ABSTRACT

CLINICO-PATHOLOGIC OBSERVATIONS OF HOG CHOLERA IN GNOTOBIOTIC PIGS

by Kenneth Duane Weide

Clinical and pathologic signs found in field cases of hog cholera often present a problem in diagnosis. Concurrent infections, infestations, and nutritional deficiencies make diagnosis difficult. In this study, investigations were made into the effects of the hog cholera virus itself, utilizing gnotobiotic pigs obtained by technics used for rearing germfree laboratory animals. It was hoped that true, uncomplicated hog cholera could thereby be reproduced.

Twenty-one 14-day-old pigs from 3 litters, obtained and raised under gnotobiotic* conditions, were each inoculated intramuscularly with 750,000 lethal doses of hog cholera virus. Six uninoculated pigs in separate isolators served as controls.

The clinical course of the disease was followed by observing rectal temperatures, total and differential leukocyte counts, hemoglobin and hematocrit values, food consumption and weight gains. Gross lesions were determined at time of death; histologic studies were made of nearly all tissues.

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Ten cholera-infected and 4 control pigs were successfully maintained free of any demonstrable bacteria throughout the experiment. Bacillus subtilis was isolated from the remaining pigs. Differences could not be detected between the monocontaminated pigs and those found to be bacteria-free. Both groups were, therefore, evaluated together.

Rectal temperatures were diphasic following virus administration, reaching 2 mean peaks of 106 F. and 106.3 F. Total circulating leukocytes decreased nearly to one-third their preinoculation levels within 24 hours. There was a reduction in hemoglobin and hematocrit levels, followed by a rise in numbers of circulating nucleated erythrocytes. Food consumption decreased several days before death, the greatest inappetence occurring 24 hours prior to death. All 21 infected pigs died within a period of 4 to 15 days after inoculation.

Consistent gross lesions were limited to hemorrhages in the lymph nodes and kidneys. Histologically, hemorrhages were found in the tonsils, tongue, diaphragm, lung, heart, lymph nodes, meninges, urinary bladder, kidney, liver and tubular digestive tract. Hemorrhages and edema were most extensive in the internal lymph nodes. Necrosis was found in the gallbladder, lymph nodes, and tubular digestive tract. Leukocytic cellular infiltrations were found in the liver and associated with necrosis in the gallbladder and portions of the tubular digestive tract. Anitschkow myocytes were found

in large numbers within the myocardium. Lymphoid depletion was common in the lymph nodes and spleen. Degenerative changes were present in the smaller blood vessels of the major tissues. These included endothelial proliferation, hyalinization and vacuolar changes in the vessel walls, and thrombosis. Frequently the blood vessel alterations in infected pigs were subtle, enabling no definitive conclusions to be reached. Livers of infected pigs were characterized by glycogen depletion and absence of extramedullary hemopoietic areas.

Correlation could not be made between the number of organs showing hemorrhage and/or the severity of these hemorrhages, with the elevated temperatures, leukopenia, survival time, or other clinical signs. Lesions were somewhat independent of the clinical signs; death, in turn, was not dependent on the severity of either clinical signs or gross lesions.

The signs and lesions found in infected pigs in this study were attributed to the effects of the virus itself, and served to emphasize the marked variation in the response of individual pigs to the hog cholera virus.

Both control and infected pigs were kept in plastic isolators within the same room, and no precautions other than those routinely employed in rearing germfree pigs were used. Cross infection between isolators did not occur. This, in

itself, was significant and suggested that the technics used may be a useful tool in the study of infectious disease of domestic animals.

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*Gnotobiotic refers to animals reared under germfree conditions or in the presence of known microbial flora.

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by

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Veterinary Pathology

1962

ACKNOWLEDGMENTS

Acknowledgment is gratefully extended to Dr. Glenn L. Waxler, research director, who contributed freely of his time, energy and skill in helping to secure and raise experimental animals used in this work, and for his invaluable assistance in the histopathologic studies. Appreciation is extended to Dr. C. C. Morrill, academic advisor, for his advice, criticism and assistance in major portions of this work. The continuous constructive criticism of Dr. C. K. Whitehair throughout the term of graduate study and the duration of this experimental work is greatly appreciated, as well as the time and physical energy which he so freely donated. Collaboration with these three individuals has indeed been a rewarding experience.

Dr. W. D. Pounden, Chairman, Department of Veterinary Science, Ohio Agricultural Experiment Station, is to be extended my sincerest appreciation for his continuous encouragement and his many efforts on my behalf. The allotment of time to pursue graduate studies was obtained entirely through his labors, to say nothing of the provision of numerous supplies and equipment for completion of this research.

To my good friends, Dr. Kenneth K. Keahey and Dr. Edward J. Bicknell, Michigan State University, and Dr. Vance L. Sanger, Ohio Agricultural Experiment Station, I extend grateful acknowledgment for their many efforts incident to this thesis.

To my wife and daughter, who
have so graciously and patiently
endured the rigors of graduate
study, this dissertation is
affectionately dedicated.

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I. INTRODUCTION

Hog cholera has been present in the United States for at least 150 years (19). Today, "it is unquestionably the most important disease of swine in the United States" (47). Although suitable prophylactic measures have been available since 1905 (12), control of any disease cannot be accomplished simply by maintaining the economic losses within acceptable limits. Actual control can only be accomplished by eradication of the disease. Eradication in turn is dependent upon knowledge of the disease including accurate diagnosis.

Hog cholera presents a confusing picture, from both clinical and pathologic standpoints. Its incubation period, duration, clinical signs, and lesions are quite variable. Concurrent infections and infestations with other microorganisms and parasites produce further variations and increase the difficulty of correct diagnosis. The literature on hog cholera is full of references to "changes due to secondary invaders". The problem of diagnosis, then, becomes one of detection of signs and lesions due to the hog cholera virus alone.

Investigations into the effects of hog cholera virus on the pig have been limited to animals living in an environment abounding in microbial life. It is doubtful if truly "uncomplicated" hog cholera has existed. The clinical and

pathologic findings present in conventional hog cholera have been described and are summarized in several recent publications (16,18,39,47).

Adaptation of equipment and technics developed at the University of Notre Dame (49,50,51) to the rearing of farm animals in a microbe-free state provided a tool heretofore not available for basic studies in animal disease (41,53,54). With them, it is now possible to study the effects of a single disease entity on the individual animal.

This report describes the results of a clinical and pathologic study of the effect of hog cholera virus on susceptible pigs maintained in an otherwise germfree or monocontaminated environment. "Germfree" refers to pigs reared in the absence of known microbial flora. "Monocontaminated" refers to pigs reared in the presence of one known microbial agent. Animals reared under germfree conditions or in the presence of known microbial flora have been called "gnotobiotes" (37).

The purpose of this study was to produce truly uncomplicated hog cholera in susceptible pigs, and to study the clinical signs and gross and microscopic lesions of the disease. It was hoped that this investigation would provide the basis for a better understanding of the clinical signs and lesions of hog cholera and, in addition, provide the framework for further studies on clinically related conditions.

II. REVIEW OF LITERATURE

The tremendous volume of literature which has accumulated since 1900 concerning the various aspects of hog cholera makes a complete literature review beyond the scope of this dissertation. An attempt has been made, however, to review those publications pertinent to the substance of this study; i.e., the clinical signs and gross and microscopic lesions in hog cholera.

A. Clinical Signs

The recognition of hog cholera as a specific disease entity with characteristic signs and lesions was first recorded by Detmers and Law (9) in 1879. Salmon prepared a thorough report which was published in 1889 (40). Subsequent reports (10,27,31,36) established the classical signs present in conventional hog cholera. Recent summations of the clinical hog cholera picture (16,18,24,34,39,47,52) tend to place less emphasis on respiratory and intestinal signs attributing them chiefly to secondary bacterial invasion.

The incubation period of the disease is from five to ten days following contact with contaminated material. The first signs observed are usually dullness, inactivity and anorexia. Inappetence is greatest near termination of the disease; animals may stagger and weave when walking. The disease is generally accompanied by diarrhea, though constipation may occur during the early stages. Sometimes

vomiting occurs. Body temperatures reach 106 F. to 107 F. within 2 to 6 days after infection. Convulsions and other signs of central nervous system involvement occur occasionally. In acute forms, death may occur in a few hours, often with few noticeable signs. In less severe attacks the affected animals may live 5 to 15 days. Under natural conditions, the morbidity and mortality are close to 100%. If the course of the disease is prolonged more than 7 days, bacterial complications may occur, consisting chiefly of pneumonia and ulcerative or necrotic enteritis (52).

It was recognized in early investigations by Dinwiddie, and others, that leukopenia was a characteristic sign of hog cholera (11,27,31). This early and marked decline in circulating leukocytes was considered the most striking cellular change (4,7,26,29,30,45,48). The decline was very evident within 48 hours following contact with the virus (26,29,30, 45). Leukopenia usually preceded any temperature reaction, and reached a maximum 5 to 6 days after infection (7,26). Circulating lymphocytes, eosinophils and basophils were reported to decline and neutrophils to increase in some investigations (26,39,45); other studies reported a neutrophil decrease (sometimes total) and a lymphocyte elevation (30); nonspecific variable changes were found by Dinwiddie to occur in differential leukocyte studies (11).

A gradual decline in circulating erythrocytes has been a frequent observation with the appearance of a moderate number of nucleated erythrocytes in the blood stream (11,26,29,30,45).

B. Gross Lesions

Early studies on the gross lesions of hog cholera (10, 23,27,31,36) included the following as characteristic: erythema of the skin with purplish discoloration of the ventral portions of the body and areas of the head. Petechial hemorrhages were found on the serosal and mucosal surfaces of the stomach and intestines, with localized or diffuse necrosis of the mucosa. Discrete to numerous hemorrhages, varying in size from petechial to ecchymotic were found on and in the lungs, heart, liver, gallbladder, urinary bladder, pharynx, brain and skin. Hemorrhages in the kidneys were constantly observed, the cortex being more frequently and more extensively involved. Hemorrhagic infarcts were common in the spleen; peripheral hemorrhages were characteristic in the lymph nodes. A summation of the gross lesions found in 3 investigations is presented in Table 1. Recent summations by Dunne (16), Runnells et al. (39), and Smith and Jones (47) do not differ significantly from the foregoing.

C. Microscopic Lesions

Proescher and Seil (35), in 1917, apparently made the first attempts to systematically record the microscopic lesions of hog cholera. Additional studies (2,8,10,13,14,15, 20,21,22,25,32,33) have provided supplementary information but the initial elucidation of the microscopic lesions of hog cholera are found in reports by Proescher (35,36) and Seifried (42,43,44).

TABLE I. Summary of Reported Gross Lesions of Hog Cholera

Location of Lesion	Per Cent Showing Lesion		
	Hoskins (23) and Proescher (36)	Kernkamp (26)	Dunne et al. (13)
Kidney	100	92.5	64.3
Urinary bladder		83.2	
Lymph nodes	100	83.5	96.0
Spleen	34	60.2	27.4
Larynx	75	59.6	23.8
Lungs			
Hyperemia		44.9	
Hemorrhage		27.2	15.7
Inflammation		40.4	58.3
Large Intestine			
Mucosal hemorrhage		24.6	10.7
Serosal hemorrhage		6.3	
Inflammation		13.8	16.8
Button ulcers			20.0
Heart		29.0	13.1
Liver		6.2	10.7
Small Intestine			
Mucosal hemorrhage	20	9.3	1.2
Serosal hemorrhage		7.2	1.2
Inflammation		.3	15.5
Stomach			
Mucosal hemorrhage	40	8.7	8.3
Serosal hemorrhage		3.9	3.6
Inflammation		3.6	45.2

The virus of hog cholera is thought to exert a direct effect on the vascular system, the lesions resulting from degenerative changes in the capillaries, precapillaries, arteries and veins (47).

1. Digestive System

In general, microscopic lesions have not been common in the liver. Occasionally infiltration of the interlobular

connective tissue with mononuclear cells has been seen. Congestion, cloudy swelling, and hemosiderosis were common. Focal necrosis of the mucosa of the gallbladder has been reported by Luedke and Dunne (32) as well as intranuclear inclusion bodies (2).

The stomach and small intestine were generally free of microscopic lesions, though occasional serosal and mucosal hemorrhages were observed. The cecum and colon frequently had degenerative changes with accumulations of mononuclear cells around blood vessels, especially in the mucosa. Blood vessel lesions consisting of hyaline changes of the walls with endothelial proliferation and occlusion of lumina were commonly encountered. If extensive, this led to general necrosis of the mucosa, or, more often, according to Dunne *et al.* (14), focal necrosis resulting in so called "button ulcers". The latter was particularly true if the disease was of longer duration than average.

2. Respiratory System

Subpleural hemorrhages were commonly found in the lungs and increased numbers of mononuclear cells were reported in the alveolar walls and around blood vessels. Vascular changes with thickened vessel walls and occluded lumina were present.

3. Genito-Urinary System

Extensive changes have been reported in the kidneys of pigs with hog cholera. Subcapsular hemorrhages were

common, extending around uriniferous tubules and frequently causing the tubules to be widely separated. Necrosis of tubular epithelium was not uncommon. Extensive hemorrhage was most often present between the proximal and distal convoluted tubules. Foci of blood frequently obliterated the renal tissue. The capillary endothelium was swollen. Capillaries of the glomeruli were often distended with erythrocytes, occluded with endothelial cells, or plugged with thrombi. The thrombi were reported as including remnants of ruptured capillaries, swollen endothelial cells and pyknotic nuclei. Necrotic endothelial cells were seen lining many capillaries. Perivascular infiltrations with macrophages and lymphocytes were seen, especially in more advanced stages of the disease. The infiltrating cells frequently showed degenerative changes.

The urinary bladder, ureters and urethra had perivascular hemorrhages and accumulations of mononuclear cells. Frequently the hemorrhages extended deeply into the mucosa.

4. Cardio-Vascular System

Frequently, the heart exhibited no microscopic changes. Occasionally subendocardial and subpericardial hemorrhages occurred; localized mononuclear cell infiltration and cloudy swelling were observed.

5. Lymphatic System

Infarcts of the spleen were frequent and arose from blood vessel lesions, namely endothelial degeneration and

hyalinization with resulting thrombosis. Focal necrosis of the spleen was widespread. In addition, lymphoid depletions of the splenic follicles, mononuclear cell infiltration and hemorrhage with numerous phagocytized erythrocytes were frequently present.

In all cases of hog cholera, various degrees of hemorrhage were present in one or more lymph nodes. Many times, all lymph nodes were grossly involved. Commonly these changes were peripheral, but medullary and diffuse hemorrhage was not infrequent. The blood vessels were found engorged, small vessels were thrombosed and phagocytized erythrocytes were abundant. Infiltration of mononuclear cells was observed. Three groups of lesions in the lymph nodes have been described by Seifried (44): (1) swelling and hyperemia, (2) peripheral hemorrhage, and (3) dense, total hemorrhage. These 3 types of lesions have been found in varying degrees in most cases of hog cholera.

6. Nervous System

The most striking microscopic lesion observed in the brain resulted from the accumulation of lymphocytes in the perivascular spaces around arteries and veins. Hemorrhages have been observed around blood vessels, especially in the cerebellum and spinal cord. Focal accumulation of microglial elements have been seen, but degenerative changes in the neurons were infrequent.

7. Musculo-Skeletal System

Hemorrhages have been found in the muscles, especially the diaphragm. In acutely infected pigs, lesions have been reported by Dunne et al. (15) at the costochondral junction of the ribs. These consisted of a mildly irregular epiphyseal line with an adjacent narrow transverse band of hemorrhage.

8. Integument

Intracytoplasmic inclusion bodies have been reported in epithelial cells of the conjunctiva (22).

The skin was observed to be hyperemic with occasional hemorrhage near the hair follicles. Degenerative changes in blood vessels were also observed.

9. Summation of Microscopic Lesions

Vascular lesions were considered responsible in great part for the characteristic pathologic picture of hog cholera regardless of which organ or tissue was involved. These were endothelial proliferation, hyalinization and degenerative changes in the vessel walls, and thrombosis. Hemorrhage and necrosis were the results. Mononuclear and lymphocytic infiltration was not uncommon, especially perivascularly. The duration of the disease in general did not determine the type or severity of the lesions. Pathologic findings in the lymph nodes, kidneys and central nervous system were thought to be of special value in diagnosis.

Proescher and Hoffman (36) described hog cholera as an "acute, specific endangitis, combined with thrombo-angitis and endarteritis production".

D. Current Status of Hog Cholera

A great amount of research has been devoted to the various facets of hog cholera in the past half-century. The excellent work of past and recent investigators has helped to understand and to alleviate the effects of the disease, but the problems of hog cholera are not all solved, nor is the complete picture elucidated.

Today, emphasis is being placed on eradication of the disease. Therefore, the possibility of reservoirs becomes important. In addition, attempts are being made to develop laboratory tests for simple identification of the disease. If eradication is to be accomplished, the effectiveness of the various vaccines becomes more critical. More study is needed on the chronicity of the disease, which has been recognized but its importance not ascertained. The effect of complications by bacterial and other organisms are under investigation and as technics improve, "new" conditions are being recognized which play a possible role in diagnosis.

Thus the overall hog cholera problem is changing. Emphasis must be placed on the elucidation of problems which obstruct the final goal - eradication of hog cholera.

III. MATERIALS AND METHODS

A. Germfree Technics

The development by Trexler (49,50,51) of equipment and procedures utilizing flexible plastic film has provided a practical and economical method of rearing animals in a germfree state. Adaptation of these technics by Waxler (53,54) and Schmidt (41) to rearing germfree pigs was utilized in this study.

Female swine, 112 days pregnant and not vaccinated against hog cholera, were anesthetized by ether or carbon dioxide and placed in lateral recumbency. A sterile surgical isolation unit (Fig. 1) was secured to the skin of the flank region by adhesive. Working through shoulder-length rubber gloves, the operators made an incision through the floor of the isolator and the abdominal wall, using cautery for the primary skin incision. The uterus was then brought through the incision and opened. The pigs were removed, the umbilical cords were ligated and severed, and the animals were passed into a sterile transportation isolator. They were then dried, weighed, and placed in individual stainless steel cages within a rearing isolator (Fig. 2). Autoclaved cows' milk with added vitamins and minerals (to compensate for those deficient in milk, or destroyed by heat and pressure) was fed every 3 hours from 8 A.M. to 11 P.M. daily. Initially 60 ml. was fed each pig at each feeding. This was increased by 15 ml. every 5 days. Bacteriologic examination

