	Lymph	Mono	Segs	Bands	Eosino	Baso
-96	11040	480	7488	0	192	0
-72	12191	334	3674	83	418	0
-48	11543	485	6790	0	388	194
-24	11009	358	6086	179	179	89
0	15780	789	8942	132	526	131
15 min.	15008	261	9787	0	783	261
30 min.	15281	389	9324	259	518	129
45 min.	14144	544	12104	408	0	0
1	14151	534	10680	534	534	267
2	10602	466	11883	233	116	0
4	10176	530	9116	954	318	106
6	11045	353	11398	352	352	0
24	10566	374	7386	187	0	187
30	11172	588	7252	0	392	196
48	11580	676	7044	0	0	0
54	13328	196	5488	196	196	196
72	11696	207	7969	207	414	207
78	11227	309	8034	0	618	412
96	12348	392	6370	98	196	196

[a]Absolute TWBC differential counts expressed in numbers per cubic mm. of blood.

Table 51. Absolute differential leukocyte counts[a] on blood smears prepared from catheterized swine inoculated with saline. Controls

		Pig 576				
Hours PI	Lymph	Mono	Segs	Bands	Eosino	Baso
-96	9000	313	3000	0	187	0
-72	8645	390	3575	130	260	0
-48	8772	194	3676	129	129	0
-24	7412	109	3161	0	109	109
0	10854	324	4455	0	324	243
15 min.	9709	146	4599	73	0	73
30 min.	8250	525	5850	75	225	75
45 min.	9734	79	5573	157	157	0
1	7674	298	6928	0	0	0
2	7050	300	7500	75	0	75
4	5957	242	9418	161	161	161
6	8775	242	6762	80	241	0
24	7598	393	4520	0	589	0
30	7937	357	5148	0	715	143
48	7934	129	4386	0	258	193
54	11022	167	5261	0	250	0
72	7770	111	2886	0	278	55
78	8618	70	4865	0	208	139
96	9335	441	4630	0	0	294

[a]Absolute TWBC differential counts expressed in numbers per cubic mm. of blood.

Table 52. Absolute differential leukocyte counts[a] on blood smears prepared from catheterized swine inoculated with saline. Controls

Pig 577

Hours PI	Lymph	Mono	Segs	Bands	Eosino	Baso
-96	9243	237	5925	158	237	0
-72	10496	328	5002	0	410	164
-48	9943	489	4646	244	734	244
-24	9335	294	4189	147	735	0
0	10150	613	5862	0	700	175
15 min.	12731	310	7038	104	414	103
30 min.	11542	398	7164	199	299	298
45 min.	9614	523	9927	314	418	104
1	11009	202	8888	101	0	0
2	12444	204	7752	0	0	0
4	13100	0	7987	0	0	213
6	8228	484	15004	363	121	0
24	8782	289	9650	97	289	193
30	9215	570	8645	0	380	190
48	9625	175	7350	0	263	87
54	7912	430	7826	86	688	258
72	8540	488	14640	488	0	244
78	8522	598	19285	449	747	299
96	8843	359	13623	0	836	239

[a]Absolute TWBC differential counts expressed in numbers per cubic mm. of blood.

Necropsy findings

Gross lesions   Renal infarcts were present in all 3 pigs. No other lesions were observed. Pig 577 had an abscess in the subcutaneous tissue where the catheter emerged from the back. The purulent exudate followed the catheter a few centimeters into the subcutaneous fat. No other changes were observed.

Microscopic lesions   The renal infarcts were similar to those described in Experiments I, II and III. No other lesions were present.

Cultural and FA results

The FA tests were negative for both HC viral antigen and salmonellae. The cultural attempts to isolate salmonellae were also negative.

