EXPERIMENTAL LEPTOSPIROSIS: THE EARLY PATHOGENESIS OF *LEPTOSPIRA POMONA* INFECTION IN YOUNG SWINE

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ABSTRACT

An experiment was conducted on the pathogenesis of experimental Leptospira pomona infection in young swine during the 14 day period following infection. Serological responses were followed using the agglutination-lysis test with L. pomona antigen. A comparison was made between pre-infection and post-infection hematological values. Only minimal changes were observed. Clinical manifestations were slight with a transient febrile response being the only recognizable symptom.

Pathological alterations were observed in the kidneys, renal lymph nodes, adrenal glands and brains of the infected animals. Gross kidney lesions consisted of grayish white foci on the cortical surface. The microscopic renal lesions consisted primarily of an intertubular leucocytic infiltration with lymphocytes predominating. The renal lymph nodes were edematous. Small areas of lymphocytic infiltration were observed in the adrenal glands. Meningoencephalitis, characterized by perivascular lymphocytic infiltration, typified the brain lesions. The chronology and severity of the lesions were determined.

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INTRODUCTION

Leptospirosis caused by *Leptospira pomona* is a disease of considerable economic importance and public health significance (31, 36, 46, 49). The role of swine is important not only because of adverse effects in infected animals (6, 7, 9, 14, 20, 37, 43, 44), but also because swine are believed to be the greatest reservoir of the disease for other livestock as well as man (7, 22, 41).

The pathogenesis of *L. pomona* infections in feeder-type hogs was investigated by Morse *et al.* (41), and the pathological alterations were described by Langham *et al.* (30). It was not in the scope of their investigations to study the early pathological manifestations.

Considerable interest was shown by those working on the leptospirosis project at Michigan State University in the chronological development of the early lesions in swine. While renal lesions had been reported as early as 7 days after infection in one animal (42), a controlled experiment on the pathogenesis of the lesions had not been undertaken. The purpose of this experiment was to study this phase of experimental leptospirosis in swine and, in addition, to make further hematological, bacteriological, serological, and clinical observations during the two-week period following infection.

REVIEW OF THE LITERATURE

The initial isolation of L. pomona was made in 1936 from the blood of a dairy farmer living near Pomona, South Queensland, Australia (12). Serological studies of this strain, later referred to as L. pomona (15), differentiated it from the then known serotypes of leptospirae (24, 25, 32). Mochtar (34), in 1940, isolated L. pomona from swine in Java, and Johnson (24) in Australia made porcine isolations in 1942. Gsell (50), in 1944, proved that "swineherds disease", known as a disease of man since 1933, is caused by L. pomona. He isolated the organism from swine as well as man.

The first cultural isolation of L. pomona from swine in the United States was made by Gochenour (21) in 1952, although Monlux et al. (35) had demonstrated the presence of leptospirae in swine kidneys as early as 1948. In 1952, Bohl and Ferguson (5) isolated the organism from porcine urine and Bryan et al. (8) isolated L. pomona from aborted swine fetuses.

Results of serological surveys indicate an incidence of from 3 to 25 per cent among the swine population and a distribution in all geographic sections of the United States (5, 7, 9, 23, 38, 45, 47). The U. S. Department of Agriculture has estimated the loss from leptospirosis in farm animals at greater than 100 million dollars per year (49). While the greatest share of this loss is due to bovine infection, a significant loss occurs in swine, primarily from abortion and the birth of weak or unthrifty pigs (36). The

economic loss in feeder-type hogs is difficult to assess, but probably does not exceed 1 to 5 per cent of the value of an infected drove (36).

Swine are apparently the natural host and reservoir of L. pomona infection for other animals as well as man (7, 22, 41). Morse (41) gives several reasons for this. First, a prolonged period of leptospiremia occurs, during which considerable numbers of leptospirae are present in the blood of essentially asymptomatic swine. Second, leptospirae are very numerous in porcine urine 20 to 30 days after infection, and contamination of feed, bedding, and water makes transmission possible. Third, hogs excrete L. pomona in their urine longer than do other animals.

The ability of swine to transmit L. pomona infection to other hogs, cattle, sheep, and goats has been shown experimentally (10, 39, 43). Human cases of leptospirosis in the United States have been traced to contact with infected swine urine as well as infected tissues (28). The public health aspects of leptospirosis in animals have been reviewed by Larson (31) and Reinhard (46).

Clinical reports on the symptoms noted in naturally occurring outbreaks of leptospirosis in feeder-type hogs vary greatly. Many infections are asymptomatic or so slight as to go unnoticed (7, 47). Bryan (9) reported the following symptoms noted by practitioners: fever, anorexia, hemoglobinuria, icterus, and anemia. Observations reported in Wisconsin included encephalitis, hyperirritability,

incoordination, and pyrexia (37). Bennett (47) has described a case with prominent convulsive and encephalitic symptoms.

The most important and often only symptom of L. pomona infection in sows is abortion or the birth of weak and unthrifty pigs (6, 14, 20, 37, 44). The number of infected sows in which abortion occurs has been reported to range from 20 to 100 per cent (43). Abortion appears to depend on the stage of gestation at time of exposure, occurring mainly during the last three weeks of the gestation period (44). Sows which abort once have not been observed to abort again due to L. pomona infection (42, 44). Bryan (9) reported abnormal milk and reduced milk flow as an occasional symptom in sows.

Workers in this country have found that pigs inoculated experimentally with L. pomona usually fail to develop clinically recognizable symptoms (10, 41, 47).

Morse et al. (41) in studies of the pathogenesis of L. pomona infections in swine found a leptospiremia of 3 to 9 days duration occurring in some animals until the tenth day following inoculation. Leptospiral antibodies were detected as early as day 7 and by day 10 there were strong reactions of 50 per cent agglutination or more at dilutions of 10^{-2} or 10^{-3}. Leptospiremia was not found to be terminated absolutely with the appearance of specific antibody. Morse postulates that this may be due to antibody neutralization due to in vivo lysis of large numbers of leptospirae.

Maximum serum antibody response of 10^{-7} to 10^{-8} usually

occurred 3 to 4 weeks following inoculation and diminished to 10^{-2} to 10^{-5} by postexposure month 4 or 5. Titers were found to persist as long as one year.

Urinary antibody levels increased gradually to a maximum of 10^{-3} at three to four months. Leptospirae were not found in urine with antibody levels higher than 10^{-1} or with a reaction lower than pH 6.3. The greatest number of leptospirae were excreted in the urine during days 20 to 30 and the maximum duration of the renal carrier state was found to be 122 days.

Burnstein and Baker (10) infected swine subcutaneously, intranasally, and by contact exposure. They observed pyrexia of 24 to 36 hours' duration 7 to 13 days following inoculation. Leptospiruria was observed as early as day 12 and as long as until day 159. The specific gravity of the urine was normal (1.025 - 1.040) until 3 weeks following inoculation at which time it became lower, usually less than 1.010 and remained low for as long as 5 months. Albumin was found in the urine concurrently with the lowering of the specific gravity.

Hematological studies made during the first two weeks of infection have revealed normal values with few exceptions (40, 47). Morse et al. (40) observed anisocytosis and reticulocytosis in 2 pigs and a decrease in erythrocytes and hemoglobin in another.

Langham et al. (36) found that the two most important macroscopic changes in L. pomona infected pigs were scattered to numerous grayish white foci in the kidneys, extending in

some cases through the cortex into the medulla, and edema of the renal lymph nodes. Burnstein and Baker (10) described similar kidney lesions which appeared no earlier than three weeks after pyrexia and progressively increased in number thereafter. As the duration of the infection progressed the kidneys appeared shrunken, with small bands of fibrous tissue extending into the parenchyma.

In pigs killed during pyrexia 7 to 13 days following exposure, Burnstein and Baker (10) observed microscopically an occasional focal area of "mononuclear" infiltration of the interstitial tissue of the kidney and areas of degeneration in the liver.

