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BRAIN LESIONS AND VIREMIA STUDIES IN SWINE INFECTED WITH A HOG CHOLERA VIRUS OF LOW PATHOGENICITY

by

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INTRODUCTION

Hog cholera is one of the few American heritages in animal diseases; there is little doubt it had its insidious origin in the United States. A United Stated Department of Agriculture report (40) for 1887-1888 indicates hog cholera was first noted in Ohio in 1833. Since then it has spread to all the major swine producing areas of the world.

Hog cholera has consistently cost the swine industry of the United States 40 to 60 million dollars annually (30). However, all the losses cannot be monetarily measured. This disease hampers interstate and international trade in swine and pork. In 1960, an act was passed by Congress and signed by the President which cleared the way for the Animal Health Division of the United States Department of Agriculture to cooperate with the various states to eradicate hog cholera. Presently most states and many foreign countries have eradication programs in effect and embargoes and other restrictive measures are imposed on swine and pork from areas where hog cholera exists.

Hog cholera has been considered an acute, febrile, highly contagious, and usually fatal viral disease affecting only swine. In the early 1950's workers in the United States began recognizing other forms of hog cholera (3, 43, 44). Presently at least two-thirds of the hog cholera in the United States

is caused by viral strains of lowered pathogenicity[a].

Hog cholera eradication programs have given new meaning and added importance to the diagnosis. These programs depend heavily upon accurate diagnostic methods. Hog cholera caused by viral strains of lowered pathogenicity presents vexing diagnostic problems. Clinical manifestations and gross lesions are not usually sufficient to make a definite diagnosis. Some of the weakly pathogenic strains of virus are not lethal and have no appreciable antigenic properties; thus animal inoculation is of limited value since the inoculated animals neither die nor develop sufficient antibodies to withstand challenge with a virulent strain of hog cholera virus. The presence of leukopenia is a valuable aid to the diagnosis of typical hog cholera, but it does not occur in all cases of low grade hog cholera (14).

Hog cholera virus will replicate *in vitro* in some cell cultures and *in vivo* in swine tissue; it can be demonstrated in either tissue with appropriate fluorescent antibody procedures (1, 29). However, attenuated live viruses used for vaccination against hog cholera cannot be differentiated from field strains with fluorescent antibody procedures. An antigenic relationship between hog cholera virus and the agent causing bovine virus diarrhea may cause misinterpretations

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[a]Tillery, M. J., U.S. Department of Agriculture, Washington, D.C. Epidemiological data from case reports. Private communication. 1966.

(28). Hog cholera antibodies conjugated to a fluorescent dye will adhere to the virus causing bovine virus diarrhea as well to hog cholera virus.

Recent studies on the diagnosis of hog cholera places heavy emphasis on brain lesions as one of the diagnostic criteria (6, 7). Several comprehensive studies relating to the incidence, type, and pathogenesis of brain lesions encountered in acute hog cholera were found in the literature, but no reports concerning systematic studies of brain lesions caused by lowered pathogenic strains of hog cholera were found. The presence or absence of brain lesions has not been correlated with the duration of virus in the blood. It was not known how long brain lesions persist and what stages of resolution they go through in nonlethal hog cholera.

The purpose of this study was to characterize lesions produced in the central nervous system throughout the course of nonlethal hog cholera infection and determine the duration of brain lesions and viremia in the experimentally infected pigs.

LITERATURE REVIEW

Encephalitis Associated with Hog Cholera

The first neurohistologic changes in hog cholera affected swine were recorded in 1922 when Hutyra and Marek (18) reported hemorrhages in the meninges and neuroparenchyma. Later, several other European workers, including Huguenin (17), Brunschwiler (4), Rohrer (39), Graf (12), and Laumen (25), published results of limited studies on brain lesions in hog cholera affected swine.

Seifried (46) was the first to publish extensively on the lesions of hog cholera in the central nervous system. He inoculated 4 strains of hog cholera virus into 39 experimental pigs and found brain lesions in 33 of the 39 animals. He divided the brain alterations into three major groups according to tissue affected, i.e., mesodermal tissues, glial cells, and nerve cells. He described mesodermal lesions as consisting of perivascular cuffing with small lymphocytes, other mononuclear cells, plasma cells, and a few polymorphonuclear leukocytes. These vascular lesions were found in the brain, meninges, and choroid plexus. He also described degeneration of the vascular walls including swelling and hydropic degeneration of the endothelial cells.

The glial cell lesions described by Seifried consisted of focal accumulations of nicroglia and other mononuclear cells. Satellitosis and neuronophagia by glial cells were noted. Seifried found glial cell foci under the ependyma of the ven-

tricles, but this finding was later questioned by Helmboldt and Jungherr (16). They found subempendymal foci in most young pigs whether affected by hog cholera or not. These focal embryonic remnants disappeared with increasing age.

A correlation between vascular lesions and neuronal degeneration was not always possible in Seifried's study (46). The affected neurons appeared to be relatively independent of each other; one neuron might be severely affected while an adjacent one appeared normal. The neuronal degeneration was characterized by denticulation of the cell membrane, vacuolation of cytoplasm, tigrolysis, peripherally located swollen nuclei, and nuclear degeneration consisting of fragmented chromatin, swollen nucleoli, and loss of nucleoli. Seifried did not feel the neuronal changes alone were diagnostic of hog cholera.

Done (6) believed the virus of hog cholera primarily affected mesodermal tissue. He stated, "The overall histological picture may best be imagined as a delicate network of susceptible and highly reactive mesoderm, spread over a matrix of ectoderm not itself susceptible to the virus but highly sensitive to any interference with its nutrition, and hence with its blood supply."

In 1950, Helmboldt and Jungherr (16) published the results of their studies on the neuropathologic diagnosis of hog cholera. Their data were derived from swine presented to the diagnostic Laboratory, Storrs, Connecticut, between 1938

and 1949. They also believed primary brain lesions in hog cholera were limited to non-nervous tissue. The lesions encountered are listed according to their frequency: vascular and perivascular cuffs, microgliosis, leptomeningeal infiltration with mononuclear cells, capillary hemorrhage, and hyalinization of the vascular wall. The last four lesions were relatively infrequent as compared to the first. They found brain lesions in 40 of 44 cases of hog cholera. The changes were not localized in any particular portion of the brain, but a predilection for the brain stem seemed apparent. The intensity of the brain lesions was not paralleled by the severity and duration of the disease.

Jones and Doyle (20) studied the encephalitides of swine with special reference to hog cholera. They examined the following groups of pigs: 100 cases presented to the Purdue Diagnostic Laboratory, 52 pigs inoculated with hog cholera virus for commercial virus production, and 12 pigs used to study encephalitis produced by simultaneous serum and virus vaccination. Of the 100 field cases, 33 were positive for hog cholera and 5 were suspicious. Of the 33 positive cases, 30 had encephalitis and 4 of the 5 suspicious cases had encephalitis. Of the 52 swine used for virus production, 28 had encephalitis 6 days post inoculation. Only 4 of 12 pigs given anti hog cholera serum and virus simultaneously had brain lesions indistinguishable from hog cholera.

In light of the work of Jones and Doyle, Helmboldt and

Jungherr (15) published the results of further observations on the neurological diagnosis of hog cholera in which they compared brain lesions in additional hog cholera cases with those in other swine encephalitides and in swine vaccinated against hog cholera. The control pigs for these studies were brought from Canada where hog cholera did not exist. Helmboldt and Jungherr tested the statistical significance of encephalitic lesions in the diagnosis of hog cholera and found no evidence that any method of immunization caused encephalitis similar to actual hog cholera infection. They concluded lesions such as Jones and Doyle found suggested vaccination failure or inapparent hog cholera and felt the vaccination failure might be due to insufficient antibodies in the anti-hog cholera serum. These workers characterized the differences between hog cholera encephalitis and other swine encephalitides including listeriosis, "lymphocytic choriomeningitis," and purulent meningitis. In addition, their work encompassed non-encephalitic diseases such as erysipelas, salmonellosis and tuberculosis as well as nutritional dificiencies and parathion poisoning. They concluded none of the lesions in the other encephalitides of swine fulfilled all the criteria for hog cholera encephalitis and the occasional encephalitic foci observed in other swine conditions could be readily differentiated from hog cholera.

In 1952, Dunne, Smith, Runnells, Stafseth, and Thorp (9) reported a highly pathogenic variant strain of hog cholera

virus. They discussed the nature and extent of the brain lesions produced by this strain of virus. Hydropic degeneration and proliferation of vascular endothelium were the first changes noted. Subsequently, perivascular cuffing and endothelial proliferation resulting in partial occlusion of the blood vessels and congestion were seen. Lesions were present in 100 per cent of the thalami studied. The severity of the lesions was greatest between the 10th and 14th day post inoculation.

In a later publication, Dunne (7) indicated brain lesions were observed in 85 to 95 per cent of the field cases of hog cholera, but he warned against complete reliance on the brain lesions in pigs under five weeks of age. He had observed that a viral encephalitis not caused by hog cholera occurs in this age group and occasionally a non-specific encephalitis occurred in older, clinically normal pigs.

According to Done (6), the virus of hog cholera should produce an unequivocal nonsuppurative panencephalitis involving at least the cerebrum, corpora quadragemina, and medulla oblongata, and characterized by vascular cuffing with marked endothelial damage and usually focal and/or diffuse microgliosis.

In 1956, Potel and Korn (36, 37, 38) reported the results of studies in which the blood vessels from the brain of hog cholera affected pigs were "washed out". They concluded the high virus titers in the tissues of the central nervous system were not solely due to increased vascularity but to

the presence of the virus in the nervous tissue as well. The virus was distributed fairly evenly in different parts of the "washed out" brain and cord. They believed virus accumulated in the brain, not because of a special affinity for brain tissue, but as the result of increased vascular permeability.

In 1960, Japanese workers (31, 32, 33) studied brain lesions in swine experimentally inoculated with virulent hog cholera. The animals were killed at daily intervals from 1 to 6 days post inoculation. These workers observed vascular changes in the neuroparenchyma and choroid plexus on the first day post inoculation. They concluded that vascular proliferation in the neuroparenchyma, meninges, and choroid plexus was the most prominent change in the central nervous system of hog cholera infected animals.

Electron microscopic studies by Schulze (45) revealed that the choroid plexus of normal pigs did not differ significantly from other animals. Edema of the plexus was noted in hog cholera infected pigs on the first day post inoculation. Later, the nuclei of otherwise unaffected choroid plexus epithelial cells were surrounded by an almost clear, structureless zone. The epithelial cells appeared otherwise healthy. Subsequently, signs of degeneration appeared in the nuclei of epithelial cells, followed by destruction of the cell membranes nearest the vascular lumen. The basement membrane was also destroyed. These studies employed virulent hog cholera

virus and did not extend beyond 5 days post inoculation. Schulze also published several electron photomicrographs illustrating the changes he described.

Fankhauser (10) in mentioning work of Saunders stated: "As a rapid test for detecting encephalitis (and presumptively, hog cholera), Saunders used the Pandy test on the cerebrospinal fluid of twenty pigs." The fluid was obtained from the cisterna magna immediately after exsanguinating the pig. All pigs with histopathologic lesions of encephalomyelitis gave at least 1-plus reading, and most gave a 2-plus or 3-plus result. Fankhauser also published a photomicrograph illustrating lymphocytic infiltration in the choroid plexus, but made no mention of this change in the text.

Hog cholera virus has been demonstrated in cerebrospinal fluid. In 1956, Gralheer and Pehl (13) studied the electrophoretic migration of hog cholera virus in serum and cerebrospinal fluid. They concluded the cerebrospinal fluid served as a pure and highly potent substrate for the virus which was suitable for demonstration of purity of the virus.

Hog Cholera Due to Viral Strains of Low Pathogenicity

The incidence and distribution of low grade hog cholera infection are rapidly coming to the forefront in the eradication program of hog cholera in the United States. Strict quarantines drastically reduced the incidence of acute hog cholera, but the type of hog cholera caused by viruses of

reduced pathogenicity is more difficult to locate, diagnose, and eradicate.

Schwarte (42, 43) demonstrated marked differences in pathogenicity and stability among field strains of hog cholera virus. Some of the low pathogenic strains possessed the same degree of pathogenicity after repeated passage in susceptible animals, but other strains increased in pathogenicity after animal passage until they were considered virulent. Some strains decreased in pathogenicity until the agent was no longer pathogenic for swine.

Problems associated with viral strains of low pathogenicity have occurred following vaccination with various products used for immunization against hog cholera (3, 35). Differences in animal susceptibility account for some of the disease problems caused by these strains of viruses. Biester and Schwarte (3) studied weakly pathogenic strains of virus in both 35 and 50 pound animals and 75 and 100 pound animals. Some of the strains were lethal for the younger animal but non-lethal for the older animal.

Subtypical hog cholera was reported by Kernkamp and Fenstermacher (24). The course of the disease was extended and the animals failed to manifest the intensity or magnitude of clinical signs and lesions usually associated with hog cholera.

Dunne et al. (8) noted cases of chronic hog cholera occurring in titration experiments when the dose approached

the MLD_{50} of the virus. However, they were unable to transmit this type of chronic hog cholera either by contact exposure or with whole blood inoculation. A virulent hog cholera virus exposed to sonic vibration also produced a chronic type of hog cholera. However, acute hog cholera developed on subsequent passage of this agent and further experiments failed to link chronicity with sonic treatment of the virus.

Sorensen et al. (48) reported chronic hog cholera constituted one-third of the cholera cases in Minnesota in 1962. Low virulent and/or chronic hog cholera has been reported in France (11, 21), Japan (14, 41), New Zealand (23), and Malaya (34). It probably occurs wherever classical hog cholera exists.

In France, several atypical or variant strains of hog cholera were reported in 1959 (21). These viruses of low pathogenicity were quite stable, appeared pneumotrophic, resulted in retarded or arrested growth, and possessed increased virulence for young pigs. Many of these observations were reconfirmed in a 1961 report in which the authors referred to the altered virus as hypocontagious and hypovirulent, but not weakened antigenically (11).

A weakly virulent strain of hog cholera virus was found in Japan in 1956 and reported in 1960 (41). The epizootic caused by this virus of reduced virulence differed from virulent hog cholera by its mild nature. Although a few pigs died following severe clinical manifestations, others had

only slight febrile reactions and recovered. The causative virus was isolated and proven to be the virus of hog cholera by hematologic findings, experimental infection of susceptible pigs, histopathologic findings, and immunization tests. The same workers had isolated two other weakly virulent strains of hog cholera viruses from insufficiently inactivated crystal violet vaccine. These two isolates were different from their naturally occurring weakly virulent viruses. They recovered their virulence on the 2nd and 4th animal passage and failed to withstand storage at -50^{o} Centigrade for 6 months. Their naturally occurring, weakly virulent strain did not regain virulence by the 7th animal passage and withstood storage at -50^{o} Centigrade for at least a year.

In a comprehensive report published in 1961, Hayashi, Okaniwa, and Sashara (14) described additional cases of chronic hog cholera in Japan. They recovered the hog cholera virus after a 72 day course in one animal and a 54 day course in another animal. In both cases the hog cholera virus was identified immunologically. However, the virus was not stable. On the second passage in susceptible animals, a dose of 10 milliliters of supernatant from a splenic emulsion administered intramuscularly caused death in 18 days. One milliliter of a 10^{-1} dilution of the same inoculum resulted in eventual recovery. One milliliter of a 10^{-2} dilution resulted in death after 23 days, and 1 milliliter of a 10^{-4} dilution caused no obvious response, but the animal was subsequently

immune to challenge with fully virulent hog cholera virus. The third animal passage produced acute hog cholera lethal in 6 to 12 days post inoculation. The authors felt the most noteworthy clinical signs were prolonged febrile reactions, constipation, anorexia, and a paralytic condition in the terminal stages. Marked leucopenia was not observed at any time.

Japanese workers described the brain changes as follows:

> In the 72 day duration pig, vascular cell infiltration extending in every part was observed in the pia mater and parenchyma. Also, small blood vessels in the lumbar cord were dilated, congested, and hemorrhagic. In the 54 day duration pig, brain changes were less marked, although vascular cell infiltration was observed sporadically. Changes indicating activation of cells in the vascular wall were extensive.

Baker and Sheffy (2) noted persisting hog cholera viremia in young pigs. The prolonged disease was produced by a lapinized strain of virus in 6 week old pigs from non-immune sows. Pigs with persistent viremia failed to grow and had elevated temperatures, but survived for periods from 6 to 17 weeks. During this interval, the virus reverted to a fully virulent strain.

Neuroparenchymal vacuolation has been associated with hog cholera in Malaya (34). Four brains were submitted from one herd of swine. Lesions typical of hog cholera were disclosed in 3, while pronounced widespread vacuolation involving both the gray and white matter was seen in the fourth. Some of the vacuoles appeared to be greatly distended pericapillary spaces, but they could not all be accounted for in this manner. A mild strain of hog cholera virus was present in

Malaya. Although the microscopic lesions were identical with those of classical hog cholera, other aspects of the mild disease are different. The generalized post-mortem lesions are less severe. The outbreaks were less explosive, mortality rates were low, and they tended to be self-limiting.

Carbrey et al. (5) reported immune tolerance and persisting viremias in pigs infected with hog cholera virus in utero. Volkert and Larsen (49) associated immune tolerance with viral agents of low pathogenicity. The brain lesions have not been studied in hog cholera infected pigs immunologically tolerant to hog cholera virus. This immunologic unresponsiveness may account for many reports of persisting viremia and the absence of brain lesions in pigs with hog cholera.

MATERIALS AND METHODS

Source of Inoculum

The strain of hog cholera virus selected for this study had to meet several critical standards. A virus of reduced pathogenicity with the following characteristics was required: the agent would have to (1) elicit an acute febrile response; (2) produce clinical manifestations and lesions characteristic of hog cholera; (3) be nonlethal so that studies of the central nervous system could be conducted during resolution of lesions and permit the duration of viremia to be determined; and (4) produce a solid immunity in recovered animals.

Several strains of hog cholera virus were investigated in swine before one meeting the above requisites was located[b]. The original isolation of the agent selected was made from a field outbreak of several months duration. In this outbreak most of the younger affected pigs died whereas most of the older pigs recovered. Blood containing virus was collected from the second animal passage in the laboratory.

The viability and pathogenicity of this strain of hog cholera virus were determined by inoculating three 2 month old, 40-50 pound, specific pathogen free (SPF) swine.

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[b]The author wishes to express his thanks to Dr. Dale Sorenson, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, for providing the virus.

Experimental Design

The animal inoculation studies were carried out in Building 3, Wing E of the National Animal Disease Laboratory (NADL). All animals were first brought into the animal quarantine area and observed 2 to 4 weeks for evidence of the disease. After passing the quarantine period they were brought into the NADL compound area and housed in Wing E of Building 3. The quarantine area is about one-half mile from Building 3. No movement of personnel, equipment, or feed occurred between the two areas. Wing E is separate from other animal holding areas and the outside air is conditioned by an air filter system designed to trap even viral particles. Only the selected strain of hog cholera virus was present in Wing E during the entire course of these experiments.

All inoculated animals were observed at least once daily, their temperatures taken, and prominent clinical signs recorded.

Sixty-nine swine, comprising four experimental groups, were used in this study. Group I consisted of three 2 month old swine of mixed Yorkshire breeding from a specific pathogen free (SPF) herd. These animals were used to determine the viability and pathogenicity of the selected strain of hog cholera virus. Each animal was inoculated intramuscularly with 1 milliliter of undiluted swine blood containing the hog cholera virus. One animal was killed 7 days post inoculation. Blood collected from this animal was used for

subsequent animal inoculations. The brain from this pig was examined histopathologically. The remaining 2 pigs succumbed.