DISCUSSION

Clinical Observations

The clinical responses resulting from inoculation with live salmonellae, killed salmonellae and extracted endotoxin all produced similar clinical signs in pigs, but varied in their intensity and duration. The clinical signs included elevated temperature, vomiting, anorexia, depression, and transient diarrhea followed by constipation. The progression of clinical signs appeared to be due to the effects of endotoxin and the severity was directly proportional to the endotoxin concentration.

Clinical signs were not observed in pigs inoculated with live salmonellae for several hours PI. It was necessary for the organisms to multiply in the body of the pig and be destroyed by the defense mechanism to liberate enough endotoxin to produce clinical signs. The effects of endotoxin were observed much sooner in pigs inoculated with the killed, washed cell suspension of salmonellae. Large numbers of cells were inoculated, but were washed to be free of any endotoxin in the media prior to inoculation. The endotoxin was a component of the bacterial cell wall and was liberated by macrophages and leukocytes as they phagocytized the foreign particulate matter from the blood. The clinical signs began to decrease after the cell walls were destroyed and the endotoxin detoxified. When extracted endotoxin was

inoculated the clinical response was immediate. One pig went into shock but recovered. The effects of the endotoxin were severe but did not persist.

The temperature response in all 3 experiments indicated that endotoxin was a pyrogenic substance. Jawetz et al. (1970) believes the pyrogen is released from leukocytes that have been damaged or destroyed by the endotoxin. Beeson (1947) found endotoxin altered the glycolytic activity of the leukocytes and caused the liberation of leukocytic pyrogens. Braude (1964) attributes the pyrogenic effect to direct action of the endotoxin on the thermal control centers in the hypothalamus. He also believed the elevated temperature was due to slowing of the peripheral blood, particularly through the skin, which did not allow excess body heat to dissipate through radiation. The findings in this study were best explained on the basis of the pyrogen being released from damaged leukocytes, as the highest temperature recorded in each case coincided with the maximum leukopenia.

The autonomic nervous system appeared to be affected by endotoxin. Clinical signs of vomiting, exaggerated peristalsis and profuse salivation were all signs consistent with parasympathetic stimulation. These signs were present in all 3 experiments, but were most severe in pigs inoculated with endotoxin. Coulter and Swenson (1968) mentioned emesis as a clinical sign in their work with pigs and endotoxins. The action of endotoxin on the parasympathetic nerves is

not known. The endotoxin may stimulate the parasympathetic nerves or it may mimic their reaction.

Cyanosis of the skin is usually described in acute salmonellosis of swine (Jubb and Kennedy, 1970; Sorensen, 1970). Cyanosis was not observed in the skins of pigs in this study, but they did not receive enough inoculum to result in death. Cyanosis of the skin is usually seen in terminal cases of salmonellosis, and results from sludging of peripheral blood and hypostatic congestion of the cutaneous vessels. Cyanosis is compatible with an unfavorable prognosis. Pigs which received live, virulent organisms were given enough of the inoculum to produce the desired clinical response of acute salmonellosis but not enough to cause death. Lesions were just part of this study with hematologic changes being the other part. The interests of this study were best served by not causing deaths and allowing the clinical course of the disease to be studied.

The pigs inoculated with live organisms were observed to start bleeding spontaneously from surgical wounds from 48 to 72 hours PI. This time corresponded with the maximum effect of endotoxin resulting from inoculation with live organisms. Pigs inoculated with endotoxin started bleeding around the surgical wounds and from the anus at about 6 hours PI. This time corresponded with the maximum effect of endotoxin in the endotoxin inoculated pigs. The clotting mechanism of the blood was definitely affected by the endotoxin.