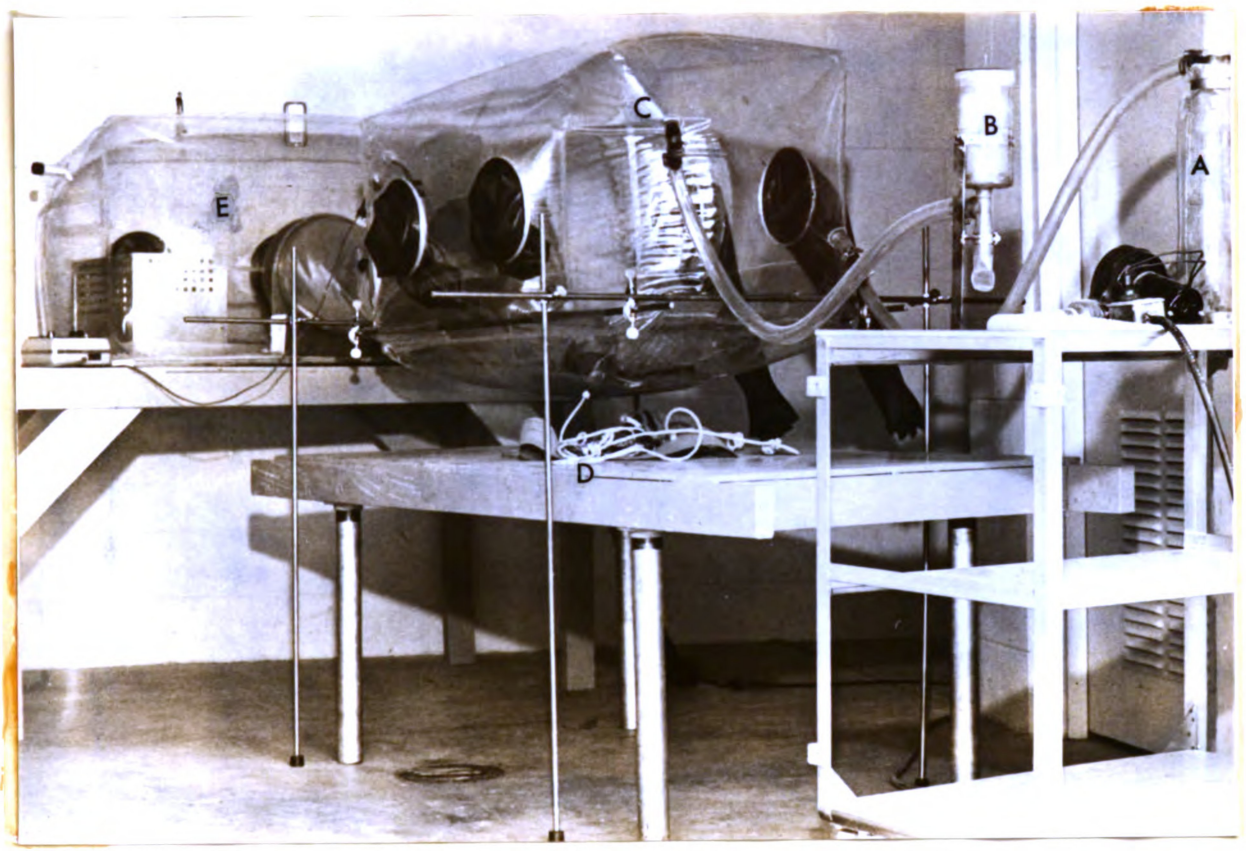


Figure 1. Surgical and Transfer Isolators

A. Air Supply and Filter

B. Air Outlet Trap

C. Surgical Isolator

D. Table and Straps for Restraining Sow

E. Transfer Isolator

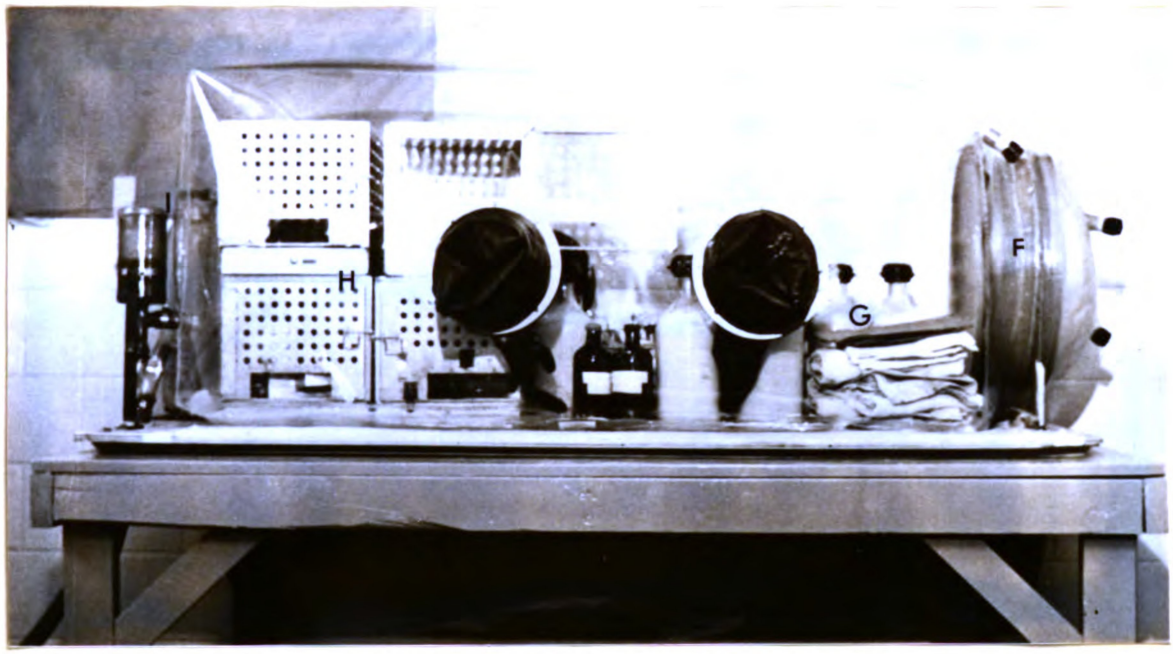


Figure 2. Rearing Isolator

F. Sterile Lock

G. Supplies for Rearing, Bleeding and Culturing

H. Rearing Cages

I. Air Inlet and Outlet Equipment

of rectal swabs and waste material from the cages was made at selected intervals to determine the microbial status. Sterilization of all equipment and supplies was by autoclave (250 F. for 30 minutes) or germicidal chemical (2% peracetic acid) (28,49,50,51).

B. Experimental Procedures

Twenty-one 14-day-old Yorkshire pigs (10 males, 11 females) from 3 litters, obtained and raised under germfree conditions, were each inoculated intramuscularly with 1 ml. of standardized hog cholera virus*. Six uninoculated pigs (3 males, 3 females) in separate isolators served as controls. All pigs were free from known microbial flora when examined at 10 days of age.

Rectal temperatures were taken twice daily both prior to inoculation and following administration of the virus. Similarly, total and differential leukocyte counts and hemoglobin and hematocrit values were determined (6). Pigs were observed regularly for signs of illness, and necropsies were performed according to routine procedures (38) as soon after death as possible. The 6 control pigs were killed by barbiturate overdose between 10 and 15 days after inoculation of the exposed pigs. Room temperature was maintained at 75 F. to 80 F.

Food consumption was determined daily for each pig. At the time of inoculation, daily food consumption was 540 ml.

*750,000 lethal doses of Station Virus 315 from the Hog Cholera Research Station, ARS, USDA, Ames, Iowa, obtained through the courtesy of Dr. J. P. Torrey.

per pig. This was increased to 630 ml. on the second day after inoculation. When anorexia appeared in inoculated pigs, the amount of milk not consumed was subtracted from the quantity fed and this adjusted amount was used in determining the volume to be fed the control pigs. Body weights were determined at the time of death.

Following necropsy, the following tissues from each pig were preserved in 10% neutral formalin: representative portions of the skin of the flank, back, sternum and thigh, the lip, cheek, and tongue (tip, middle, and posterior portion), and middle portions of the external ear. The following external lymph nodes were preserved: external inguinal, prefemoral, prescapular, parotid, mandibular and supra-pharyngeal. Both right and left tonsillar areas were preserved as well as the parotid and mandibular salivary glands.

Portions of the digestive system preserved included: 3 equi-distant portions of the esophagus, the esophageal, cardiac, fundic and pyloric portions of the stomach, the pyloric valve, 9 equi-distant levels of the small intestine, the ileocecal valve, the tip and body of the cecum, 8 equi-distant levels of the large intestine, and anal tissue. Pieces of all 4 major lobes of each liver were taken together with the gallbladder, pancreas, thymus, thyroid, and 4 equi-distant levels of the spleen.

Preservation was made of portions of the walls of all 4 chambers of the heart, a portion of the posterior aorta, the

epiglottis, trachea, diaphragm, and representative samples of all 7 lobes of the lungs.

The pituitary, adrenals, testicles, spermatic cord, body of the uterus and 3 levels of the horns, oviducts, ovaries, body and neck of the urinary bladder, ureters, 3 levels of the pelvic urethra and 2 of the external urethra, prepuce, and portions of the anterior, middle and posterior parts of both kidneys were preserved.

Internal lymph nodes taken included: mediastinal, gastric, splenic, hepatic, mesenteric, renal, cecal, colic and internal inguinal.

Paraffin sections of each tissue were stained with hematoxylin and eosin according to routine laboratory procedures (1). Special stains were used on selected tissues.

The brain, spinal cord, bone marrow, ribs, and eye were preserved but are not included in this study because of time limitations.

Bacteriologic examination of rectal swabs and waste material from the cages was made when pigs were 10 days of age and again at the time of death, samples being taken before pigs were removed from isolators. Samples were streaked on blood agar plates which were then incubated both aerobically and anaerobically at room temperature, 37 C. and 55 C. Thioglycollate medium was also inoculated and incubated at these same temperatures.

To detect the presence of the hog cholera virus in inoculated pigs, fluid extracts of the spleen of 3 pigs were

frozen for subsequent inoculation into farm raised, hog cholera susceptible pigs. Six pigs were given the extracts only, and 6 pigs were given the extracts plus commercial anti-hog cholera serum.

IV. RESULTS

Ten cholera-infected and 4 control pigs were successfully maintained free of any demonstrable bacteria throughout the experiment. Bacillus subtilis was isolated from 11 infected and 2 control pigs. At no time were monocontaminated and bacteria-free pigs found within the same isolation unit. When bacteria gained entrance to an isolator, all pigs in that unit became contaminated. Since differences in clinical signs, gross necropsy findings, and microscopic lesions between the monocontaminated and bacteria-free groups could not be detected, both were evaluated together.

A. Clinical Signs

A summary of maximum and minimum body temperatures is presented in Table II. Rectal temperature curves (Fig. 3) were diphasic. The first peak was reached at a mean time of 2.5 days (range, 2.0-3.5 days) and the second peak at a mean time of 6 days (range, 3.5-7.0 days). Near normal temperatures were present between the peaks at a mean time of 4 days (range 3.0-5.0). The rectal temperatures at the first peak ranged from 104.8 F. to 107.0 F. (mean 106.0 F.) and temperatures at the second peak ranged from 105.4 F. to 107.0 F. (mean, 106.3 F.). Following the second peak, the temperatures remained elevated and later considerable individual variation was recorded. Deaths occurred at various times after inoculation; the time of death did not cause variation

TABLE II. Summary of Maximum and Minimum Body Temperatures of Infected Pigs After Inoculation with Virus

Pig No.	Day of First High Temp.	Peak 1 Temp. F.	Day of Low Temp.	Valley Temp. F.	Day of Second High Temp.	Peak 2 Temp. F.
3	2.5	105.6	4.0	103.6	6.0	106.6
4	2.5	104.8	4.5	104.1	5.5	106.0
5	2.5	105.7	3.0	104.6	3.5	105.6
6	3.5	105.0	4.0	103.8	6.0	105.8
7	2.5	105.1	4.0	103.4	5.5	106.4
8	2.5	106.2	4.0	104.1	5.5	106.5
9	3.0	106.0	4.0	104.6	6.0	106.6
10	3.0	106.5	4.0	105.2	5.5	106.7
11	3.0	105.9	4.0	104.0	5.5	106.4
14	2.0	106.4	4.5	102.0	6.0	105.9
15	3.5	105.8	4.5	104.4	6.0	105.8
16	1.5	105.4	4.5	103.8	6.0	105.4
17	2.5	106.4	4.0	103.6	6.0	106.0
18	2.5	105.8	5.0	104.0	6.5	106.2
19	2.5	107.0	4.0	102.0	6.5	107.0
21	2.5	106.6	4.5	104.2	5.0	107.0
22	2.5	106.4	5.0	103.4	7.0	106.0
23	2.5	105.8	4.0	102.0		
25	2.5	106.8	3.5	102.8		
26	2.0	106.4	4.0	104.0	6.0	106.6
28	2.0	106.4	4.0	104.2		
Mean	2.5	106.0	4.1	103.7	6.1	106.3
Range	2.0-3.5	104.8-107.0	3.0-5.0	102.0-105.2	3.5-7.0	105.4-107.0

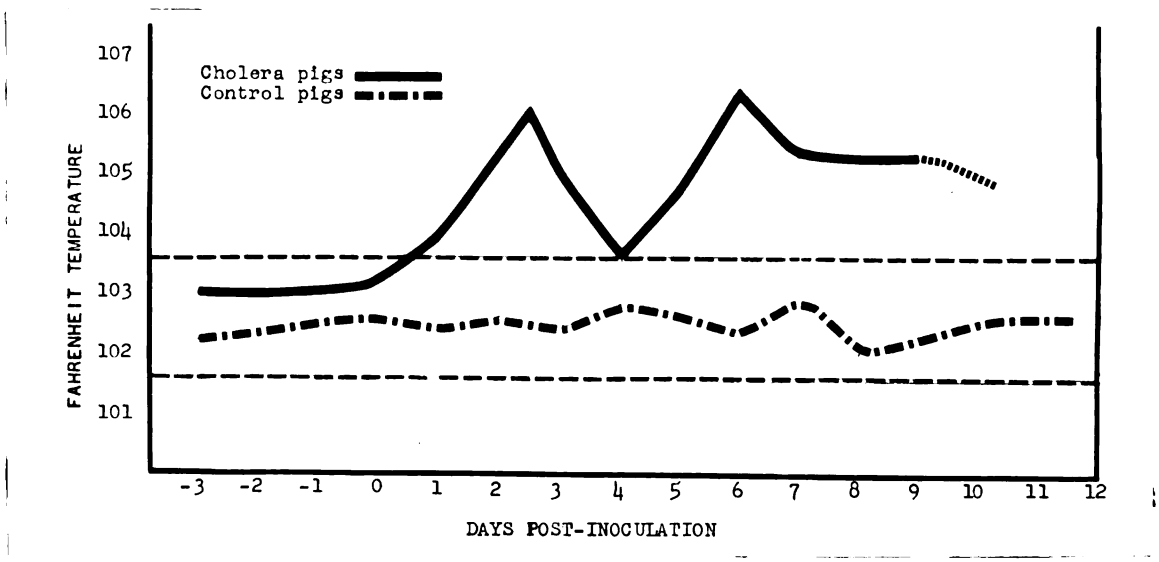


Figure 3. Mean Rectal Temperatures Following Inoculation With Virus. (Area between dotted lines represents normal range.)

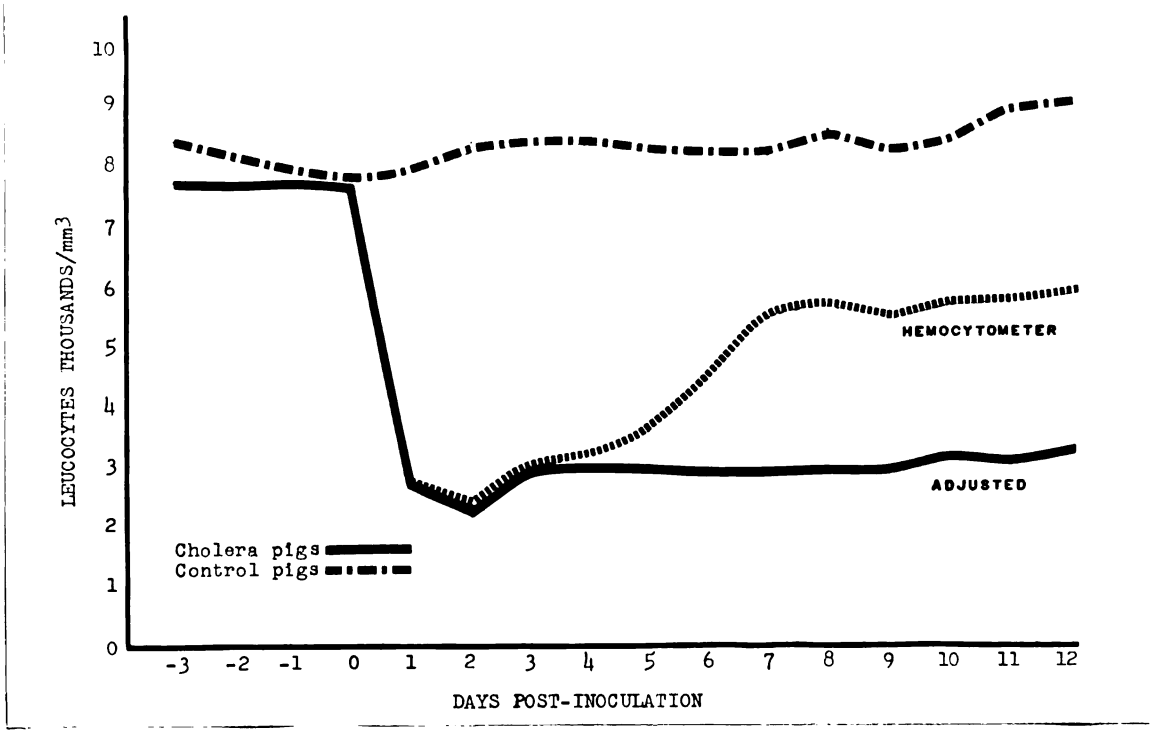


Figure 4. Total Mean Circulating Leukocytes Following Inoculation With Virus. (Dotted line representing hemocytometer counts includes nucleated erythrocytes. Solid line represents actual numbers of leukocytes.)

in the diphasic temperature curve, except for the fact that a subnormal temperature frequently developed a few hours before death.

A summary of total leukocyte numbers following inoculation with virulent hog cholera virus is presented in Table III. Total circulating leukocyte numbers (Fig. 4) were decreased to nearly one-third the preinoculation levels (mean, 7700/cmm.) within 24 hours. A mean low of 2347 cells was recorded 2 days after inoculation. Hemocytometer counts of leukocytes indicated a gradual rise to approximately two-thirds the preinoculation levels by the seventh day. However, subsequent examination of blood smears revealed this increase to be almost entirely due to nucleated erythrocytes which appeared in large numbers by the fifth day after inoculation (Fig. 5). The nuclei of the red blood cells were not destroyed with the mature erythrocytes in the leukocyte counting procedure. Since these nucleated cells could not be distinguished from leukocytes in the hemocytometer count, their number was inadvertently included in the total leukocyte count. Adjustments of total leukocyte counts is presented in Table IV. At no time did total leukocyte counts change to indicate impending death of the infected pigs.

Differential leukocyte counts remained constant in both control and cholera infected pigs. Lymphocytes varied from a mean of 62.1 to 65.3% and neutrophils ranged from a mean of 33.0 to 37.1%. Monocytes and eosinophils each represented 1% or less of the total differential leukocyte count and basophils represented one half per cent or less of the total count.

TABLE III. Hemocytometer Leukocyte Counts of Infected and Control Pigs (cells/cmm.)

Pig No.	Days After Inoculation							
	0	1	2	3	4	5	6	7
Control								
1	**10550**	8100	9100	8350	8800	8250	7950	7800
2	**6600**	7150	7750	9300	6250	9500	7850	6350
12	**11250**	10150	11650	10150	10450	12000	9950	11250
20	**5450**	6550	6950	7150	8500	8900	7150	6950
24	**5300**	7750	6950	7000	8100	7550	7700	7850
27	**7200**	7300	7750	8050	7950	7900	8650	8500
Infected								
3	**7400**	2400	1950	2100	2400	2100	2100	4850
4	**7500**	2900	2200	2250	2400	2000		
5	**7550**	2500	2250	3950	5300	3100	1950	6400
6	6900	4400	3300	2000	1650	2000	1600	4500
7	8300	3400	1350	3450	2950	2900	2000	3250
8	8150	3100	2150	3950	4200	3600	5750	
9	9000	2950	2100	3300	3150	4200	4500	7100
10	6750	3000	1950	4000	3350	5500	7050	6550
11	7050	2450	2200	5850	5150	5550	7550	9000
14	8550	2150	1900	2700	4250	4650	7700	
15	6750	1950	3450	2850	4200	3550	5400	5750
16	7050	2100	1700	2150	3050	3400	3950	4000
17	9050	1550	2750	3700	1800	3650	4200	4000
18	7850	2500	1800	2650	2450	2900	4550	5000
19	7700	2750		2900	4400	2750	4650	7550
21	7950	7100	1450	3150	1550	3400	3950	6200
22	8100	800		1900	1550	3750	3600	3450
23	7750	1500		1750	4250			
25	6900	3200	1900	2900				
26	6950	2850	2550	2850	1500	3800	5500	6250
28	8050	1600	5900	3700	2350			

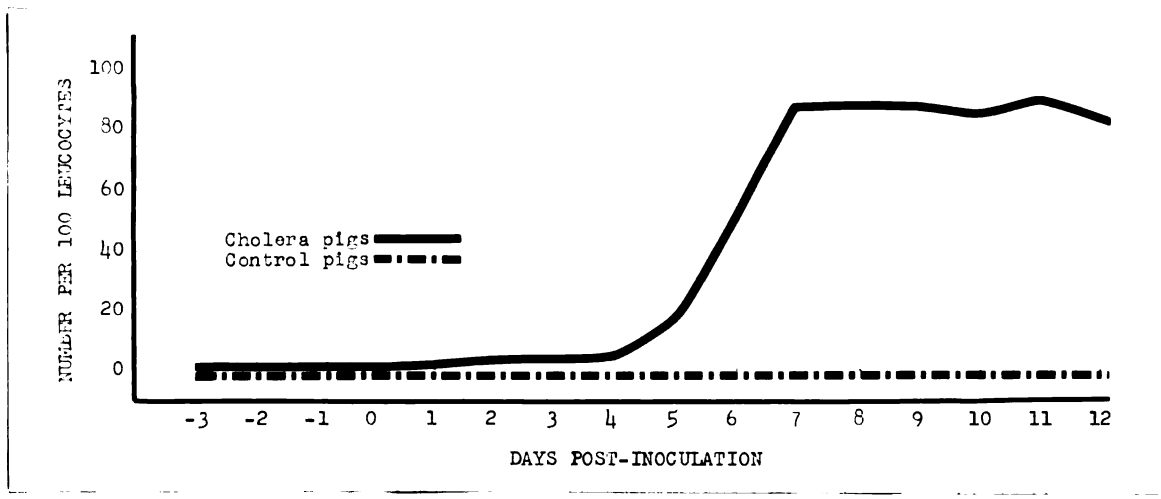


Figure 5. Total Mean Circulating Nucleated Erythrocytes Following Inoculation With Virus (cells/100 leukocytes).