Group II consisted of twenty-six 3 month old swine of mixed breeding. They were farm raised animals moved to NADL at 8 weeks of age and held in quarantine 4 weeks. No evidence of illness was noted during the quarantine period. When the pigs were 3 months old, 19 were randomly selected and inoculated intramuscularly with 1 milliliter of a 10^{-3} saline dilution of blood collected from the Group I pigs. The remaining 7 served as controls. The controls were maintained in the animal quarantine area until the day they were killed. At this time they were moved to Wing E. The inoculated and control animals were killed between 5 and 106 days post inoculation.

This phase of the study was designed to provide maximum information from each experimental animal by allowing flexibility in the schedule for killing the inoculated and control animals. Inoculated pigs were killed on post inoculation days 5, 8, 12, 15, 19, 20, 21, 22, 23, 26, 29, 32, 36, 45, 58, 74, and 106. Only one pig at a time was killed through day 74, but 3 were killed on the 106th day. This provided more frequent examinations at shorter intervals during critical stages when brain lesions were resolving. Blood was collected from incised axillary blood vessels and stored at -75° to -80° Fahrenheit until inoculated into test animals. Five control pigs were killed at times corresponding to post inoculation days 5, 22, 36, 74, and 98. At 98 days post inocula-

tion the 2 remaining controls and the 3 remaining experimental inoculated animals were challenged with an intramuscular injection of 1 milliliter of a virulent strain of hog cholera virus maintained under laboratory conditions for many years and known as the Bureau of Animal Industry (BAI) strain.[a]

Group III consisted of twenty-five 8 week old SPF swine of mixed Yorkshire breeding. They were used to detect hog cholera virus in blood from Group II pigs. Three group III pigs were employed for each Group II pig killed between 8 and 20 days post inoculation. Two pigs were inoculated and 1 served as a control. Subsequently it was decided 1 animal was sufficient for this determination, and 1 pig was used for virus detection for all Group II pigs killed between 20 and 74 days post inoculation. Each inoculated animal was given intramuscularly 1 milliliter of blood collected from a Group II animal. All test animals surviving inoculation of blood from Group II animals together with the controls were challenged with the BAI strain of hog cholera virus. The challenge dose was 1 milliliter of swine blood containing the BAI strain of hog cholera virus administered intramuscularly.

Group IV consisted of 15 animals similar to Group II animals in age and breeding. They were inoculated with the same dilution of the same virus used in Group II animals. These animals were killed 45 days post inoculation. They were used to evaluate the method of brain fixation used for

[a]This strain of virus is also known as the Ames strain.

Groups I and II animals and to determine if residual lesions indicative of hog cholera were present in the central nervous system 45 days post inoculation.

Swine Husbandry

All animals were fed and tended by regularly employed animal caretakers. Each of the two groups of inoculated and the control pigs had different caretakers. These individuals showered prior to entering and leaving the animal room. Only unopened sacks of feed delivered directly from the commercial supplier to individual animal holding areas were used. Each animal room and the equipment used in the room such as feeders, waterers, catch gates, and thermometers were thoroughly cleaned and disinfected 24 hours before the pigs were moved into the rooms.

The lighting, environmental temperature, and humidity were maintained at an optimum level. A commercially balanced hog ration was fed in pelleted form. Self-feeders and automatic waterers were used.

Necropsy Procedure

All animals killed prior to post inoculation day 36 were electrocuted with 110 volts of alternating current. One electrode was placed on the ear near the auditory canal and the other on the anus or vulva. Each animal was exsanguinated as soon as the electrodes were removed by incising the axillary blood vessels. This method was not entirely satisfactory

since it was thought to occasionally produce petechial hemorrhage on the surface of the lungs and serosa of the urinary bladder. Therefore, animals killed after post inocula tion day 36 were anesthetized with pentobarbital sodium and the axillary blood vessels incised. This method of killing did not appear to produce hemorrhage or other lesions. Changes attributable to either electrocution or pentobarbital sodium were not detected on histopathologic examination of tissue from the central nervous system.

Blood from all clinically sick pigs was cultured for bacteria. A sterile swab was dipped into the blood running from the incised axillary vessels, streaked on blood agar, then broken into a tube containing nutrient broth and incubated at 37° Centigrade. The culture media were examined after 24 and 48 hours. Significant bacterial growth was not obtained from any of the pigs.

The head was removed at the atlanto-occipital junction. A dorsal midline incision was made through the skin from the occipital region rostrally to the muzzle. The skin was reflected laterally to the level of the maxillae. A Stryker saw[c] was used to cut through the bones. The first cut was dorso-ventrally approximately 1 inch anterior to the medial canthus, passing ventrally to the molar teeth of the maxillae. Next a horizontal cut approximately one-half inch ventral to

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[c]Orthopedic Frame Co., Kalamazoo, Michigan.

the base of the floor of the calvarium disjoined the maxillae and mandible from the head.

The bones and dura mater forming the dorsal and lateral boundaries of the cranial vault were removed. Projections of bone, cartilage and soft tissue were trimmed away so approximately three-fourths of the surface of the brain was exposed and the remaining fourth retained its seat in the base of the cranial vault. The brain in its natural base was immersed in 10 per cent formo-saline buffered to pH 7.

Four segments of the spinal cord were collected from each animal and fixed in 10 per cent buffered formo-saline. They were taken at the level of the following vertebrae: cervical - 3; thoracic - 3; thoracic - 12; and lumbar - 3.

The cervical, thoracic, and abdominal organs were examined and each change recorded. There was no evidence of complicating conditions which might interfere with the manifestation and course of hog cholera.

Processing of Tissue

Each brain from pigs in Groups I and II, still partially surrounded by bone, was transferred to a Technicon[d] tissue basket and placed in one of the upper receptacles of an Autotechnicon. All the receptacles in the top section were filled with a 10 per cent neutral buffered formalin. A brain was allowed to agitate approximately 16 hours. The Autotechnicon[d]

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

automatically changed the brain to a different formalin solution every hour. After this preliminary fixation, the brain was firm enough to maintain its natural shape, and the osseous tissue around the base was removed (Figure 1).

Tissue blocks some 3 millimeters thick were cut from the brains after approximately 16 hours fixation. Each block was an entire frontal cross section of the brain. A Lipshaw brain sectioning guide[e] was used to insure uniform thickness and bilateral symmetry of the blocks. The brain was placed on the sectioning guide with the dorsal surface down. The anterior poles of the cerebral cortex were removed to the level of the corpora callosum so blocks consisting of the two halves of the brain would be held together. Two frontal sections were taken in this position. Because of the normal flexed shape of the pig brain, it was impossible to continue cutting frontal sections of uniform thickness. Therefore, a wedge-shaped portion of the cerebral cortex and basal ganglia was removed. The thickest part of the wedge was the dorsal surface of the cerebral cortex. This permitted the ventral surface of the brain to lay flat on the base of the sectioning guide and the cut surface of the brain formed approximately a 90^{o} angle with the base of the brain. The cross sectioning of 3 millimeter blocks continued through the entire length of the brain. Eleven to 15 blocks were taken from each brain as well as

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[e]Lipshaw Manufacturing Company, Detroit, Michigan.

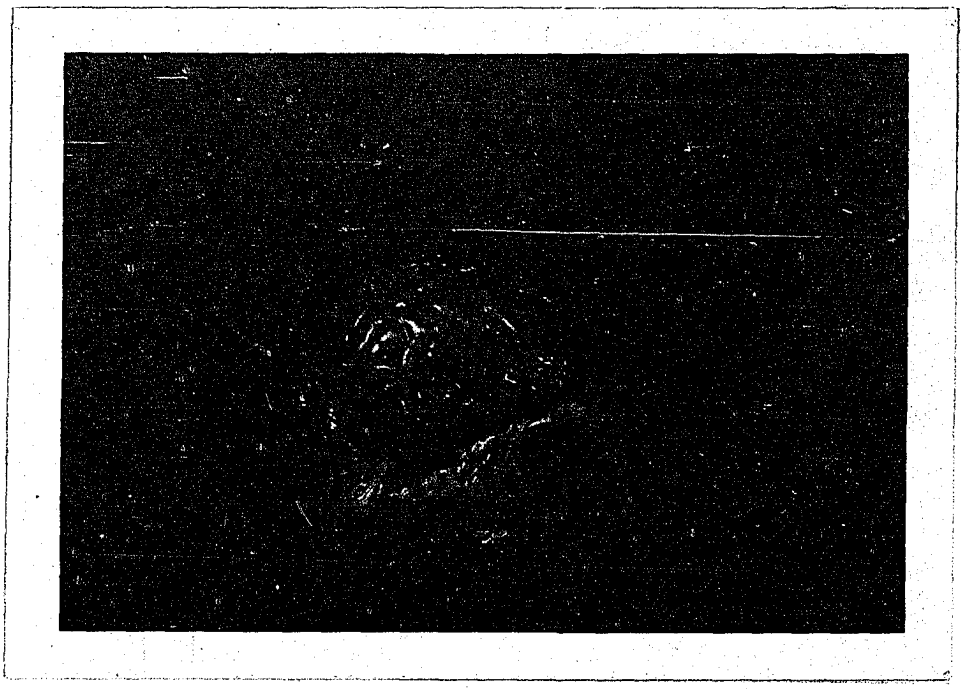


Figure 1. This brain, fixed in situ 16 hours, is ready to be removed from its osseous fossa and cut into tissue blocks.

blocks from the pituitary gland and olfactory lobes. One block was taken from each of the four segments of the spinal cord (Table 1). All tissue blocks were fixed an additional 24 hours to one week in 10 per cent neutral buffered formalin.

The 15 Group IV animals were killed with pentobarbital sodium and exsanguinated 45 days post inoculation. Each brain was removed immediately and cut into frontal blocks 3 to 4 centimeters thick. A thin sharp knife warmed in hot water facilitated cutting the soft brains. Each block was immediately placed into gallon glass jars previously filled with 10 per cent buffered formo-saline solution. Approximately 1 inch of

Table 1. Tissue blocks and paraffin sections taken from animals in Group II[a]

Animal Number	Spinal Cord L3	Spinal Cord T12	Spinal Cord T3	Spinal Cord C3	Pituitary Gland	Medulla	Medulla and Cerebellum
23	2-1	2-1	2-1	2-1	2-1	4-2	4-2
22	2-1	2-1	2-1	2-1	2-1	8-4	2-1
27	2-1	2-1	2-1	2-1	2-1	17-4	5-2
10	2-1	2-1	2-1	2-1	2-1	3-3	4-2
19	2-1	2-1	2-1	2-1	2-1	8-4	3-1
14	2-1	2-1	2-1	2-1	2-1	4-2	2-1
24	2-1	2-1	2-1	2-1	2-1	18-5	3-1
04	2-1	2-1	2-1	2-1	2-1	6-3	2-1
17	2-1	2-1	2-1	2-1	2-1	6-3	2-1
05	2-1	2-1	2-1	2-1	2-1	12-4	2-1
18	2-1	2-1	2-1	2-1	2-1	8-4	3-2
08	2-1	2-1	2-1	2-1	---	4-2	2-1
30	2-1	2-1	2-1	2-1	2-1	4-2	2-1
11	2-1	2-1	2-1	2-1	---	3-2	2-1
28	1-1	1-1	1-1	1-1	4-2	10-3	2-1
15	2-1	2-1	2-1	2-1	---	4-2	2-1
16	2-1	2-1	2-1	2-1	2-1	4-2	2-1
21	2-1	2-1	2-1	2-1	2-1	4-2	1-1
25	2-1	2-1	2-1	2-1	2-1	6-3	4-2
12[b]	2-1	2-1	2-1	2-1	---	8-4	2-1
07[b]	6-1	6-1	6-1	6-1	6-2	16-3	9-2
02[b]	2-1	2-1	2-1	2-1	2-1	6-3	2-1
13[b]	2-1	2-1	2-1	2-1	4-2	6-3	2-1
26[b]	2-1	2-1	2-1	2-1	4-2	6-3	2-1

[a]Each preparation of spinal cord and brain consisted of frontal sections through the entire organ. The first figure designates the number of sections, the second the number of different blocks.

[b]Control animals.

Table 1. (Continued)

Animal Number	Pons and Cerebellum	Mesencephalon and Cerebral Cortex	Thalamus, Hippocampus and Cerebral Cortex	Basal Ganglia	Olfactory Bulbs
23	---	---	8-4	2-1	---
22	2-1	2-1	10-5	4-2	4-2
27	4-1	1-1	12-5	11-3	8-2
10	5-2	---	8-3	8-3	6-3
19	2-1	---	7-3	11-4	4-2
14	4-1	---	6-3	4-2	4-2
24	6-2	2-1	8-3	8-3	6-2
04	4-2	2-1	7-3	7-3	4-2
17	4-2	---	9-4	6-3	4-2
05	2-1	6-2	6-3	5-2	4-2
18	3-1	---	11-4	4-2	4-2
08	2-1	---	10-4	7-3	4-2
30	2-1	---	8-4	6-3	4-2
11	2-1	2-1	7-3	7-3	6-3
28	3-1	2-1	8-4	3-1	4-2
15	3-2	---	8-4	5-2	6-3
16	2-1	2-1	10-5	6-3	2-1
21	4-2	2-1	9-4	6-2	4-2
25[b]	2-1	2-1	8-4	8-4	4-2
12[b]	2-1	4-2	8-4	4-2	3-2
07[b]	7-2	2-1	20-5	11-3	8-2
02[b]	2-1	---	8-4	8-4	4-2
13[b]	4-2	2-1	10-5	8-4	4-2
26[b]	4-2	---	10-5	4-2	4-2

cotton covered the bottom of each jar. The blocks were arranged so they did not touch and all surfaces were exposed to formalin. After fixation, the tissue blocks were loaded into an Autotechnicon for conventional dehydration, clearing, and infiltration with paraffin.

Whole frontal sections were mounted in paraffin blocks and cut on a rotary microtome. The microtome was first set at 7 microns, but if the tissue did not appear to hold together or curled excessively, the thickness was increased to 10 microns. The thin sections were affixed to glass slides with mounting fluids containing 1 per cent egg albumin in glycerin. Celloidin was used to assure suitable adhesion of tissue sections to the slides during staining. The slides were dipped into a solution of 0.5 per cent celloidin in equal parts ethanol and ether prior to staining.

Seven staining procedures were followed according to methods outlined in the Manual of the Armed Forces Institute of Pathology (26). One slide from each block was stained with hematoxylin and eosin, and a companion slide from each block was stained with thionine. Periodic acid-Schiff, Weil-Weigert Stain for myelin sheaths, Prussian blue for hemosiderin, and Holtzer's stain for glial cell processes were employed when appropriate. Frozen sections of selected blocks of formalin fixed tissue from animals 24, 30, and 28 were cut in a cryostat and stained with Cajal's gold sublimate and examined for astrocytosis.

RESULTS

Preliminary Studies in Group I Animals

Table 2 lists the daily temperatures of Group I animals inoculated with the selected hog cholera virus of low pathogenicity.

A marked temperature response was first noted on post inoculation day 2. High temperatures continued until death. Animal 6924 was killed on post inoculation day 7. The other two inoculated animals succumbed to the disease.

The incubation period, clinical manifestations, duration of clinical signs, mortality rate, and gross lesions in all three animals were indistinguishable from manifestations observed in classic hog cholera.

The brain from animal 6924 was examined histopathologically. Microscopic lesions characteristic of hog cholera were found. Vascular cuffs composed of mononuclear cells apparently resulted from proliferation of adventitial cells in the vascular wall, migration of microglia from the neuroparenchyma, and migration of lymphocyte-like cells from the blood. These cells were distributed throughout the thickness of the vascular walls (Figures 2 and 3). It was difficult to identify endothelial cells lining the lumen. Cells undergoing mitosis were seen in some of the vascular lesions (Figure 4).

Evidence of edema was detected around some of the vascular lesions. Edematous spaces separated blood vessels and

Table 2. Daily temperatures for Group I animals (age 2 months)

Days Post Inoculation	Animal Numbers 6924	6937	6922
0[a]	104.0° F	104.8° F	103.8° F
1	104.0° F	104.0° F	103.6° F
2	105.0° F	106.4° F	106.6° F
3	105.2° F	106.0° F	104.5° F
4	106.8° F	106.8° F	105.2° F
5	106.0° F	105.4° F	105.2° F
6	105.4° F	107.6° F	107.0° F
7	107.6° F[b]	104.5° F	105.0° F
8		105.6° F	104.0° F
9		105.2° F[c]	104.2° F
10			106.0° F
11			106.5° F
12			105.4° F
13			106.0° F
14			105.0° F[c]

[a]Pigs were inoculated on 0 day.

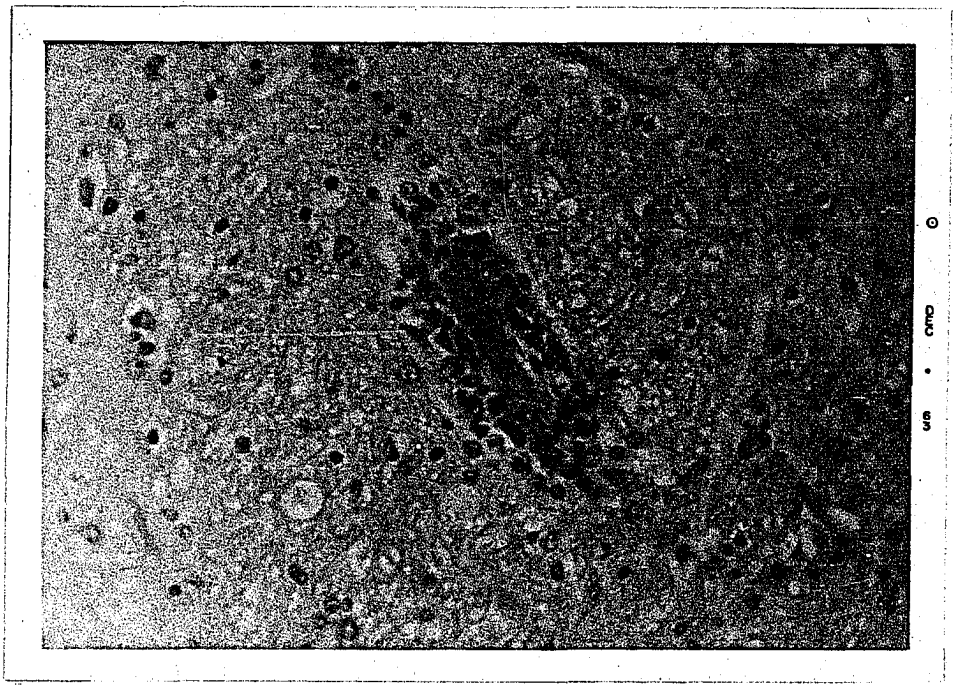
[b]Killed.

[c]Died.

neurons from the surrounding neuroparenchyma. Foot pad protoplasmic strands of astrocytes traversing the distended space gave it a lobulated appearance (Figure 5).

Figure 2. A typical vascular cuff composed of mononuclear cells. Pig 6924. (7 days post inoculation). H and E. X250.

Figure 3. Note the variety of mononuclear cells and a few eosinophils in this vascular lesion. The vascular wall is not discernable. Pig 6924. (7 days post inoculation). H and E. X250.