Milner et al. (1971) attributed the reduced ability of the blood to clot to destruction of thrombocytes. He believes endotoxin causes a clumping of the thrombocytes and that it increases their osmotic fragility resulting in a thrombocytopenia. If this is true, it might appear a total thrombocyte count would be beneficial to detect the presence of endotoxins. Thrombocyte counts were not conducted in this study, however, as it is very difficult to do accurate counts and the margin of error is quite large even with technicians who have a great deal of proficiency in doing these counts. In addition, a thrombocytopenia would not be a good point of differential diagnosis between salmonellosis and hog cholera as a thrombocytopenia is also a consistent finding in hog cholera (Dunne, 1970). In hog cholera the thrombocytes are removed from the circulating blood by adhering to the roughened intima of the vasculature which is a result of damage to the endothelial cells by the virus.

Using clinical signs as a basis for the diagnosis or for the differential diagnosis of acute salmonellosis would be very difficult. Astute observations of clinical signs coupled with hematologic and pathologic changes are all necessary for the diagnosis of salmonellosis. Laboratory confirmation would still be necessary in most cases to confirm the presumptive diagnosis.

What happens when large quantities of antibiotics are given to pigs with acute salmonellosis? This study proves

definite intensification of clinical signs when large numbers of killed salmonellae are released into the general circulation. This additional stress may be sufficient to cause deaths in pigs that might have otherwise survived with no treatment. Abernathy and Spink (1958) while working with *Brucella abortus* in humans found a definite increase in temperature following treatment with antibiotics. They attributed the rise in temperature to the action of endotoxin released from the killed *Brucella* organisms.

The indwelling arterial catheters served their purpose very well, as handling the pigs for the blood collections did not cause any significant elevations of temperatures or other undesirable clinical changes. The control animals did not become hyperthermic at any time during the experiments.

Hematology

Erythrocyte sedimentation rates have not been studied in porcine salmonellosis as a sequential study during the course of the disease. The ESRs are good hematologic tests to determine the general state of health in pigs. Erythrocytes in the blood of pigs have a tendency for rouleau formation and this fact results in a moderately fast sedimentation rate. The state of health of the pig affects the colloidal osmotic pressure exerted against the erythrocytes and the ESR will vary making it a valuable test in hematologic

studies. Schalm (1965) lists the average normals for both sexes of pigs of the Duroc-Jersey crossbreeds as 2.6 mm. of sedimentation of erythrocytes in 1 hour (Wintrobe method). Many factors influence these determinations but mainly the number and maturity of erythrocytes and the buoyant density of the plasma. The ESR is naturally variable in pigs and sometimes quite high results are expected.

The pigs in this study were not normal pigs as they were surgically prepared for frequent blood collections by inserting indwelling arterial catheters. Following surgery the ESRs were elevated but decreased after a few days recovery. The ESRs never became stabilized to a point that normal values could be established. Each pig reacted differently to surgery and the ESRs did not all reach the same levels prior to inoculation.

All 3 inoculations produced definte changes in the ESRs. The live and killed organisms produced changes as early as 2 hours PI, and the values nearly doubled by 4 hours PI. The endotoxin inoculated pigs did not have elevated ESRs until 6 hours PI. The cause of this delayed response is not known. The endotoxin reaction evidently resulted in capillary plugging in the microcirculation of the parenchymatous organs and skin, keeping the serum and plasma proteins in suspension and maintaining constant buoyant density. Hemoconcentration would also keep the ESRs from increasing, but no evidence of this change occurred as the total erythro-

cyte counts and the PCVs did not increase.

Packed cell volume determinations are also important in hematologic studies to detect anemias, hemoconcentrations and to assess the extent of damage to erythrocytes in hemolytic diseases. Schalm (1965) gives the ranges of normal values on the sex and age of pigs used in these studies as a minimum of 38 and a maximum of 44 with an average as 40 volume percent. Weide and Twiehaus (1959) reported the PCV values on 12 control pigs, from 10 to 94 days of age, with a range of 20.4 to 32.9 volume percent. There is an obvious wide range of normals and many factors influence the so-called normals. Factors such as breed, sex, age, state of health, state of nutrition and many other environmental and physical conditions can affect PCVs.