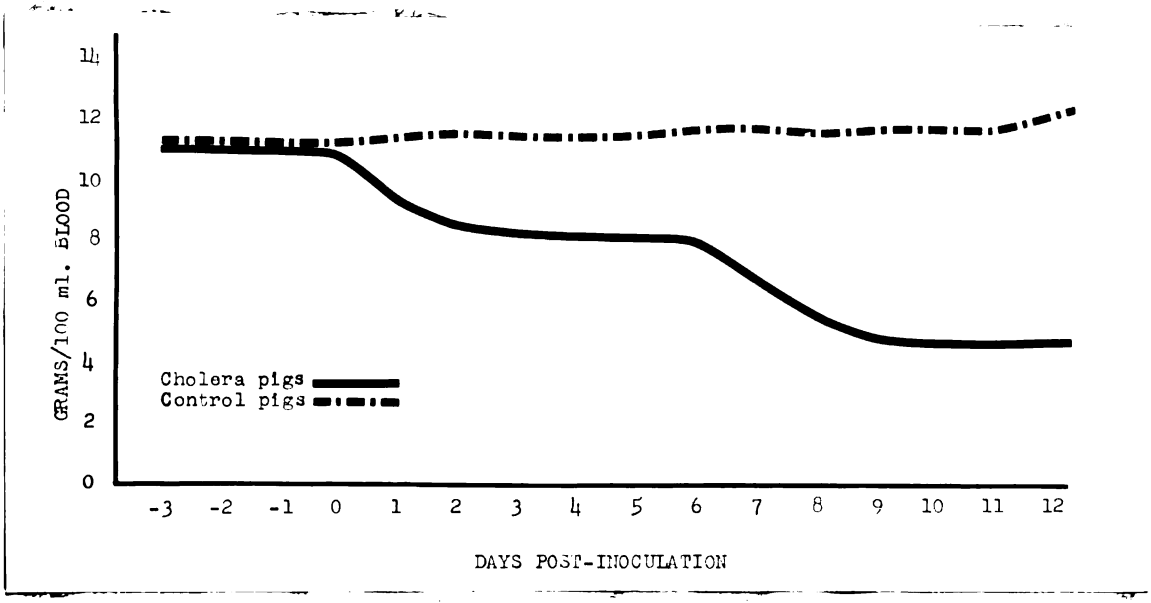


Figure 6. Mean Hemoglobin Values for Infected and Control Pigs.

TABLE IV. Relationship of Hemocytometer Leukocyte Counts to Actual Numbers of Circulating Leukocytes in Infected Pigs. (cells/cmm.)

Days After Inoculation	Mean Hemocytometer Counts	Mean No. Nucleated RBC*/100 WBC	% Nucleated RBC in Hemocytometer Counts	Corrected Mean WBC** Count	Mean Control WBC
-3	7733			7733	8283
0	7679			7679	7725
1	2721			2721	7833
2	2383	1.7	1.5	2347	8358
3	3050	2.0	2.0	2989	8333
4	3055	2.6	2.1	2990	8325
5	3500	17.1	14.6	2989	8200
6	4471	50.1	33.4	2978	8208
7	5590	87.6	46.7	2979	8117
8	5644	88.8	47.0	2991	8492
9	5472	86.8	46.5	2928	7842
10	5721	83.1	45.4	3124	8392
11	5700	89.0	47.1	3015	8863
12	5825	82.0	45.1	3198	8900
13	5900	85.0	45.9	3192	9025
14	6150	84.0	45.6	2804	9500

* RBC - erythrocytes
** WBC - leukocytes

A summary of hemoglobin levels following inoculation with virulent virus is presented in Table V. Both hemoglobin and hematocrit determinations (Figs. 6 and 7) were closely parallel. A decrease in both occurred within 24 hours after inoculation with the virus. This decrease was temporarily stabilized until the sixth day when a second decline reduced levels to approximately 40% of their original values. Although circulating nucleated erythrocytes reached their peak on the seventh day after inoculation, they did not prevent this second decline.

Food consumption decreased several days before death, with the greatest inappetence occurring 24 hours prior to death. Decreased vigor and interest in consuming milk were observed 2 to 5 days after inoculation with the virus. Table VI summarizes food consumption prior to death of infected pigs. The time required for complete food consumption increased from 2 to 3 minutes to nearly 3 hours. The anticipated food consumption (Fig. 8) was based on the usual increase in consumption encountered with previous groups of pigs raised under similar conditions.

Diarrhea occurred 3 to 9 days after inoculation; the character of the feces changed from a dark, tarry consistency initially to a very fluid state just before death. Pigs dying early in the experiment did not develop as severe a diarrhea as did those living a longer period of time. It was difficult to detect the exact onset of scouring since the germfree control pigs had semisolid to fluid stools starting at about 1 week of age.

TABLE V. Hemoglobin levels of infected and control pigs. (mg./100 ml.)

Pig No.	Days After Inoculation									
	0	1	2	3	4	5	6	7	8	9
Control										
1	11.9	10.9	11.5	11.5	10.8	11.6	12.0	12.6	11.5	11.9
2	11.3	12.1	11.5	10.6	11.6	10.8	11.0	10.6	11.7	11.1
12	10.4	11.5	11.7	12.0	11.5	11.8	11.5	11.5	10.9	11.5
20	11.2	10.9	12.0	11.5	11.3	10.9	11.2	11.0	11.6	11.5
24	10.9	10.8	11.5	11.7	11.6	11.4	12.0	12.5	11.5	11.9
27	10.9	11.5	10.9	10.5	11.2	11.4	11.6	11.7	10.9	12.0
Infected										
3	10.6	9.0	8.5	8.3	8.0	8.0	7.9	7.8		
4	10.3	9.2	8.3	8.6	8.1	8.2				
5	11.5	9.5	8.7	8.4	8.2	7.9	8.0	7.6		
6	10.3	9.2	8.4	8.1	7.8	7.9	7.9	7.6	5.0	
7	11.3	9.7	8.6	8.0	8.4	8.3	7.9			
8	10.5	9.1	8.5	8.1	8.1	8.1	7.9			
9	10.7	9.5	8.2	8.1	8.0	8.0	8.0	7.5	6.6	4.4
10	11.5	9.2	8.0	8.0	8.0	8.1	8.2	6.7	5.5	5.3
11	10.9	9.1	8.1	8.1	8.0	7.6	8.0	6.1	5.8	5.2
14	10.6	8.5	8.5	8.3	7.9	7.6				
15	10.9	9.1	8.5	8.3	8.0	8.1	7.8	6.8	5.9	5.0
16	11.9	9.1	8.1	8.0	7.8	8.0	7.9	6.5	5.6	4.6
17	10.9	9.1	8.3	8.0	8.0	8.1	7.9	6.2	5.0	
18	12.0	9.6	8.7	8.5	8.3	7.9	8.0	6.6	5.4	4.9
19	11.5	9.4	8.6	8.1	8.1	8.0	7.8	6.9	5.6	4.8
21	11.9	8.9	8.4	8.2	8.1	7.9	7.9	6.3	5.0	
22	11.0	9.0	8.5	8.3	8.2	8.1	8.0	6.1	5.3	4.4
23	10.9	9.1	8.6	8.3						
25	11.7	9.5	8.7	8.3						
26	11.0	9.2	8.0	7.9	7.7	7.9	8.1	6.5	5.1	4.5
28	12.0	9.6	8.1	8.0	8.0					
Control Mean	11.1	11.3	11.5	11.3	11.3	11.3	11.6	11.7	11.4	11.7
Infected Mean	11.1	9.2	8.4	8.2	8.0	8.0	8.0	6.7	5.5	4.8

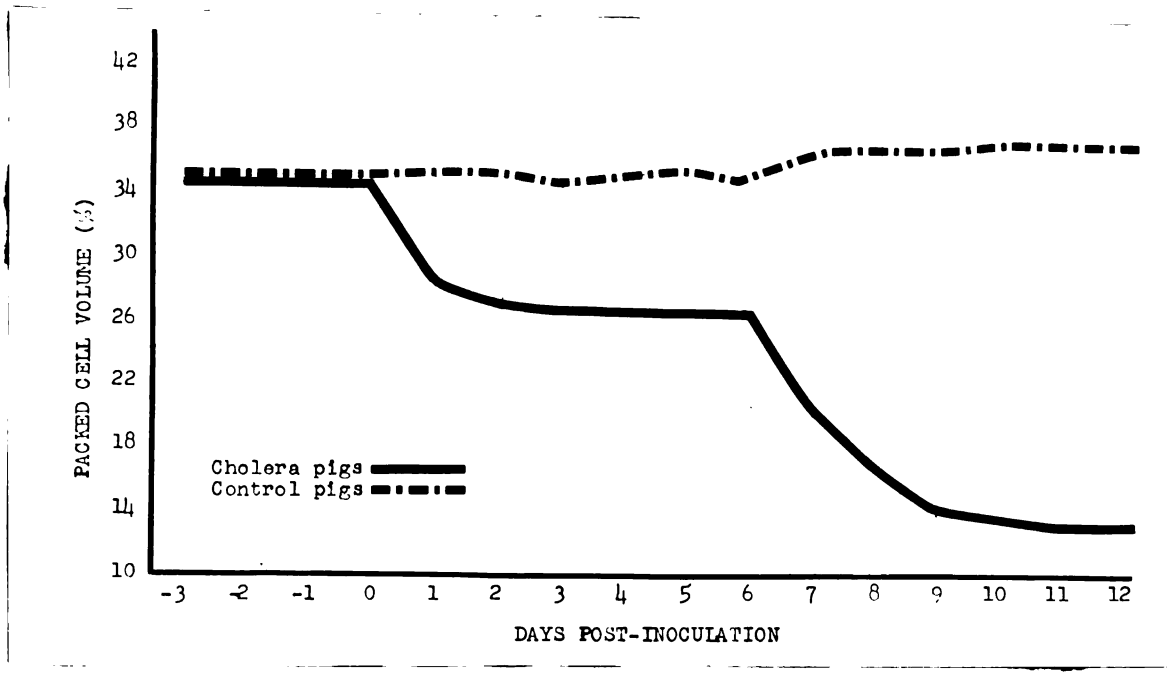


Figure 7. Mean Hematocrit Values for Infected and Control Pigs.

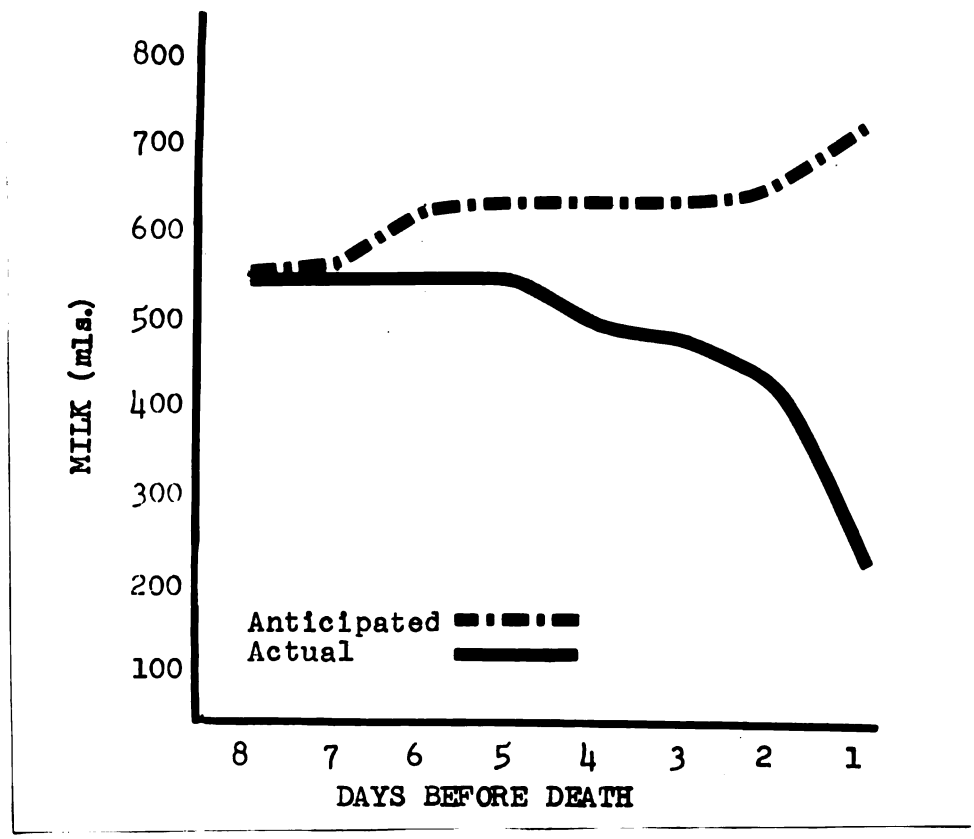


Figure 8. Mean Food Consumption of Infected Pigs Prior to Death.

TABLE VI. Food Consumption of Infected Pigs (ml.)

Pig No.	Days Prior to Death						
	7	6	5	4	3	2	1
3	555	630	548	455	455	565	280
4			555	630	561	400	200
5	555	630	615	430	375	265	155
6	630	630	620	395	575	445	185
7		555	630	496	455	380	380
8		555	630	500	495	465	200
9	630	525	520	555	545	470	230
10	455	530	630	630	630	500	230
11	630	630	630	580	540	450	210
14			555	555	445	500	260
15	430	540	510	470	430	440	210
16	630	510	510	550	490	440	300
17	630	630	465	440	410	400	195
18	495	465	425	350	300	210	150
19	465	550	600	410	400	350	210
21	630	510	480	480	550	490	310
22	630	550	540	400	350	340	210
23	480	540	540	540	570	630	330
25	540	540	540	540	570	610	355
26	410	400	385	350	390	200	150
28				570	630	630	475
Mean	550	551	549	492	484	437	249
Anticipated Mean	555	630	630	630	630	645	720

Inactivity, depression, and weakness increased as the disease progressed. Signs of central nervous system involvement, starting 1 to 3 days prior to death, were observed in 5 pigs. These signs consisted of muscular spasms with twitching of the head and limbs, paddling movements of the legs, and hyperexcitability or depression. Posterior paresis was present in nearly all of those pigs surviving 9 days or longer.

Initial and final body weights are summarized in Table VII. Mean birth weights were 2.76 pounds for control pigs and 2.71 pounds for cholera-infected pigs. Mean death weights were 5.83 pounds and 4.53 pounds, respectively. This indicated a net weight gain of 40% less for the cholera-infected pigs.

Splenic extracts from 3 infected pigs, inoculated into 6 farm-raised, hog cholera-susceptible pigs, produced death with temperatures, blood counts, and gross lesions typical of those seen in hog cholera. Six hog cholera-susceptible littermates, given commercial anti-hog cholera serum simultaneously with the splenic extract, developed a transitory leukopenia but otherwise remained normal.

B. Gross Lesions

All 21 pigs died within 4 to 15 days after virus inoculation (Fig. 9), with 76% of the deaths occurring from 6 to 12 days after inoculation. Necropsies were performed as soon after death as possible.

Consistent gross necropsy findings (Table VIII) included petechial and ecchymotic hemorrhages in the cortex and medulla of the kidney (Fig. 10). Hemorrhages were

TABLE VII. Body Weights of Infected and Control Pigs (lb.)

Pig No.	Birth Weight	Death Weight	Gain
Control			
1	2.8	5.8	3.0
2	2.7	5.8	3.1
12	2.8	5.9	3.1
20	2.7	5.9	3.2
24	2.6	5.6	3.0
27	3.0	6.0	3.0
Infected			
3	3.1	4.9	1.8
4	2.9	4.8	1.9
5	2.9	4.1	1.2
6	2.8	4.8	2.0
7	2.5	4.4	1.9
8	2.6	4.4	1.8
9	2.3	4.2	1.9
10	2.6	4.5	1.9
11	3.0	5.0	3.0
14	2.4	4.2	1.8
15	2.4	4.8	2.4
16	2.6	4.8	2.2
17	2.6	4.4	1.8
18	2.8	4.5	1.7
19	2.9	4.2	1.3
21	3.1	5.0	1.9
22	2.9	4.2	1.3
23	2.5	4.4	1.9
25	2.3	4.1	1.8
26	3.0	4.9	1.9
28	2.4	4.5	2.1
Control Mean	2.76	5.83	3.06
Infected Mean	2.71	4.53	1.82

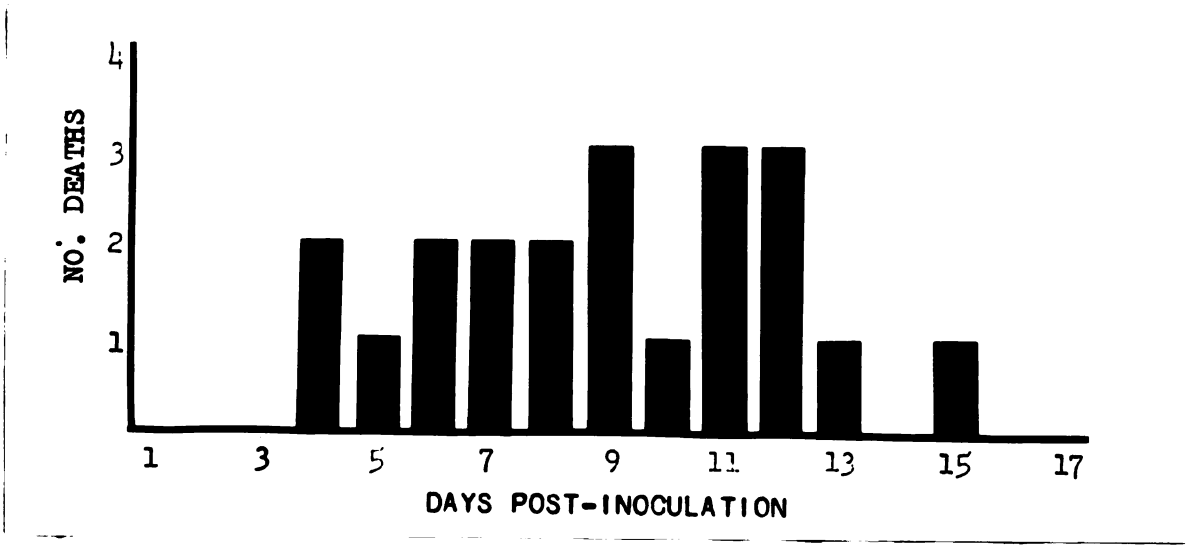


Figure 9. Time of Death of Infected Pigs Following Inoculation with Virus.

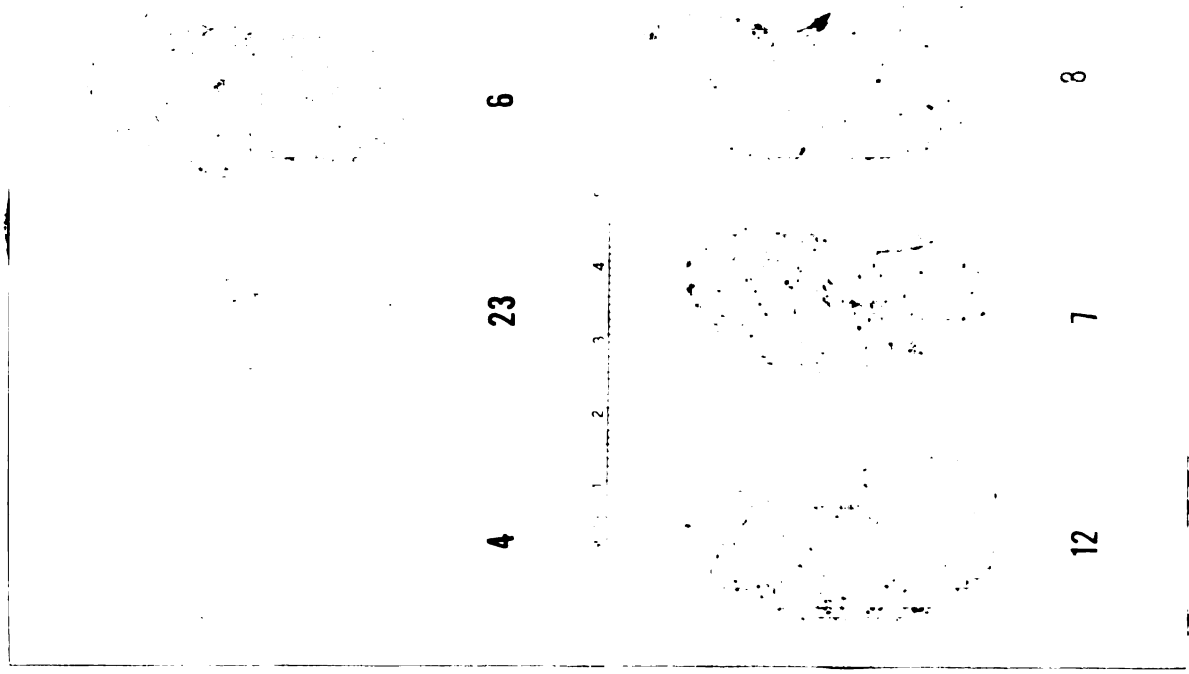


Figure 10. Variations in Kidney Hemorrhages Observed in Infected Pigs.