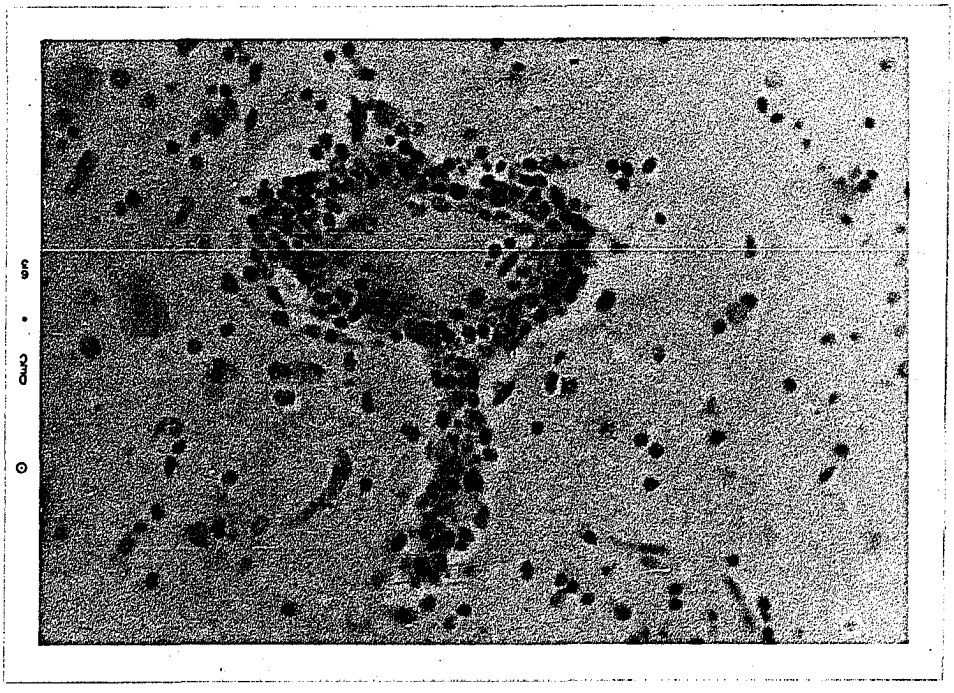
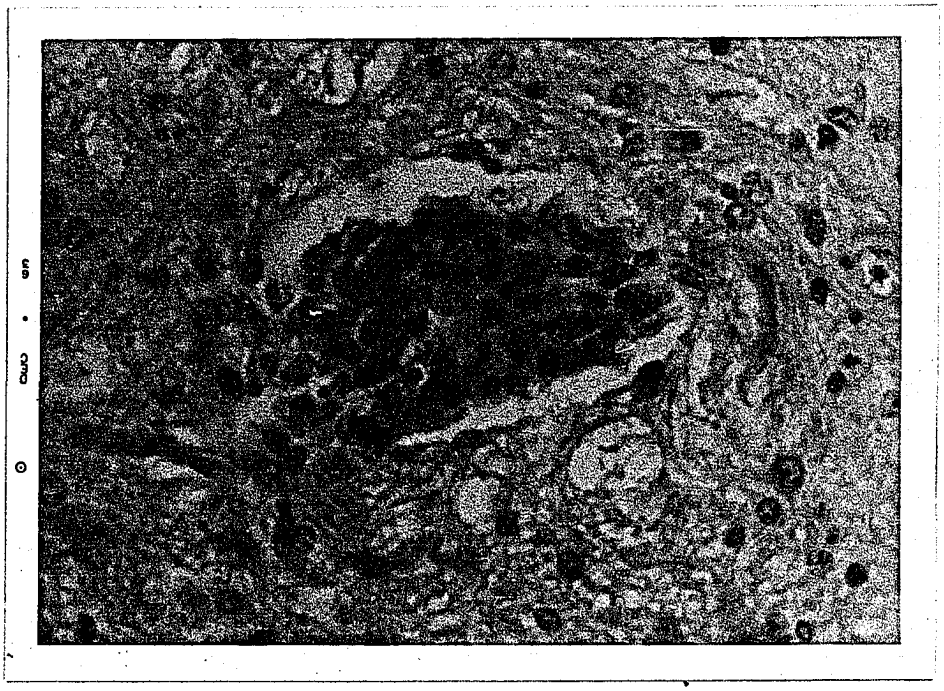
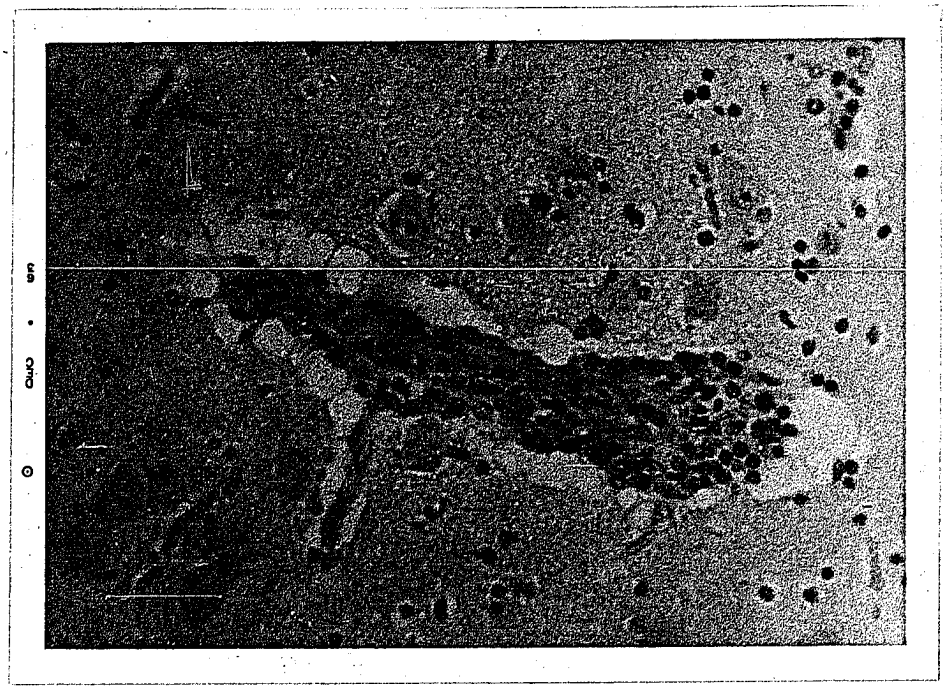


Figure 4. Inflammatory cells appear to have obliterated the vascular lumen in this brain lesion. Note two dividing cells in the lesion. Pig 6924. (7 days post inoculation). H and E. X450.

Figure 5. Note marked vascular cuffing and the lobulated edematous perivascular space. The strands of tissue traversing the edematous space are probably protoplasmic extensions of astrocytes with foot pads attached to the blood vessel. Pig 6924. (7 days post inoculation). H and E. X250.





Focal gliosis, sometimes referred to as glial stars or glial nodes was noted (Figures 6 and 7). Degenerating neurons encircled by mononuclear cells were found occasionally throughout the brains (Figures 8 and 9). Focal areas of nonsuppurative meningitis characterized by mononuclear cell infiltration were widespread throughout the pia and arachnoid, but were most severe in the sulci of the cerebral cortex (Figure 10). Congestion and cuffing involved blood vessels within the meninges as well as the branches of meningeal vessels entering the brain tissue.

Lesions were found in the choroid plexus. These histopathologic alterations consisted primarily of lymphocyte-like cells and pial cells forming aggregates within the stroma near blood vessels (Figure 11). They often partially or completely encircled blood vessels, but they did not symmetrically surround the vessels. The lesions did not involve the entire thickness of the vascular wall, and the greatest part of them were peripheral to the vessel.

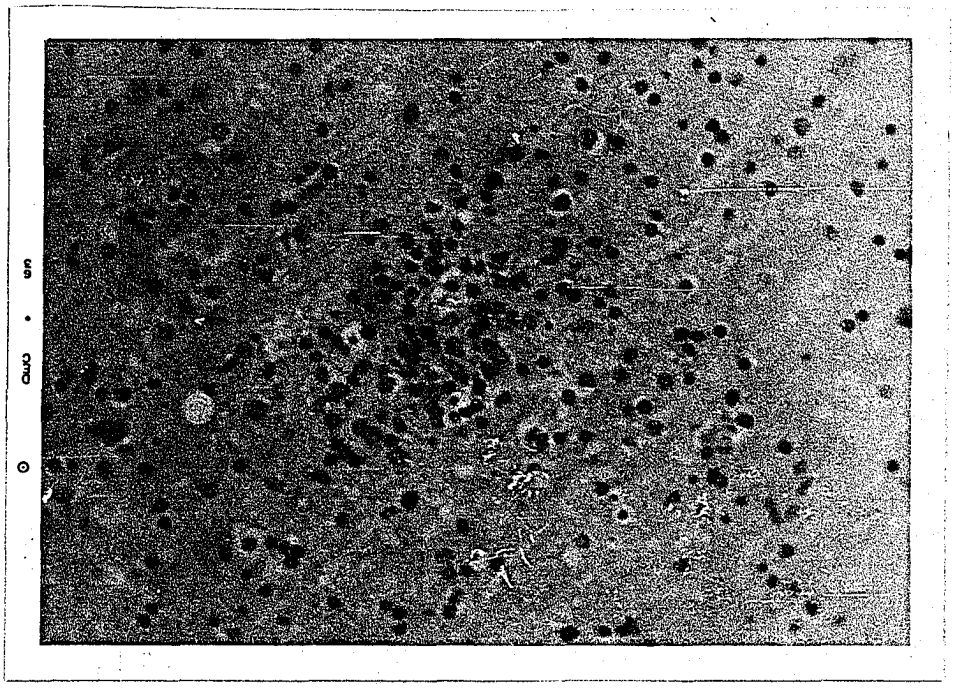
In summary the lesions in the brain of animal 6924 at 7 days post inoculation were characteristic of hog cholera and typical of those observed in animals affected with virulent strains of hog cholera virus.

Clinical Observations in Group II Animals

Clinical manifestations typical of hog cholera were observed in the 19 inoculated experimental animals in Group II

Figure 6. Group of microglia and oligodendroglia in white matter. Pig 6924. (7 days post inoculation). H and E. X250.

Figure 7. Several hyperchromatic cells in this glial focus are apparently undergoing division. This tissue is grey matter. Pig 6924. (7 days post inoculation). H and E. X250.



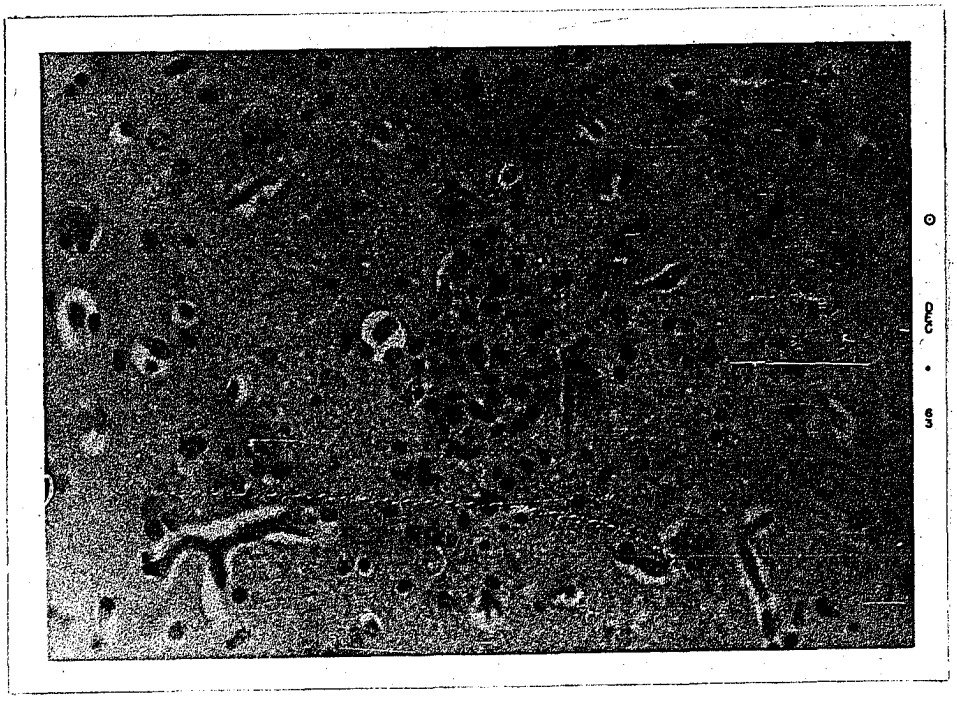
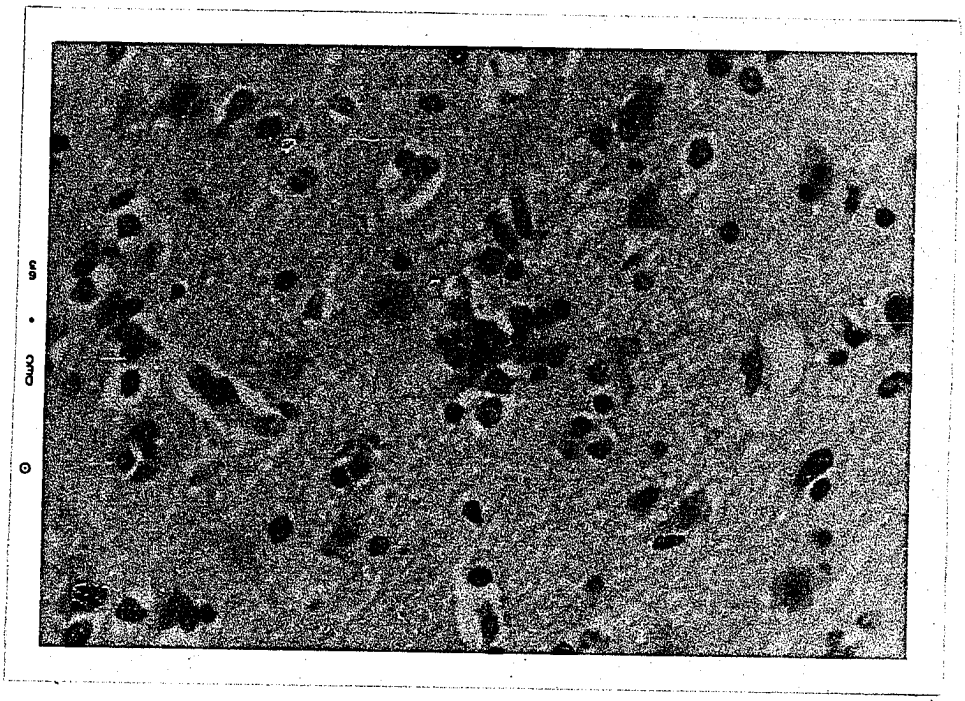


Figure 8. Satellitosis of an eosinophilic degenerating neuron with a large nucleolus. Pig 6924. (7 days post inoculation). H and E. X450.

Figure 9. A small fragment of a neuron is discernible in this glial nodule. Mononuclear cells are filling the perineuronal space. Pig 6924. (7 days post inoculation). H and E. X250.



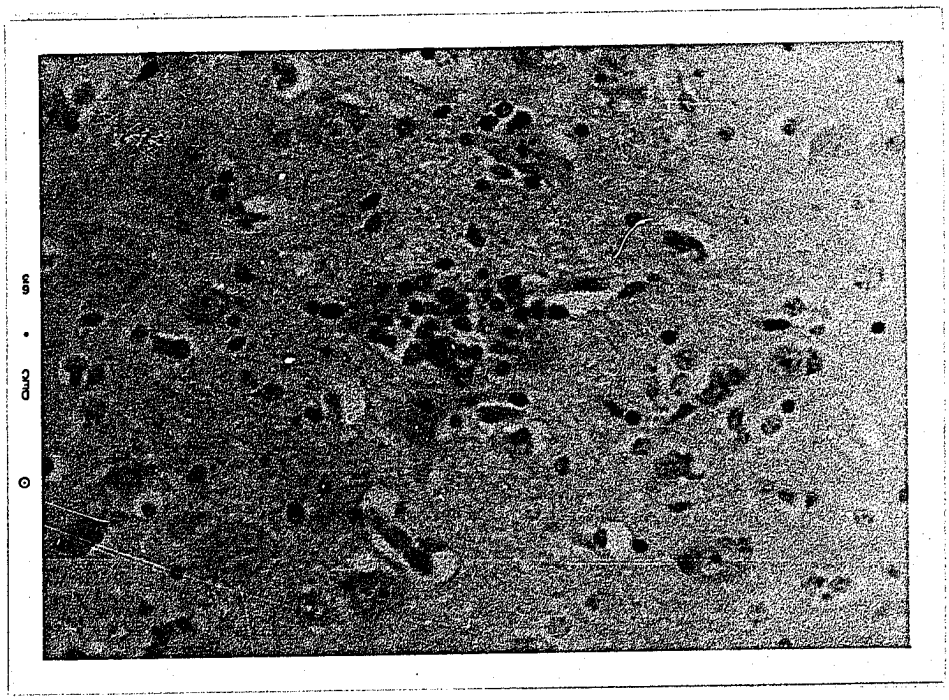
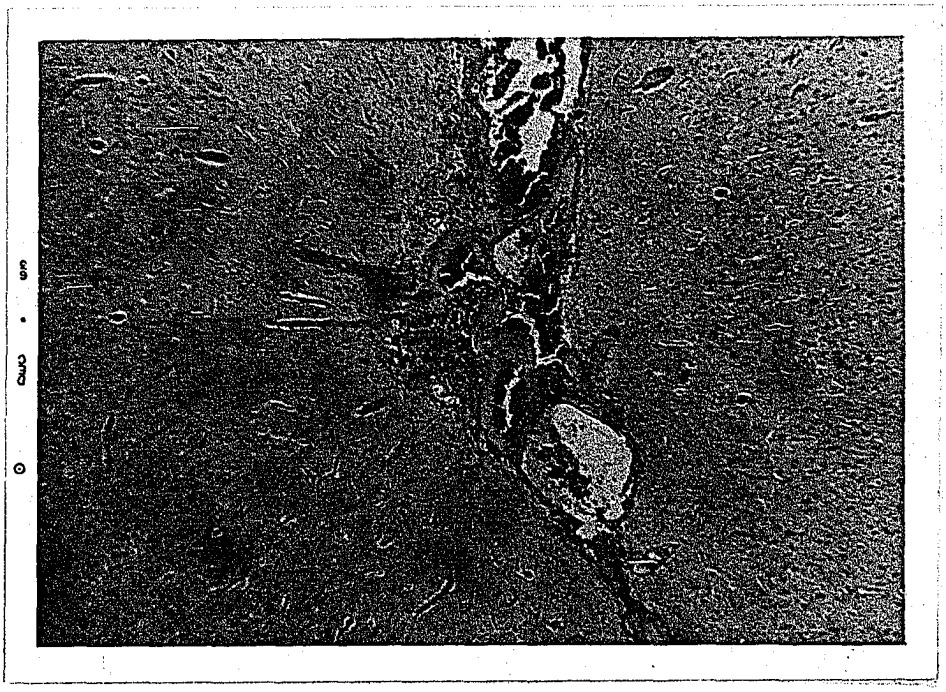
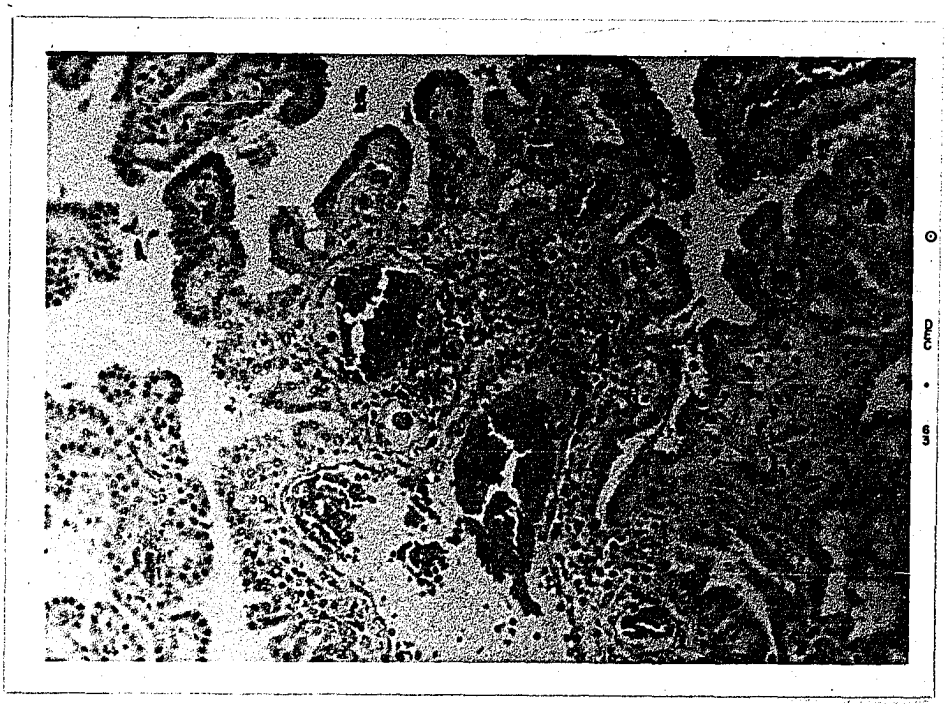


Figure 10. Meningitis and congestion are prominent in the sulci of the cerebral cortex. Note the cuffed vessels penetrating the brain. Pig 6924. (7 days post inoculation). H and E. X35.