Upon microscopic examination, the grayish white foci in the kidneys have been found to consist of an intertubular and perivascular infiltration of numerous lymphocytes, plasma cells and some macrophages (10, 30). Langham et al. (30) observed varying degrees of degeneration and necrosis in the proximal and distal convoluted tubules in the inflammatory areas. An intertubular proliferation of fibroblasts and increase of collagenous fibers, although not extensive, was also noted. Minor changes were occasionally observed in the renal corpuscles. The pigs in Langham's experiment were killed 35 to 214 days after exposure.

Morter, Morse, and Langham (42) have found that the active inflammatory processes in the porcine kidney persist for as long as 10 to 14 months after initial infection and for 8 to 10 months after leptospirae can be demonstrated in

the urine. They also observed grossly a few grayish white foci and, upon microscopic examination, small areas of lymphocytic infiltration in kidneys of two swine killed 7 and 13 days after inoculation.

MATERIALS AND METHODS

Thirty, apparently healthy, crossbred pigs, 3 months of age, were used as experimental animals. By random number assignment, 24 of the pigs were divided into three groups of 8, and 6 were selected to serve as controls, each group being assigned separate pens. The killing date for each animal was determined by random numbers before inoculation. Two infected pigs were killed daily starting on post-infection day three. A control pig was killed on alternate days.

All serological studies were performed by the modified microscopic agglutination-lysis test (18, 40, 41) using L. pomona (strain Johnson) live antigen. Two pre-infection serum samples per pig were obtained at 21 day intervals. No pre-infection antibody reactions were demonstrated.

The animals were fed free choice a standard starter ration without antibiotics. Water was provided ad libitum. No significant level of intestinal parasites was found on examination of representative fecal samples.

To establish normal hemoglobin, packed cell volume, erythrocyte, leucocyte, differential leucocyte (13), blood non-protein nitrogen (13, 26, 27, 48), and blood creatinine levels (13, 19, 20, 48), at least two blood samples were taken from each pig before inoculation.

Two pre-inoculation rectal temperatures were taken on each pig. Post-inoculation temperatures were recorded daily.

The 24 pigs to be infected were inoculated with 2 ml. of L. pomona (strain Ohio) infected cavian blood (40). Titration using ten-fold serial dilutions and guinea pig inoculation established the presence of at least 10^4 organisms per ml. (40). The strain of L. pomona used was isolated from porcine urine by workers at the Ohio Agricultural Experiment Station, Wooster, Ohio, approximately two years ago and has been maintained in our laboratory by continual hamster or guinea-pig passage. The six control animals were inoculated with 2 ml. of non-infected cavian blood each.

All animals were given sodium pentobarbital intravenously to effect before necropsies were performed.

Five consecutive daily blood samples for hematological studies were obtained from each infected pig killed after and including post-infection day 6, the fifth bleeding being made on the day of necropsy. As bleedings were started on post-infection day 2, those pigs killed on post-infection days 3, 4, and 5 were bled 2, 3, and 4 times respectively. Control animals were bled terminally.

Modified microscopic agglutination-lysis tests for L. pomona antibodies were made on the terminal blood samples of all pigs killed up to and including post-infection day 6. On days 7 and 8, the ten samples taken for hematological studies were tested, and on day 9 and each day thereafter all remaining pigs were tested daily. Ten-fold serial dilutions were used to determine the end point of the reaction.

On post-infection days 4 through 7, approximately

0.1 ml. of blood from each pig was inoculated into each of 3 tubes of modified Chang's medium (11, 37). The cultures were incubated at 30°C. and examined for the presence of leptospirae by dark field microscopy at 14 and 28 days.

Terminal urine samples were obtained from 17 pigs and were clinically tested for albumin, occult blood, pH (4), specific gravity, and urine sediment (13).

Tissues were saved from the kidneys, liver, spleen, renal lymph nodes, heart, adrenals, skeletal muscle, brain, spinal cord, and pituitary gland of each pig. Immediately after necropsy all tissues were placed in one or more of the following fixatives: Zenker's fluid, 10 per cent neutral formal-saline solution, and Carnoy's fluid (32).

The following staining procedures were used: hematoxylin and eosin, Sudan IV for fat, Best's carmine stain for glycogen, and Steiner and Steiner's method for spirochetes (33).

Samples of both kidneys, liver, spleen and brain were obtained for guinea pig inoculation from each pig killed through post-infection day 8. Tissues were homogenized in 0.85 per cent sterile sodium chloride solution to give approximately a ten per cent tissue suspension (40). Three weanling guinea pigs were inoculated with 2 ml. kidney suspension, two with 2 ml. liver and spleen suspension, and two with 2 ml. of brain suspension for each pig killed. On post-infection day 9 and thereafter, kidney suspensions only were used and three guinea pigs were inoculated per

pig. Two guinea pigs were inoculated with kidney suspension from each control animal. The original inoculum was considered to have contained leptospirae if the serum of the guinea pigs contained antibody for L. pomona at a dilution of 10^{-2} or higher three to four weeks after inoculation (40, 41).

EXPERIMENTAL RESULTS

The only significant clinical symptom attributable to L. pomona infection was a transient febrile response. The average daily temperature recordings are summarized in Table 1. Individuals showing febrile response and the post-infection day on which the response occurred are given in Table 2. Sixteen infected animals had temperatures of 104.0°F. or higher (Table 2) during the observation period with the highest reading being 105.6°F.

No blood or blood pigments were visible in the urine. However, 8 of 17 urine specimens examined were positive to the occult blood test. Clinical examinations for urine albumin, pH, and sediment gave essentially normal results.

Hematological data obtained during the course of the experiment is summarized in Table 3. Average normal values for the 6 control animals and pre-infection determinations for the 24 infected pigs are given. Daily post-infection averages for the pigs bled each day together with a comparison of normal values for these animals are also listed in Table 3.

The average erythrocyte, packed cell volume, and blood non-protein nitrogen values did not significantly change after the animals were infected. Average hemoglobin determinations did not vary more than 0.6 gms. per 100 ml. except on day 10 when there was a fall of 1.15 gms. per 100 ml. from normal values.

Leucocyte counts were subject to considerable individual variation. However, the average values indicated an initial slight leucocytosis of approximately 3,000 leucocytes per cu. mm. until day 4. Daily averages fell progressively until day 7 when a slight leucopenia of about 2400 leucocytes per cu. mm. below normal values was evident. The leucopenia was followed by a second rise until a slight leucocytosis again occurred by day 9 and lasted until day 14. One animal (3-7) showed a consistent marked leucocytosis on all 5 days that values were determined. As this animal was the only one showing this extremely elevated leucocyte count, leucocyte averages excluding 3-7 are given in Table 3.

Blood creatinine levels did not vary markedly after infection except for 2 animals on day 7 which had levels of 2.5 mg. per 100 ml.

Results of differential leucocyte counts are given in Table 4. In 5 animals a post-infection decrease in heterophils with a corresponding increase in lymphocytes occurred (Table 4).

Significant serum titers appeared first on day 8. The maximal agglutination-lysis reactions that occurred were present in serum dilutions ranging from 10^{-3} to 10^{-8} with L. pomona antigen. Table 5 summarizes the post-infection agglutination-lysis test results. Terminal blood samples from the 6 control animals were negative.

Leptospira were demonstrated in tissues of the infected swine by inoculation of guinea pigs with homogenized

tissues (Table 6). These findings indicate that L. pomona was present in the kidneys of the infected swine from day 4 until the experiment was concluded, in the liver and/or spleen at least from day 4 until day 8, and in the brain tissue from day 6 at least until day 8. Serum from guinea pigs inoculated with homogenized kidney tissue from the control animals was negative indicating that L. pomona was not present in the non-infected swine.

Macroscopic lesions were observed in the kidneys of all infected pigs killed on day 7 and thereafter. The kidney lesions consisted of grayish white areas or foci located principally in the cortex and occasionally in the medulla. The size of the lesions varied from the limit of visibility to approximately 5 mm. in diameter (Fig. 1). An indication of the individual variation in the severity and extent of the gross lesions is given in Table 7. The first kidney lesions were slight, with the edges of the affected areas being poorly defined and the lesion itself being slightly raised and swollen. However, by day 9, marked gross kidney lesions were observed in pig 4-1 which had more than 50 per cent of the cortical surface affected. Moderate to marked lesions occurred in 8 of 12 infected pigs killed during the last 6 days of the experiment (Table 7).