TABLE VIII. Summary of Gross Necropsy Findings in 21 Infected Pigs

Lesion	Number Showing Lesion
Kidney Hemorrhages	21
Cortex	21
Medulla	17
Hemorrhagic Lymph Nodes	21
External Inguinal	21
Prefemoral	12
Prescapular	15
Parotid	21
Mandibular	21
Suprapharyngeal	18
Mediastinal	8
Gastric	9
Splenic	15
Hepatic	10
Mesenteric	18
Renal	21
Cecal	15
Colic	19
Internal Inguinal	21
Petechial Hemorrhages of Urinary Bladder (Musoca)	15
Body	15
Neck	6
Petechial Hemorrhages of Small Intestine (Mucosa)	12
Duodenum	6
Jejunum	10
Ileum	12
Petechial Hemorrhages of Small Intestine (Serosa)	8
Duodenum	8
Jejunum	8
Ileum	8
Petechial Hemorrhages of Colon (Mucosa)	8
Petechial Hemorrhages of Colon (Serosa)	6

TABLE VII. (Continued)

Lesion	Number Showing Lesion
Hemorrhages on Lungs	6
Apical Lobe	6
Cardiac Lobe	1
Diaphragmatic Lobe	2
Intermediate Lobe	6
Hemorrhages on Meninges	6
Hemorrhages on Diaphragm	5
Hemorrhages on Heart	5
Ventricle	5
Atrium	2
Infarcts in Spleen	3
Petechial Hemorrhages on Parietal Pleura	2
Petechial Hemorrhages in Thymus	1
Gastric Serosal Hemorrhage	1

consistently found in the lymph nodes, either at the periphery or throughout the tissue. Petechiae were found less commonly in the mucosa of the urinary bladder and the mucosa and serosa of the intestine. Gross hemorrhages were also seen in the lungs, meninges, diaphragm, heart, parietal pleura, thymus, and stomach; but the appearance of these lesions was inconsistent from one animal to another. Gross hemorrhages on the epiglottis and larynx, ulcers of the intestine, hyperemia and discoloration of the skin, and prepucial ulcers were not observed. The severity of the lesions was not dependent on the length of survival time. Renal hemorrhages amounting almost to suffusions were present in 1 pig dying 5 days after inoculation while 2 pigs dying 11 and 12 days, respectively, after inoculation had only a few petechial hemorrhages in the kidneys.

C. Microscopic Lesions

Histologically, the lesions found in hog cholera infected pigs were congestion, edema, and hemorrhage, accompanied by thrombosis and necrosis, and degenerative changes in blood vessel walls.

1. Skin, Lip, Cheek, and Ear

Microscopic lesions were not observed in the skin, lip, cheek, or ear of hog cholera-infected pigs. A few areas of congestion were present in the skin of 3 infected pigs.

2. Tonsils

Though the lymphatic tissue of germfree pigs proved to be considerably less well developed than that in

conventionally reared pigs (41,53,54), the tonsils were apparently better developed than other lymphatic structures.

Various degrees of hemorrhage and thrombosis of underlying vessels were found in the tonsillar areas in 15 of 21 infected pigs. These lesions varied from slight, focal hemorrhage within the lymphatic tissue to extensive hemorrhage, necrosis, and thrombosis of vessels with total to partial occlusion (Figs. 11 and 12). Hemorrhages were observed also in the musculature underlying the tonsillar areas.

Suggestions of endothelial proliferation, endothelial pyknosis, and degenerative alterations in blood vessel walls were observed, but comparison with tonsils from control pigs yielded no definitive conclusions.

3. Tongue and Diaphragm

Numerous changes in the blood vessels of the tongue were observed in all infected pigs. The muscular elements and other structures were apparently normal.

Endothelial proliferation (Figs. 13 and 14) and hyaline changes (Fig. 15) were seen in blood vessels. Pyknosis of endothelial cells (Fig. 16) was found. Congestion and hemorrhage were associated with these changes (Fig. 17). Most of these degenerative vascular changes and the accompanying hemorrhage were in the mid portion or base of the tongue. Lesions were not observed in the anterior third of the tongue.

Hemorrhages and edema of the diaphragm were found in 12 of 21 infected pigs (Fig. 18). The hemorrhages were sub-

Figure 11. Hemorrhage in Tonsillar Area.

Hematoxylin and Eosin. x 50

J. Epithelium

K. Erythrocytes

L. Lymphocytes

Figure 12. Hemorrhage, Edema and Thrombi Underlying Tonsillar Area.

Hematoxylin and Eosin. x 105

M. Thrombus

N. Erythrocytes

O. Edema

Figure 13. Endothelial Proliferation in Blood Vessel of Tongue.

Hematoxylin and Eosin. x 540

Figure 14. Proliferation of Endothelial Cells in Blood Vessel of Tongue.

Hematoxylin and Eosin. x 540

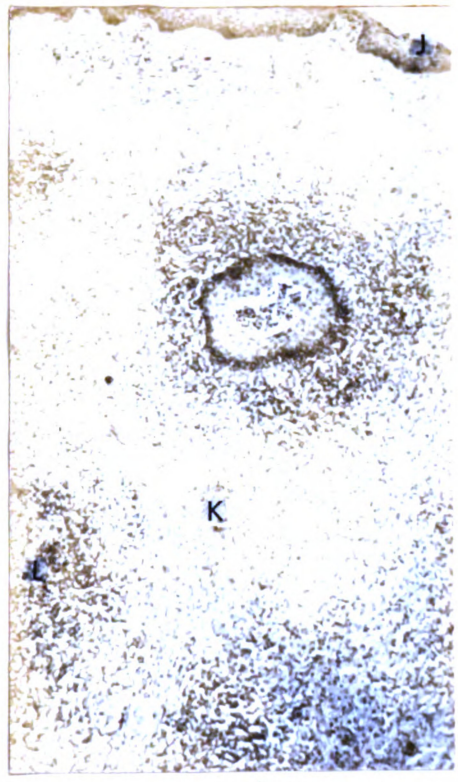
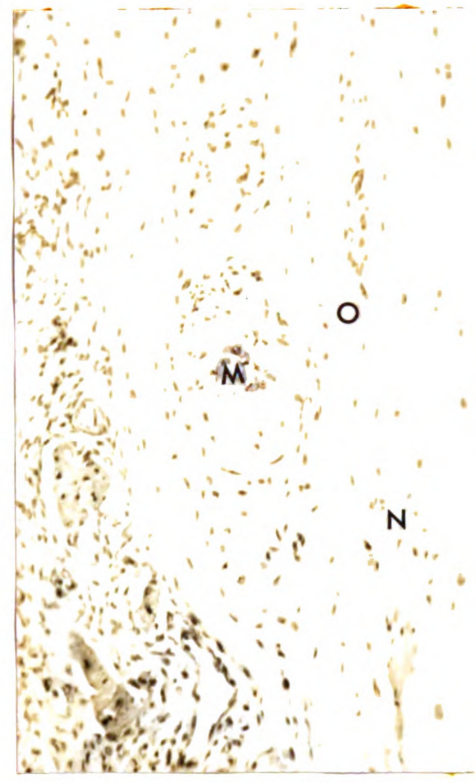


Figure 11



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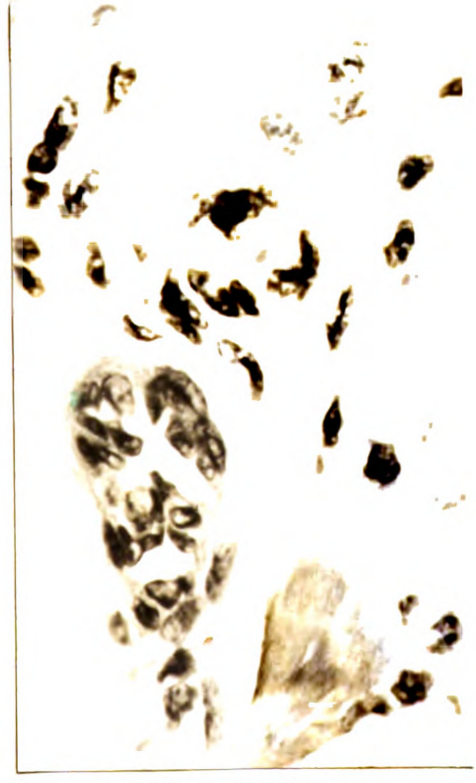


Figure 13

Figure 14

Figure 15. Hyalinization of Blood Vessel Wall in Tongue.

Hematoxylin and Eosin. x 540

P. Hyalin Material

Q. Tongue Muscle

Figure 16. Pyknosis of Endothelial Cells of Blood Vessel in Tongue.

Hematoxylin and Eosin. x 540

Figure 17. Hemorrhage in Papilla of Tongue.

Hematoxylin and Eosin. x 196

R. Stratified Squamous Epithelium

S. Erythrocytes

T. Propria Mucosae

Figure 18. Hemorrhage and Edema of the Diaphragm.

Hematoxylin and Eosin. x 28

U. Serosa

V. Erythrocytes

W. Muscle

Figure 15

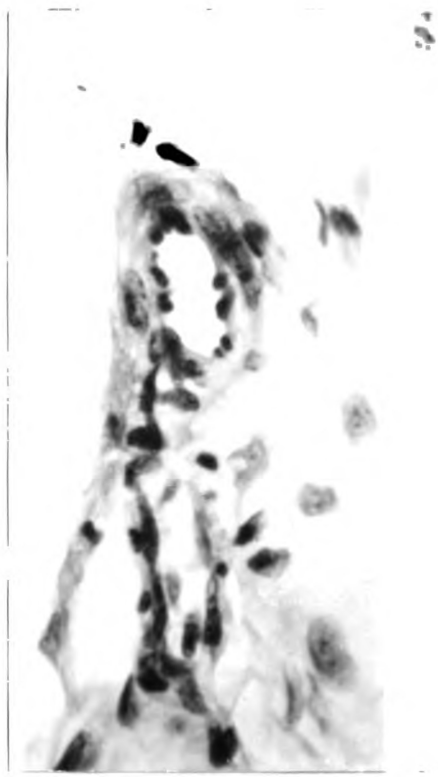


Figure 16

Figure 17

Figure 18

serosal and generally on one side only. It could not be determined if this was the thoracic or abdominal sides, or both. Occasionally hemorrhages were found within the musculature. Suggestions of blood vessel degeneration were observed.

4. Epiglottis, Trachea and Lungs

No lesions were observed in or on the epiglottis or trachea of any infected pig. The surrounding structures were commonly congested, but no other lesions were noted.

Congestion of blood vessels was common in most lungs from infected pigs. Frequent and extensive hemorrhages were found in 10 of 21 lungs; smaller hemorrhages were found in lungs from another 5 infected pigs. The apical and intermediate lobes appeared to be more commonly involved.

Hemorrhages in the lungs were commonly found associated with blood vessels and infiltrating the interlobular connective tissue (Fig. 19). Free blood in the alveoli was not uncommon (Fig. 20). Pleural and subpleural hemorrhages were also observed (Fig. 21). Edema of the lung with numerous alveoli filled with proteinaceous exudate was found in the lungs of 12 of 21 infected pigs (Fig. 22). Occasionally interlobular edema was found (Fig. 23).

5. Salivary Glands and Pancreas

Comparison of salivary glands of hog cholera infected and control pigs did not reveal essential differences. It appeared that more glands were collapsed (especially mucous glands) in infected pigs than in non-infected control animals, but it could not be determined if this was

Figure 19. Perivascular and Interlobular Hemorrhage in Lung.

Hematoxylin and Eosin. x 32

X. Artery

Y. Erythrocytes

Z. Interlobular Area

A. Alveoli

Figure 20. Hemorrhage in Alveoli of Lungs.

Hematoxylin and Eosin. x 224

B. Erythrocytes

C. Alveolus

Figure 21. Hemorrhage and Edema of Visceral Pleura.

Hematoxylin and Eosin. x 105

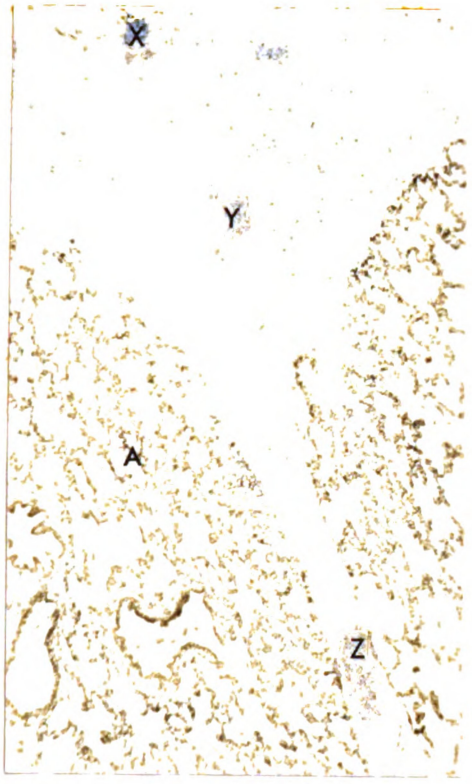
D. Erythrocytes

E. Edema

F. Alveoli

Figure 22. Proteinaceous Fluid in Alveoli of Lung.

Hematoxylin and Eosin. x 140



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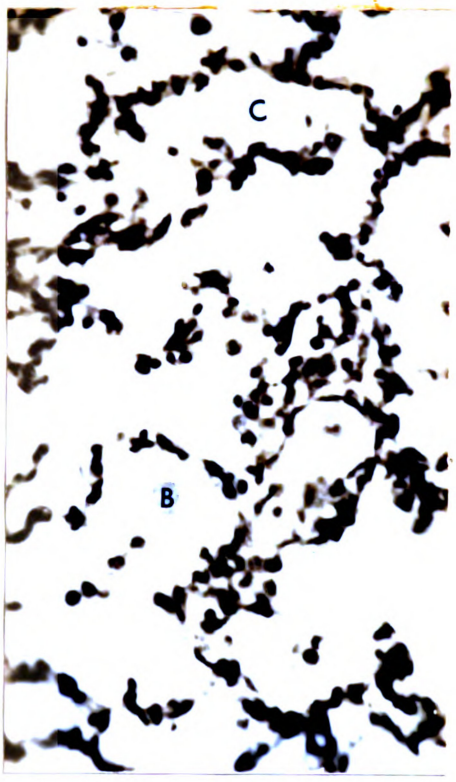

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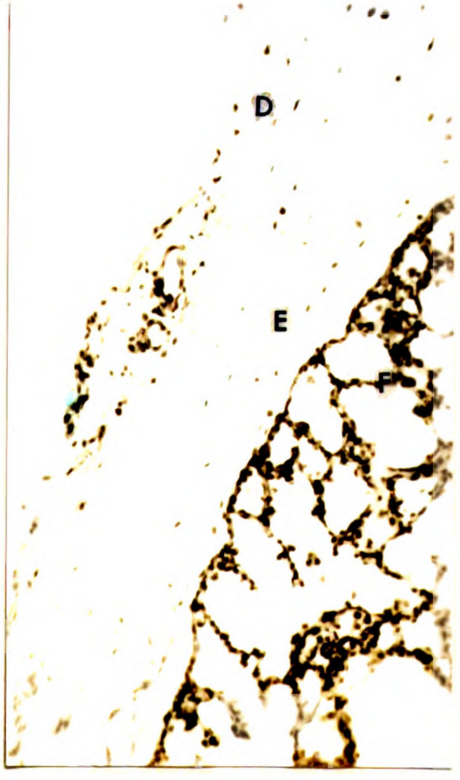

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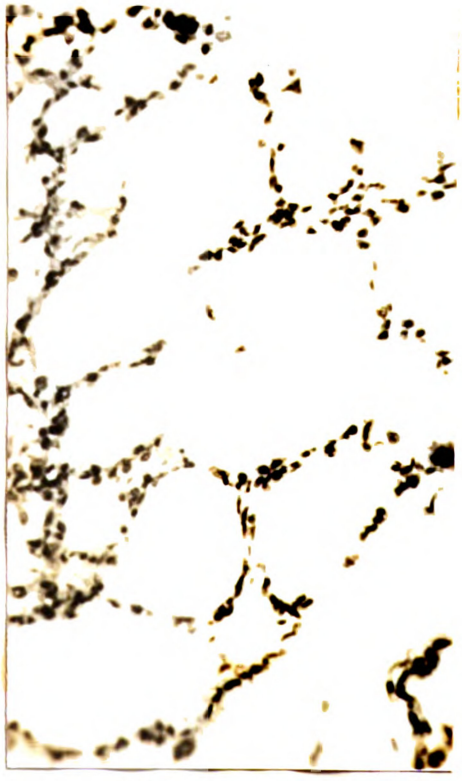


Figure 22

Figure 23. Interstitial Edema of Lung.

Hematoxylin and Eosin. x 105

G. Alveolus

H. Interstitial Edema

Figure 24. Hemorrhage in Salivary Gland.

Hematoxylin and Eosin. x 140

I. Mucous Gland

J. Erythrocytes

K. Duct

Figure 25. Hemorrhage in Myocardium.

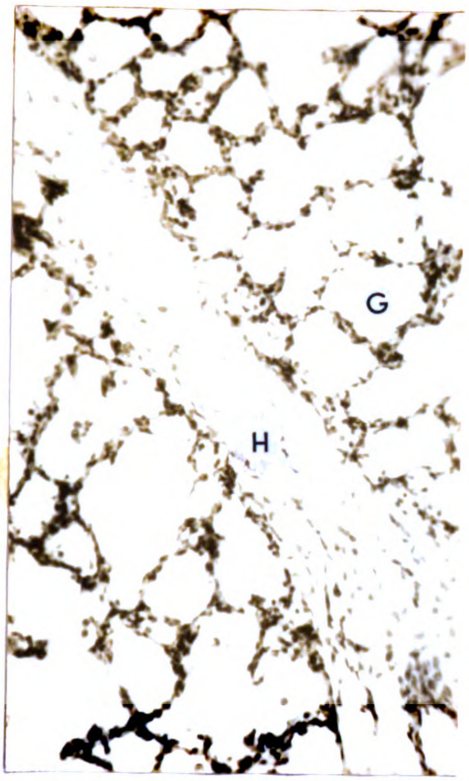
Hematoxylin and Eosin. x 140

Figure 26. Epicardial Hemorrhage.

Hematoxylin and Eosin. x 140

L. Erythrocytes

M. Myocardium.



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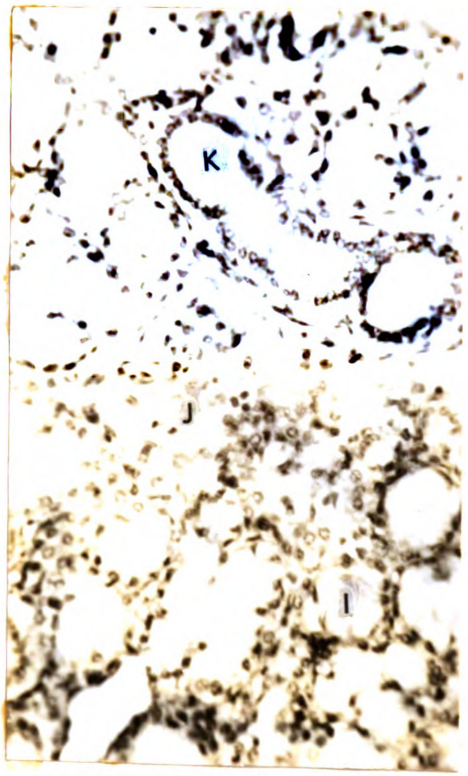

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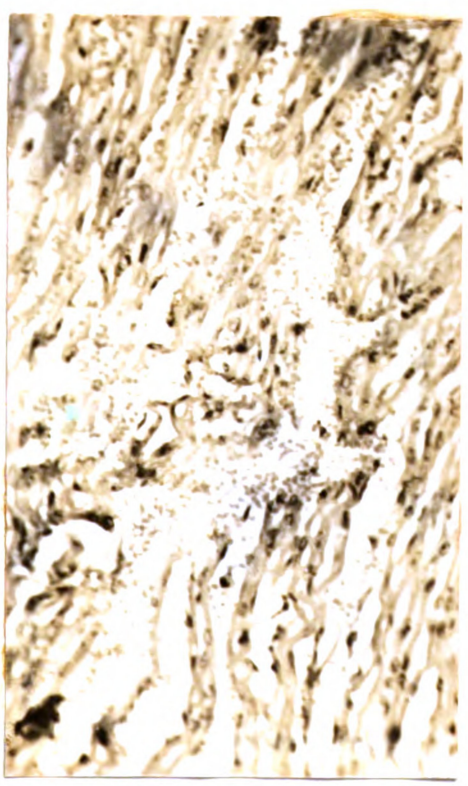


Figure 25

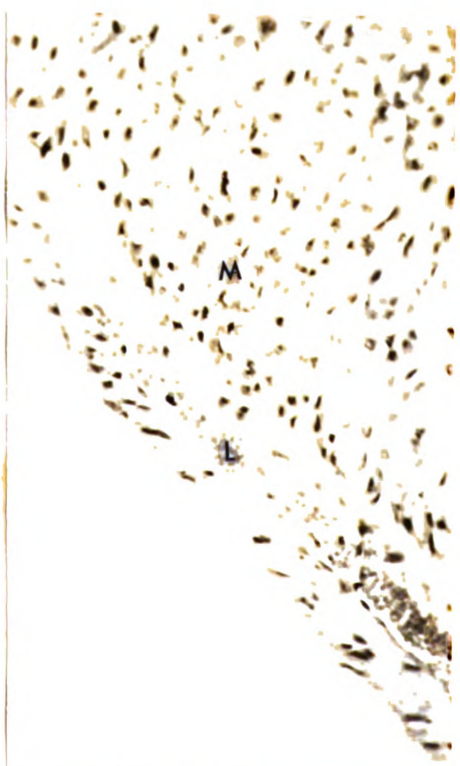


Figure 26

absolute. An occasional small hemorrhage was observed in the salivary glands of 6 infected pigs (Fig. 24), and congestion was not uncommon.