Figure 11. A variety of mononuclear cells adjacent to congested blood vessels in the choroid plexus. Pig 6924. (7 days post inoculation). H and E. X125.





during the first 12 to 14 days post inoculation. The animals were listless and developed anorexia, conjunctivitis, and sticky tenacious ocular and nasal exudation. Pyrexia occurred in all animals with temperatures averaging 4° Fahrenheit above normal 5 to 7 days post inoculation. Peak temperatures greater than 107° Fahrenheit occurred in several pigs. As judged by clinical manifestations the pigs were most severely ill between post inoculation days 10 and 12. All animals not previously killed began eating 11 to 13 days post inoculation and recovered clinically from the infection. Pyrexia had subsided by post inoculation day 13 in all pigs except number 10. Fever persisted in pig 10 until it was killed 15 days post inoculation; however, the clinical appearance and appetite had improved. Table 3 lists the daily temperatures observed in each animal from one day prior to inoculation to 30 days post inoculation.

Histopathologic Brain Changes in Group II Animals

Table 4 lists significant changes found on histopathologic examination of each animal in Group II.

Significant lesions were not found in the central nervous system at post inoculation day 5 (pig 23). At post inoculation day 8 (pig 22) perivascular edema and endothelial degeneration were prominent and vascular cuffing was distinctly noticeable, but not yet extensive. By post inoculation day 12 (pig 27) many lesions were extensive; perivascular edema, endothelial degeneration, margination of lymphocyte-like

Table 3. Daily temperatures for Group II animals[a]

Days	Animal Numbers																		
	23	22	27	10	19	14	24	04	17	05	18	08	30	11	28	15	16	21	25
-1	2.0	2.8	2.4	2.0	2.0	2.6	2.0	2.2	1.2	2.2	0.8	2.0	2.0	2.4	2.6	1.8	2.8	2.8	1.8
0[b]	2.8	3.0	2.0	3.0	3.0	2.6	3.0	2.8	2.2	2.6	1.0	2.8	2.8	2.4	2.0	2.6	3.2	2.6	2.2
1	4.0	3.4	3.2	2.6	3.6	2.8	3.2	3.0	3.4	3.8	3.2	3.4	3.2	3.6	2.8	3.6	2.4	3.0	3.2
2	5.4	3.8	4.6	4.0	4.8	3.6	4.0	3.8	3.6	3.8	3.6	4.0	5.0	4.4	4.0	4.2	4.8	4.2	5.6
3	6.0	3.8	5.6	4.0	4.2	4.6	5.4	5.2	4.2	4.0	4.0	5.2	5.8	5.4	3.6	5.0	5.4	5.0	4.0
4	6.6	5.0	4.8	4.8	4.0	5.8	6.2	6.0	4.0	4.2	6.4	5.2	6.8	6.0	4.6	5.8	5.6	4.2	5.2
5[c]	7.2	5.0	5.0	5.6	4.4	6.2	6.4	5.0	5.0	5.6	5.8	6.4	6.8	5.2	4.8	6.2	5.0	6.0	5.0
6	---	3.0	6.0	7.0	4.6	5.0	6.0	6.2	5.0	5.8	4.8	6.2	6.8	4.8	6.2	6.0	4.0	6.0	6.4
7	---	4.0	7.0	7.2	4.2	4.0	4.6	5.0	4.6	4.6	6.0	5.0	5.6	4.0	5.6	6.2	4.8	6.4	5.0
8	---	4.6	4.0	3.2	3.2	3.2	4.8	4.2	3.8	4.0	4.2	3.6	4.2	5.0	5.4	3.8	3.0	6.0	5.7
9	---	---	2.4	4.4	4.3	3.6	4.0	3.4	3.2	5.2	3.4	4.8	4.6	4.0	4.8	3.4	3.0	4.0	4.5
10	---	---	2.0	7.8	4.0	2.8	3.8	4.0	1.2	4.4	2.8	3.2	4.0	3.6	2.6	4.2	3.0	4.0	3.8
11	---	---	3.8	5.8	3.4	3.2	3.0	3.0	2.6	3.2	4.2	3.6	4.0	3.4	2.8	3.6	2.0	4.0	4.0
12[c]	---	---	1.8	5.8	2.0	3.2	3.2	3.6	4.0	3.2	5.4	3.2	3.8	3.0	3.4	3.0	3.0	2.8	3.0
13	---	---	---	5.4	2.6	2.8	3.0	4.0	3.8	3.6	4.8	3.4	4.0	2.6	3.2	3.0	3.0	3.0	3.4
14	---	---	---	5.2	3.4	4.0	3.8	2.0	2.0	2.0	3.2	3.2	2.0	3.0	4.0	2.8	2.4	2.8	2.4
15[c]	---	---	---	6.0	2.6	2.6	2.6	3.0	2.0	3.0	2.2	2.4	2.0	2.0	2.2	2.0	2.2	2.6	2.4
16	---	---	---	---	3.2	2.4	3.0	3.0	2.8	2.8	3.2	2.4	3.0	2.4	2.6	2.0	2.4	2.6	2.8
17	---	---	---	---	4.0	2.6	3.0	2.6	3.4	3.0	4.2	3.0	4.2	3.2	3.8	2.8	2.4	3.0	4.0

14	---	---	---	5.2	3.4	4.0	3.8	2.0	2.0	2.0	3.2	3.2	2.0	3.0	4.0	2.8	2.4	2.8	2.4
15[c]	---	---	---	6.0	2.6	2.6	2.6	3.0	2.0	3.0	2.2	2.4	2.0	2.0	2.2	2.0	2.2	2.6	2.4
16	---	---	---	---	3.2	2.4	3.0	3.0	2.8	2.8	3.2	2.4	3.0	2.4	2.6	2.0	2.4	2.6	2.8
17	---	---	---	---	4.0	2.6	3.0	2.6	3.4	3.0	4.2	3.0	4.2	3.2	3.8	2.8	2.4	3.0	4.0
18	---	---	---	---	3.6	1.8	3.8	2.8	3.4	3.8	4.0	2.6	3.2	4.0	2.6	2.2	4.0	2.0	3.4
19[c]	---	---	---	---	3.4	2.2	4.0	2.4	3.2	4.2	4.2	2.4	3.0	3.8	2.6	2.8	4.0	2.4	3.4
20[c]	---	---	---	---	---	4.2	3.2	4.0	3.0	3.8	3.4	2.8	3.0	3.4	3.8	3.2	4.2	3.4	3.4
21[c]	---	---	---	---	---	---	3.0	2.0	2.8	2.6	2.2	2.0	1.0	2.0	1.0	3.0	2.4	3.0	2.4
22[c]	---	---	---	---	---	---	---	3.2	2.0	2.6	3.2	3.0	3.0	3.0	3.2	3.0	3.0	3.2	2.2
23[c]	---	---	---	---	---	---	---	---	2.0	2.0	2.8	2.4	3.0	2.0	2.6	2.2	2.0	2.0	1.6
24	---	---	---	---	---	---	---	---	---	4.0	2.8	3.0	3.4	3.4	3.6	2.6	3.0	3.2	4.5
25	---	---	---	---	---	---	---	---	---	4.8	3.2	3.0	4.4	3.8	3.4	4.0	4.6	4.0	3.6
26[c]	---	---	---	---	---	---	---	---	---	4.2	2.8	3.2	3.6	3.6	3.4	3.6	4.0	3.4	3.8
27	---	---	---	---	---	---	---	---	---	---	2.2	3.0	3.4	2.0	0.8	2.8	2.4	2.4	3.2
28	---	---	---	---	---	---	---	---	---	---	2.2	2.8	2.6	3.0	2.4	1.8	2.4	2.6	2.0
29[c]	---	---	---	---	---	---	---	---	---	---	3.0	2.4	3.2	3.0	2.2	2.0	2.0	2.2	1.8
30	---	---	---	---	---	---	---	---	---	---	---	2.6	3.4	3.2	2.4	2.0	2.4	2.6	1.6

[a]All temperatures are 100 plus the given figure and expressed in degrees Fahrenheit.

[b]All animals were inoculated with a strain of hog cholera virus of low pathogenicity on day 0.

[c]Each animal was killed on the last day a temperature is listed.

Table 4. Lesions noted in the brains from animals inoculated with hog cholera virus of low pathogenicity

Days Post Inoculation	5	8	12	15	19	20	21	22	23	26	29	32	36	45	58	74	106	106	106
Animal Number	23	22	27	10	19	14	24	04	17	05	18	08	30	11	28	15	25[a]	21[a]	16[a]
Perivascular edema		x[b]	x							x'[c]								x'	
Endothelial degeneration		x	x	x'								x'							
Margination of lymphocyte-like cells			x									x'							
Increased cellularity of vascular wall			x'	x			x'	x'		x'	x'	x'							
Vascular cuffing		x'	x	x	x	x'	x'	x'											x'
Satellitosis and neuronophagia			x				x	x		x	x								
Cell lining periphery of perivascular space							x	x			x		x				x		x
Meningitis		x'		x'		x'				x'									

[a]Challenged with BAI strain of hog cholera virus 8 days before necropsy.

[b]x - marked lesions.

[c]x' - less severe lesions.

cells and vascular cuffing were marked. Some vessels not affected by typical cuffing appeared thickened and contained more cells than expected. Neuronal degeneration was also noted in Pig 27. Extensive lesions in the pig killed 15 days post inoculation (Pig 10) included vascular cuffing and increased cellularity in vessels not typically cuffed. Endothelial degeneration could still be detected in some vessels, but evidence of perivascular edema was not noteworthy. At 19 days post inoculation (Pig 19) vascular lesions were still considered diagnostic of hog cholera even though the pig appeared clinically healthy. The peak occurrence of vascular lesions seemed to take place between 15 and 19 days after injection. Lesions seen at post inoculation days 20, 21, and 22 (Pigs 14, 24, and 4) were suspicious of hog cholera, but not diagnostic. Vascular lesions were regressing and some had apparently disappeared by 19 days post inoculation. Non specific changes interpreted as an aftermath of hog cholera encephalitis occurring in animals killed after post inoculation day 19 include neuronal degeneration and oligodendroglia-like cells lining the periphery of perivascular spaces.

Lesions found in remaining pigs killed through post inoculation day 74 did not provide evidence for differentiating hog cholera from other encephalitides. Significant lesions were not found in the 5 control pigs killed at times corresponding to post inoculation days 5, 22, 36, 74, and 98.

At 98 days post inoculation 2 control and 3 experimen-

tally inoculated animals were challenged with 1 milliliter of whole swine blood containing virulent hog cholera virus. The controls died, but the experimentally inoculated pigs were not clinically affected by the challenge.

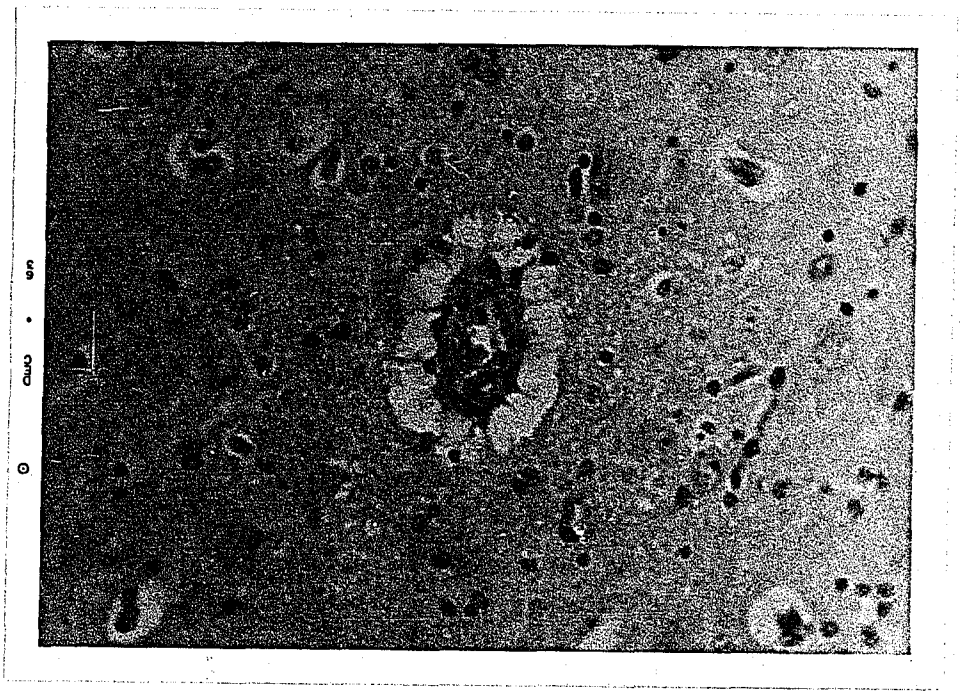
Additional significant lesions were not found in spinal cord sections taken at the following levels: cervical - 3, thoracic - 3, thoracic - 12, and lumbar - 3. Vascular cuffing was found in all 4 cord sections from animal 10 only (15 days post inoculation). Pig 22 (8 days post inoculation) had one small cuff in the lumbar section, and pig 4 (22 days post inoculation) had one vascular lesion in the thoracic - 3 section. Significant cord lesions were not found in any other animals.

Marked evidence of edema, characterized by distention of perivascular and perineuronal spaces, was encountered in experimental animals 22 and 27 (post inoculation days 8 and 12). Similar lesions, but focal and less severe, occurred in animals 5 and 12 (Figures 12, 13, 14, and 15).

Vascular endothelial cells were undergoing degenerative changes characterized by enlarged hyperchromatic nuclei extending into the lumen (Figures 13, 14, and 16). These endothelial changes were pronounced in most blood vessels throughout the brain of animals 22 and 27 at 8 and 12 days post inoculation. Similar lesions, but fewer in number, were found in animals 10 and 8 at post inoculation days 15 and 32 respectively. Occasionally the endothelial cytoplasm appeared to

Figure 12. Evidence of edema can be seen around this arteriole. Pig 27. (12 days post inoculation). H and E. X250.

Figure 13. The endothelial nuclei are swollen and hyperchromatic. Edematous distention is apparent around the venule. Pig 27. (12 days post inoculation). H and E. X250.



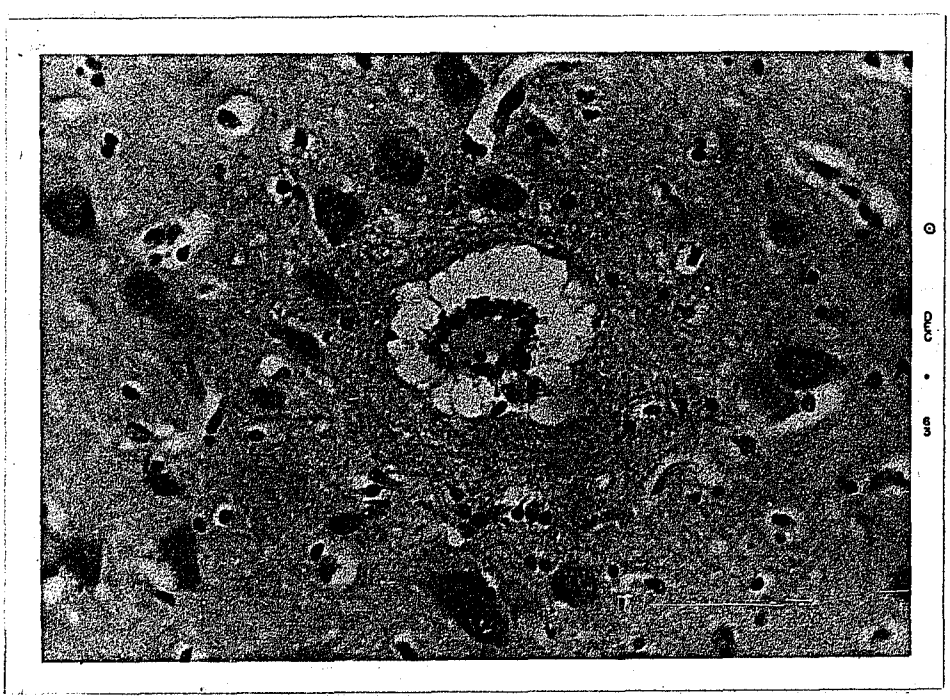
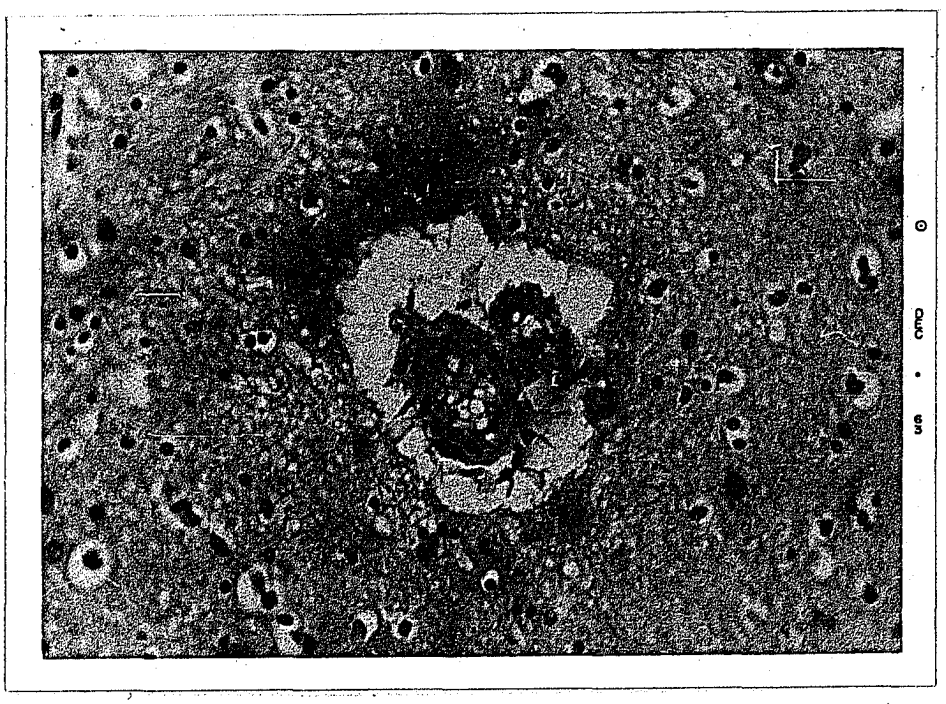


Figure 14. Margination of lymphocytes, evidence of edema, and increased eosinophilic staining of the surrounding neuroparenchyma can be seen in this lesion in the cerebral cortex. Pig 27. (12 days post inoculation). H and E. X250.

Figure 15. Note the asymetric increased eosinophilic staining of the neuroparenchyma, evidence of edema, and vacuolar degeneration of endothelial cells. Pig 25. (12 days post inoculation). H and E. X250.