Edematous and enlarged renal lymph nodes were a consistent finding on day 9 and thereafter (Fig. 1).

There were no other gross lesions observed which could be attributed to infection with L. pomona, nor were any

significant gross lesions observed in the control animals (Fig. 2).

In Table 7, an indication of the chronological appearance and extent of the microscopic lesions is given. The first renal lesions were observed in the cortex of pig 3-4 killed on day 4 (Figs. 3 and 4). The majority of these early lesions were rather discrete with infiltrations of small numbers of lymphocytes between the tubules (Fig. 3). An occasional lesion was rather diffuse with intertubular infiltrations of heterophils, some plasma cells, and numerous lymphocytes (Fig. 4). Similar lesions to those described above with marked individual variations were observed in kidney sections from all animals killed on day 5 to day 14. Figure 5 shows an extensive intertubular infiltration of leukocytes in the kidney of animal 4-2 killed on day 8. Marked leucocytic accumulations were found in the medullary portion of the kidney of pig 4-1 killed on day 9 (Fig. 6). Occasionally, the infiltrations would surround the renal corpuscle (Fig. 7). A few degenerative changes were observed. An occasional tubule had pyknotic nuclei (Fig. 8). Hyaline casts were found in a few tubules (Fig. 9) and hydropic degeneration of some proximal convoluted tubules was evident (Fig. 9). A slight increase in collagenous fibers was observed in a few areas in the kidney of pig 4-6 killed on day 14 (Fig. 10).

Areas of lymphocytic infiltration, not as extensive as the renal lesions, were observed in sections from the adrenal glands of 6 animals (Fig. 11).

Microscopic examination of the renal lymph nodes verified the grossly observed edematous condition (Fig. 12).

Brain lesions, located principally in the cerebrum, consisted of meningoencephalitis (Figs. 13 & 14) characterized by perivascular infiltration and hemorrhage (Figs. 15, 16 and 17) and microencephalomalacia (Fig. 18). Lymphocytes were the predominant inflammatory cell present. The brain lesions, though not extensive, were present in all animals killed after day 11 (Table 7). No lesions were observed in brain sections of any of the non-infected control animals.

Upon examination of kidney sections taken from the control animals, an accumulation of lymphocytes in the medullary portion, in close proximity to the hilus, was observed in 5 of 6 animals. These infiltrations were not seen in the cortical portion and the lymphocytes were less concentrated than similar lesions in the region of the hiluses of the infected animals.

No leptospirae could be demonstrated in the Steiner and Steiner stained sections.

Microscopic lesions were not observed in the liver, spleen, heart, sketetal muscle, or spinal cord of either the infected or control animals.

DISCUSSION

Experimental L. pomona infection in this group of young swine was a very mild clinical infection with a slight febrile reaction being the only recognizable symptom. This concurs with previous experimental cases of L. pomona infection in swine reported in the United States (10, 41, 47). While naturally occurring infections in swine apparently may produce more severe symptoms such as marked anorexia, hemoglobinuria, icterus, anemia, incoordination and convulsions, such outbreaks are typically asymptomatic (7, 9, 36, 46). The diversity in reported symptoms may be due to variations in the virulence of the infecting organism or its serotype, host susceptibility, and the type of management practices. Factors which could be expected to increase host susceptibility were noticeably absent in this experimental group of animals. The animals were fed a balanced ration, water ad libitum, and housed in a heated building. No apparent parasitism or concurrent bacterial or viral infections were present. While the animals were readily infected, good husbandry practices may have been important in the mildness of the clinical infection.

The febrile period in experimental L. pomona infections in swine has been observed to occur from approximately the 4th to the 9th day after infection (16, 41). While the temperatures of the infected pigs in this experiment were not markedly elevated, the increases that were recorded generally did occurduring this period (Table 2).

The slight leucopenia may be related to the concurrent pyrexia and leptospiremia. However, Ferguson and Powers (16) observed a mild leucocytosis during pyrexia in a group of experimentally infected gilts. Morse et al. (41) observed no significant alterations in the leucocyte counts. While variations from normal values may occur during the febrile period, these changes are so subject to group and individual variation that they are of little diagnostic value.

Terminal samples from 5 of the last 14 pigs killed showed an absolute and relative decrease in heterophils and an increase in lymphocytes. There did not appear to be any significant alterations in the differential leucocyte counts in those animals killed during the period considered to be the pyrexic phase with but two exceptions (4-1 and 4-2). This contrasts with the findings of Ferguson and Powers (16) in that changes they observed were mainly an absolute increase in heterophils during pyrexia. It would seem, in view of present knowledge, that unlike many infectious diseases, L. pomona infection in swine produces no predictable alteration in the differential leucocyte determinations.

The absence of marked alterations in the erythrocyte, the packed cell volume, and the hemoglobin values may be due to the relative resistance of porcine erythrocytes to the hemolytic effects of L. pomona (1, 2, 3). Experimental work with hemolysins, which have been concentrated from the supernates of broth cultures of L. pomona, indicate that the ovine and bovine erythrocytes are highly susceptible to

the hemolysins while the pig erythrocytes are relatively resistant (2). Hemoglobinuria, icterus, and anemia are common findings in experimental and natural ovine and bovine L. pomona infections (2, 17, 39). Such manifestations are rarely observed in swine. The infrequent occurrence and mild nature of the hemolytic features undoubtedly are among the factors which contribute to the usual asymptomatic course of L. pomona infection in immature swine.

There was some evidence that a slight degree of hemolysis was taking place in the infected pigs. Urine samples were positive for occult blood in 8 instances, and on day 10 hemoglobin values were depressed 1.15 gms. per 100 mls. from normal determinations. It is possible that at the period of the marked increase in antibody titers, which occurred from days 8 to 10, destruction of the leptospiral organisms liberated sufficient hemolysins to slightly alter the hemoglobin values (17).

The antibody responses of the infected pigs were similar to those in the experiment of Morse et al. Titers rose rapidly after day 7 to levels of 10^{-8} in some cases by day 11. The period of leptospiremia was not established in this experiment due to the contamination of media when making blood inoculations. It cannot be definitely stated that leptospiremia terminated with the appearance of antibody. However, the organisms were still present in liver, spleen, and brain tissue of pigs killed on day 8 which indicates that viable organisms were present in these organs after the

appearance of circulating antibody. Unfortunately, due to a lack of guinea pigs, it could not be determined at what point the organisms were no longer present in liver, spleen, and brain and only present in the kidney.

The characteristic grayish white foci in the kidney, which were observed as early as day 7, have been described by many workers (10, 30, 41, 47). The significant features of the gross renal lesions were the early appearance and relative severity. Burnstein and Baker (10) observed no gross lesions until approximately 3 weeks after pyrexia. Langham and Morter (42) observed small lesions in each of two pigs killed 7 and 13 days after infection. All of the infected animals in this experiment showed gross renal lesions on day 7 through day 14 and 8 pigs showed moderate to marked lesions. The early appearance of renal lesions in contrast to the report of Burnstein and Baker, may have been due to a strain difference in the infective organism. The lack of early marked lesions in Langham's and Morter's work was probably due to relatively few animals being observed prior to day 14.

Even though the gross renal lesions were extensive, kidney function was evidently normal. Total blood non-protein nitrogen values and blood creatinine levels were in the normal range and albuminuria was not evident. The microscopic appearance of the kidney sections helped explain this lack of kidney malfunction. There were very few degenerative changes that could be observed. Occasional

pyknosis and a slight degree of hydropic degeneration were the only degenerative changes seen, even in areas of extensive infiltration.

The termination of this experiment at 14 days did not enable the period of initial kidney malfunction to be determined, but Burnstein and Baker (10) previously observed that disturbances in renal function apparently begin to take place about 20 days after infection.

The edematous renal lymph nodes, previously described by Langham et al. (30), appeared nearly concurrently with the gross renal lesions. This edema apparently may persist for long periods of time as Langham observed extensive edema in the lymph node of one pig killed 146 days after exposure. The edema in the renal lymph node is likely a response to the inflammatory reaction in the kidney and may aid in the localization of the organism.