Pigs used in these studies had pre-inoculation PCVs that ranged from 27.0 to 40.0 between surgery and inoculation. The PCV values varied considerably in each pig. After inoculation with live and killed salmonellae very little effect was noticed, but a large rise was detected 15 to 30 minutes after inoculation with endotoxin. This change was probably due to contraction of the spleen as a result of endotoxin stimulation or sensitization to the action of adrenalin. The spleen is an organ with considerable smooth muscle contained in the capsule and interstitial trabeculae. Zweifach (1964) stated that endotoxin sensitizes smooth muscle to become hyperreactive to the effects of adrenalin.

The PCV values in all pigs including the controls declined throughout the hematologic studies of each experiment. This may have been in part due to loss of blood resulting from the frequent collections. On the day of inoculation a pre-inoculation blood sample was collected. Following inoculation of the pigs, blood samples were collected 7 times in the next 6 hours. Approximately 16 ml. of blood was taken at each bleeding, amounting to 128 ml. on the first day. On subsequent days there were 2 blood collections amounting to 32 ml. of blood each day. This amount of blood loss was not excessive from an approximately 35 to 40 pound pig, but certainly was enough to affect the PCV values. The results of the PCVs in this study did not indicate that they were of much importance in the diagnosis of acute salmonellosis.

Total red blood cell counts were similar among the groups of pigs and nearly paralleled the results of the PCVs. Schalm (1965) listed the normal range for pigs as 5 million erythrocytes per cmm. of blood as a minimum to 8 million as the maximum with an average of 6.5 million. Braude (1964) states that some 4 hours after inoculation with endotoxin there was generally an outpouring of RBCs into the circulation from reserve supplies throughout the body. This study did not disclose such an increase at any time postinoculation with the exception of the PCV increase which followed inoculation with endotoxin and caused contraction of the spleen. He also stated that many erythro-

cytes are immature forms, but in this study only a few nucleated erythrocytes were noted at 24 and 48 hours PI.

Generally in an acute condition, unless there is hemolysis, there are no sudden or drastic changes in the total erythrocyte counts because of the long life of the erythrocytes. Bush et al. (1955) using C_{14} labeling determined the mean life span for the erythrocytes as 62 days. Jensen et al. (1956) using Fe_{59} found the mean life span as 63 days. An insult to the hematopoietic system is not evident for several days after injury. As with the findings in the PCVs, the total erythrocyte count does not appear to be important for the diagnosis of salmonellosis as determined by this study.

Glutamic oxaloacetic transaminase is an enzyme present in most cells of the body. Serum GOT determinations are not organ specific tests to detect damage to any single organ. Muscle tissue is high in GOT content and serum GOT will become high if there is much muscle damage. The enzyme is usually found intracellular, therefore, a significant increase of this enzyme in the serum is a good indication of cellular destruction. Serum GOT levels of activity are also increased in the diseases of the liver if there is much hepatic necrosis as GOT is present in the hepatocytes in high levels. The SGOT determinations are not considered a specific test for liver destruction.

Increases in levels of activity of SGOT were apparent in all 3 experiments of this study. These determinations

were a good indication of tissue damage and the highest levels appeared to follow the maximum effects of the endotoxin. The effect of the endotoxin on the microcirculation throughout the body caused some tissue damage and resulted in an increase of the SGOT levels of activity.

Lactic dehydrogenase is an enzyme that catalyzes the reversible oxidation of lactic acid to pyruvic acid and is widely distributed in tissues of the body. In human medicine, patients with myocardial infarctions or malignant neoplasms have an increased amount of LDH activity.

There are 5 known isoenzymes of LDH that have been identified by electrophoresis; they are LD_1, LD_2, LD_3, LD_4 and LD_5. These isoenzymes of LDH have different rates of migration on electrophoresis, with LD_1 having the highest negative charge and migrates the farthest toward the anode. The purpose of isoenzyme studies is to identify the specific damaged organ or tissue. No isoenzyme studies were conducted during this study, but only total levels of LDH activity were determined.