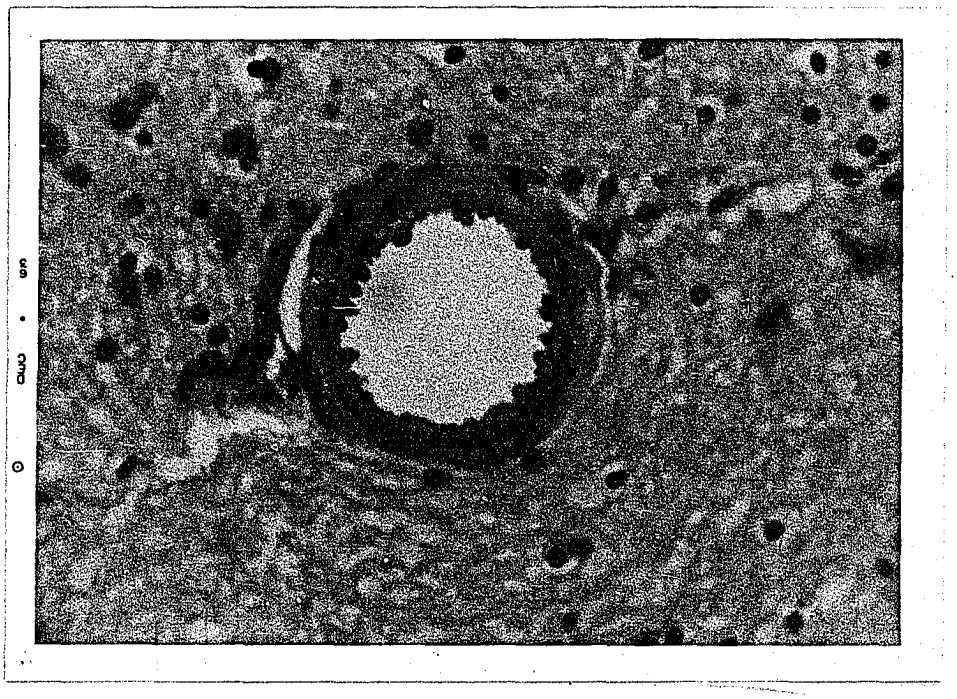


Figure 16. Several lymphocytes are attached to the roughened endothelium in this cerebral blood vessel. Pig 8. (32 days post inoculation). H and E. X450.

be distended and vacuolated. This change resulted in occlusion of some blood bessels (Figure 15). The absence of blood cells in the lumen of affected vessels may be an indication of occlusion above the level of the tissue section.

Endothelial degeneration was apparently responsible for the roughened appearance of the lumen and attachment of lymphocytes. Endothelial degeneration and margination of lymphocytes were noted in animals 27 and 8 killed on post inoculation days 12 and 32 respectively (Figures 13, 14, and 16). Both changes were marked throughout the brain from animal 28 and focal in the brain from animal 8. However, the two

changes were not consistently noted in the same areas.

An increased number of lymphocytes and adventitial cells was noted within the walls of arterioles which otherwise appeared normal (Figure 17). The infiltrating cells appeared either in uniform rows or diffusely throughout the wall. This alteration was most notable in the brain from animal 10 killed post inoculation day 15; however, it was also present in the brains from animals 27, 24, 4, 5, 18, and 8 at post inoculation days 12, 21, 22, 26, 29, and 32 respectively.

Vascular cuffing was the most outstanding lesion seen in the brain tissue. It was observed in all seven animals killed

Figure 17. Endothelial and adventitial cells are swollen and apparently undergoing proliferation. Pig 10. (15 days post inoculation). H and E. X250.

8 to 22 days post inoculation. The cuffing, involving the entire thickness of the vessel wall, consisted predominantly of lymphocytes, round or oval cells resembling histiocytes, and microglia. Eosinophils were rarely observed in the cuffs. Intact endothelial cells were difficult to identify when the lumens were surrounded by well developed cuffs. Lesions were most numerous in animals 27, 10, and 19 killed on post inoculation days 12, 15, and 19 respectively (Figures 13, 14, 18, 19, 20, and 21). Vascular cuffing was not noted in either the animal killed 5 days post inoculation or any of the 8 animals killed between post inoculation days 22 and 74.

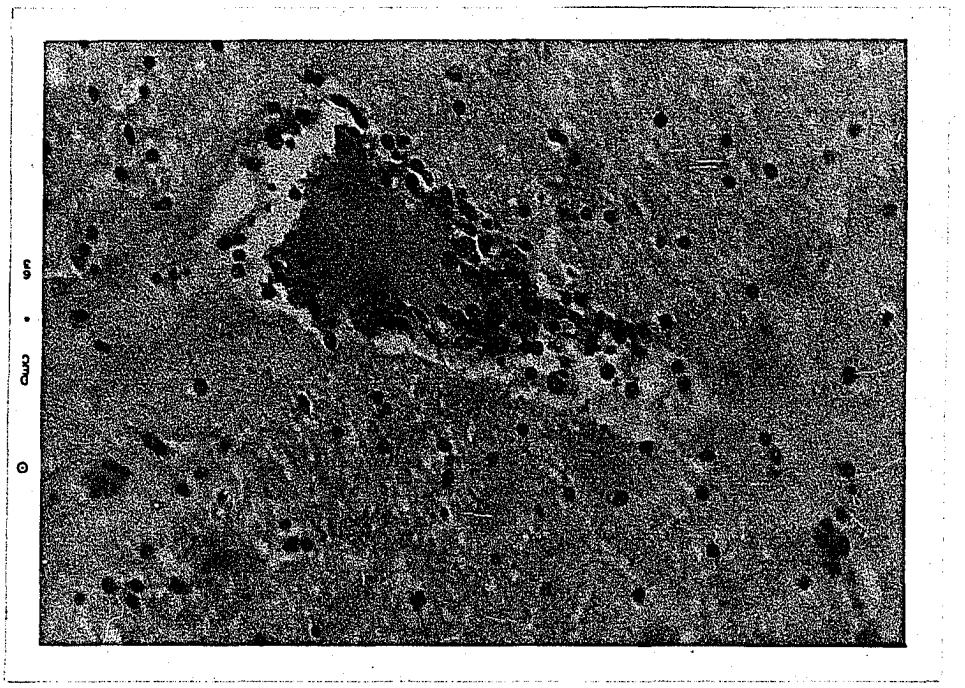
Several prominent vascular cuffs were noted near the junction of the left anterior dorsal part of the thalamus and the internal capsule from animal 16 killed 106 days post inoculation. However, this animal had been challenged with the BAI strain of hog cholera virus 8 days before necropsy.

Some of the vascular cuffs in the brain from animal 19 at 19 days post inoculation were unusual (Figure 21). The lesions were confined to veins and venules in the anterior cerebral cortex and the individual lesions were extensive. Lymphocytes, adventitial cells, eosinophils, and undifferentiated mononuclear cells comprised the cuffs.

With the exception of animal 16, vascular cuffing was widely disseminated. The frequency and distribution of the lesions did not differ significantly between the white and grey matter.

Figure 18. The cellular infiltration has obliterated the entire vascular wall. Pig 10. (15 days post inoculation). H and E. X250.

Figure 19. Some of the inflammatory cells appear to be migrating through the perivascular space, but the greater portion of the lesion is within the vascular wall. Pig 10. (15 days post inoculation). H and E. X250.



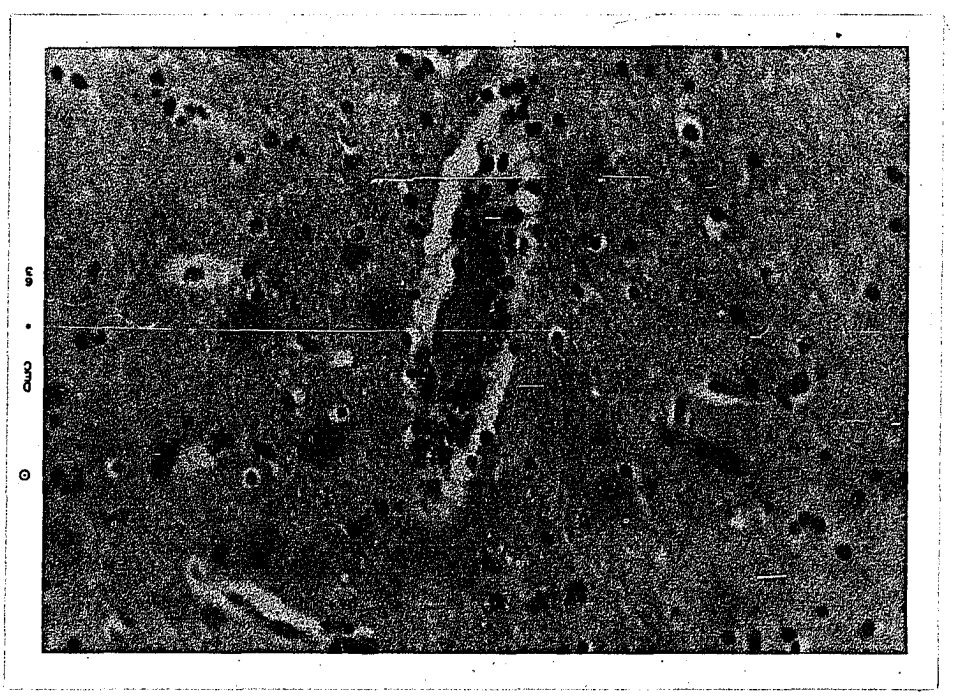
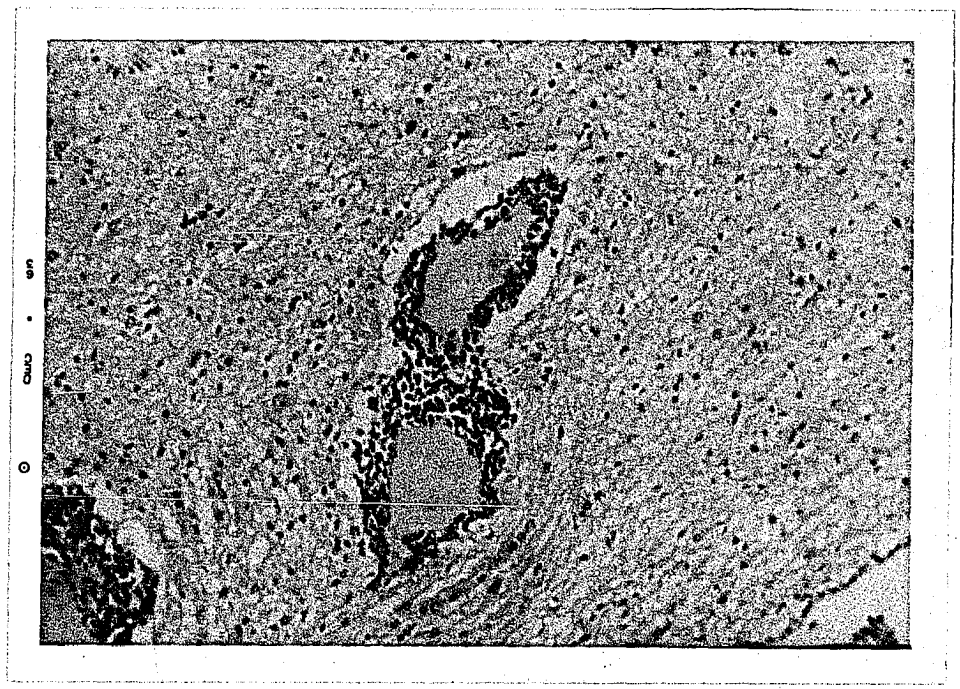
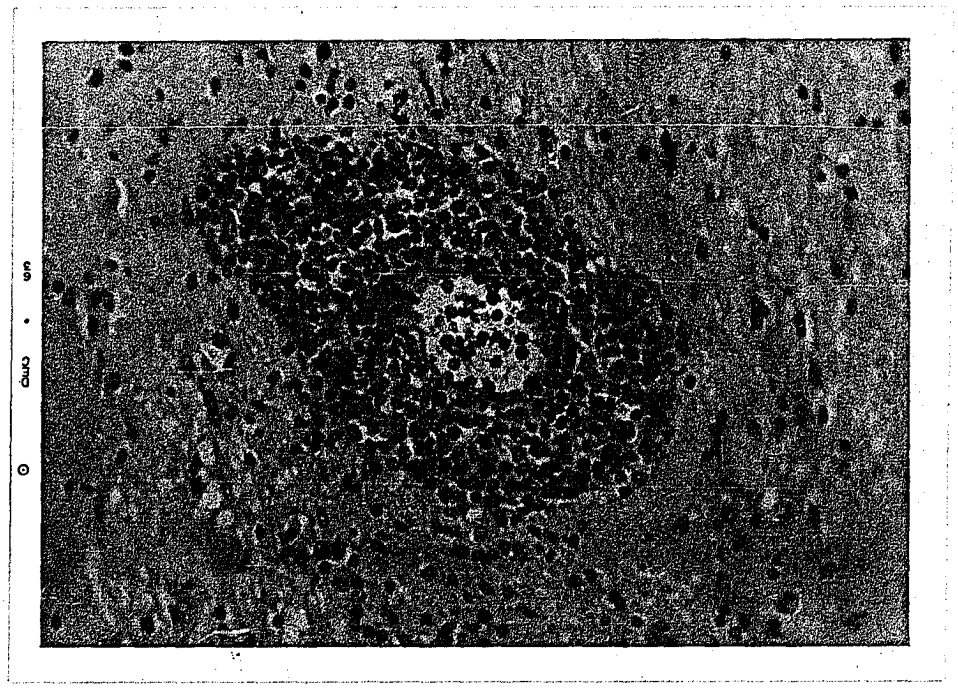


Figure 20. The cellular infiltration extends throughout the vascular walls and endothelial cells cannot be differentiated lining the lumen. Thalamus. Pig 10. (15 days post inoculation). H and E. X125.

Figure 21. This vascular cuff is much thicker than most cuffs found in hog cholera. Pig 19. (19 days post inoculation). H and E. X250.





Focal gliosis such as that illustrated in Figures 6 and 7 was not encountered in the brain from any animal in Group II.

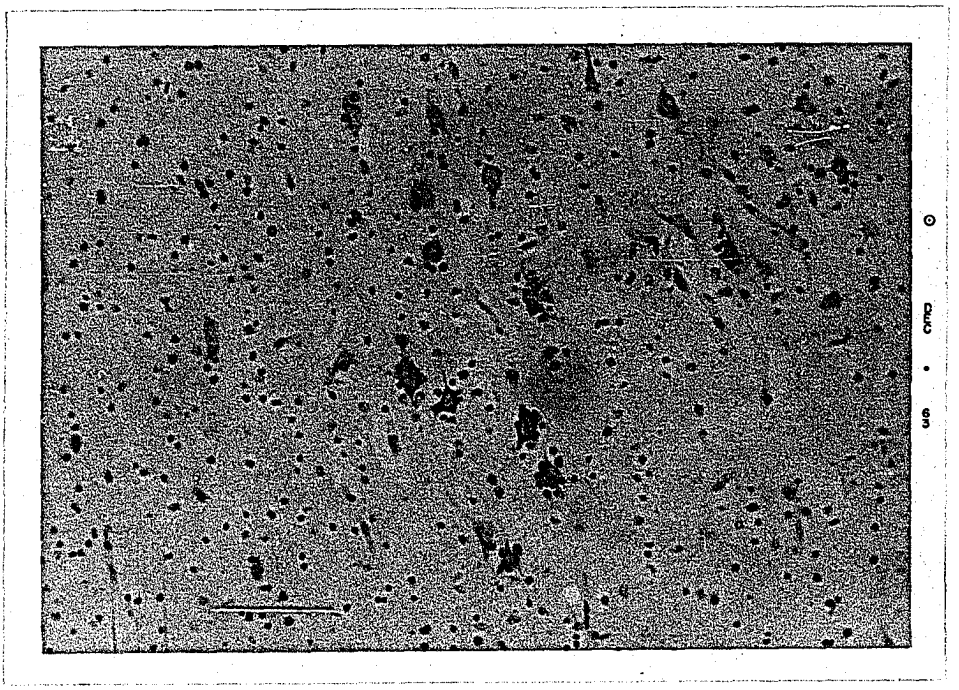
Satellitosis consisting of microglia and oligodendroglia surrounding neurons with eccentric hyperchromatic staining nuclei and enlarged nucleoli was noted in animals 27, 24, 4, 5, and 18 at post inoculation days 12, 21, 22, 26, and 29, respectively (Figures 22 and 23). This change was not as marked in any Group II animals as in animal 6924 from Group I.

Small dark cells resembling microglia and oligodendroglia were observed lining the periphery of perivascular spaces (Figures 23 and 24). This change was not observed in animals killed before post inoculation day 21. It was noted in animals 24, 4, 18, 30, 25, and 16 killed on post inoculation days 21, 22, 29, 36, and 106, respectively. However, pigs 25 and 16 received a challenge dose of virulent hog cholera virus 8 days before they were killed.

Meningitis was not an outstanding or consistent lesion in any Group II animal. It was present focally and to a limited extent in four animals -- 22, 10, 14, and 5 at post inoculation days 8, 15, 20, and 26, respectively. The lesions consisted of small foci of mononuclear cells usually in close association with congested blood vessels.

Figure 22. Satellitosis of degenerating neurons. Pig 27. (12 days post inoculation). Thionine. X125.

Figure 23. Hyperchromatic mononuclear cells line outer border of perivascular spaces in the cerebrum. Satellitosis of a neuron can also be seen. Pig 24. (21 days post inoculation). H and E. X125.



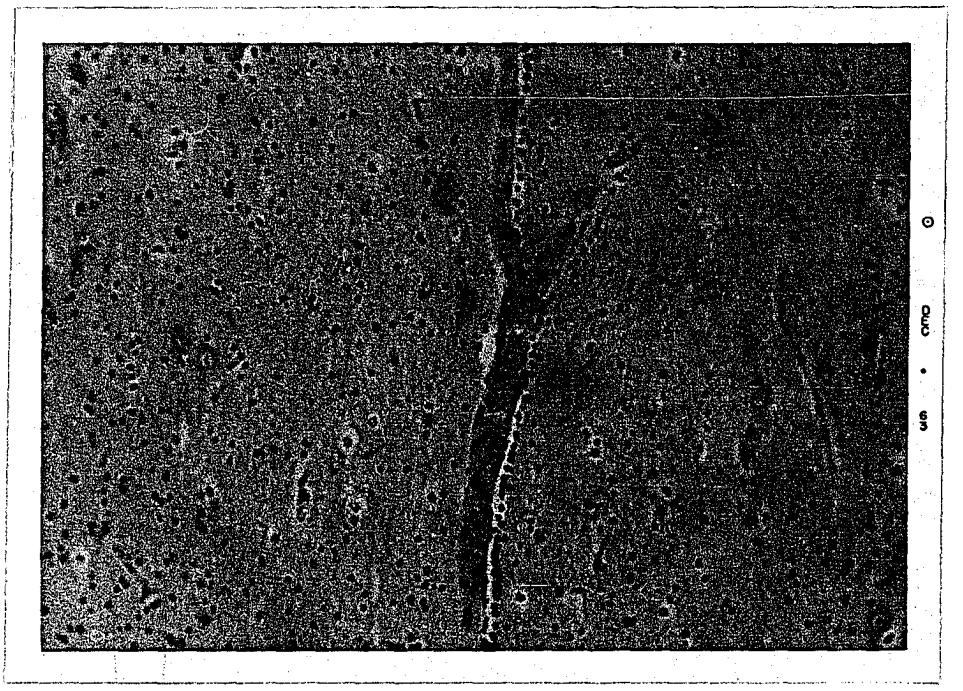


Figure 24. Perivascular satellites are lining the periphery of the perivascular space. Pig 4. (22 days post inoculation). H and E. X125.

Histopathologic Changes in the Choroid Plexus in Group II Animals

Changes in the choroid plexus are summarized in Table 5. Edema, congestion, and hemorrhage, not apparent in control animals, were the first changes observed (Figures 25, 26, 27, and 28).