The appearance of microscopic lesions in the kidney on day 4 indicates that lesions develop very early,during the leptospiremic and pyrexic phase of the disease. While a small portion of the kidney was affected in the earliest lesions, by days 8 and 9 marked infiltrations of leucocytes, predominantly lymphocytes, were present (Figs. 5 & 6). This lymphocytic reaction during the acute phase of the disease and the persistence of this reaction during the chronic stage of leptospirosis (30, 42) may be due to a distinctive antigen-antibody relationship, the precise characteristics of which have not yet been determined. It is hoped that

fluorescent antibody techniques will help clarify this correlation between leptospira infection and lymphocytic infiltration.

The lesions observed in the adrenal glands were very discrete and probably would cause very little functional disturbance in this organ.

The brain lesions consisting of meningoencephalitis and characterized by perivascular infiltration and hemorrhage were similar to the lesions described by Morse et al. (39) in ovine leptospirosis and by Koppisch and Bond (29) in human cases. These porcine lesions were observed primarily in the cerebrum while the human and ovine lesions were seen throughout the brain.

Nervous symptoms have been described in cases of leptospirosis in swine and this fact, together with the positive animal inoculations from brain tissue, made logical the finding of these brain lesions. While the lesions in this group of pigs were not extensive, it is not difficult to visualize that with a multiplicity of the lesions observed nervous manifestations could appear.

Lesions have been described in the liver of L. pomona infected swine by Burnstein and Baker (10). The lesions consisted of "mononuclear" infiltration with areas of degeneration. Similar lesions were not seen in this group of pigs. It is probably that, although the liver may be slightly affected in some cases, the damage is usually not severe in L. pomona infection in swine.

Carditis, splenitis, and myositis have been described

in human cases of leptospirosis (29) but lesions in these areas were not observed in this experiment.

The inability to observe leptospirae in specially stained tissue sections emphasizes the need for improved techniques in this field. While the use of serological methods using laboratory animals is relatively satisfactory in the verification of infection, much more information could be obtained about the host-parasite relationship if improved methods for observation of the leptospirae in tissues could be developed. Fluorescent antibody techniques offer considerable promise and future research is being formulated along this line.

SUMMARY

A controlled experiment using 30 pigs was conducted to study the pathogenesis of L. pomona infection in young swine during the 14 day period following inoculation with live organisms.

Demonstrable microscopic renal lesions were present by day 4. These early lesions consisted primarily of an intertubular leucocytic infiltration with lymphocytes the predominant inflammatory cell. Only minor degenerative changes were observed, and kidney function was apparently normal.

Gross kidney lesions, consisting of grayish white foci on the cortical surface, appeared on day 7 and were extensive by day 9.

Microscopic brain lesions were present by day 11. The meningoencephalitis was characterized by perivascular lymphocytic infiltrations in the cerebrum.

Hematological values were only slightly altered from normal determinations. Serological and clinical findings substantiated previous experimental evidence.

Swine appear clinically to adapt quite readily to L. pomona infection and this further enhances their ability to serve as carrier animals.

TABLE I

AVERAGE DAILY TEMPERATURES*

	Controls	Infected pigs
Pre-infection	102.7	102.8
Day 1	103.3	103.2
Day 2	103.1	103.2
Day 3	103.3	103.2
Day 4	103.2	103.6
Day 5	103.0	103.5
Day 6	103.0	103.6
Day 7	102.8	103.4
Day 8	103.2	103.6
Day 9	103.5	103.9
Day 10	103.1	102.9
Day 11	102.8	103.2

*Temperatures are expressed in degrees Fahrenheit.

TABLE 2

PERIOD OF HIGHEST TEMPERATURE RESPONSE

Pig Number	Highest Temperature*	Day Recorded
2-4	103.9	4
1-1	103.5	3
3-6	104.2	5
1-6	103.9	6
3-8	104.3	4
2-3	103.7	4
4-2	104.4	5
1-9	103.7	1
**4-1	105.6	7
3-7	105.5	8
1 no ear	104.4	5 & 8
2-9	104.1	6
3-3	104.6	2
4-4	104.8	7
1-7	104.6	9
4-5	104.7	9
1-8	104.3	6
3-2	104.4	7
1-5	104.4	2
4-6	105.0	9

*Temperatures are expressed in degrees Fahrenheit.
**Highest temperature recorded.

KEY

Hgb. = hemoglobin in grams per 100ml. blood

Hct. = packed cell volume expressed as per cent.

Rbc. = erythrocytes in millions per cmm.

Wbc. = leucocytes per cmm.

Npn. = non-protein nitrogen in mg. per 100 ml. blood

Creat. = creatinine in mg. per 100 ml. blood

TABLE 3

SUMMARY OF HEMATOLOGICAL DATA

AVERAGE VALUES

		Hgb	Hct	Rbc	Wbc	Npn	Creat.
Controls	Pre. Inoc.	11.78	39.25	6.57	20200	42.3	1.39
	Post Inoc.	12.23	39.58	7.12	20066	39.25	1.44
Infected Pigs	Pre. Infection Normals	11.20	37.19	6.57	17944	40.86	1.36
Day 2	Pre. Inf.	11.27	37.10	6.49	18105	38.45	1.40
	Post Inf.	11.07	38.20	6.72	2100	40.60	1.42
Day 3	Pre. Inf.	11.27	37.10	6.49	18105	38.45	1.40
	Post Inf.	11.60	36.20	6.86	21320	37.90	1.19
Day 4	Pre. Inf.	11.25	37.75	6.48	18235	39.65	1.43
	Post Inf.	10.92	37.30	6.42	21060	38.90	1.05
Day 5	Pre. Inf.	11.15	37.75	6.40	*18250	37.83	1.37
	Post Inf.	11.80	36.60	6.64	*17850	39.30	1.29
Day 6	Pre. Inf.	11.16	37.90	6.54	*18245	41.47	1.35
	Post Inf.	10.46	36.20	6.52	*17140	43.40	1.46
Day 7	Pre. Inf.	11.00	37.45	6.48	*17595	42.77	1.35
	Post. Inf.	11.07	37.10	6.43	*15150	43.96	1.67
Day 8	Pre. Inf.	11.00	37.40	6.48	*17915	42.37	1.34
	Post Inf.	11.34	38.00	None	*17180	43.22	1.35
Day 9	Pre. Inf.	10.96	37.35	6.63	*17445	41.70	1.37
	Post Inf.	10.90	36.50	6.34	*18580	37.28	1.58
Day 10	Pre. Inf.	11.16	36.90	6.74	17290	42.00	1.33
	Post Inf.	10.01	36.00	6.83	18930	38.56	1.33
Day 11	Pre. Inf.	11.12	37.75	6.63	17140	42.10	1.36
	Post Inf.	10.71	35.85	6.40	21420	36.86	1.35
Day 12	Pre. Inf.	11.30	37.90	6.79	18300	41.90	1.32
	Post Inf.	11.71	35.08	6.35	20850	44.90	1.42
Days 13 & 14	Pre. Inf.	11.67	38.25	6.87	17933	43.25	1.29
	Post. Inf.	11.67	36.33	6.90	22160	40.67	1.32

*Indicates that values for pig 3-7 were excluded.

TABLE 4

DIFFERENTIAL LEUCOCYTE COUNTS

Pig number	Day of count	E	B	S	L	M
4-7	Pre-inf.**	2	5	39	62	1
	4***	0	16	21	54	9
3-4	Pre-inf.	1	11	19	55	4
	5	3	17	32	44	4
1-1	Pre-inf.	4	6	24	60	6
	5	0	7	26	63	4
3-6	Pre-inf.	1	14	18	62	5
	6	1	10	20	63	6
1-6	Pre-inf.	1	4	33	57	5
	6	1	8	32	56	3
3-8	Pre-inf.	2	5	21	68	4
	7	2	3	18	73	4
2-3	Pre-inf.	3	6	26	61	4
	7	3	3	21	67	6
*4-2	Pre-inf.	2	3	36	54	5
	8	4	2	22	69	3
1-9	Pre-inf.	0	4	34	59	3
	8	5	4	25	60	6
*4-1	Pre-inf.	3	9	29	51	8
	9	3	5	7	80	5
3-7	Pre-inf.	3	11	20	60	6
	9	6	19	17	55	3
1 no ear	Pre-inf.	1	2	21	73	3
	10	3	3	17	71	6
2-9	Pre-inf.	1	5	19	69	6
	10	2	5	16	74	3
*3-3	Pre-inf.	2	7	46	43	2
	11	3	6	22	66	3

4-4	Pre-inf.	2	9	19	65	5
	11	3	4	19	72	2
1-7	Pre-inf.	1	4	23	67	5
	12	4	1	22	73	0
*4-5	Pre-inf.	1	4	23	67	5
	12	6	3	14	72	5
1-8	Pre-inf.	1	4	25	62	8
	13	3	3	22	66	6
3-2	Pre-inf.	0	6	27	61	6
	13	6	3	20	69	2
4-6	Pre-inf.	3	3	16	70	8
	14	3	9	17	71	0
*1-5	Pre-inf.	1	4	29	64	2
	14	3	3	15	76	3

**Average of counts made before animal considered to be significant.
***Refers to the day after infection that counts were made.