Suggestions of hyaline changes in blood vessels of the pancreas were present; no other microscopic lesions were observed in the pancreas of infected pigs.

6. Aorta and Heart

Histological alterations could not be detected in the aorta of infected pigs. Thrombi were seen in smaller vessels near the aorta.

Hemorrhages were common both within the musculature of the heart and subepicardially in 16 of 21 infected pigs (Figs. 25 and 26). Subendocardial hemorrhages were found in only 3 pigs. These were commonly in the same areas as Purkinje fibers (Fig. 27). The hemorrhages were more common and more extensive in the ventricles; attempts to trace hemorrhages to blood vessel lesions were unsuccessful. Endothelial proliferation in blood vessels or degenerative changes in blood vessel walls were not observed in the hearts of infected pigs.

An outstanding feature of the histopathologic lesions in the heart, in addition to hemorrhages, was the great increase in numbers of Anitschkow myocytes (Fig. 28). Not only were their numbers increased, but the size of the individual nuclei was considerably greater. Increased amounts of nucleoplasm with very distinct prominent central bars of chromatin and accompanying strands radiating to the

Figure 27. Subendocardial Hemorrhage Associated with Purkinje Fibers.

Hematoxylin and Eosin. x 140

N. Myocardium

O. Purkinje Fibers

P. Erythrocytes

Q. Endocardium

Figure 28. Anitschkow Myocytes Associated With Myocardial Hemorrhage.

Hematoxylin and Eosin. x 315

R. Anitschkow Myocyte Nuclei

S. Myocardial Cell Nucleus

T. Erythrocytes

Figure 29. Anitschkow Myocytes in Myocardium.

Hematoxylin and Eosin. x 1200

Figure 30. Anitschkow Myocytes in Myocardium.

Hematoxylin and Eosin. x 1200

U. Anitschkow Myocyte Nucleus

V. Myocardial Cell Nucleus

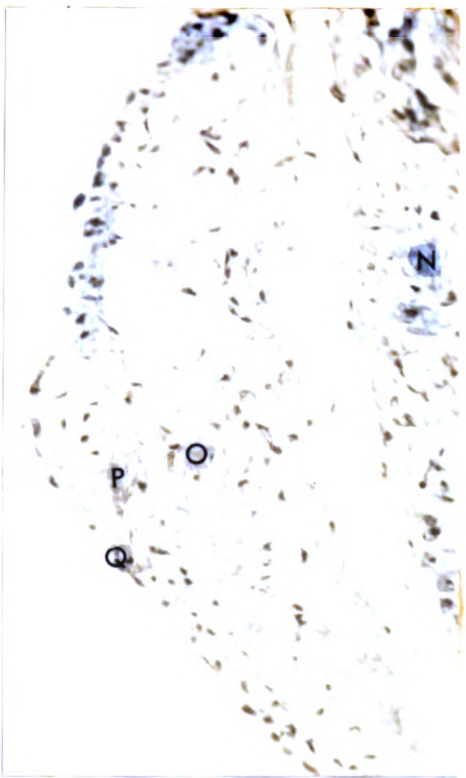
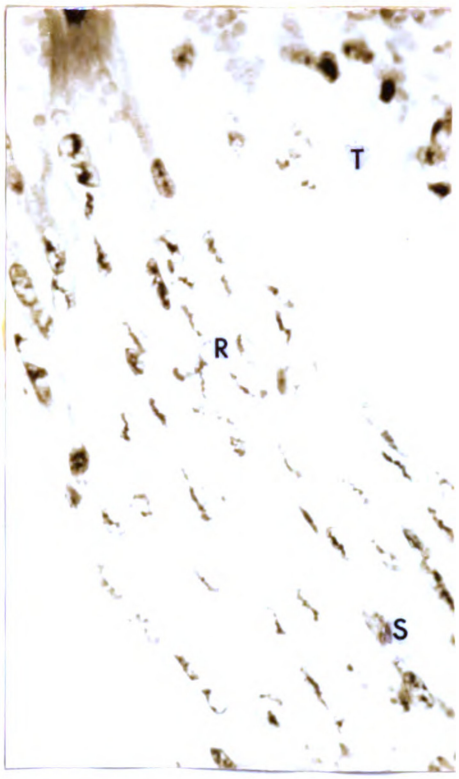


Figure 27



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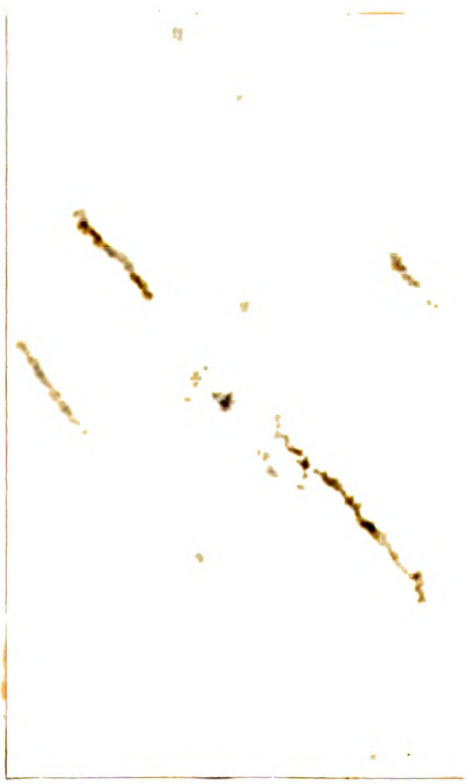


Figure 29

Figure 30

nuclear membrane were found in numerous Anitschkow myocytes of infected pigs (Figs. 29 and 30). These cells were found in the hearts of control pigs, but they were not nearly so numerous or so prominent nor did they show the large amounts of nucleoplasm and the distinct central bars (Fig. 31).

The Anitschkow myocytes were found most commonly in the ventricles, especially in the basal and middle portions. Few were found in the apex. The atria were nearly devoid of these cells. Frequently the Anitschkow myocytes were associated with the intramuscular focal hemorrhages. Myocytes were commonly found without adjacent hemorrhage, but few hemorrhages were found without adjacent Anitschkow myocytes.

7. Spleen and Lymph Nodes

Hemopoietic areas were commonly observed in the spleens of control pigs. These foci were absent or their numbers greatly reduced in infected pigs. Increased numbers of erythrocytes were found in the cortex of the spleens of infected pigs; uniform lymphocytic depletion was common in infected pigs (Figs. 32 and 33); splenic corpuscles were not distinct in all spleens.

Hemorrhages were present on the surface of the spleens of infected pigs with accumulations of erythrocytes frequently distending the capsules (Fig. 34). Some of these foci appeared to be intracapsular. Definite infarctions were not found histologically, but were observed in 3 of 21 infected pigs on gross examination.

Figure 31. Anitschkow Myocyte in Heart of Control Pig.

Hematoxylin and Eosin. x 1200

W. Myocardial Cell Nucleus

X. Anitschkow Myocyte Nucleus

Figure 32. Spleen of Control Pig.

Hematoxylin and Eosin. x 42

Y. Trabecula

Z. White Pulp

Figure 33. Spleen of Infected Pig.

Hematoxylin and Eosin. x 34

A. Trabecula

B. White Pulp

C. Capsule

Figure 34. Hemorrhages on Surface of Spleen.

Hematoxylin and Eosin. x 140

D. Erythrocytes

E. Parenchyma

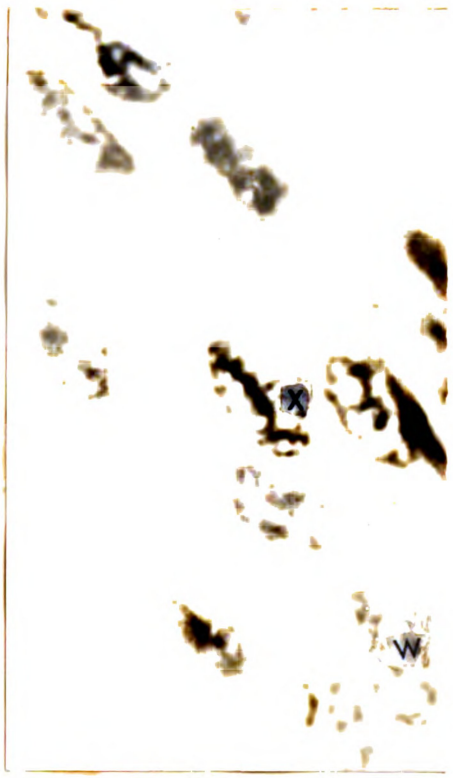


Figure 31

Figure 32

Figure 33

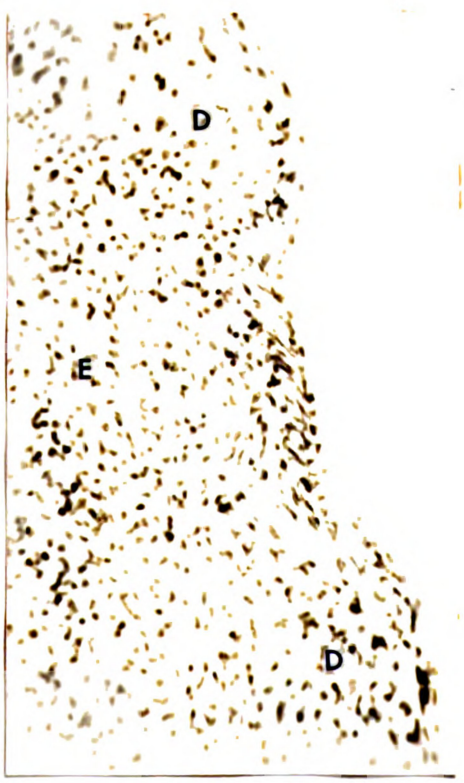


Figure 34

The white pulp of the spleen of infected pigs had numerous pyknotic and degenerating lymphocytes (Fig. 35). These were quite evident when compared with spleens of control pigs (Fig. 36).

Changes in blood vessels were observed in spleens of all infected pigs. These consisted of endothelial proliferation and swelling (Fig. 37), hyaline changes (Fig. 38), and vacuolar degeneration of the subendothelial and medial layers of blood vessel walls (Fig. 39).

A summary of lesions found in lymph nodes of infected pigs is presented in Table IX. Edema was consistent in lymph nodes of infected pigs (Fig. 40). Congestion was common but not consistent. Hemorrhages were present in nearly all lymph nodes of all infected pigs. For convenience, these hemorrhages were arbitrarily classified as (1) moderate peripheral, (2) extensive peripheral, (3) moderate diffuse, and (4) extensive diffuse. Examples of these are shown in Figs. 41, 42, 43, 44 and 45.

Congestion was of similar occurrence in both internal and external lymph nodes of infected pigs. Edema tended to be more common in external nodes; hemorrhages were more extensive in internal nodes.

Lesions were found in the blood vessels of most lymph nodes of all infected pigs. These consisted of endothelial proliferation, endothelial swelling (Fig. 46), hyalinization of blood vessel walls (Figs. 47 and 48), and thrombosis of some smaller blood vessels. All blood vessel lesions

Figure 35. White Pulp of Spleen of Infected Pig.
Hematoxylin and Eosin. x 540

F. Central Artery

G. Pyknotic Lymphocytes

Figure 36. White Pulp of Spleen of Control Pig.
Hematoxylin and Eosin. x 540

H. Central Artery

I. Lymphocytes

Figure 37. Blood Vessel in Spleen Occluded with Endothelial Cells.
Hematoxylin and Eosin. x 540

J. Endothelial Cells

K. Lymphocytic Cells

Figure 38. Hyalinization of Wall of Blood Vessel in Spleen.
Hematoxylin and Eosin. x 540

L. Hyaline Material

M. Immature Lymphocytes

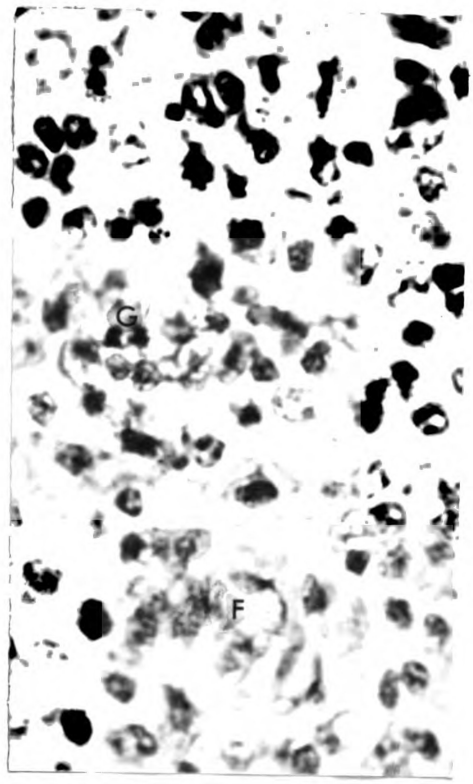


Figure 35

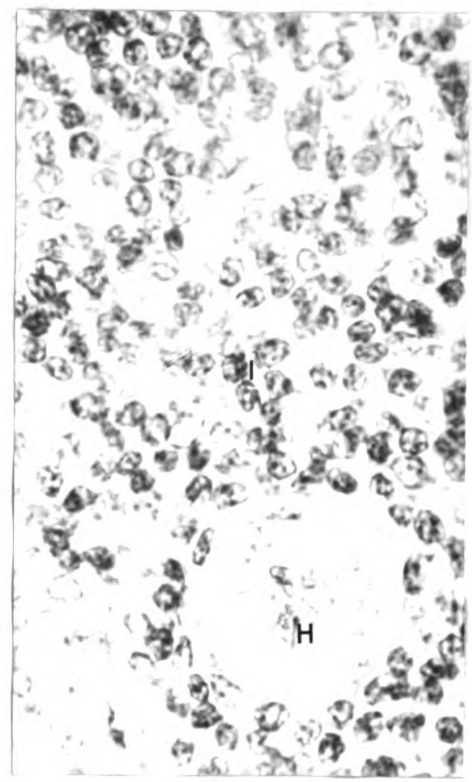


Figure 36

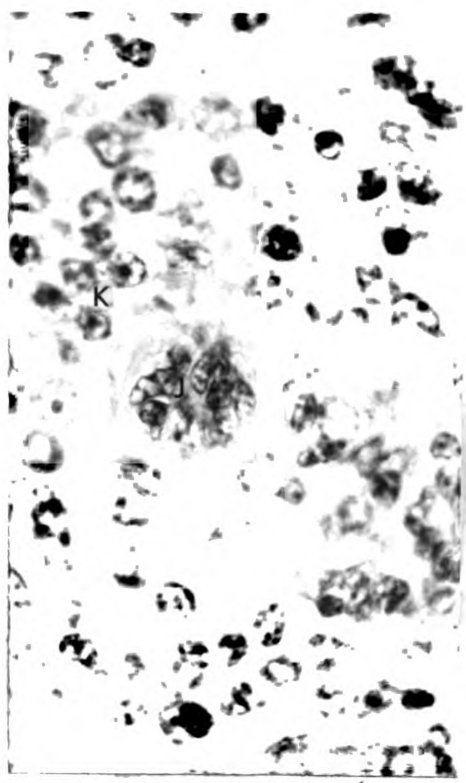


Figure 37

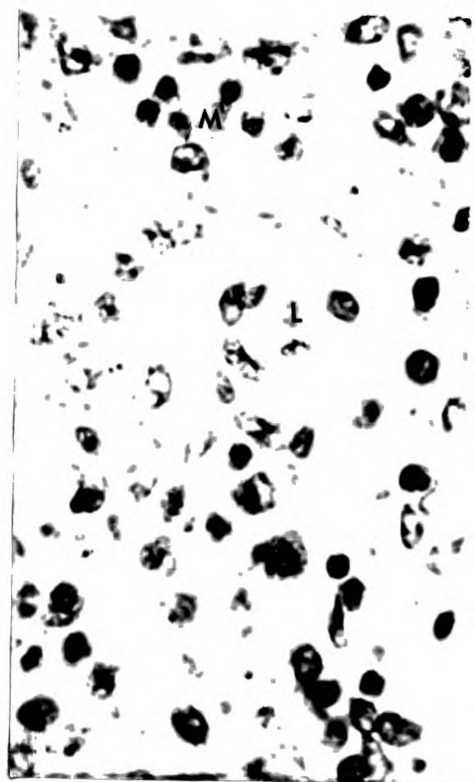


Figure 38

Figure 39. Vacuolar Degeneration of Blood Vessel in Spleen.

Hematoxylin and Eosin. x 315

N. Lumen

O. Vacuoles

P. Smooth Muscle

Figure 40. Edema in Cortex of Lymph Node.

Hematoxylin and Eosin. x 140

Q. Stroma

R. Macrophage with Engulfed Erythrocytes

S. Edema

Figure 41. Lymph Node of Control Pig.

Hematoxylin and Eosin. x 32

Figure 42. Lymph Node with Moderate Peripheral Hemorrhage.

Hematoxylin and Eosin. x 32

T. Erythrocytes

U. Lymphocytic Cells

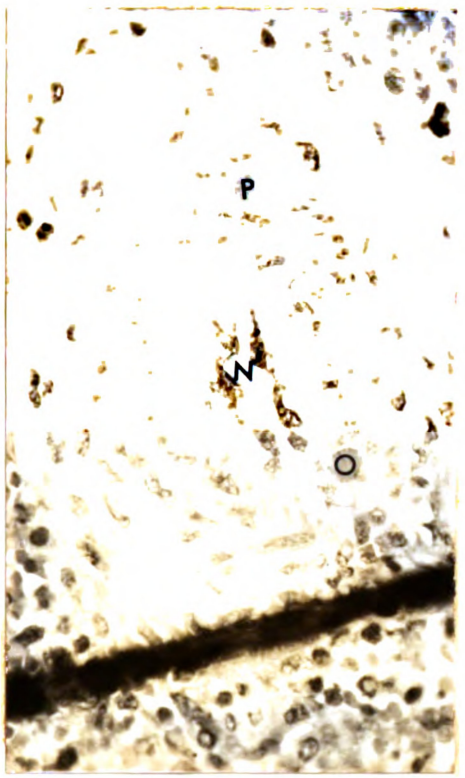


Figure 39

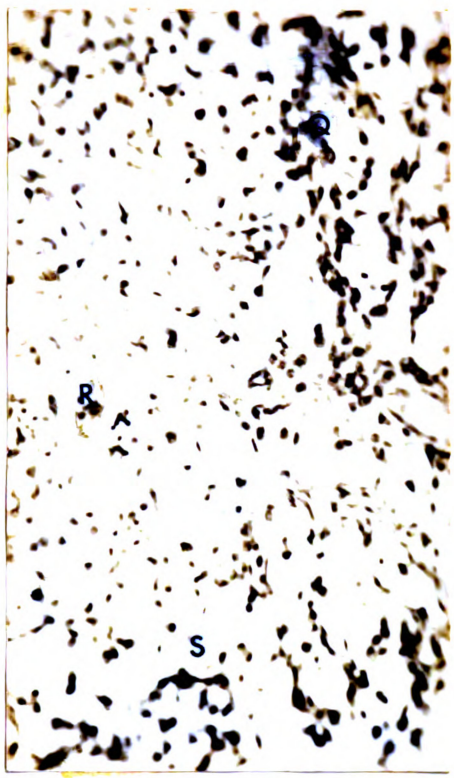


Figure 40

Figure 41

Figure 42

TABLE IX. Summary of Microscopic Lymph Node Lesions in 21 Infected Pigs. (Number of pigs showing each lesion.)