The most significant lesion, and probably the only lesion in the choroid plexus specifically indicative of hog cholera, was increased cellularity of the stroma. Hog cholera virus apparently has a specific affinity for endothelium of blood vessels in the stroma as well as pial cells comprising the

Table 5. Lesions noted in the choroid plexuses from animals inoculated with hog cholera virus of low virulence

Days post Inoculation	5	8	12	15	19	20	21	22	23	26	29	32	36	45	58	74	106	106	106
Animal Number	23	22	27	10	19	14	24	04	17	05	18	08	30	11	28	15	16[a]	21[a]	25[a]
Edema		x[b]	x			x													
Hemorrhage			x'[c]	x		x				x	x								
Desquamated epithelial			x'	x	x	x	x	x	x	x	x			x	x		x'		
Increased cellularity of stroma		x	x	x	x	x	x	x	x	x	x	x'		x'	x'		x'		
Plasma cells in stroma			x'			x			x	x	x	x							

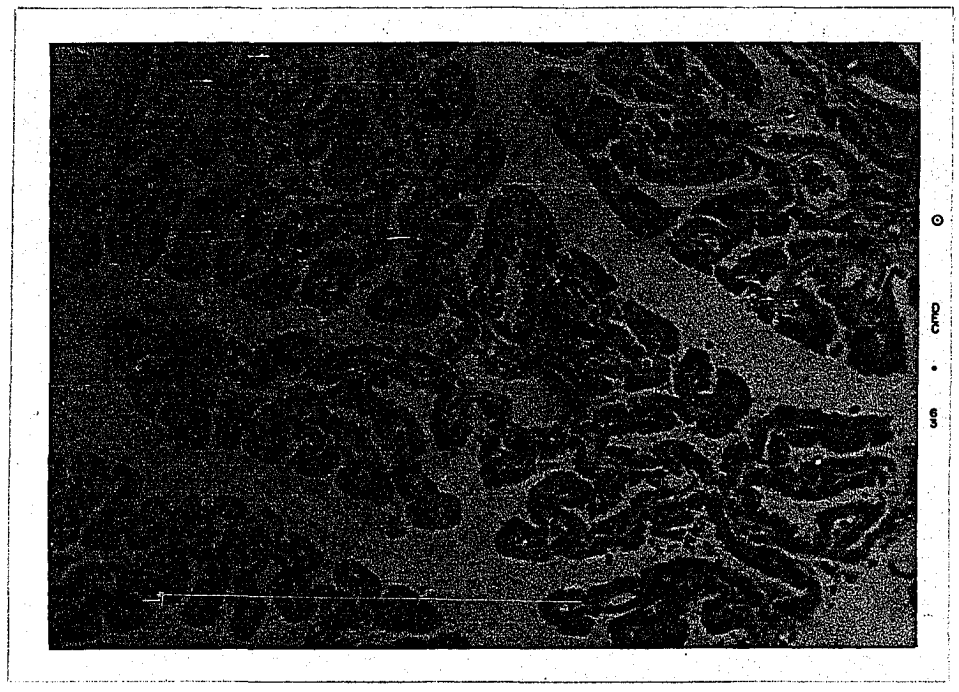
[a]Challenged with BAI strain of hog cholera virus 8 days before necropsy.

[b]x - marked lesions.

[c]x' - less severe lesions.

Figure 25. Choroid plexus from animal 12, a noninoculated control killed on the day corresponding to post inoculation day 8. Note the laminae covered with villi, a structural arrangement well developed in swine to increase surface area. Also note the small number of cells in the stroma. H and E. X125.

Figure 26. These laminae and villi from the choroid plexus of animal 22 were edematous, thus villi are not as apparent on the surface as in Figure 25. Diffusely increased number of pial cells in the stroma can be seen. The epithelial cells are stretched so they resemble mesenchymal cells rather than cuboidal or columnar epithelium. (8 days post inoculation). H and E. X250.



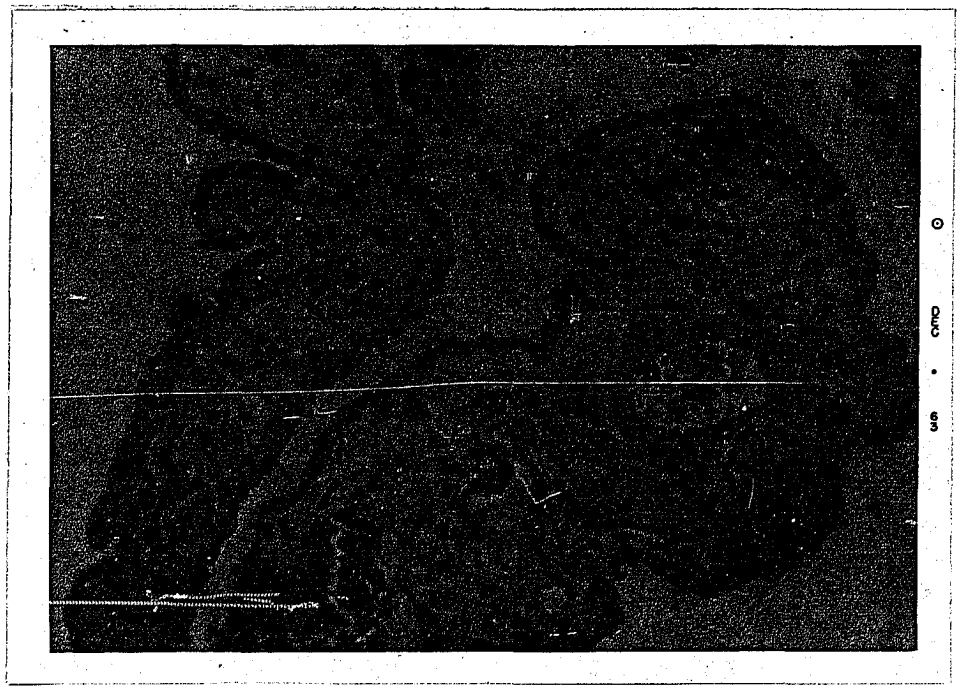
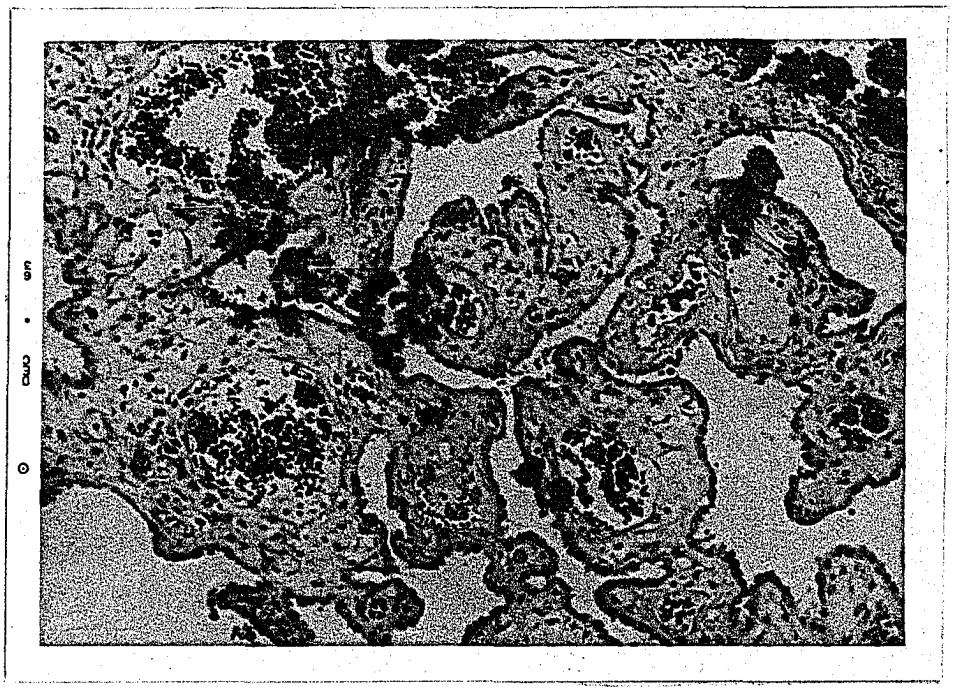
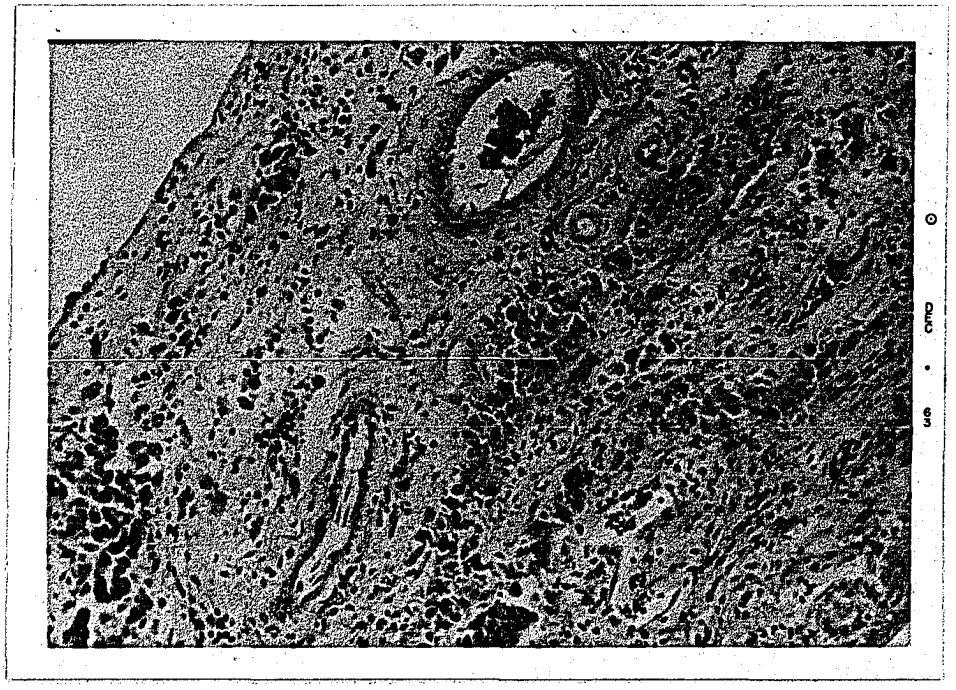


Figure 27. Congestion, edema, and stromal cell proliferation can be seen in this choroid plexus. Note the beginning of perivascular cellular infiltrations. Pig 22. (8 days post inoculation). H and E. X125.

Figure 28. This degenerating choroid plexus is characterized by complete loss of organization. Groups of desquamated epithelial cells, pial cells, pial fibrils, and lymphocytes can be seen. Pig 5. (26 days post inoculation). H and E. X125.





stroma since the latter cells are part of the reticulo-endothelial system. The pial cells are elongated with long fibrillar extensions. Diffusely increased numbers of these cells together with their extensions were seen first. Then lymphocytes and undifferentiated mononuclear cells appeared to migrate into the stroma from the blood and together with the pial cells formed focal lesions usually near blood vessels. However, these lesions did not typically involve the vascular wall in the same manner as vascular cuffing in the neuroparenchyma.

Lesions were not found in the choroid plexus 5 days post inoculation (pig 23). At 8 days post inoculation (pig 22) evidence of edema, diffuse proliferation of pial cells, and increasing numbers of pial fibrils were noted in the stroma. By post inoculation day 12 (pig 27) hemorrhage and sloughing of epithelial cells were noted in addition to edema. Focal lesions appeared to be forming near blood vessels in the stroma by this time. Plasma cells were first found in the stroma on post inoculation day 12.

The size and number of choroid plexus lesions increased and the stroma became more dense through approximately 19 days post inoculation. It was difficult to assess edematous changes between post inoculation days 12 through 19 because of severe disorganization and degeneration of the choroid plexus. Regeneration of choroid plexus and resolution of focal lesions became evident 20 to 23 days post inoculation (pigs 14, 24, 4,

and 17). However, significant focal lesions and groups of plasma cells persisted through 32 days post inoculation (pig 8), but most of the choroid plexus tissue appeared normal and functional after post inoculation day 23.

Edema was present in the stroma of the choroid plexus from animals 22, 23, and 14 at post inoculation days 8, 12, and 20 (Figures 26 and 27). The fluid distended tissue spaces were most apparent in the stroma between the blood vessels and the epithelium. The cuboidal or columnar epithelial cells stretched by edema appeared flattened like mesenchymal epithelium. Congestion and hemorrhage were observed in the choroid plexus from animals 27, 10, 14, 5, and 18 at post inoculation days 12, 15, 20, 26, and 29, respectively (Figure 27). Evidence of hemorrhage consisted of red blood cells leaving the blood vessels or trapped in the stroma.

The vasculature, leptomeningeal tissue, and epithelium comprising the normal choroid plexus were disrupted to some extent in all 7 experimental animals killed between post inoculation days 15 and 26, but the change was more severe in pigs killed at post inoculation days 15 and 19. Epithelial cells which had sloughed from the basement membrane were usually grouped in clusters of 4 to 20 cells. Most of the epithelial cells appeared viable. Their staining properties and membranes indicated they were living although removed from their basement membrane. Increased numbers of pial cells, lymphocytes, and other mononuclear cells added to

the disorganized appearance of these tissues.

Large disorganized masses of tissue composed predominantly of epithelial cells, stromal tissue, mononuclear cells, and erythrocytes, held together by a fibrillar matrix, were floating freely in the cerebrospinal fluid.

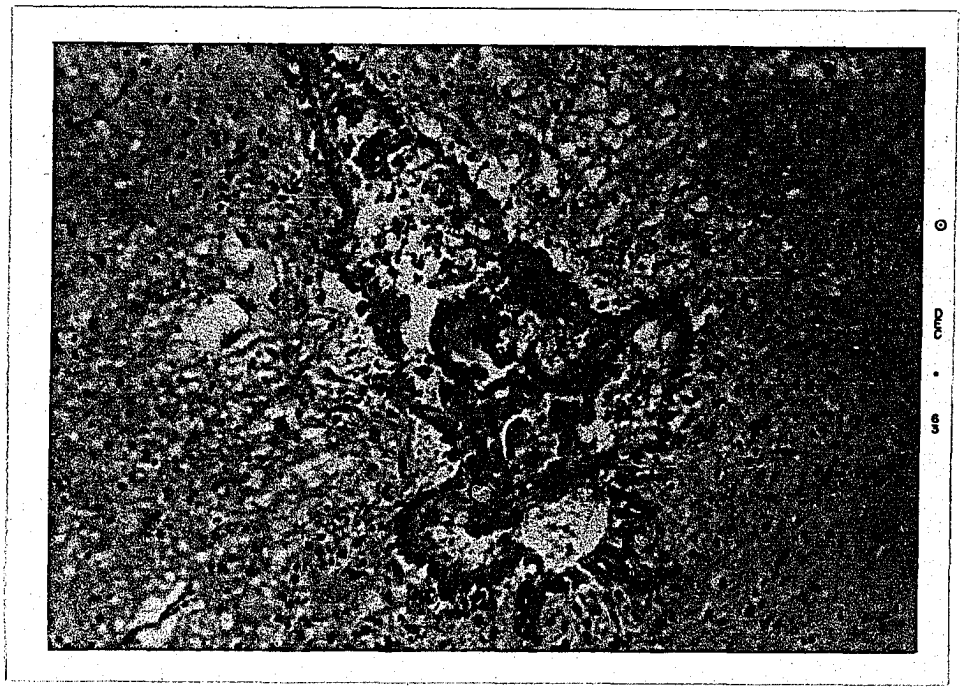
Detached epithelial cells were carried by cerebrospinal fluid to locations removed from the choroid plexus, i.e., meningeal spaces, central canal of the medulla, and spinal cord (Figures 29 and 30). Even at distant locations the epithelial cells still appeared viable. Displaced epithelial cells were noted in animals 27, 10, 19, 24, 4, 18, 11, 28, and 16 at post inoculation days 12, 15, 19, 21, 22, 29, 45, 58, and 106.

Cellularity of the stroma was distinctly increased in all animals killed from 8 through 29 days post inoculation and slightly increased in animals 8, 11, 28, and 16 at post inoculation days 32, 45, 58, and 106. Some focal lesions composed of pial cells, lymphocytes, and undifferentiated cells surrounded blood vessels while others were adjacent to vessels (Figures 31, 32, 33, and 34). Vessel walls were usually not affected, although occasionally lesions extended into the lumens (Figure 35). Individual focal lesions had no distinct peripheral boundary.

Increased numbers of pial fibrils resulted in more intense eosinophilic staining of the stroma. Pial fibrils occurring singularly and in groups were observed (Figures

Figure 29. Epithelial cells from the choroid plexus were seen in the central canal of the medulla. Pig 24. (21 days post inoculation). H and E. X125.

Figure 30. Detached epithelial cells from the choroid plexus are "packed" into the central canal near the junction of the medulla and spinal cord. The separation can be seen between the white and grey matter. Pig 4. (22 days post inoculation). H and E. X125.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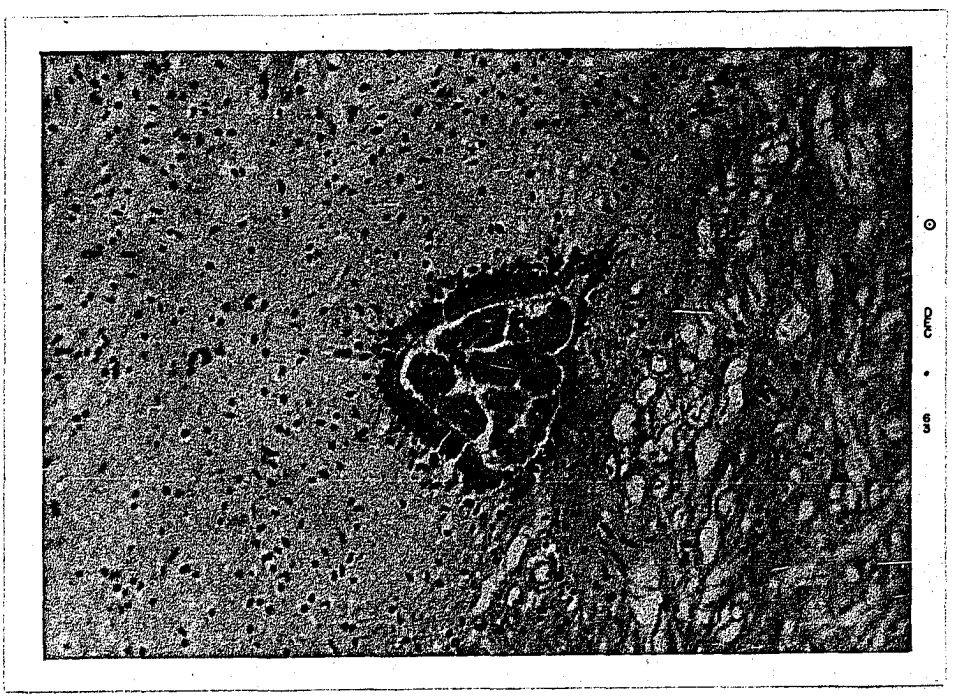
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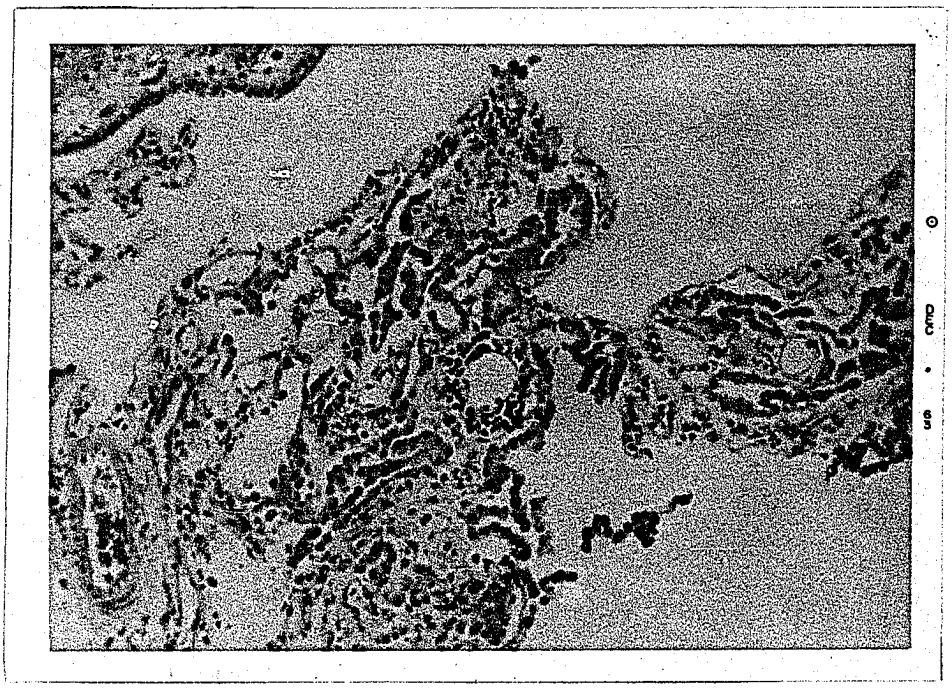
Figure 31. Note a relatively narrow cuff of loosely arranged lymphocyte-like cells encircling a capillary in this disrupted choroid plexus. Many bead-like strands of detached epithelial cells can be seen. Pig 10. (15 days post inoculation). H and E. X125.