E = eosinophils

B = immature heterophils

S = segemnted heterophils

L = lymphocytes

M = monocytes

TABLE 5

ANTIBODY TITERS* FOR L. POMONA IN SERA OF INFECTED PIGS

Pig Number	Days after infection									
	5	6	7	8	9	10	11	12	13	14
1-1	± (Terminal)									
1-6		± (Terminal)								
3-7			-	-	2					
4-1			-	-	2					
2-9			-	-	-	3				
1 no ear			-	1	1	3				
4-4				-	1	6	7			
3-3				-	1	3	4	6		
4-5				-	2	4	8	8		
1-7				2	3	6	8	6		
1-8					-	1	3	4	6	
3-2					1	4	6	8	6	
4-6					2	5	6	8	8	7
1-5					-	1	3	5	8	7

*The titers are expressed as the negative exponents of the highest serum dilutions showing 50 per cent agglutination-lysis.

± Indicates 25 per cent agglutination-lysis in the 10^{-1} serum dilution.

TABLE 6

SUMMARY OF GUINEA PIG INOCULATIONS WITH HOMOGENIZED TISSUES FROM INFECTED PIGS

Pig No.	Day after Infection	Brain	Liver and Spleen	Kidney
1-4	3	-	-	-
2-5	3	-	-	-
4-7	4	-	+	+
3-4	4	-	+	+
2-4	5	-	+	+
1-1	5	-	+	+
3-6	6	+	+	+
1-6	6	+	+	+
3-8	7	+	+	+
2-3	7	+	+	+
4-2	8	+	+	+
1-9	8	+	+	+
4-1	9	Not Attempted		+
3-7	9	"	"	+
1 no ear	10	"	"	+
2-9	10	"	"	+
4-4	11	"	"	+
3-3	11	"	"	+
1-7	12	"	"	+
4-5	12	"	"	+
1-8	13	"	"	+
3-2	13	"	"	+
1-5	14	"	"	+
4-6	14	"	"	+

+ indicates that the serum of the guinea pigs contained antibody for L. pomona at a dilution of 10^{-2} or higher three to four weeks after inoculation with the respective tissues of the infected pigs.

- indicates serum of guinea pig was negative.

TABLE 7

PATHOLOGICAL CHANGES IN L. POMONA INFECTED SWINE

Fig No.	Days after infection	Gross Kidney Lesions	Leucocytic Infiltration in Kidney	Tubular Casts	Pyknosis in Nuclei of Tubular Cells	Increased Collagenous Fibers in Kidney	Edema of Renal Lymph Nodes	Brain Lesions	Adrenal Lesions
1-4	3	-	-	No	No	No	No	NO	No
2-5	3	-	-	No	No	No	No	No	No
4-7	4	-	-	No	No	No	No	No	No
3-4	4	-	+	No	No	No	No	No	No
2-4	5	-	+	No	No	No	No	No	No
1-1	5	-	+	No	No	No	No	No	No
3-6	6	-	+	No	No	No	No	No	No
1-6	6	-	+	No	No	No	No	No	No
3-8	7	+	++	No	No	No	No	No	No
2-3	7	+	++	No	No	No	Yes	No	No
4-2	8	+	+	No	No	No	No	No	No
1-9	8	+	+++	No	No	No	Yes	No	No
4-1	9	+++	+++	Yes	Yes	No	Yes	No	No
3-7	9	+	+++	Yes	Yes	No	Yes	No	No
1 no ear	10	+++	++	No	No	No	Yes	No	No
2-9	10	++	++	No	Yes	No	Yes	No	Yes
3-3	11	+++	++	No	No	No	Yes	PI&M	Yes
4-4	11	+	+	No	No	No	Yes	PI,PH,&M	No
1-7	12	+++	+	No	No	No	Yes	PI&M	Yes
4-5	12	+++	++	No	No	No	Yes	PI	Yes
1-8	13	+	+	No	No	No	Yes	PI&M	Yes
3-2	13	+++	+++	No	No	Yes	Yes	M	Yes
1-5	14	++	+++	No	No	Yes	Yes	M	No
4-6	14	+	+++	Yes	No	No	Yes	PI&M	No

\+ = Slight
++ = Moderate
+++ = Extensive

M = Meningitis
PI = Perivascular infiltration
PH = Perivascular hemorrhage

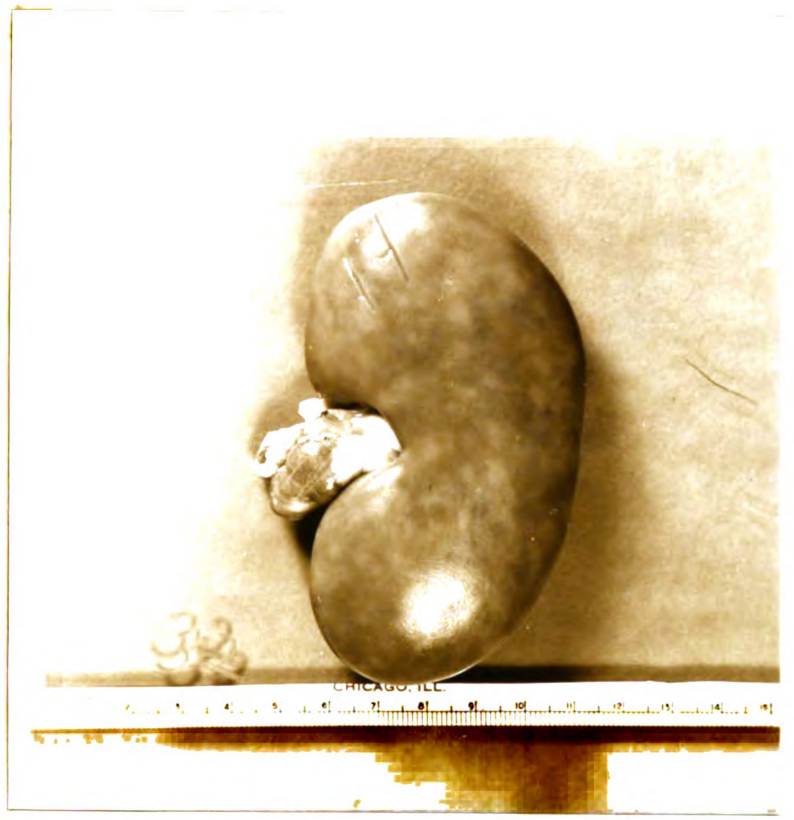

FIGURE 1. Kidney of pig 13 days after infection with L. pomona. x$\frac{1}{2}$.