The LDH levels of activity were quite variable during the course of this study. Surgery for arterial catheterization was important to consider in pre-inoculation normal LDH values as considerable tissue damage caused an elevation of LDH activity. At the time of inoculation with the different inoculums the LDH activity was beginning to stabilize or decrease.

There was no sudden or drastic change detected in LDH activity following inoculation. The pigs inoculated with live organisms did not reveal significant changes in LDH activity until late in the course of the disease and then doubled their pre-inoculation values. Tissue damage appeared to occur after the maximum effects of endotoxin and later in the course of the disease. If the pigs had progressed to death the LDH activity would probably have been significantly higher. LDH activity could be used to determine the prognosis. High LDH levels of activity would indicate much tissue damage and the prognosis would be unfavorable.

When extracted endotoxin was inoculated into pigs, a change in LDH activity was demonstrated as early as 4 hours PI, and reached the highest levels between 24 and 48 hours PI. Very little permanent tissue damage occurred as a response to the endotoxin. Endotoxin exerts its action mainly on the microcirculation. Although capillary circulation was impaired, extensive tissue death did not occur.

Total white blood cell counts are important in determining the state of health of animals. Not only is the total number of leukocytes important but also the cell type and stage of maturation. All these factors are involved in various disease conditions. Results of this study demonstrated that salmonellosis produced rather characteristic hematologic changes.

Following inoculation with both the live and killed

salmonellae, a marked leukopenia developed in the experimental pigs. When endotoxin was inoculated, a severe leukopenia soon followed. As the pigs began to recover, a leukocytosis was apparent. Athens *et al*. (1961) have done considerable work on granulocyte kinetics to explain the leukopenia following inoculation with endotoxin. He described the compartmentalization of the total body granulocyte pool (TBGP) into 2 sub-compartments, the circulating granulocyte pool (CGP) and the marginal granulocytic pool (MGP). Bacterial endotoxins were found to produce a shift of the cells from CGP to the MGP without an increase in the TBGP. Herion *et al*. (1965) found the granulocytopenia produced by endotoxins resulted in the granulocytes leaving the CGP and entering the MGP; most did not recirculate. Mulholland and Cluff (1964) attributed the leukopenia to be a result of damage to leukocytes by endotoxin. Affected leukocytes did not have the ability to migrate from the MGP back into the CGP by diapedesis. Braude (1964) stated that granulocytes are the first to leave the CGP followed by a gradual decline in lymphocytes. As the leukocytes disappear from the blood they appear in increasing numbers in the tissues of the lungs and other organs.

In this study the leukopenia persisted for 6 to 8 hours after inoculation with endotoxin followed by a leukocytosis. The endotoxin stimulated the bone marrow to start producing granulocytes. The mature cells held in reserves were re-

leased into the circulation and their leaving stimulated the bone marrow to start producing large numbers of granulocytes which were forced into circulation before they had a chance to mature. The result was that most of the circulating leukocytes during the leukocytosis were immature forms. The leukocytosis persisted for 48 to 72 hours after inoculation with endotoxin and then returned to normal.

The percentage values for each cell type in normal swine is quite variable. The surgery for catheterization altered the leukocyte counts in these animals so normal differential values could not be established prior to inoculation. Most were within the normal limits however.

Necropsy Findings

Gross lesions were minimal as most changes were the result of the endotoxin and had disappeared by the time the pigs were killed at the conclusion of the experiment. If enough pigs had been used to allow a pig to be necropsied at time sequences during the study, more definite lesions would probably have been encountered.

One unfortunate sequella of equipping the pigs with indwelling catheters was the development of infarcts which occurred in the kidneys of all pigs in the project including controls. Numerous cortical infarcts were present in various stages of development in both kidneys of all animals. Although the infarcts were extensive no clinical evidence

of uremia was noted in any of the pigs.