Node	Edema	Congestion	Extent of Hemorrhage* 1	2	3	4
External						
External Inguinal	16	6	12	4	1	4
Prefemoral	16	4	8	4	1	2
Prescapular	12	2	6	6	6	
Parotid	14	10	9	7	4	1
Mandibular	18	12	17	4		
Suprapharyngeal	18	11	12	4	1	1
Internal						
Mediastinal	7	4	1	1	2	4
Gastric	4	3		2	2	5
Splenic	6	4	1	2	7	5
Hepatic	7	5	1	2	4	5
Mesenteric	9	6	4	3	4	7
Renal	10	1	4	4	6	7
Cecal	5	3	3	3	4	5
Colic	4	1	2	7	4	6
Internal Inguinal	6	4	5	2	7	7

*Extent of Hemorrhage
1 - Moderate Peripheral
2 - Extensive Peripheral
3 - Moderate Diffuse
4 - Extensive Diffuse

Figure 43. Lymph Node with Extensive Peripheral Hemorrhage.

Hematoxylin and Eosin. x 32

V. Erythrocytes

W. Lymphocytic Cells

Figure 44. Lymph Node with Moderate Diffuse Hemorrhage.

Hematoxylin and Eosin. x 32

X. Erythrocytes

Y. Lymphocytic Cells

Figure 45. Lymph Node with Extensive Diffuse Hemorrhage.

Hematoxylin and Eosin. x 32

Z. Erythrocytes

A. Lymphocytic Cells

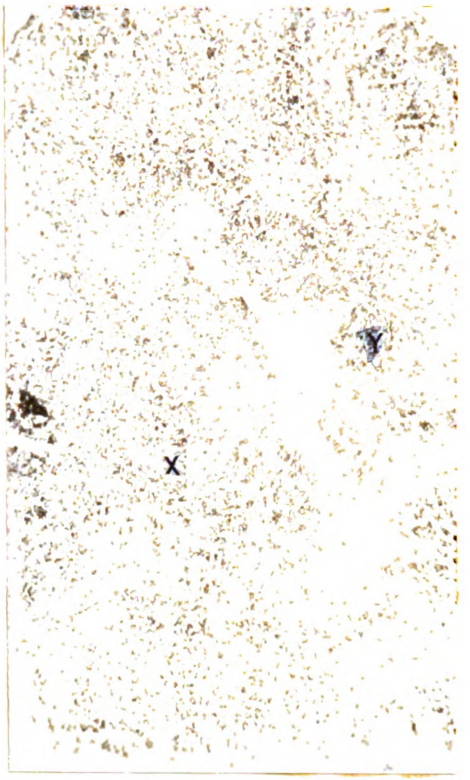
Figure 46. Swollen Endothelial Cells in Blood Vessel of Lymph Node.

Hematoxylin and Eosin. x 1200

B. Lumen

C. Endothelial Cells

Figure 43



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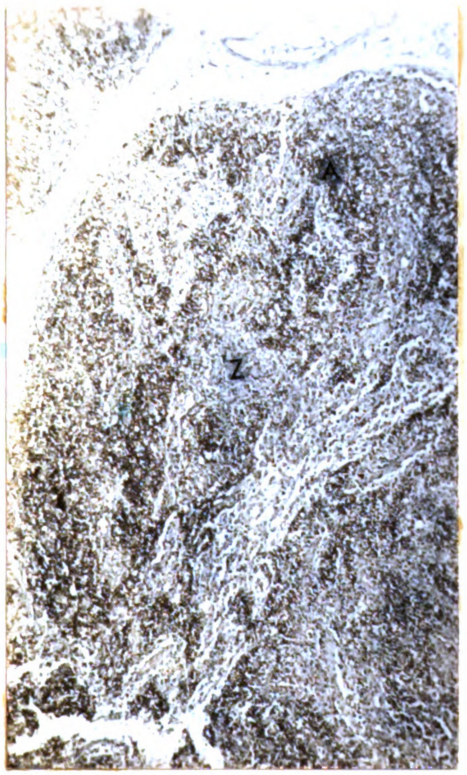

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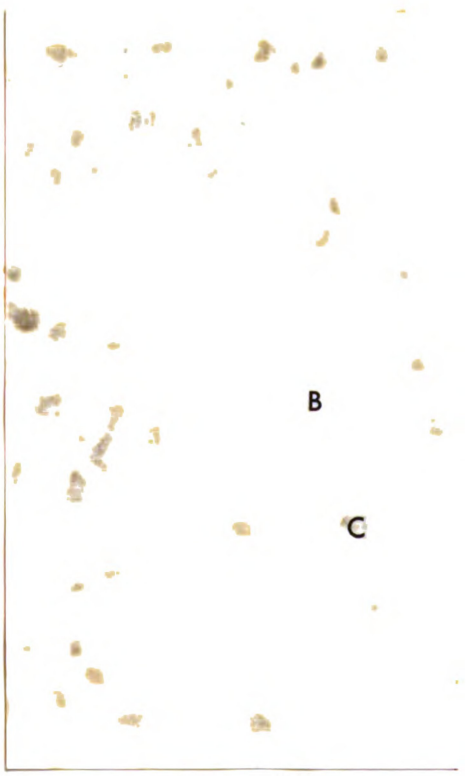

Figure 46

Figure 47. Hyalinization of Wall of Blood Vessel in Cortex of Lymph Node.

Hematoxylin and Eosin. x 540

D. Hyaline Material

E. Stroma

Figure 48. Hyalinization of Wall of Blood Vessel in Medulla of Lymph Node.

Hematoxylin and Eosin. x 540

F. Hyaline Material

G. Degenerate Lymphocytes

Figure 49. Macrophages in Lymph Node Laden with Erythrocytes

Hematoxylin and Eosin. x 540

H. Erythrocytes

I. Nucleus of Macrophage

Figure 50. Subcapsular and Intratrabecular Hemorrhage in Thymus.

Hematoxylin and Eosin. x 32

J. Erythrocytes

K. Thymocytes

L. Capsule

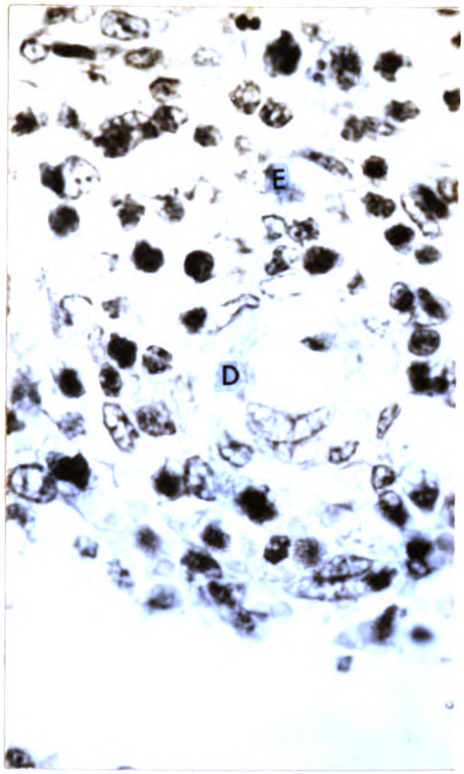

Figure 47

Figure 48

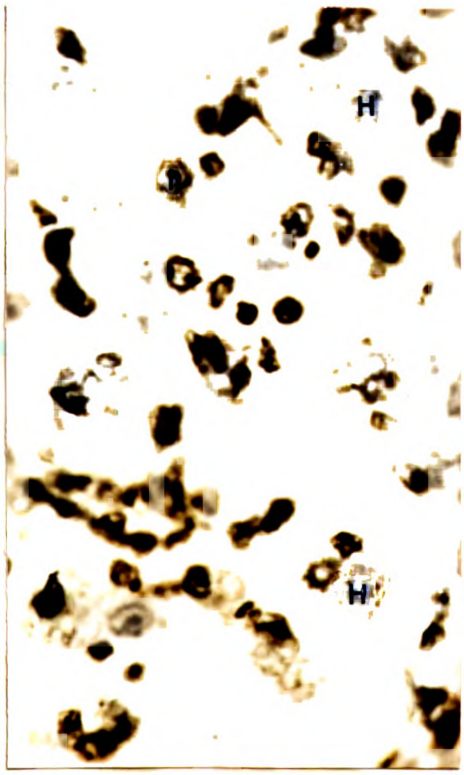

Figure 49

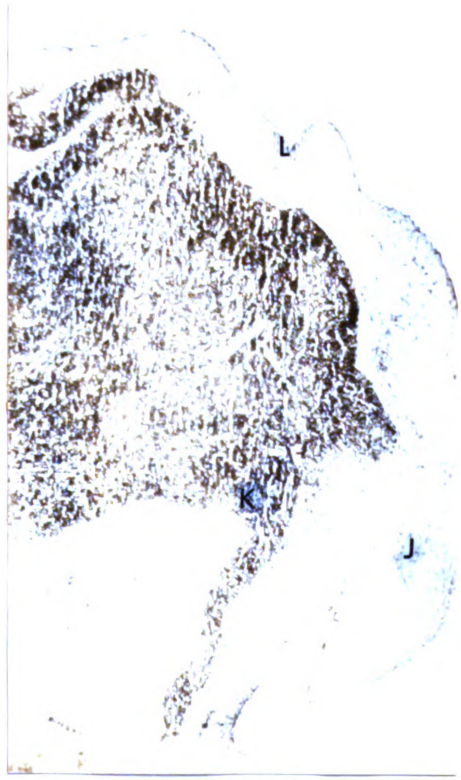

Figure 50

were similar to those previously described in other organs. These were not always distinct nor specific.

Large numbers of pyknotic cells were observed within the lymph nodes of infected pigs, especially in the cortex of internal lymph nodes. These were thought to be degenerating lymphocytes.

Macrophages were prominent within the cortex of each lymph node. They were commonly engorged with erythrocytes (Fig. 49).

8. Endocrine Organs

The thymus of all infected pigs had areas of hemorrhage, varying from focal accumulations of erythrocytes to large subcapsular suffusions (Fig. 50). Hemorrhage frequently extended into the interlobular connective tissue. Edema of the subcapsular tissue and congestion of blood vessels was a common observation.

The adrenals, thyroids and pituitary glands of hog cholera-infected pigs had no observable microscopic lesions.

9. Meninges

Extensive submesothelial hemorrhage was present in the pachymeninges of 6 infected pigs (Fig. 51). Five other pigs had less, but nevertheless distinct, submesothelial hemorrhages. Sufficient lymphocytic and neutrophilic infiltration was present to justify a diagnosis of hemorrhagic pachymeningitis. Congestion and edema were consistently found in the pachymeninges of infected pigs. Some indications of endothelial

Figure 51. Hemorrhage and Edema of Pachymeninges.

Hematoxylin and Eosin. x 140

M. Mesothelium

N. Erythrocytes

O. Edema

Figure 52. Hemorrhage and Edema in Propria Mucosae of Urinary Bladder.

Hematoxylin and Eosin. x 140

P. Surface Epithelium

Q. Erythrocytes

R. Edema

Figure 53. Hemorrhages and Dilated Tubules in Cortex of Kidney.

Hematoxylin and Eosin. x 38

S. Erythrocytes

T. Dilated Tubule

Figure 54. Hemorrhage in Medulla of Kidney.

Hematoxylin and Eosin. x 38

U. Hemorrhagic Area

V. Normal Tubules

Figure 51

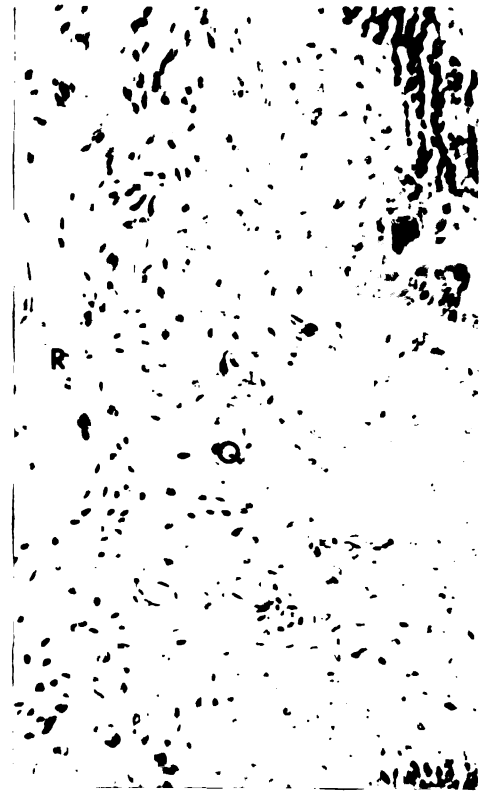

Figure 52

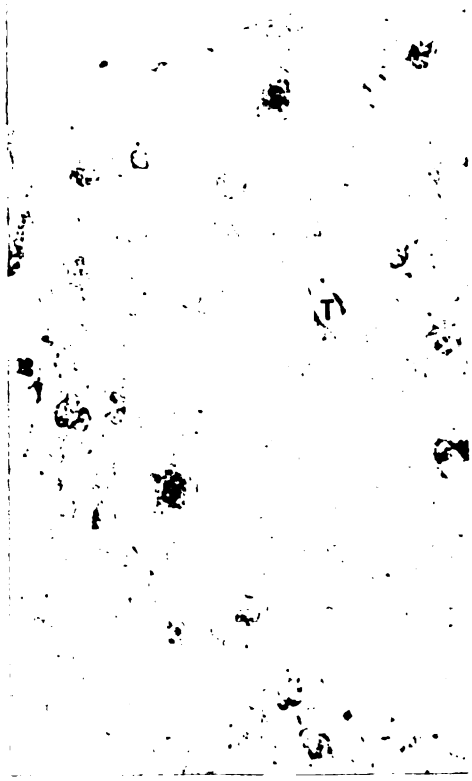

Figure 53

Figure 54

proliferation were present, as well as paravascular* accumulations of lymphocytes.

10. Urinary Ducts and Bladder

The urinary bladder of infected pigs consistently had microscopic submucosal hemorrhages with considerable variation in size (Fig. 52). Vessels of the lamina propria were consistently congested. Edema frequently accompanied this hemorrhage and congestion. A few subserosal hemorrhages were also observed.

Subtle changes were noted in blood vessel walls, but endothelial changes and degenerative alterations in blood vessel walls were indistinct.

Changes were not observed in the ureters of hog cholera-infected pigs. The corpus cavernosum of the abdominal urethra in the male was consistently congested, but no evidence of degenerative changes in blood vessel walls or hemorrhages were observed.

11. Kidney

Hemorrhages were observed in the cortex of all kidneys of all infected pigs and in the medulla of 15 of 21 infected pigs. The hemorrhages were quite variable in size and location (Figs. 53 and 54). Many subcapsular hemorrhages were found as well as extensive accumulations of erythrocytes between tubules in both medulla and cortex.

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*Paravascular, meaning beside the blood vessel. Perivascular accumulations of lymphocytes (meaning around the blood vessel) were seen only occasionally in the various organs of infected pigs.

Those kidneys with extensive accumulations of erythrocytes between collecting tubules frequently had dilated or cystic tubules in the cortex of the kidney (Fig. 53).

Proteinaceous casts (Fig. 55) were present in many tubules, together with hyaline changes in the blood vessels (Fig. 56). The glomeruli did not seem to be affected; inflammatory infiltration was not present; degenerative changes in the tubules were not observed.

12. Liver and Gallbladder

The lobular appearance, commonly seen in livers of conventionally reared pigs, was indistinct in all animals; the interlobular connective tissue was not well developed.

Hepatic cells of all non-infected pigs were heavily laden with what appeared to be glycogen, in many cells to such an extent as to render morphologic detail unrecognizable (Fig. 57). Hemopoietic areas were numerous throughout the tissues (Fig. 58). The livers of hog cholera-infected pigs contained little glycogen (Fig. 59) and few active hemopoietic foci. Occasionally, near a central vein of an infected pig liver, a limited fatty change was noted (Fig. 60).

Focal areas of coagulation necrosis with characteristic pyknosis and accompanied by hemorrhage were observed throughout all livers (Figs. 61 and 62). These areas were not limited to any specific location, and varied in size from a few liver cells to areas covering nearly all a high power field (250 microns). Infected pigs had similar lesions except the accompanying hemorrhage was of considerably less volume.

Figure 55. Hemorrhage and Proteinaceous Casts in Cortex of Kidney.

Hematoxylin and Eosin. x 105

W. Cast

X. Erythrocytes

Figure 56. Hyaline Changes in Blood Vessel Wall and Degeneration of Endothelial Cells. Cortex of Kidney.

Hematoxylin and Eosin. x 540

Y. Hyaline Material

Z. Tubule Cells

Figure 57. Large Amounts of Glycogen in Liver of Control Pig.

Hematoxylin and Eosin. x 315

Figure 58. Hemopoietic Foci in Liver of Control Pig.

Hematoxylin and Eosin. x 315

A. Hemopoietic Focus

B. Glycogen-laden Hepatic Cells

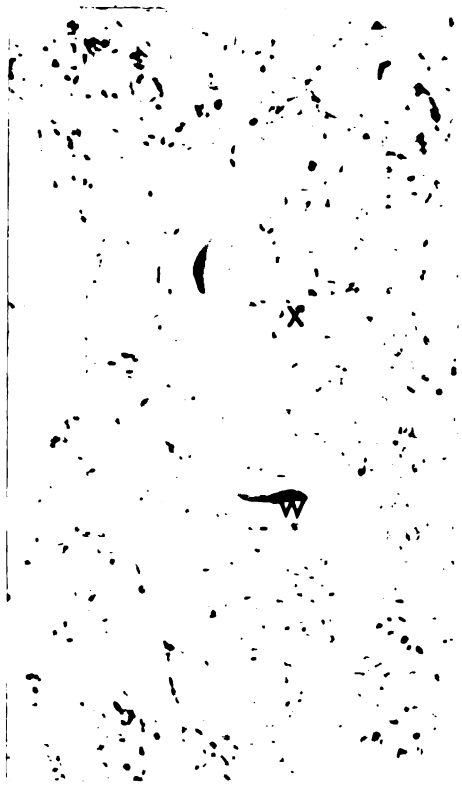


Figure 55

Figure 56

Figure 57

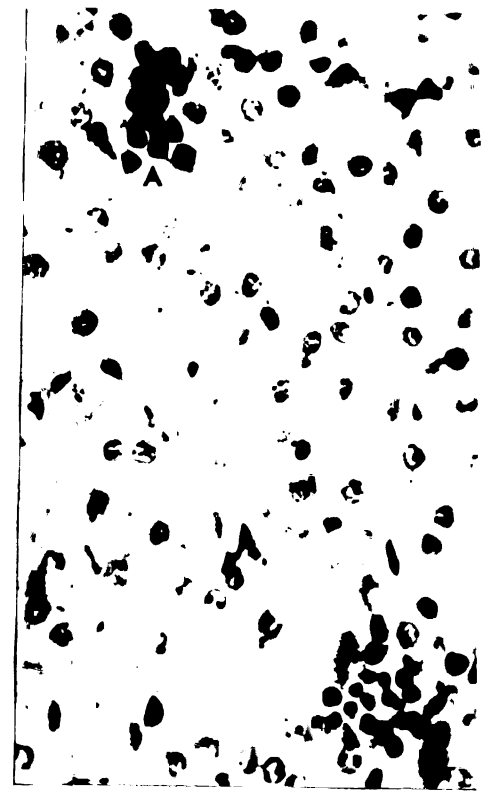


Figure 58

Figure 59. Absence of Glycogen in Hepatic Cells of Infected Pig.

Hematoxylin and Eosin. x 140

Figure 60. Fatty Changes Near Central Vein of Liver.

Hematoxylin and Eosin. x 315

C. Vein

D. Fat Droplet in Hepatic Cell

Figure 61. Coagulation Necrosis Near Central Vein of Liver of Control Pig.

Hematoxylin and Eosin. x 140

E. Hepatic Cells

F. Necrotic Area

G. Lumen of Vein

Figure 62. Coagulation Necrosis in Liver of Control Pig.