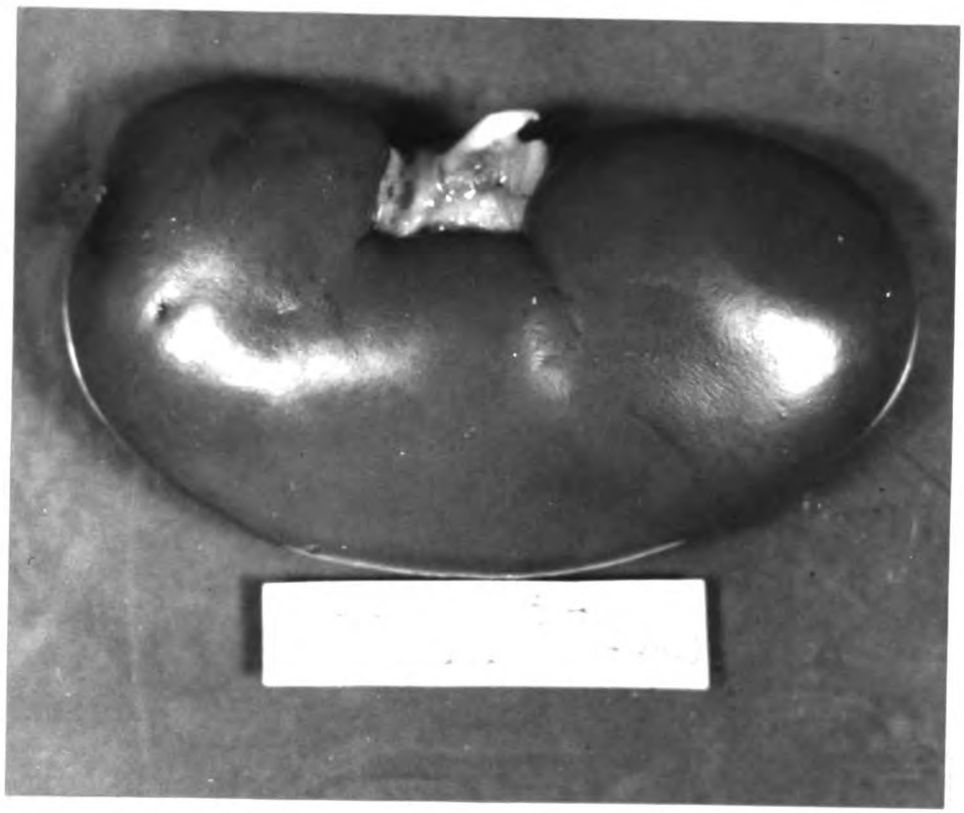

FIGURE 2. Kidney of normal control. x1$\frac{1}{4}$.

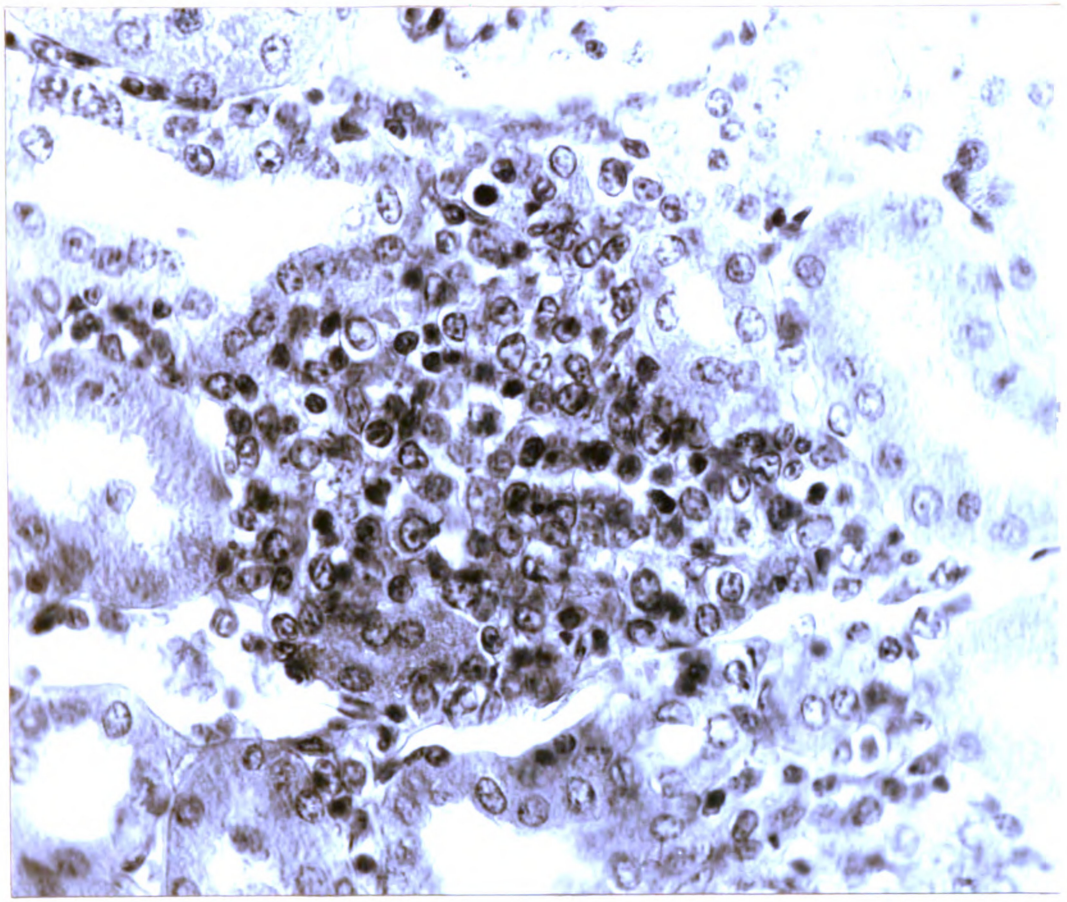

FIGURE 3. Section through cortex of kidney at day 4 to show early infiltration of lymphocytes between tubules. x600.

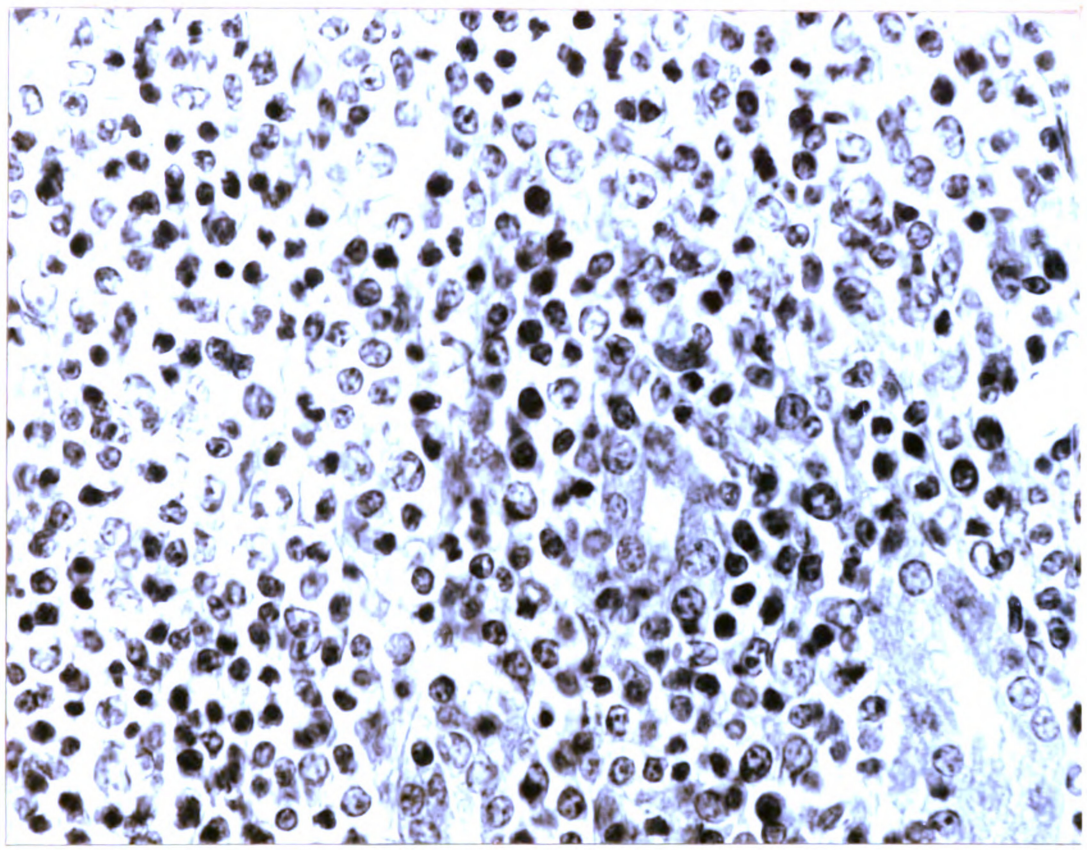

FIGURE 4. Same kidney as Figure 3 showing a more diffuse infiltration with heterophils, some plasma cells, and numerous lymphocytes. x600.