The catheters were inserted into the femoral arteries until the ends of the catheters were in the aorta to a point just posterior to the renal arteries. On necropsy the catheters were found to be almost level with the renal arteries. Small clots formed on the end of the catheters, dislodged and became emboli that occluded the arcuate arteries at the cortical-medullary junction of the kidneys.

The catheters were necessary to allow for the frequent bleedings in sequential hematologic studies but had an adverse effect by producing the renal infarcts. Damage to the kidney tissue probably affected some of the hematologic results, especially the ESR, serum enzyme activities and possibly the total leukocyte counts.

Microscopic lesions are more or less predictable when what is already known of the effects produced by endotoxins on the body are applied to the pathogenesis study. Endotoxins, regardless from what Gram-negative bacteria they are extracted and into what species of animals they are inoculated, react essentially in the same characteristic manner. Unlike other bacterial toxins, extracts of Gram-negative organisms do not appear to be directly cytopathogenic. Braude (1964) stated the mode of action by endotoxins inside the body is still largely a mystery, and for this reason it is an extremely fascinating, and currently one of the most interesting fields of research in bacteriology and of medicine

in general.

In this study a single inoculation of endotoxin was used to avoid producing lesions of the generalized Schwartzman reaction (GSR), and to produce lesions that would occur most likely in natural clinical infections. It was unfortunate that the kidneys were so extensively damaged by infarcts that the lesions of GSR could not have been observed even if present in histopathologic sections. The infarcts masked any lesions that might have been produced by a single injection of the inoculum in each experiment. The kidney changes were generally disregarded in this study. Thomas and Good (1952) and Brunson _et al_. (1955) all agree that a single injection of endotoxin usually does not produce renal cortical necrosis in normal animals, regardless of the dosages.

Zweifach (1964) working on the pathogenesis of endotoxins attributed the main action to be against the vasculature of the body. Following inoculation with endotoxin there was a gradual slowing of the flow of blood through the microcirculation, especially through the efferent venules. The muscular vessels became very responsive to the constrictor action of adrenalin. This hyperactivity persisted for 30 to 60 minutes and involved both the arterial and venular side of the circulation. The peak of hyperactivity was reached in 10 minutes in the arterioles and precapillaries then slowly declined. The venules continued to have an

exaggerated response for an hour or more. He did not believe the vascular endothelium was damaged or that the capillary permeability was affected. Later, Gaynor et al. (1970) found that endotoxin entered the endothelial cells and many detached leaving exposed basement membranes. Using electron microscopy it was determined that small microthrombi originated from the damaged endothelial lining of the vessels. This also explained the thrombocytopenia which occurred when the platelets clumped to these microlesions. Endothelial cells were actually demonstrated in the circulating blood by special techniques.

Histopathologic changes observed in the lungs of the experimental pigs were rather consistent with all 3 forms of inoculation. Lesions in the lungs from animals inoculated with live organisms were most obvious apparently because they had more time to form and persisted until the necropsy. The lesions were mainly interstitial hemorrhages as a result of vascular damage. Erythrocytes were also present in the alveolar lumina. Jubb and Kennedy (1970) described more pulmonary edema than was observed in these animals. No edematous fluid was present in the alveolar lumina. There was considerable cellular infiltration into the interstitial spaces with mononuclear leukocytes, plasma cells and a few neutrophils. Vascular damage was evident in the very terminal vascular bed. There appeared to be 2 types of occluded capillaries, one type was occluded with leukocytes and the

other was occluded with leukocytes and hypertrophied endothelial cells lining the capillaries.