Hematoxylin and Eosin. x 140

H. Hepatic Cells

I. Necrotic Area

Figure 59

Figure 60

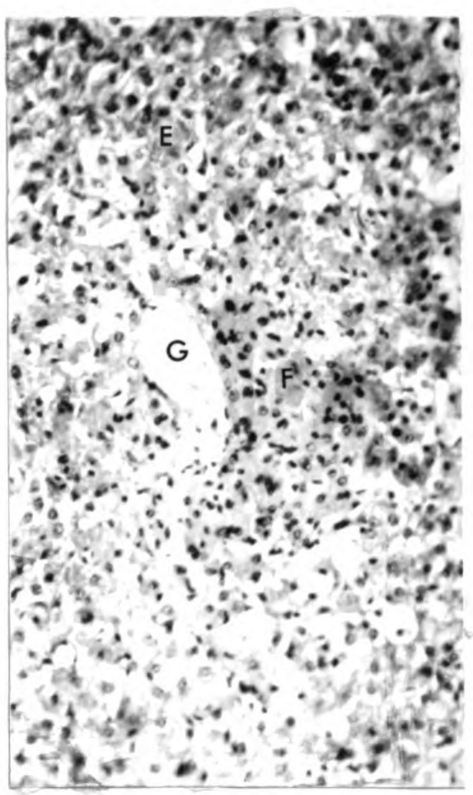


Figure 61

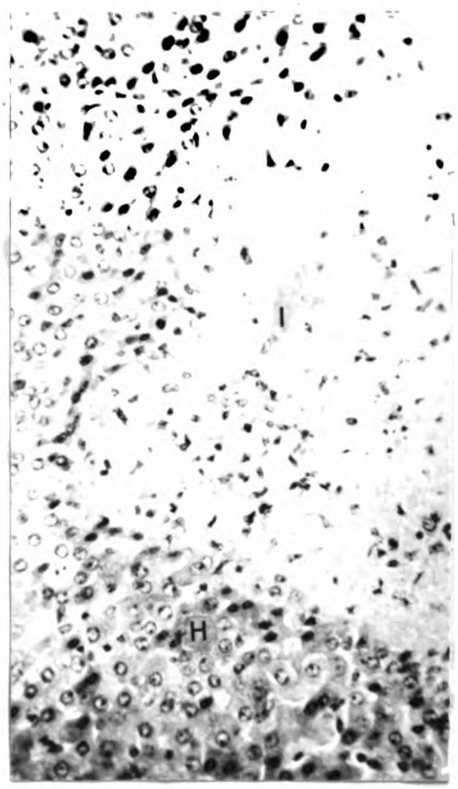


Figure 62

Interlobular veins in livers of infected pigs frequently had paravascular accumulations of cells, predominately lymphocytes (Figs. 63,64,65 and 66). Endothelioid cells, eosinophils and an occasional basophil were also observed. In several instances, the paravascular infiltration was such that it caused a projection into the lumen of the vessel (Figs. 67 and 68). Some lymphocytic infiltrations were found in the connective tissue accompanying large blood vessels and occasionally near bile ducts (Fig. 69). A tendency toward actual "cuffing" of blood vessels was noted only in the liver of one infected pig. The paravascular infiltration had little effect on surrounding hepatic cells; an occasional area was noted with some pressure degeneration of liver cells.

Vascular changes in infected pigs were indistinct and non-specific. Some suggestion of endothelial swelling and proliferation and degenerative changes in blood vessel walls was present, but not sufficient for positive conclusions. Some subcapsular hemorrhages were noted (Fig. 70), as well as hemorrhages in the connective tissue near large vessels. Moderate congestion of the hepatic sinusoids was a consistent finding in infected pigs.

Vessels in the lamina propria of the gallbladder were consistently congested. Fifteen of 21 infected pigs had subepithelial edema of the gallbladder with accompanying congestion, some paravascular infiltration with lymphocytes, and early degenerative changes (Fig. 71). Distinct

Figure 63. Focus of Lymphocytes in Interlobular Area of Liver.

Hematoxylin and Eosin. x 140

Figure 64. Focus of Cells, Predominantly Lymphocytes, in Interlobular Area of Liver.

Hematoxylin and Eosin. x 140

J. Hepatic Cells

K. Blood Vessel

L. Cellular Infiltration

Figure 65. Paravascular Infiltration of Lymphocytes Near Interlobular Vein.

Hematoxylin and Eosin. x 140

M. Vein

N. Lymphocytes

O. Hepatic Cells

Figure 66. Paravascular Infiltration of Lymphocytes in Liver.

Hematoxylin and Eosin. x 168

P. Hepatic Cells

Q. Vein

R. Lymphocytes

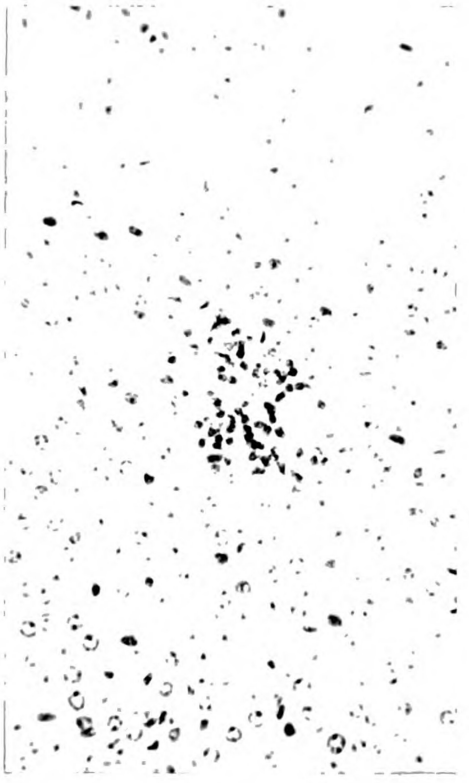


Figure 63

Figure 64

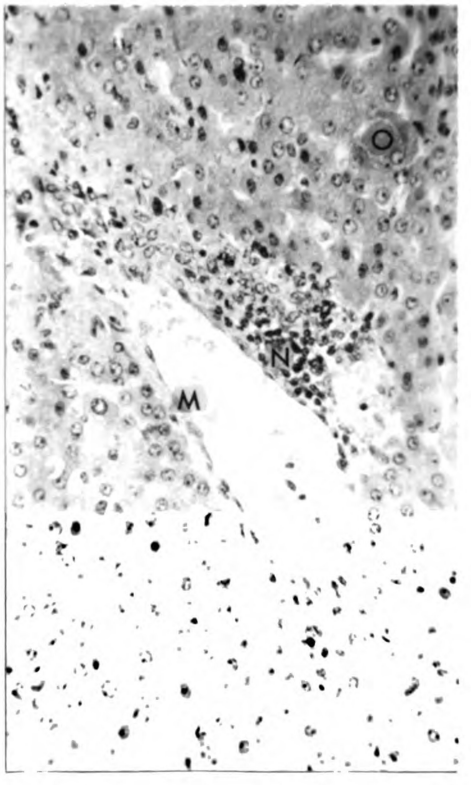


Figure 65

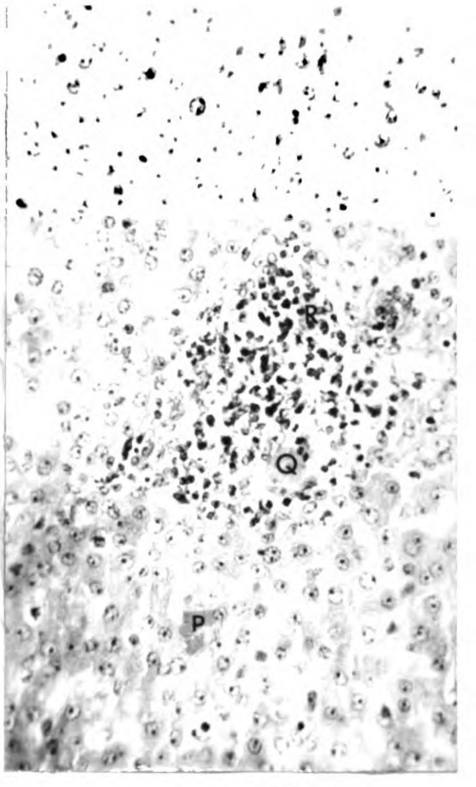


Figure 66

Figure 67. Mass of Lymphocytes Protruding into an Interlobular Vein of the Liver.

Hematoxylin and Eosin. x 140

S. Hepatic Cells

T. Bile Duct

U. Lymphocytes

V. Lumen of Vein

Figure 68. Paravascular Infiltration of Lymphocytes in Liver.

Hematoxylin and Eosin. x 140

W. Hepatic Cells

X. Lymphocytes

Y. Lumen of Vein

Figure 69. Lymphocytic Infiltration Around Interlobular Bile Ducts in Liver.

Hematoxylin and Eosin. x 140

Z. Vein

A. Bile Duct

B. Lymphocytes

Figure 70. Capsular Hemorrhage of Liver.

Hematoxylin and Eosin. x 140

C. Erythrocytes

D. Hepatic Cells

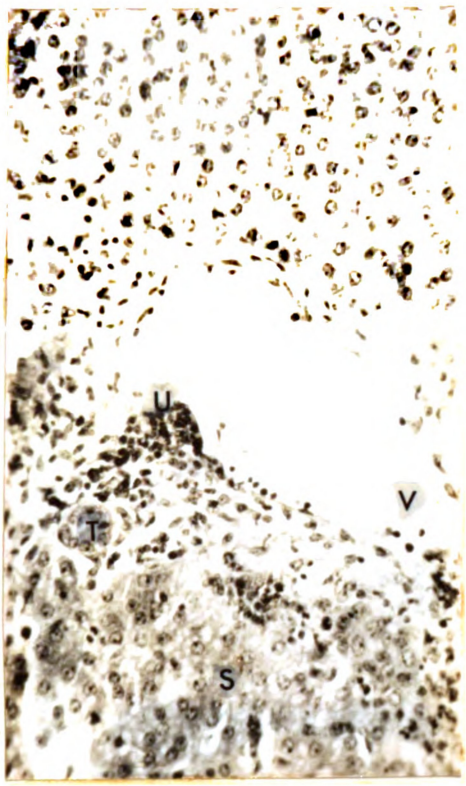


Figure 67

Figure 68

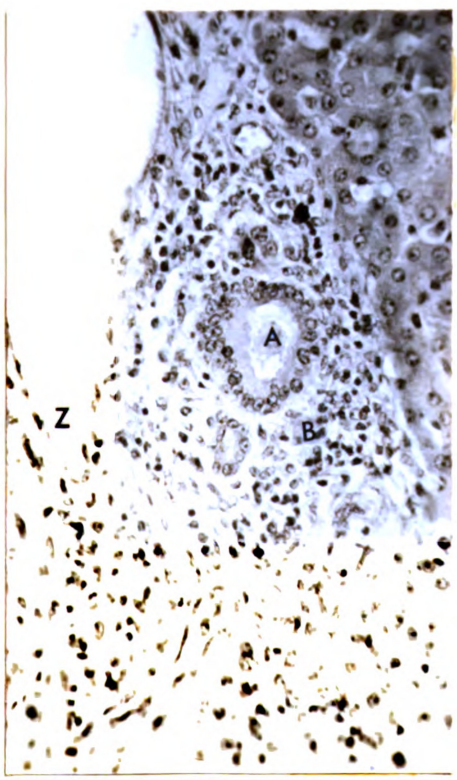


Figure 69

Figure 70

Figure 71. Edema of Gallbladder Mucosa.

Hematoxylin and Eosin. x 70

E. Edema

F. Congestion

G. Leukocytic Infiltration

Figure 72. Focal Necrosis of Gallbladder Mucosa.

Hematoxylin and Eosin. x 70

H. Necrotic Area

I. Leukocytes

J. Edema

Figure 73. Necrosis of Mucosa of Esophagus.

Hematoxylin and Eosin. x 98

K. Normal Epithelium

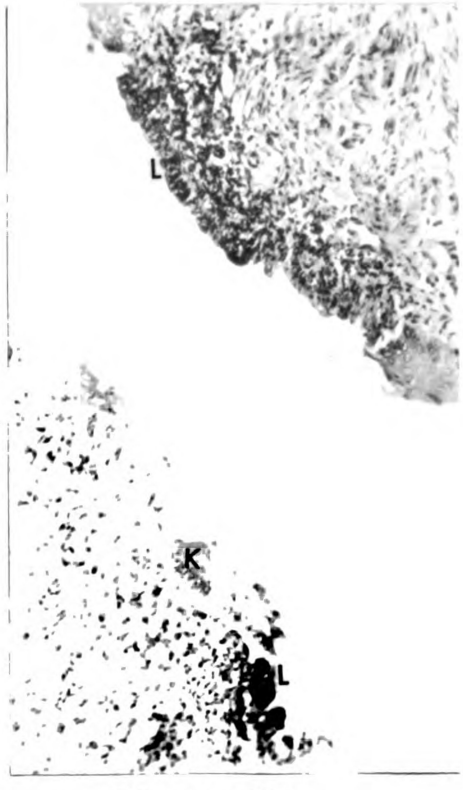
L. Necrotic Areas

Figure 74. Degenerative Changes in Wall of Blood Vessel. Submucosa of Esophagus.

Hematoxylin and Eosin. x 504

Figure 71

Figure 72



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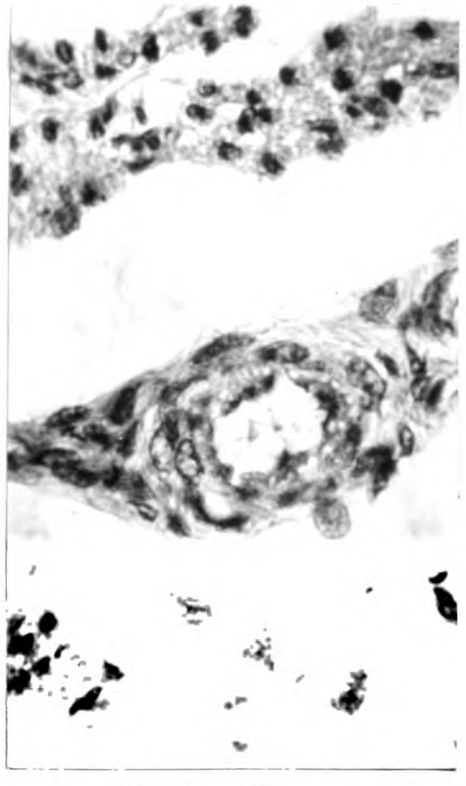


Figure 74

focal necrosis of the gallbladder mucosa was observed in 5 infected pigs (Fig. 72). The base of the necrotic areas consisted of degenerated fibroblasts and epithelial cells, with neutrophilic infiltration. Degenerative changes in blood vessels of the lamina propria were not distinct.

13. Esophagus and Stomach

The thoracic portions of the esophagus from 8 of 21 infected pigs had focal areas of necrosis in the epithelium with slight extension into the lamina propria (Fig. 73). Moderate lymphocytic and neutrophilic infiltration accompanied these degenerative changes. Congestion was present in the esophagus of nearly all infected pigs. Hyaline and vacuolar changes were present in some blood vessels (Fig. 74). Subserosal hemorrhages were found in several areas near the thoracic inlet. These were frequently accompanied by hemorrhage in the surrounding musculature.

Frequently, blood vessels within the musculature of the stomach were surrounded by lymphocytes. These accumulations were present in both control and infected pigs.

Rather extensive areas of necrosis were found in the mucosa of the stomach in 12 of 21 infected pigs (Fig. 75). These were accompanied by underlying infiltrations of neutrophils and lymphocytes (Fig. 76). Areas of hemorrhagic gastritis with erosion of surface epithelium and only a slight leukocytic infiltration were found in the stomach of 3 of 21 infected pigs (Fig. 77). Lymphocytic foci were found commonly in the submucosal areas of the stomach of infected

Figure 75. Necrosis of Mucosa of Stomach.

Hematoxylin and Eosin. x 38

M. Normal

N. Necrotic

Figure 76. Necrotic Area of Stomach Mucosa with Leukocytic Infiltration.

Hematoxylin and Eosin. x 140

O. Necrotic Epithelium

P. Neutrophils and Lymphocytes

Q. Submucosa

Figure 77. Necrosis and Hemorrhage of Stomach Mucosa.

Hematoxylin and Eosin. x 98

R. Mucosa

S. Erythrocytes

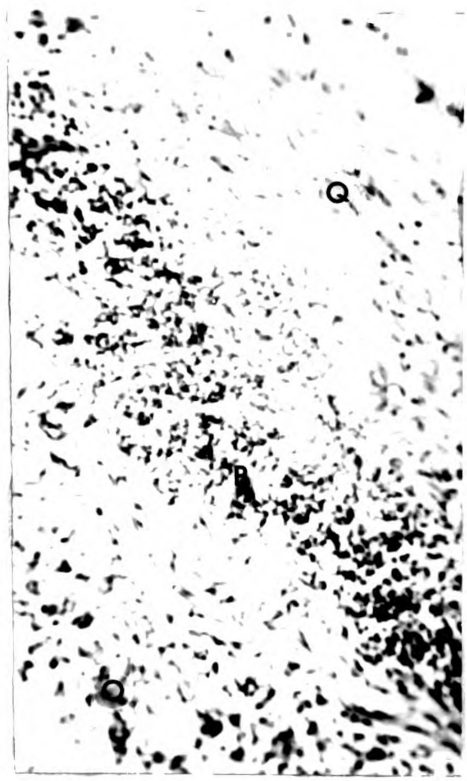
T. Necrotic Epithelium

Figure 78. Hemorrhage in Propria Mucosae of Small Intestine.

U. Epithelium

V. Erythrocytes

Figure 75



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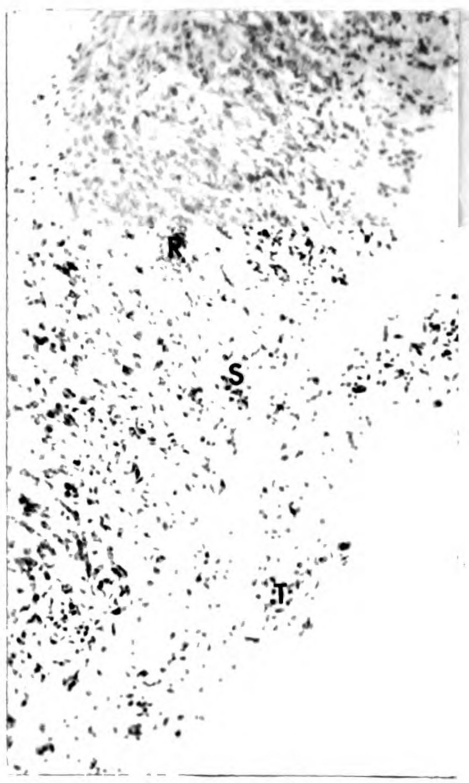

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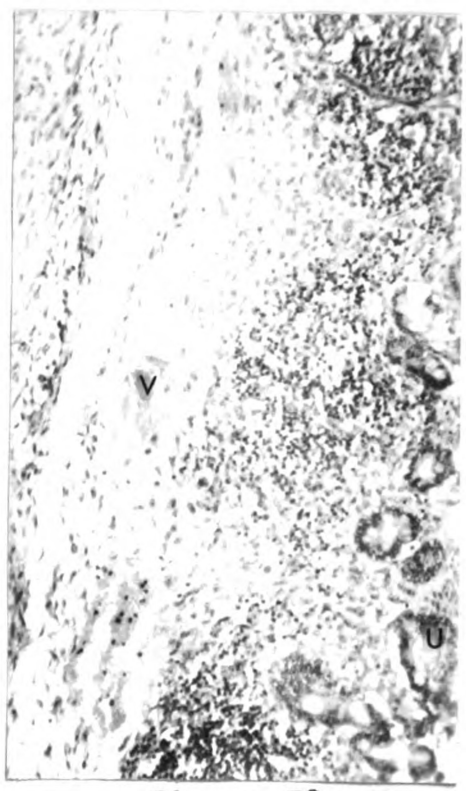


Figure 78

pigs. Generally congestion of blood vessels was not a common finding.

14. Small Intestine, Cecum and Colon

The epithelium of portions of the small intestine (especially the terminal portions) of control pigs contained many vacuoles. These vacuoles were much less numerous and smaller in size in infected pigs.

The submucosa and adjacent mesentery of the small intestine were congested in nearly all infected pigs. Mucosal, submucosal, and serosal hemorrhages varying greatly in size and location were found in 15 of 21 infected pigs (Fig. 78). Hemorrhages were common near Peyer's patches and other lymphocytic aggregations (Fig. 79). Areas quite suggestive of thrombosis were found (Fig. 80).

Necrosis and sloughing of the mucosa were observed in 10 of 21 infected pigs (Fig. 81). The degenerative changes were limited chiefly to the epithelium with only slight accumulations of inflammatory cells.

Some suggestion of increased lymphocytes was presented in the lamina propria of the mucosa of infected pigs, but interpretation was difficult because of normally occurring lymphocytes in this area.

Edema, congestion and possible thrombosis (Fig. 82) were found in the cecum of some infected pigs. Necrosis was not observed within the mucosa. Hemorrhages of various size and in various locations were found in 12 of 21 infected pigs (Figs. 83 and 84).

Figure 79. Hemorrhage in Peyer's Patch of Small Intestine.

Hematoxylin and Eosin. x 140

W. Erythrocytes

X. Lymphocytic Focus

Figure 80. Thrombus in Blood Vessel of **Propria Mucosae of Small Intestine.**

Hematoxylin and Eosin. x 140

Y. Thrombus

Z. Propria Mucosae

Figure 81. Necrosis, Hemorrhage, and Congestion of Mucosa of Small Intestine.