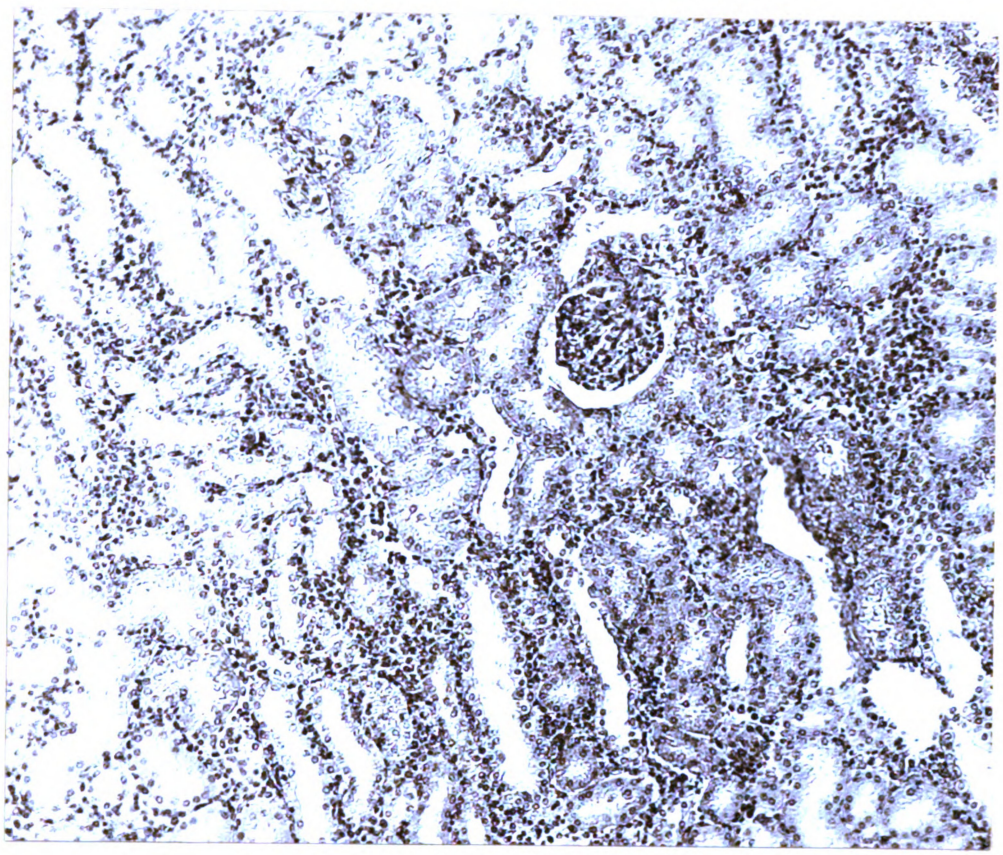

FIGURE 5. Kidney at day 8 demonstrating an extensive intertubular infiltration of leukocytes. x98.

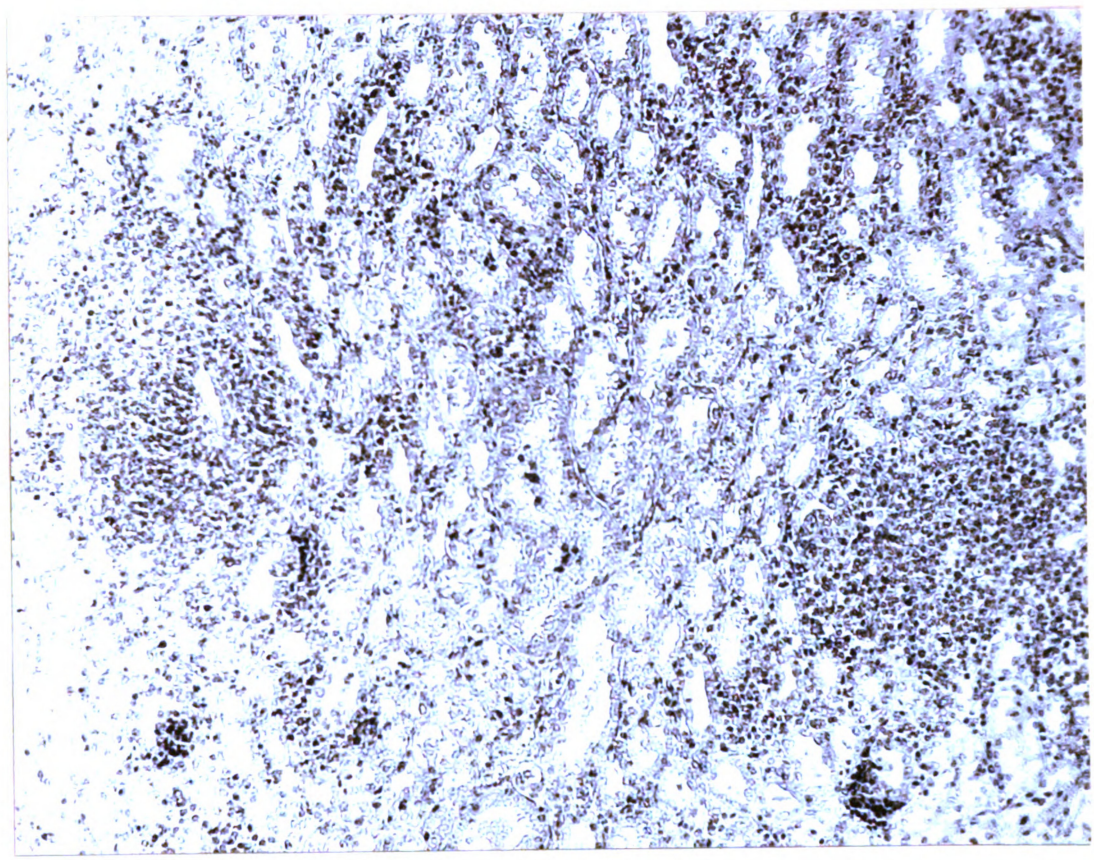

FIGURE 6. Kidney at day 9 with marked leukocytic accumulation in the medulla. x98.