The pulmonary clinical signs produced in the endotoxin inoculated pigs were the most severe, however, lesions were absent at necropsy. Probably most of the damage was reversible and had disappeared by the time euthanasia of the pigs occurred. Dade (1966) found that intravenous inoculation of bacterial endotoxins into swine produced many intravascular thrombi in the lungs but rarely did they result in necrosis. Lawson and Dow (1966) while working with field cases of salmonellosis in swine found pulmonary lesions present in varying degrees of severity in almost every case. Hemorrhages were the most constant finding and ranged from scattered petechiae involving a few alveoli to diffuse hemorrhages of lobular extent. They believed the lesions were due to vascular damage at the capillary level. They also described the infiltrating cells as primarily mononuclear cells and stated that neutrophils were rare. These findings were in agreement with the description presented by Jubb and Kennedy (1970). Nordstoga (1970) described lung lesions observed microscopically after inoculation of *S. choleraesuis* var. *kunzendorf* in experimental pigs as edema of the alveolar walls which were also thickened because infiltrations of mononuclear cells. He also found fibrin thrombi in the alveolar capillaries. The lung lesions in this study essentially agreed with the findings of other workers who have

experimented with salmonellosis or endotoxins (Dade, 1966; Lawson and Dow, 1966; Nordstoga, 1970; Jubb and Kennedy, 1970).

The liver lesions were very interesting in this experimental study. The livers of the pigs inoculated with the live organisms had marked focal necrotic areas known as "typhoid nodules". Grossly the livers were mottled and had 2-3 mm. sized focal areas of necrosis. Jubb and Kennedy (1970) described the formation of the liver lesions very clearly. The endotoxin was known to have entered the Kupffer cells and other cells of the reticuloendothelial system. The Kupffer cells hypertrophied and histocytic granulomas developed, crowding the surrounding hepatic cord cells until a focal area of coagulative necrosis developed. These lesions were essentially acellular and there was no attempt at encapsulation. These lesions were present in the endotoxin inoculated pigs as well as in the ones that received the live organisms in this study. When killed bacterial cell suspension was inoculated the liver lesions were not observed. The Kupffer cells were hypertrophied but there was no evidence of coagulation necrosis in these pigs. The cytoplasm of the hepatocytes was very foamy in appearance and the sinusoids were almost undetectable. The liver lesions in the endotoxin inoculated pigs were few in numbers as compared to the ones that occurred in the pigs that received the live organisms. The cord cells in the livers

of pigs which received the live organisms and the endotoxin were shrunken and appeared to have lost their store of glycogen. Endotoxin definitely interferes with glycolysis. Milner *et al*. (1971) found the level of blood sugar to begin rising shortly after the intravenous administration of endotoxin and reached a maximum, which may be several times the normal level, within 2 hours PI. Thereafter, the level declined and a hypoglycemia ensued, which may be profound in shocked animals.

Holmes and Smith (1969) reported PAS positive inclusion bodies in the hepatic cytoplasm of dogs and rats after inoculation with endotoxin. The inclusions were attributed to hepatic injury associated with endotoxin possibly due to hypoxia and congestion. No such inclusion bodies were demonstrated in this study.

Similar microscopic changes were present in the spleen regardless of type of inoculum. The main change was a diffuse reticuloendothelial lymphoid hyperplasia. Jubb and Kennedy (1970) described an enlarged spleen with a bluish discoloration as being typical of salmonellosis in swine, but this was not observed in this study. The spleens in all pigs in this study, including the controls, were relatively normal in both gross and microscopic appearance.

Many workers have described heart lesions in animals inoculated with salmonellae (Jubb and Kennedy, 1970; Seghetti, 1946; Sorensen, 1970). No evidence of cardiac involvement

was noted in this study other than one endotoxin inoculated pig which had an endocarditis on microscopic examination. The myocardium was not involved, only the endothelium. A *Streptococcus sp*. was isolated from the heart tissues in this case. These findings appear to be of incidental significance and the portal of entry for the bacteria was probably through the catheter system.