Hematoxylin and Eosin. x 140

A. Necrosis of Epithelium

B. Erythrocytes

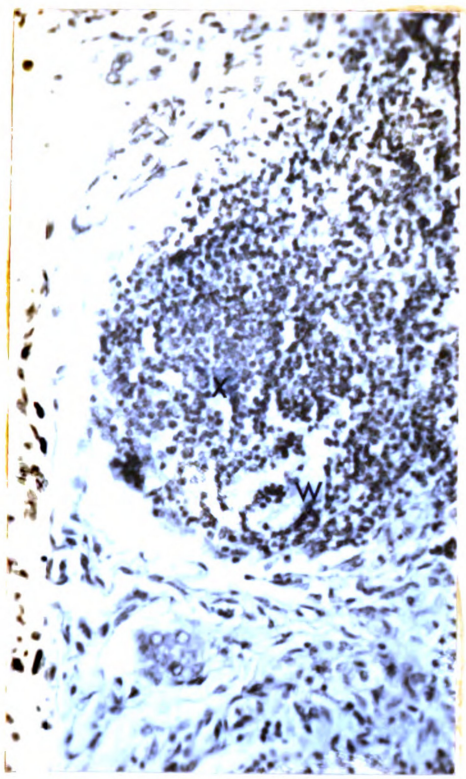
Figure 82. Hemorrhage, Edema and Thrombi in **Submucosa of Cecum.**

Hematoxylin and Eosin. x 91

C. Erythrocytes

D. Edema

E. Thrombus



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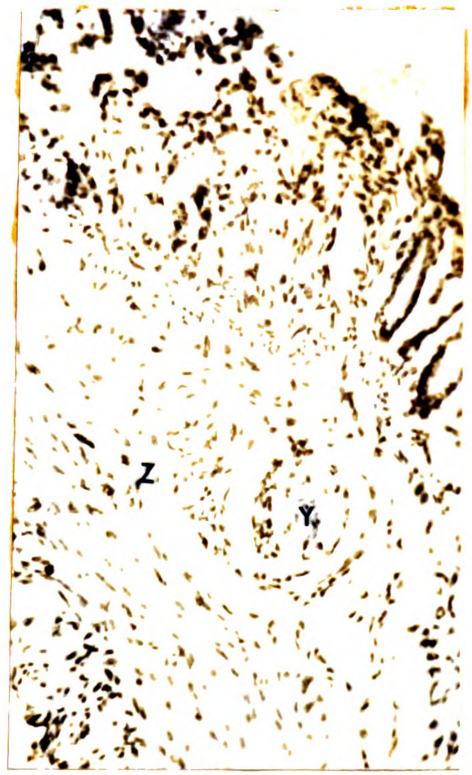

Figure 80

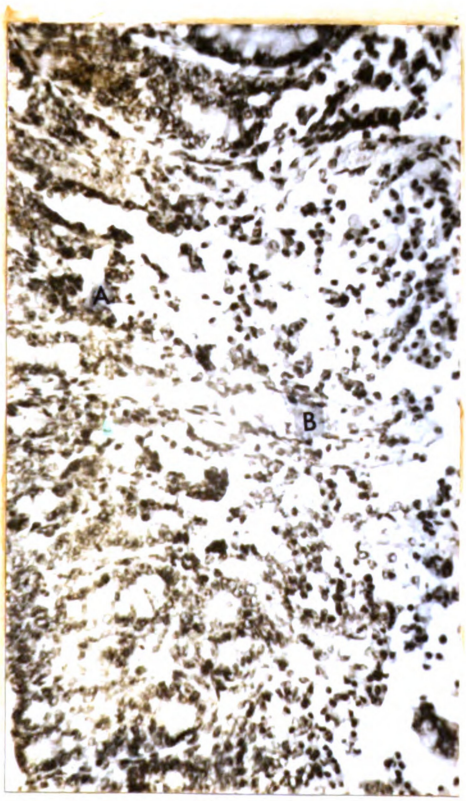

Figure 81

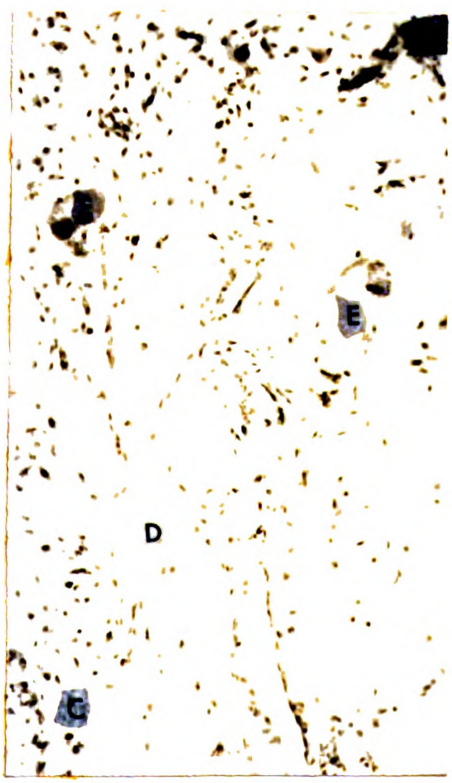

Figure 82

Figure 83. Hemorrhage in Submucosa of Cecum.
Hematoxylin and Eosin. x 91
F. Erythrocytes
G. Lymphocytic Focus

Figure 84. Hemorrhage in Propria Mucosae of Cecum.
Hematoxylin and Eosin. x 91
H. Erythrocytes
I. Globlet Cells

Figure 85. Hemorrhage in Fold of Submucosa of Colon.
Hematoxylin and Eosin. x 116
J. Epithelium
K. Submucosa
L. Erythrocytes

Figure 86. Thrombi in Submucosa of Colon.
Hematoxylin and Eosin. x 140
N. Submucosa
O. Thrombi
P. Mucosa

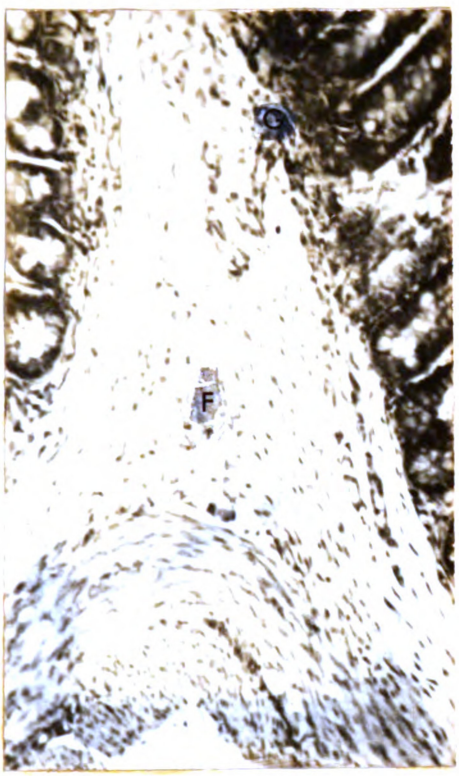

Figure 83

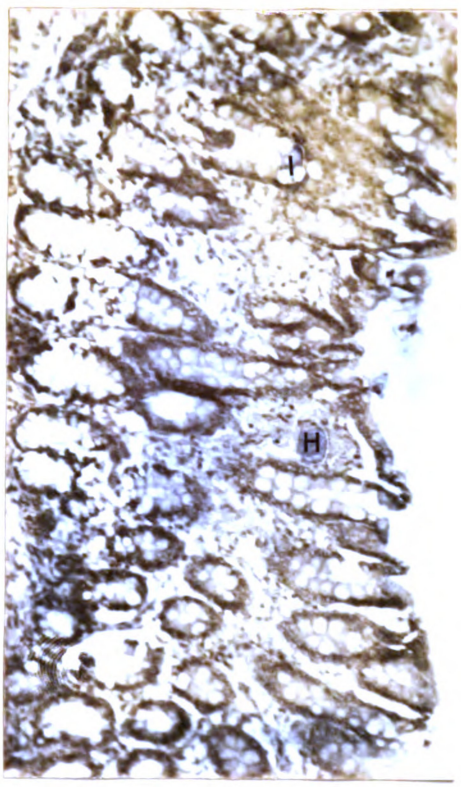

Figure 84

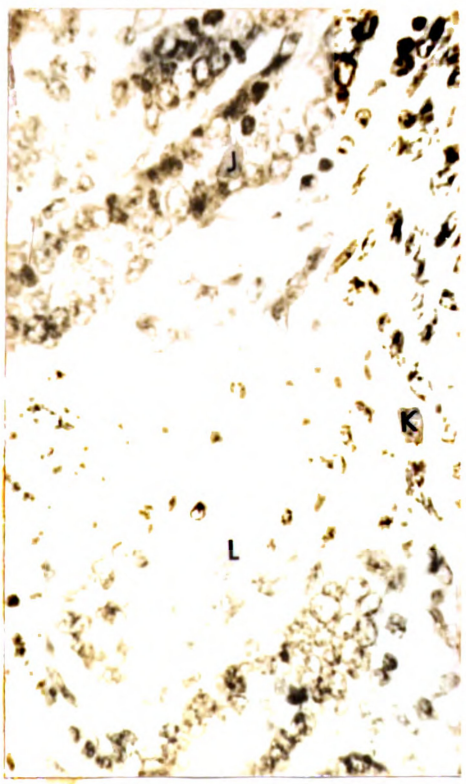

Figure 85

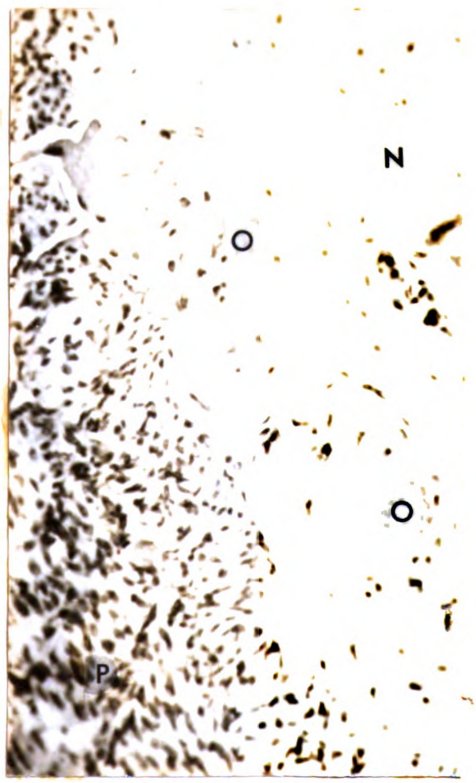

Figure 86

Hemorrhage and congestion were present in the large intestine of 14 of 21 infected pigs (Fig. 85). Necrosis was not seen. Suggestions of thrombosis were found (Fig. 86), and possible degenerative changes in blood vessel walls with some endothelial proliferation were observed.

15. Reproductive Organs

Histopathologic examination of male and female reproductive organs failed to reveal any abnormalities, except for occasional moderate congestion of the male accessory glands and the female uterus.

V. DISCUSSION

The clinical signs and lesions in the hog cholera-infected pigs were attributed to the hog cholera virus alone. The findings from this experiment discount the role of Salmonella species and other bacteria in contributing to the clinical syndrome, mortality rate, and gross lesions of hog cholera as seen under field conditions.

Bacillus subtilis is a common laboratory contaminant (3). Its mode of entrance into the isolators was not determined but the possibility exists that the organism was not destroyed during autoclaving of the diet. B. subtilis was apparently of no importance as a complicating factor in the cholera-infected pigs because clinical and gross pathologic differences could not be detected between germfree and monocontaminated animals.

The diphasic temperature curve was quite distinct for each cholera infected pig in this experiment. No deaths occurred until after the first peak was passed. The 3 pigs which died at 4, 4.5, and 5 days, respectively, after inoculation had all passed the first temperature peak and were at the "valley" stage or moving upward toward the second peak. The remaining pigs died during or after the second peak temperature.

An anemia existed from the second day after inoculation. This anemia increased in severity as the disease progressed, with a noticeable drop in both hemoglobin and hematocrit values after the sixth day. This suggested a deleterious effect of the hog cholera virus on erythrocytes. While

determining the differential leukocyte counts, there was revealed large numbers of nucleated red blood cells. A close relationship was found to exist between the unadjusted total leukocyte count (Fig. 4) and the numbers of nucleated erythrocytes (Fig. 5). The apparent recovery in numbers of leukocytes originally observed was actually due to these nucleated cells; the leukopenia itself persisted. Comparison of the hemoglobin and hematocrit values (Figs. 6 and 7) with the appearance of nucleated erythrocytes (Fig. 5) revealed a delay of approximately 5 days between the decline in hemoglobin-hematocrit values and the appearance of large numbers of circulating nucleated erythrocytes. These nucleated cells suggested hyperplasia of the bone marrow or premature release of these cells from the bone marrow as a result of the existing anemia. Differential leukocyte counts were not noticeably different from preinoculation levels or from values obtained from control pigs, suggesting that the leukopenia was absolute and the virus had similar effects on all types of leukocytes.

The most noticeable effect of the virus on food consumption was the loss of interest and enthusiasm in eating. The actual decrease in the quantity of food consumed was not marked until the day before death, but the time required to consume the milk was greatly extended.

The exact onset of diarrhea was difficult to detect. The feces of infected pigs living beyond 7 days were fluid to semisolid in consistency; pigs surviving the longest had

the most severe diarrhea. This undoubtedly caused some dehydration and may have accounted for a portion of the lower body weights of infected pigs. Blood findings did not reveal, nor did they exclude, the possibility of hemoconcentration.

Gross pathologic findings were hemorrhages of various sizes, shapes, and in numerous locations. They were consistently found only in the kidneys and lymph nodes. The cortex of the kidney of infected pigs was always involved, the medulla less often and less severely affected. Of the 15 groups of lymph nodes examined in this study, the minimum number showing gross lesions in one pig was 10. Most infected pigs had 12 to 15 of these nodes grossly involved. In paired nodes, bilateral involvement was commonly, but not always, found. The external and internal inguinal nodes, renal, parotid, and mandibular nodes always had some degree of hemorrhage. The mediastinal, gastric, and hepatic nodes were the least frequently involved, with the incidence of hemorrhages in other nodes being between these two levels.

Correlation could not be made between the number of organs showing hemorrhage and/or the severity of these hemorrhages, with the elevated temperatures, leukopenia, survival time, or other clinical signs. Apparently the lesions were somewhat independent of the clinical signs; death, in turn, was probably not dependent on severity of either clinical signs or gross lesions.

Microscopic lesions of hog cholera in gnotobiotic pigs consisted of congestion, edema, and hemorrhage accompanied by necrosis and degenerative vascular alterations (endothelial

swelling and proliferation, hyalinization, vacuolar changes, and thrombosis). Lymphocytic elements were uniformly depleted in lymph nodes and in the spleen of infected pigs; the tonsils appeared to maintain their lymphocytes. Varying degrees of edema and hemorrhage were common in these 3 structures, as well as degenerative blood vessel changes. Pyknotic cells, thought to be degenerate lymphocytes, were found in the cortex of lymph nodes and in the white pulp of the spleen of infected pigs. Lymphatic tissue within the intestinal tract appeared to be neither stimulated nor depleted.

The respiratory and urinary systems were characterized by hemorrhages while the digestive system had, in addition, focal areas of necrosis with cellular infiltration. Paravascular infiltration of the liver was mostly with lymphocytes, although large numbers of paravascular endothelioid cells were found. The exact nature of these endothelioid cells was not determined.

Necrosis and edema found in the gallbladder and tubular portions of the digestive tract of infected pigs were accompanied by infiltrations of neutrophils, with a small number of lymphocytes. Dilatation of the renal tubules appeared to be dependent on the amount of hemorrhage in the medulla. This dilatation was, perhaps, merely a mechanical condition.

The presence of focal necrosis of the liver in both infected and control pigs was uniform and persistent. The

cause of this condition was not determined. Similar lesions have not been observed in other germfree pigs (41,53,54). A dietary exclusion could be considered in view of the high temperatures and pressure used to assure sterility of the diet.

The significance of the Anitschkow myocytes in the heart was not ascertained. They were, however, a consistent finding, commonly associated with myocardial hemorrhage, and were mostly ventricular in location. They were considered by some (5,17) to be histiocytes, part of the reticulo-endothelial system, and evidence of inflammatory processes within the heart.

Attempts to trace hemorrhages to blood vessel lesions were unsuccessful. Frequently the blood vessel alterations were subtle, and comparisons with similar structures in control pigs often permitted no definitive conclusions.

Apparent glycogen depletion of the liver was observed in infected pigs. Unfortunately material was not preserved in fixative suitable for glycogen stains. Fat stains showed that the material in the liver was not fat. Previous work (41,54) showed similar material to be present in livers of gnotobiotic pigs, and this was proven to be glycogen. It was thought, therefore, that it could be rather safely assumed that this material in control pig livers was actually glycogen.

Though control pigs were fed a similar quantity of food (reduced as anorexia developed in infected pigs), glycogen depletion was not evident. Indeed, much of the architecture of the liver cells was obscured by the large amounts

of glycogen in the livers of control pigs. Apparently the metabolic rate of hog cholera infected pigs was altered so that the glycogen was needed and the liver depleted early in the course of the disease. It was thought the associated fever may have required the utilization of this glycogen. Pigs dying 5 days or earlier after inoculation with virulent virus had glycogen depletion similar to that found in animals surviving 15 days.

Erythropoietic foci were consistently absent in the livers and spleens of infected pigs. When present, they were very small and contained only a few nucleated cells. Control pigs, on the other hand, had numerous large focal areas of hemopoiesis, especially in the liver. Megakaryocytes were not uncommon. It would appear that the virus is capable of either depleting or depressing (or both) the extramedullary hemopoietic centers in the young pig. The large numbers of nucleated erythrocytes found in the circulating blood may have partially arisen from depletion of the extramedullary centers. It seems unlikely, however, that the great numbers of nucleated erythrocytes found in the blood could have been present without bone marrow hyperplasia, in addition to the depletion of the extramedullary centers. Certainly no hyperplasia was evident in these extramedullary hemopoietic centers, but it could not be determined if the virus actually "depressed" these areas. Bone marrow studies have not been included.

Comparison of gross and microscopic lesions of lymph nodes indicated that congestion, edema and moderate hemorrhage were not always detected grossly. Microscopically, the external lymph nodes had hemorrhages of less extent and severity than the internal lymph nodes.

The results of this experiment emphasized the marked variation in the response of individual pigs to hog cholera virus. An attempt was made to hold the number of variables in this work to an absolute minimum by using littermate pigs whenever possible, by maintaining the pigs in a bacteria-free or monocontaminated environment, and by giving a standardized dose of virus to each pig. In spite of these rigidly controlled conditions, the gross lesions varied from one pig to another, as did the survival time. It is not surprising, therefore, that epizootics of hog cholera are characterized by such marked variability of clinical signs, lesions and mortality rate.

The assumption was made that changes were due to the hog cholera virus since bacteria were absent and similar lesions were not found in control pigs.

While birth weights were within normal limits, it was evident that the final weights, even in control pigs, were considerably less than expected for pigs reared to this age under conventional conditions. This undoubtedly reflected the need for a more adequate ration for pigs raised from birth under artificial conditions. The net weight gain of the infected pigs was no doubt influenced by the fact that

some of these pigs died early in the experiment. If the control pigs had been killed at comparable times, the deficiency in weight gain of the infected pigs might not have been as noticeable.

Both control and infected pigs were kept in plastic isolators within the same room, and no precautions other than those routinely employed in rearing germfree pigs were used. Cross infection between isolators did not occur. This, in itself, was significant and suggested that the technique used may be a useful tool in the study of infectious disease of domestic animals.

VI. SUMMARY AND CONCLUSIONS

Germfree techniques were employed to study the clinical signs and lesions caused by the hog cholera virus. Ten germfree and 11 monocontaminated pigs were inoculated at 14 days of age with hog cholera virus. Six uninoculated pigs (4 germfree and 2 monocontaminated) were raised under similar conditions and served as controls. Rectal temperature curves were diphasic following virus administration, reaching 2 mean peaks of 106 F. and 106.3 F. Total circulating leukocytes decreased to approximately one-third their preinoculation levels within 24 hours. There was a reduction in hemoglobin and hematocrit levels, followed by a rise in numbers of circulating nucleated erythrocytes. Death occurred in all 21 inoculated pigs, with the time of death ranging from 4 to 15 days after inoculation. Necropsies consistently revealed hemorrhagic lymph nodes and petechial and ecchymotic hemorrhages in the kidneys.

Microscopically, hemorrhages were found in the tonsils, tongue, diaphragm, lungs, heart, lymph nodes, meninges, urinary bladder, kidneys, liver and tubular digestive tract. Hemorrhages were most extensive in the internal lymph nodes. Necrosis was found in the gallbladder, spleen, lymph nodes, and tubular digestive tract. Leukocytic infiltrations were found in the liver and in association with necrotic foci of the digestive system. Anitschkow myocytes were found in large numbers in the heart. Lymphocytic depletion was

common in the lymph nodes and spleen. Degenerative changes were present in the smaller blood vessels of the major tissues. Livers of infected pigs were characterized by glycogen depletion and absence of extramedullary hemopoietic areas.

These signs and lesions are attributed to the effects of the virus itself, and serve to emphasize the marked variation in the response of individual pigs to the hog cholera virus.

The ability of routine germfree procedures to contain a highly infectious agent within the same room as susceptible animals, and not infect these animals, was significant.

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