Figure 32. Thin walled vascular sinuses are partially encircled by loosely arranged cells resembling lymphocytes in this choroid plexus. Pig 10. (15 days post inoculation). H and E. X125.



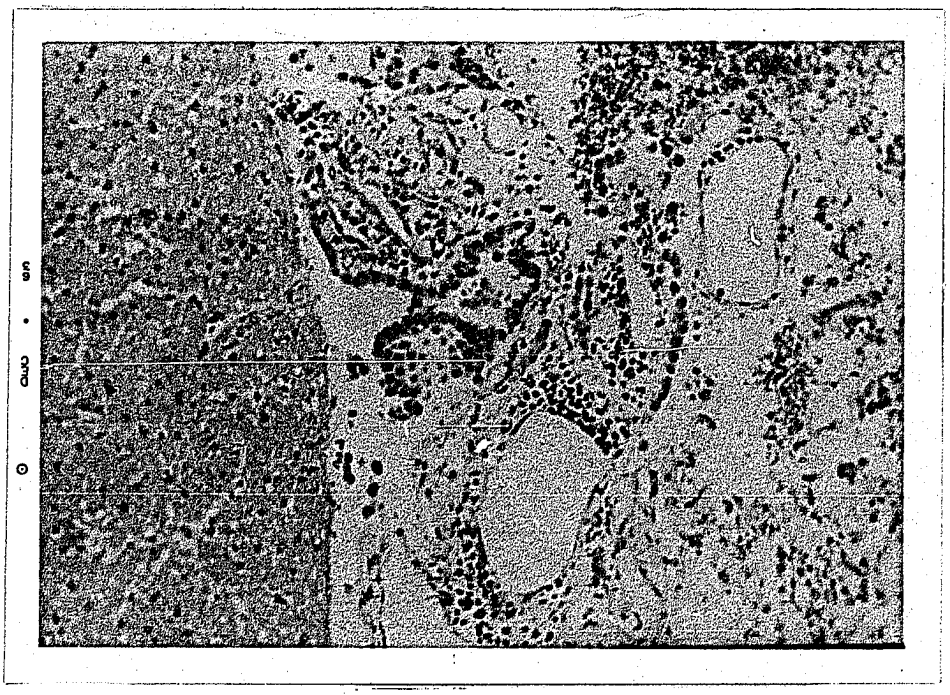
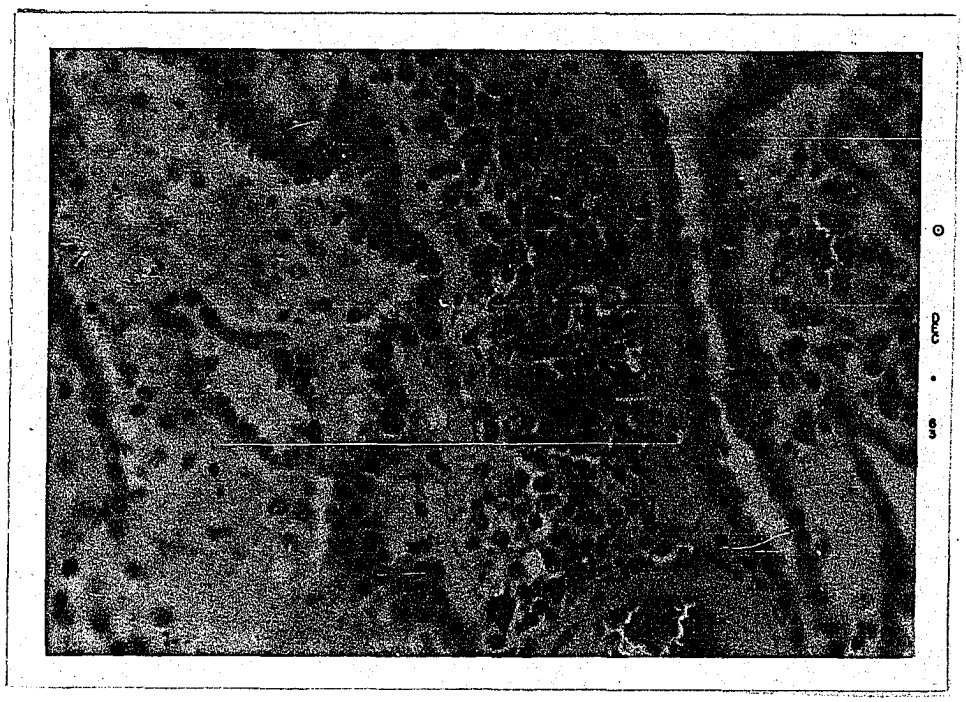
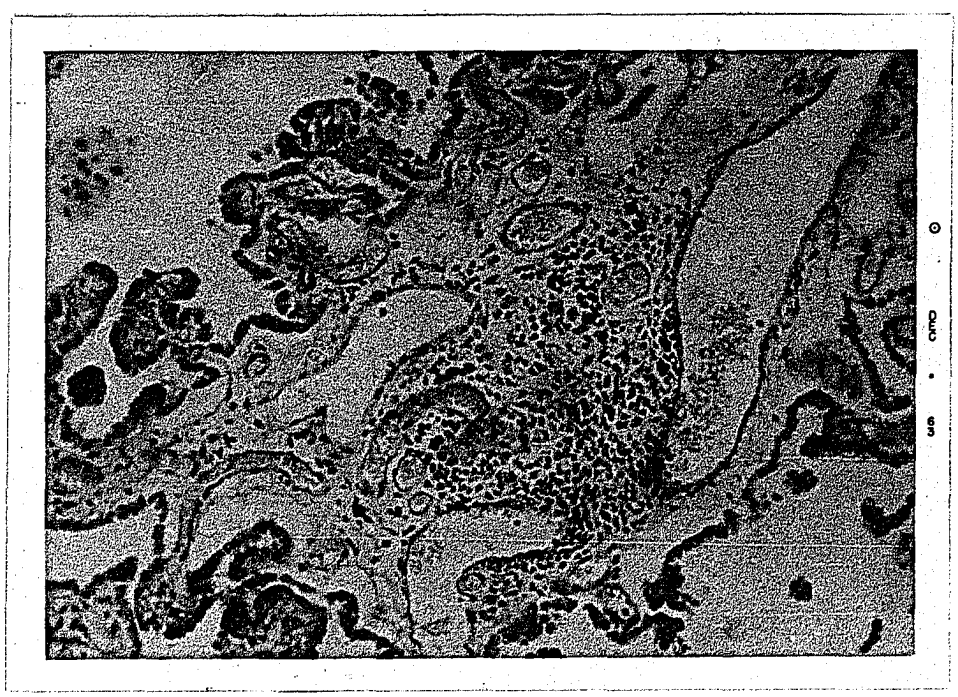


Figure 33. A lesion composed of lymphocytes, pial cells, and a number of unidentified cells can be seen in the choroid plexus from animal 10. The epithelial cells are stretched. Pig 10. (15 days post inoculation). H and E. X250.

Figure 34. This focal lesion contains pial cells, melanophores, lymphocytes, and histiocytes. Pig 17. (23 days post inoculation). H and E. X125.





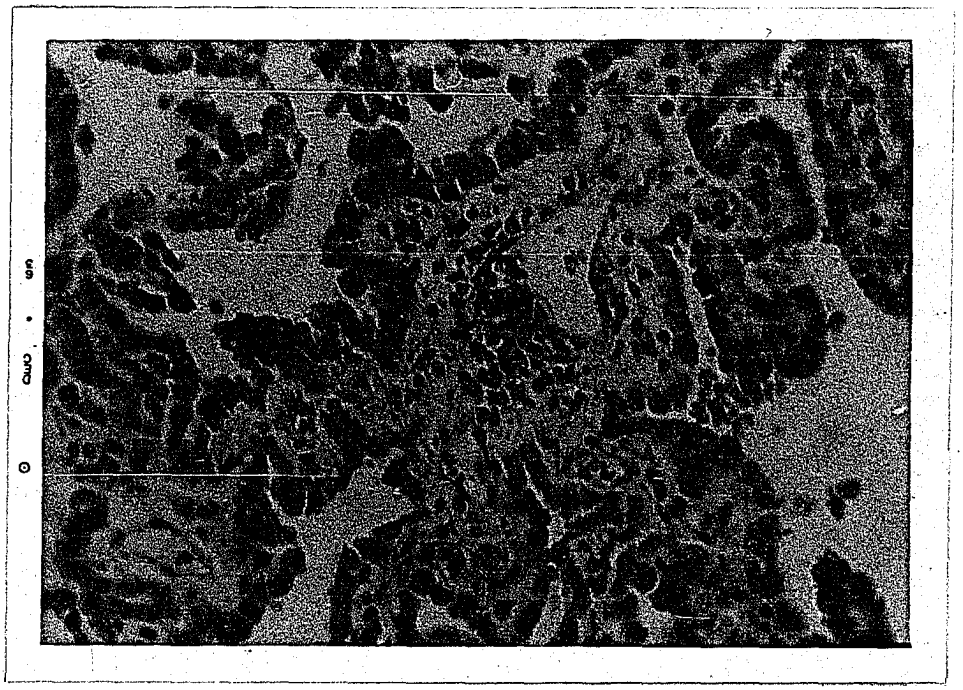


Figure 35. The lesion has penetrated a blood vessel wall and appears to be proliferating within the lumen. Pig 17. (23 days post inoculation). H and E. X250.

26 and 27). Inflammatory cells situated within the interstices of the stroma did not appear to interrupt the continuity of the pial fibers. Cell size and morphology in this conglomerate of mononuclear cells were extremely variable. Some were small round hyperchromatic cells resembling lymphocytes. The pial cells were large, elongated vesicular cells with abundant streaming cytoplasm. All gradations of undifferentiated cells between lymphocytes and pial cells could be found.

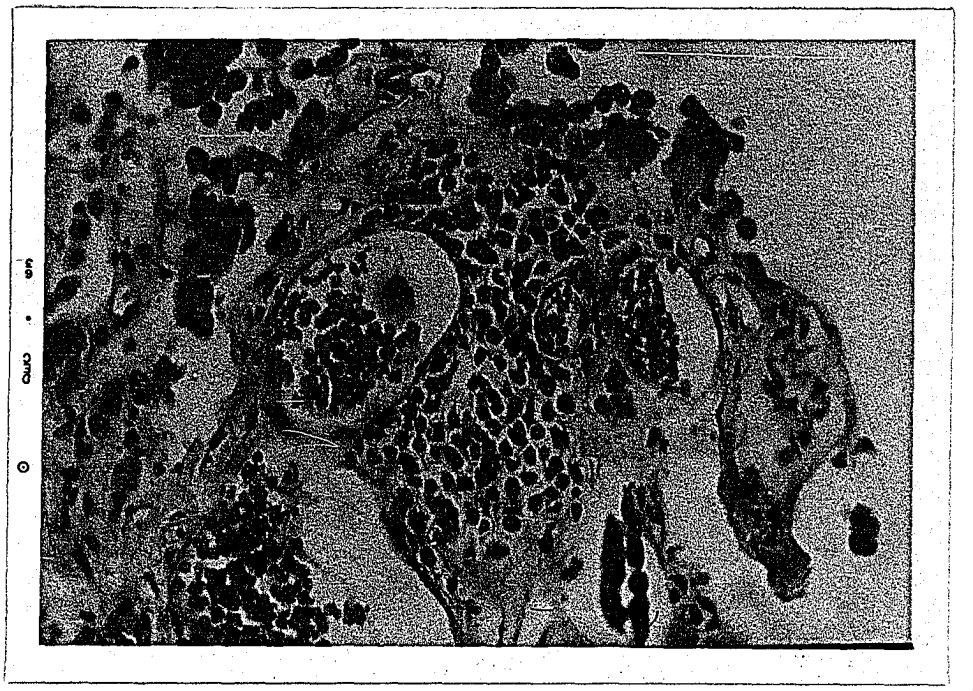
Plasma cells were encountered in the stroma of the choroid plexuses from animals 27, 14, 17, 5, 18, and 8 killed on post

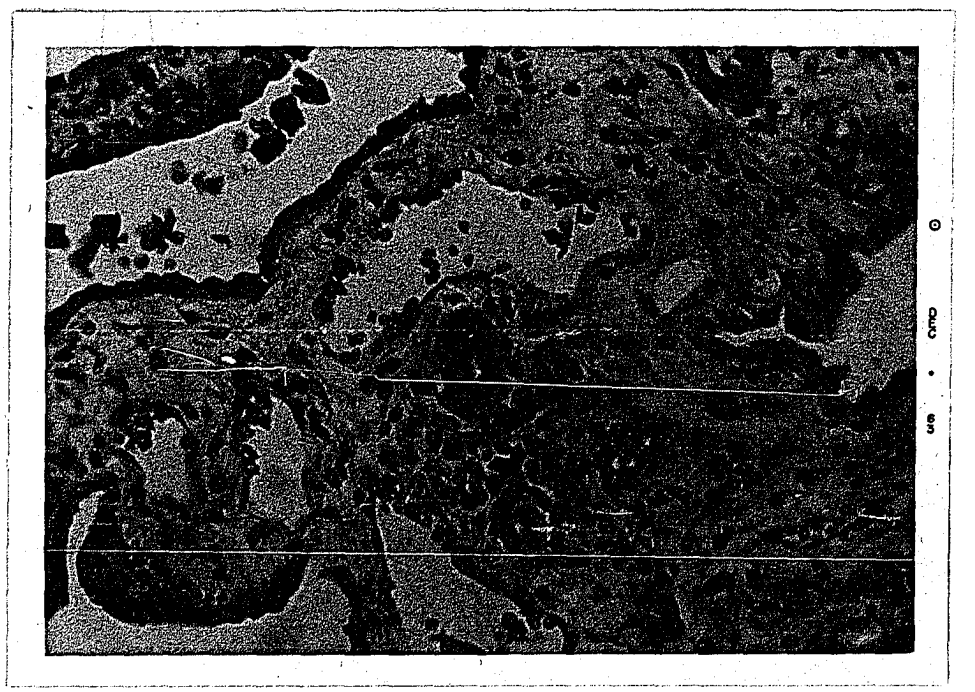
inoculation days 12, 20, 23, 26, 29, and 32, respectively. They were far more prominent in 4 pigs killed from post inoculation days 21 to 29 (Figures 36, 37, and 38). They usually occurred in small or large groups, but singly occurring plasma cells were seldom encountered in this study. The individual plasma cells were somewhat unusual; their borders were not smooth and oval, but rather polyhedral and formed points. Thin fibrils of cytoplasm extending from these points suggested the cells were transformed into plasma cells from pre-existing pial cells (Figures 37 and 38). The plasma cells were easily detected by intense hyperchromatic nuclei and abundant eosinophilic staining cytoplasm.

Figure 36. A focus of plasma cells and diffuse pial cell proliferation can be seen in this edematous choroid plexus. Pig 18. (29 days post inoculation). H and E. X250.

Figure 37. A large group of plasma cells was seen in this choroid plexus. Pig 18. (29 days post inoculation). H and E. X250.

Figure 38. A group of plasma cells between two arterioles is discernible in the choroid plexus. Diffuse pial cell proliferation is apparent in the finely fibrillar edematous stroma. Pig 8. (32 days post inoculation). H and E. X250.





Viremia Determinations

Table 6 indicates whether or not hog cholera virus was detected in blood from Group II pigs when they were killed.

Hog cholera susceptible animals were used in each test for viremia. Animal 23, killed 5 days post inoculation, was assumed to have a viremia since it had a temperature of 107.2° Fahrenheit on the day killed, and viremia of less than 7 days is not expected in any type of hog cholera. The absence of hog cholera virus in blood from pig 27 killed 12 days post inoculation points out the variability of the duration of viremia in non-lethal hog cholera. Hog cholera virus was present in blood from pig 10 killed 15 days post inoculation. There was no indication of viremia in any Group II animal after 15 days post inoculation.

Animals 25, 21, and 16 were challenged on post inoculation day 96 with a virulent hog cholera virus. They were not susceptible. At the same time two Group II controls were challenged with the same virulent strain of hog cholera virus; they were fully susceptible and died within 12 days after challenge. The three immune experimental animals were killed on post inoculation day 108.

Histologic Examination of Brains from Group IV Animals

The 15 pigs in this group were similar to Group II animals and were given the same inoculum by the same route.

Table 6. Viremia status of Group II animals

Animal Number	Days Post Inoculation	Presence of Virus in Blood
23	5	Not determined
22	8	Yes
27	12	No
10	15	Yes
19	19	No
14	20	No
24	21	No
04	22	No
17	23	No
05	26	No
18	29	No
08	32	No
30	36	No
11	45	No
28	58	No
15	74	No
25[a]	106	Not determined
21[a]	106	Not determined
16[a]	106	Not determined

[a]Challenged 8 days prior to death with virulent cholera virus.

The subsequent clinical manifestations also simulated the Group II animals. Significant lesions were not found histopathologically in any tissue from the central nervous system. The results obtained from study of this group of animals conclusively proved residual lesions indicative of hog cholera were not present 45 days post inoculation in 3 month old pigs injected with the selected strain of hog cholera virus.

The methods of fixation used for brains in Group IV animals proved superior to the method used for fixing brains of animals in Groups I and II.

DISCUSSION

Schwarte (42, 43, 44) pioneered studies in non-lethal hog cholera. He demonstrated pigs could carry hog cholera virus for long periods without manifesting clinical sings indicative of hog cholera. He found that pathogenicity of some strains of naturally occurring hog cholera virus could be altered by passage in susceptible pigs. Some viral strains of reduced pathogenicity remained stable throughout animal passages, others increased in pathogenicity until they were considered fully virulent, and still others decreased in pathogenicity until the hog cholera virus could not be detected. This work clearly demonstrated non-apparent hog cholera infected swine were the most important reservoir of virus for subsequent outbreaks.

Hog cholera caused by viruses of low pathogenicity presents a serious threat to the eradication of this disease by interfering with early recognition and effective quarantine procedures. It creates potential foci of virus for additional outbreaks. Presently two-thirds of the hog cholera reported in the United States is due to viral strains of reduced pathogenicity. Previous information indicating the time interval between exposure of swine to a low pathogenic strain of hog cholera virus and development of brain lesions was not found. Studies on duration and resolution of brain lesions in non-lethal hog cholera was not disclosed.

The non-lethal disease produced by the strain of hog cholera virus selected for this study appears representative of the majority of cases occurring throughout this country. The disease produced in the experimental swine suggests that the agent used in this study may serve as a model virus for laboratory investigations of this problem. The agent killed young, highly susceptible animals in Group I, but caused less severe clinical manifestations in older Group II and IV animals which subsequently recovered. This syndrome is similar to field reports in which young animals succumb and older animals recover after manifesting varied clinical signs.

Pyrexia, viremia stage, clinical signs, and lesions indicative of hog cholera were present, but all 3 month or older animals not killed during clinical manifestations recovered from the experimentally produced disease. This provided an opportunity to study the pathogenesis, duration, and resolution of the lesions in the nervous system and to correlate the neuropathologic changes with the viremic state of the animals at selected intervals during recovery.