The brains, spinal cords, and meninges were thoroughly examined and no lesions were demonstrated in any of the experimental pigs. This fact was surprising as cellular infiltrations of the vasculature of the meninges have been described by others (Jubb and Kennedy, 1970; Sorensen, 1970). Meningeal lesions must develop in the final stages of the disease or be reversible and disappear as the animals recover. None of the experimental pigs died during this study, therefore, it is not known if these lesions would be present in terminal cases of acute salmonellosis.

SUMMARY

The objective of this study was to determine the role of endotoxin extracted from S. choleraesuis var. kunzendorf in the clinical, hematologic, and pathologic manifestations of acute salmonellosis.

Live salmonellae, killed salmonellae and extracted endotoxin were inoculated into 3 different groups of pigs which were surgically prepared with indwelling vinyl catheters. The catheters were inserted into the femoral arteries and were necessary for the frequent blood collection regimen. Control pigs were catheterized and subjected to identical procedures as the experimental pigs.

Clinical similarities included elevated temperatures, vomiting, transient diarrhea followed by constipation, depression, spontaneous bleeding and profuse salivation. The effect of endotoxin was definitely pyrogenic. It also affected the parasympathetic side of the autonomic nervous system, and had a marked effect on the clotting ability of the blood. Large doses of endotoxin often resulted in shock.

The hematologic changes were studied and characterized. The injection of endotoxin resulted in increases in the ESRs. The PCV values and TRBC counts were not significantly altered. Serum enzyme studies revealed increased levels of activity of both SGOT and LDH. The main effects of endotoxin were on the TWBC counts and differential leukocyte counts. A

severe leukopenia was produced in the early stages following injection with endotoxin. The TWBC counts of approximately 15,000 cells/cmm. of blood decreased to approximately 1,000 cells/cmm. by the 4th hour PI. Differential leukocyte counts revealed segmented neutrophils to be almost totally driven from the main stream of the circulation. A leukocytosis was evident 24 hours PI, with the TWBC count elevated 3 to 4 times pre-inoculation values. Immature cells of the neutrophilic granulocytic series predominated during the leukocytosis.

Lesions were similar in all pigs although each group received a different form of the inoculum. Characteristic "typhoid nodules" were found in the endotoxin injected pigs as well as in the ones that received live salmonellae. A diffuse reticuloendothelial cellular hyperplasia was a consistent finding in the spleens. The lungs had foci of cellular infiltrations associated with damaged alveolar capillaries. Some capillaries were actually occluded with neutrophils, lymphocytes and hypertrophied endothelial cells of the capillaries. The lung lesions persisted for some time after the pigs began to recover.

No lesions were noted in the central nervous system. No intestinal damage was demonstrated in the acute form of salmonellosis.

Only single doses of organisms and endotoxin were given to avoid the production of lesions associated with the gen-

eralized Schwartzman reaction, which would complicate the findings of simulated field cases of acute salmonellosis.

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ACKNOWLEDGMENTS

Sincere appreciation is expressed to Dr. John P. Kluge for his guidance and counsel extended during the progress of the experimental studies. Special appreciation is also expressed for his patience and help with editing the final preparation of the manuscript.

I also wish to express my appreciation to Dr. Frank K. Ramsey for all his help and encouragement during my graduate studies.

Appreciation is expressed to Drs. E. M. Ellis, B. O. Blackburn, R. W. Dougherty and K. R. Rhoades who contributed advice or technical knowledge to this study. Also gratitude is expressed to Drs. F. K. Ramsey, C. J. Maré and D. R. Cassidy for their participation as members of my graduate committee.

I am indebted to P. A. Clark, K. L. Mead, C. K. Ringham and B. J. Mohrman for their technical assistance, to W. A. Minert, J. F. Beattie, J. Fitzgerald and F. E. Ives for their help and care of the experimental animals.

My thanks are also extended to the NADL Photographic Services personnel for their preparation of the illustrations.

To my wife Rosemary and to my children Ann and David, special thanks are in order for their tolerating many weeks and months of interrupted family activities.