The results of this study provided several significant findings. The highest fever, most severe depression, and most marked lesions in the central nervous system did not occur at the same time during infection. Pyrexia began about 48 hours post inoculation and persisted approximately 8 days with peaks occurring 5 to 7 days after the initial temperature rise. Clinical manifestations indicated the pigs were most

severely ill between 10 and 12 days post inoculation. Central nervous system lesions diagnostic of hog cholera were found in 3 pigs killed 12 to 19 days post inoculation. This is in contrast to the general concept of classical virulent hog cholera where temperature peaks, symptomatology and brain lesions are expected to develop concurrently 5 to 8 days post inoculation.

Lesions persisted longer in the central nervous system than virus was recoverable from blood. Hog cholera virus was not detected in pig 27 at 12 days post inoculation; however, it was found in animal 10 killed 15 days post inoculation. The duration of viremia in animal 10 (15 days) probably represents an extreme duration since this animal was the only one in Group II with significant fever persisting longer than 13 days after inoculation (Table 3). Pigs killed 12 through 19 days after inoculation had brain lesions diagnostic of hog cholera. Lesions in the brains and choroid plexus from pigs killed 20 to 29 days post inoculation would have been considered suspicious of hog cholera, but they were not sufficient to make a conclusive diagnosis. Classical vascular cuffing was not found.

Okaniwa et al. (31) studying brain lesions produced by virulent hog cholera virus found minimal changes on post inoculation day 1 and widespread encephalitis by day 4. On the other hand, Jones and Doyle (20) found significant brain lesions in 29 out of 52 swine used for commercial

production of fully virulent hog cholera virus. These 52 animals were killed 6 days post inoculation. Dunne (8) reported brain lesions were found in 85 to 95 per cent of field cases of hog cholera. Typical vascular cuffing did not persist indifinitely in this study as suggested by several workers (47, 48). Vascular lesions typical of hog cholera were not found in any Group II animal killed after 22 days post inoculation or in any Group IV animals which were all killed 45 days post inoculation.

Information provided by this study has made diagnostic work in hog cholera easier and more meaningful. With this knowledge it is easier to explain laboratory results when histopathological findings in the central nervous system are not in agreement with virological findings. It is apparent that virus must be present before lesions develop, but lesions also persist longer than virus is recoverable.

Evidence of perivascular and perineuronal edema in the neuroparenchyma and diffuse edema and pial cell proliferations in the stroma of the choroid plexus were the first lesions noted in the brain and choroid plexus. These changes were marked in the pig killed 8 and 12 days post inoculation, but not noteworthy in the pig killed 5 days post inoculation. Severe depression appeared to be associated far more with edema in the central nervous system than vascular cuffing in the neuroparenchyma or cellular lesions in the choroid plexus. Animals necropsied 8 and 12 days after inoculation

were markedly depressed when killed and evidence of edema was widespread throughout the nervous system. Animals killed 15 and 19 days post inoculation appeared normal clinically, evidence of edema was difficult to detect, but vascular cuffing in the neuroparenchyma and lesions in the choroid plexus were as severe as in animals killed previously.

Vascular cuffing extending throughout the vessel walls was the most outstanding lesion in the neuroparenchyma and probably provides the most important single criterion for differentiating hog cholera from other swine encephalitides. Vascular lesions in other viral encephalitides of swine are more or less truly perivascular cuffs since the majority of the cells forming the lesions are found in the perivascular spaces and at least the endothelium of the blood vessel remains intact. Vascular cuffing apparently follows endothelial degeneration and perivascular edema. Hog cholera virus appears to have an affinity for vascular endothelium.

Vascular cuffs typical of hog cholera consist of a heterogenous mixture of cells. Lymphocytes, adventitial cells, microglia and undifferentiated cells are the usual cellular components. Proliferation of adventitial cells and migration of microglia to the site of the lesions probably occur more as a response to the endothelial degeneration and perivascular edema than as a direct response to hog cholera virus (Figure 39). Hyalinization of vessels in the brain has been reported, but this change was not found in this study. This change may

Figure 39. Cells resembling microglia appear to be migrating between the vascular lesion and the neuroparenchyma. Pig 4. (22 days post inoculation). H and E. X250.

be an indication of relatively high viral pathogenicity. As the cuff began undergoing regression about 19 days post inoculation, the cells began disappearing. Some probably went to the blood and others to the neuroparenchyma. Evidence of previous vascular cuffing consisted of mostly oligodendroglia-like cells together with some microglia and pial cells lining the peripheral boundary of perivascular spaces (Figure 24). This alteration was first noted 21 days post inoculation and thereafter occasionally throughout the remaining Group II pigs.

Lesions persist longer in the choroid plexus than in the

neuroparenchyma. Evidence of edema and diffuse proliferation of stromal cells were the first changes detected in the choroid plexus. Later distinct aggregates of lymphocytes and undifferentiated mononuclear cells developed in the stroma, usually near blood vessels. However, these lesions did not typically involve the vascular walls like the vascular lesions in the neuroparenchyma. These focal lesions became more dense as lymphocyte and undifferentiated cells increased in focal areas. With increasing age some of the pial cells appeared to transform into plasma cells. Plasma cells were first detected in this location in the pig killed 12 days post inoculation. Foci of plasma cells were found in pigs killed 12, 20, 23, 26, 29, and 32 days post inoculation. Jubb and Kennedy (22) stated increasing numbers of plasma cells occurred in non-suppurative inflammation in the central nervous system persisting more than one week. However, they do not indicate the origin of the plasma cells. Focal choroid plexus lesions which did not contain plasma cells also persisted through 32 days post inoculation.

Epithelial sloughing and hemorrhage were also noted in the choroid plexus, but these changes are probably not as specifically indicative of hog cholera as the cellular lesions. Post mortem changes and artifacts produced by tissue manipulations may have caused some epithelial sloughing.

Hog cholera virus probably has a specific affinity for the stroma of the choroid plexus which is composed of pial tissues and blood vessels. Maxwell and Pease (27) stated

that pial cells of the choroid plexus were a part of the reticulo-endothelium system and repeated injections of trypan blue in rats over a 10 day period resulted in brilliant staining of pial cells. Choroid plexus changes were detected in all the experimental inoculated animals killed between 8 and 58 days post inoculation except one killed on post inoculation day 36, but lesions were minimal after 29 days post inoculation.

The histologic differences between normal blood vessels in the choroid plexus and the brain may account for some of the differences between the vascular lesions. According to Maxwell and Pease (27) there is no distinct adventitial layer in the walls of the blood vessels of the choroid plexus and large blood sinuses, 50 or more microns in diameter, are surrounded by a very thin fenestrated sheet of endothelial cells. An ultra thin layer of acellular cementing substance is the only continuous structure that separated the blood from the ventricular spaces. There is not enough connective tissue in most of the vascular walls to provide framework for the type of vascular lesions seen in the neuroparenchyma. It is probably easier for toxins or infectious agents to invade the stroma of the choroid plexus than the neuroparenchyma of the brain.

The histologic features of the pial cells forming the stroma of the choroid plexus shed some light on the pathogenesis of the perivascular lesions in the choroid plexus of

hog cholera affected animals. Maxwell and Pease (27) found the pial cells were flattened and possess long extensions of their plasma membranes. These extensions invest the thin walled blood vessels and form concentric layers of laminae around the vessels (Figure 27). These laminae do not present a complete barrier to diffusion between the blood vessels and epithelial cells but form baffels estimated to be 85 per cent complete. As lymphocytes migrate from the blood they appear to get through the vascular wall without difficulty, but they are soon trapped by the concentric laminae. Therefore, the most dense accumulations of cells occurred near blood vessels.

The histologic details of the choroid plexus in the fourth ventricle were often preserved better than in other ventricles when the brains were fixed intact. This portion of the choroid plexus is separated from the fixative only by the anterior and posterior medullary vellum. The choroid plexus is extensive in the fourth ventricle of swine.

Deposits of dark brown pigment granules resembling hemosiderin were found in some of the choroid plexuses (Figure 40). However, Prussian blue staining did not give the light blue color characteristic of hemosiderin. Therefore, it was concluded these deposits were melanin. Pial cells can transform into melanophores capable of elaborating melanin. There was no good explanation why melanin deposits were found in the brain, meninges, and choroid plexus of some animals and not

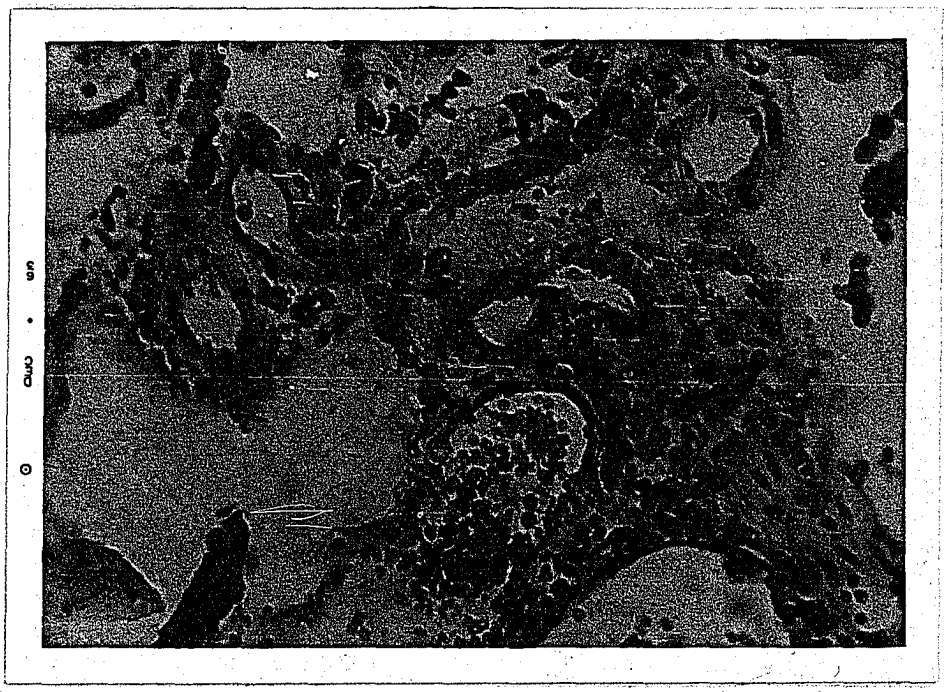


Figure 40. Melanin deposits around blood vessels and in the stroma of the choroid plexus. Pig 14. H and E. X250.

others.

Cells resembling oligodendroglia and microglia were observed as satellites around a few neurons (Figures 22 and 23). Some of the encircled neurons were undergoing degenerative changes characterized by swollen acentric nucleoli, karyorrhexis and increased eosinophilic staining of their cytoplasm. Other normal appearing neurons were encircled in a similar manner. A lesser number of degenerating neurons was seen in control animals. There is no indication that hog cholera virus has any affinity for neurons. The absence of widespread neuronal degeneration is good evidence to differentiate hog cholera from the swine encephalitides

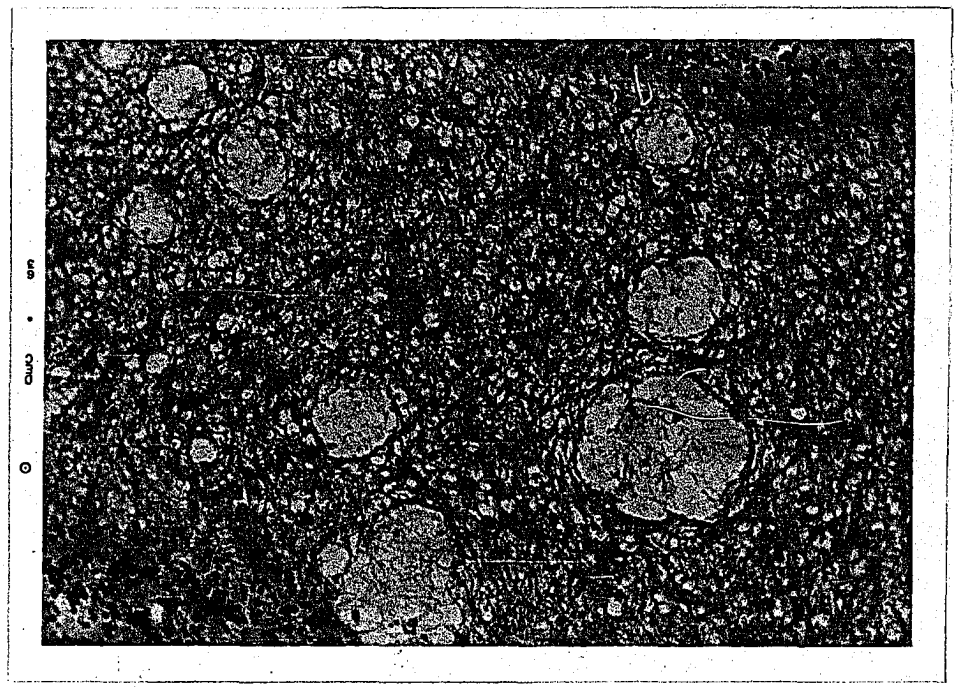
caused by enteroviruses. Vascular lesions interrupting the metabolic interchange between neurons and blood may cause secondary neuronal degeneration in hog cholera. Jubb and Kennedy's (22) explanation for degenerating neurons in normal animals is interesting. They indicate a scattering of degenerate neurons can be found in any brain since more neurons develop in fetal life than are necessary and the superfluous ones degenerate -- the degeneration beginning in fetal life and extending well into the postnatal period. Although neuronal degeneration may occur in hog cholera, this change alone should not be considered indicative of the disease.

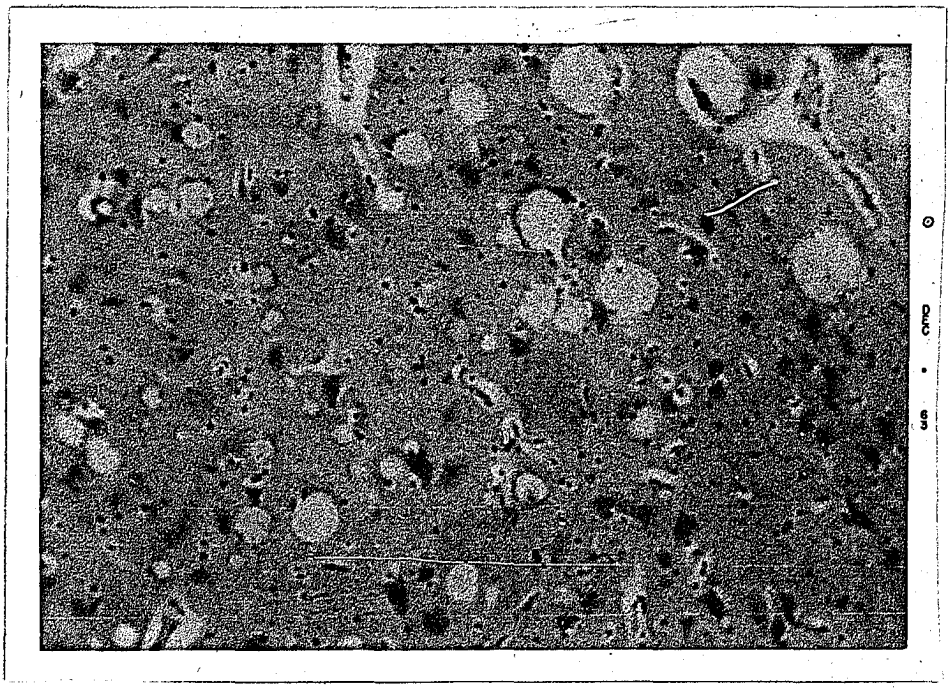
Omar's (34) report indicated cystic spaces in brain tissue were produced by hog cholera virus of reduced pathogenicity (42). Similar changes were not attributed to hog cholera virus during this study. However, the method of fixation used for Group I and II pigs failed to produce complete fixation before some post mortem changes occurred. Figures 41 and 42 are similar to published photomicrographs depicting changes attributed to hog cholera. However, the cystic spaces illustrated in these figures are due to post mortem liquefactive necrosis. Changes of this type were not found in any brains from Group IV pigs. These brains were blocked immediately after removal so more surface area was exposed to fixing fluids.

The examination of more than 1250 sections of tissue from

Figure 41. These cystic spaces are apparently due to post mortem autolysis. Pig 11. (45 days post inoculation). H and E. X125.

Figure 42. The distended perivascular spaces and vacuoles in the neuroparenchyma are likely the results of post mortem autolysis. Pig 30. (36 days post inoculation). X125.





655 different locations of the central nervous system leaves little doubt that examination of frontal sections through the following areas of the brain are sufficient for detection of lesions associated with this disease: (1) anterior medulla, (2) pons and cerebellum, including choroid plexus in the fourth ventricle, (3) midbrain, and (4) thalamus, hippocampus, lateral ventricles, and cerebral cortex.

Additional pathological studies are needed to determine whether or not lesions occur in the central nervous system in hog cholera infected swine which are immunologically tolerant to hog cholera virus. Carbrey et al. (5) reported immune tolerance could develop if dams were infected during gestation. This phenomenon could elucidate additional undetermined sources of virus responsible for hog cholera outbreaks.

SUMMARY

1. Necropsy reports and epidemiological investigations indicate: at least two-thirds of the hog cholera presently occurring in the United States is due to viral strains of reduced pathogenicity.
2. The hog cholera virus selected for this study was lethal for 2 month old pigs in Group I, but all 3 month old pigs in Groups II and IV recovered following severe illness.
3. Sixty-nine swine divided into four groups were used in this study to characterize lesions produced in the central nervous system throughout the course of non-lethal hog cholera infection and to determine the duration of brain lesions and viremia in experimentally infected pigs.
4. In pigs that recovered, pyrexia occurred between 2 and 9 days post inoculation with peaks higher than 107° Fahrenheit between 5 and 7 days after injection. The most severe depression was noted 10 to 12 days after inoculation. However, by 14 days post inoculation the pigs were eating and appeared clinically healthy.
5. More than 1250 sections from 655 locations in the central nervous system were prepared and examined microscopically.
6. Neurological lesions diagnostic of hog cholera were

found in pigs inoculated 12, 15, and 19 days before necropsy, but suspicious lesions were found as early as 8 days and persisted until 22 days post inoculation.

7. The most significant lesions in the neuroparenchyma were vascular cuffing extending throughout the entire thickness of the vessel walls. Perivascular edema and endothelial degeneration preceded cuffing. Cells forming the cuffs were primarily lymphocytes, adventitial cells and microglia.
8. Lesions persisted longer in the central nervous system than virus was recoverable from blood. Virus was detected on the 8th and 15th days but not on the 12th day post inoculation.
9. Neurological lesions were essentially confined to the vascular system and reticulo-endothelial tissue.
10. Severe lesions occurred slightly later in the reticulo-endothelial stroma of the choroid plexus than in the brain but persisted longer. Distinct choroid plexus lesions were found in all 11 pigs killed 8 days through 32 days post inoculation. Edema and pial cell proliferation occurred first in the stroma of the choroid plexus. Later, lymphocytes and undifferentiated mononuclear cells increased and formed aggregates usually near blood vessels. As the lesions regressed, some pial cells appeared to be transformed into plasma cells. However, choroid plexus lesions did not typically involve blood

vessels as did lesions in the neuroparenchyma.

11. Histopathologic examination of transverse sections through the following areas of the brain should be sufficient to detect lesions associated with this type of hog cholera: (1) anterior medulla; (2) pons and cerebellum including choroid plexus in the 4th ventricle; (3) mid brain; and (4) thalamus, hippocampus, lateral ventricles and cerebral cortex.

12. The results of this study indicate that hog cholera virus and neurological lesions should not always be expected to occur simultaneously. Therefore, diagnostic work in hog cholera is easier since differences in virological and histopathological findings can now be logically explained.

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