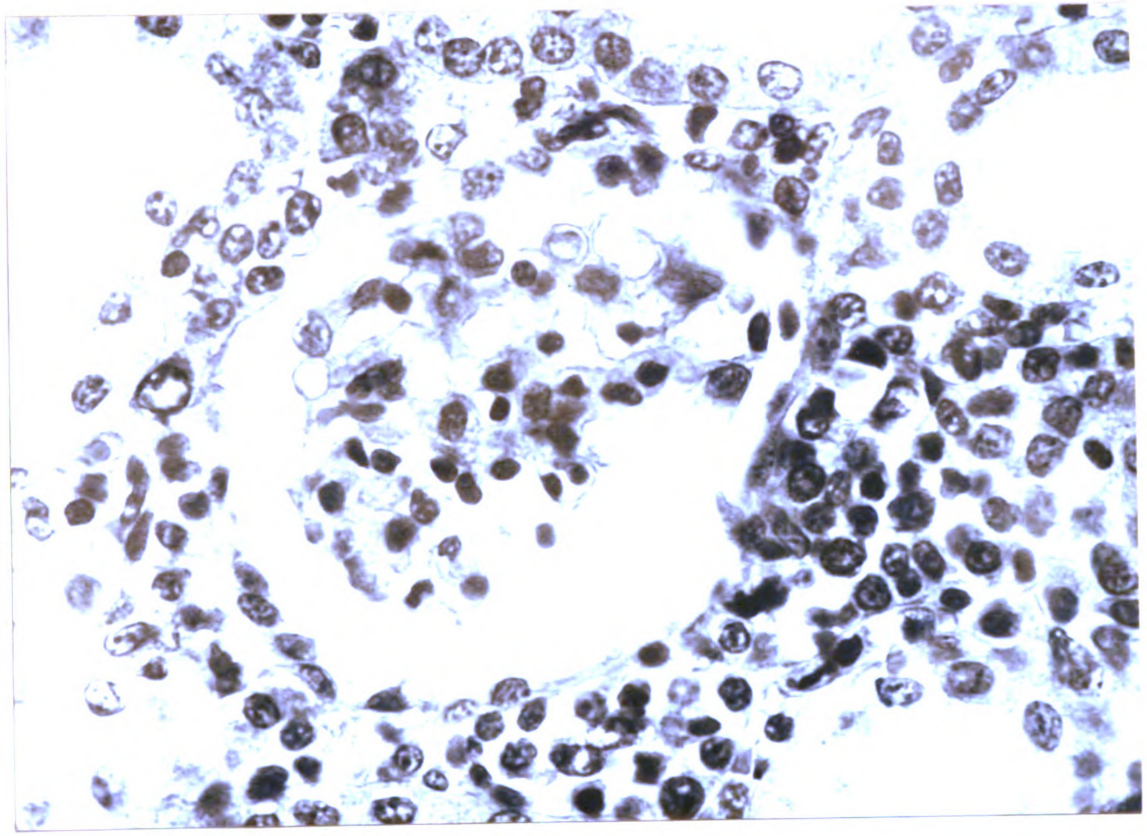

FIGURE 7. Kidney at day 9 showing peri-renal-corpuscular infiltration with lymphocytes. x720.

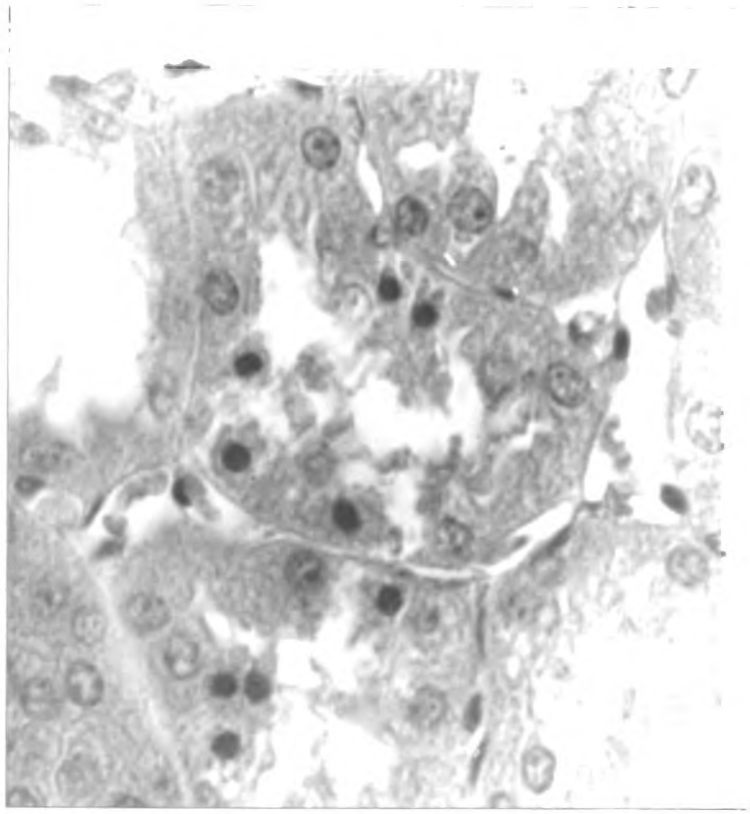

FIGURE 8. Kidney at day 9 to show pyknotic nuclei in tubular cells. x600.

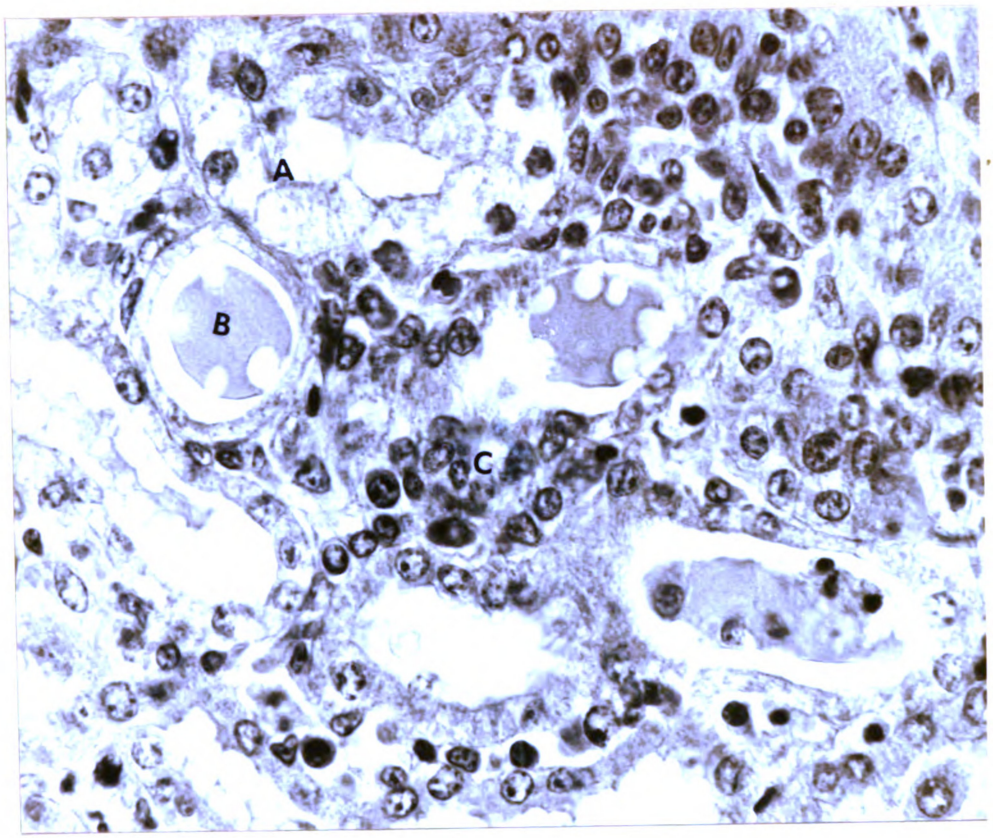

FIGURE 9. Kidney at day 9. A. Hydropic degeneration of proximal convoluted tubule. B. Hyaline casts in lumen of tubules. C. Lymphocytes in inter-tubular areas. x600.

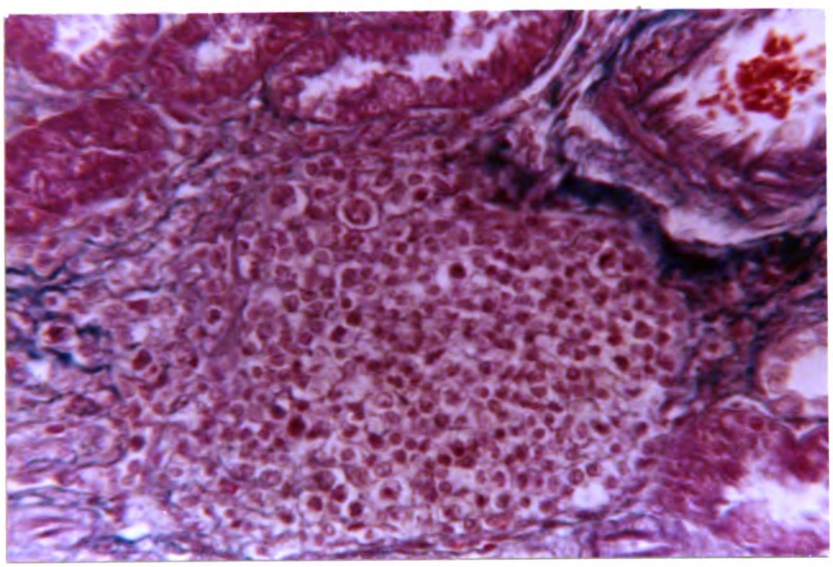

FIGURE 10. Kidney at day 14 to show collagenous fibers in area of leukocytic infiltration. x320. Aniline blue.

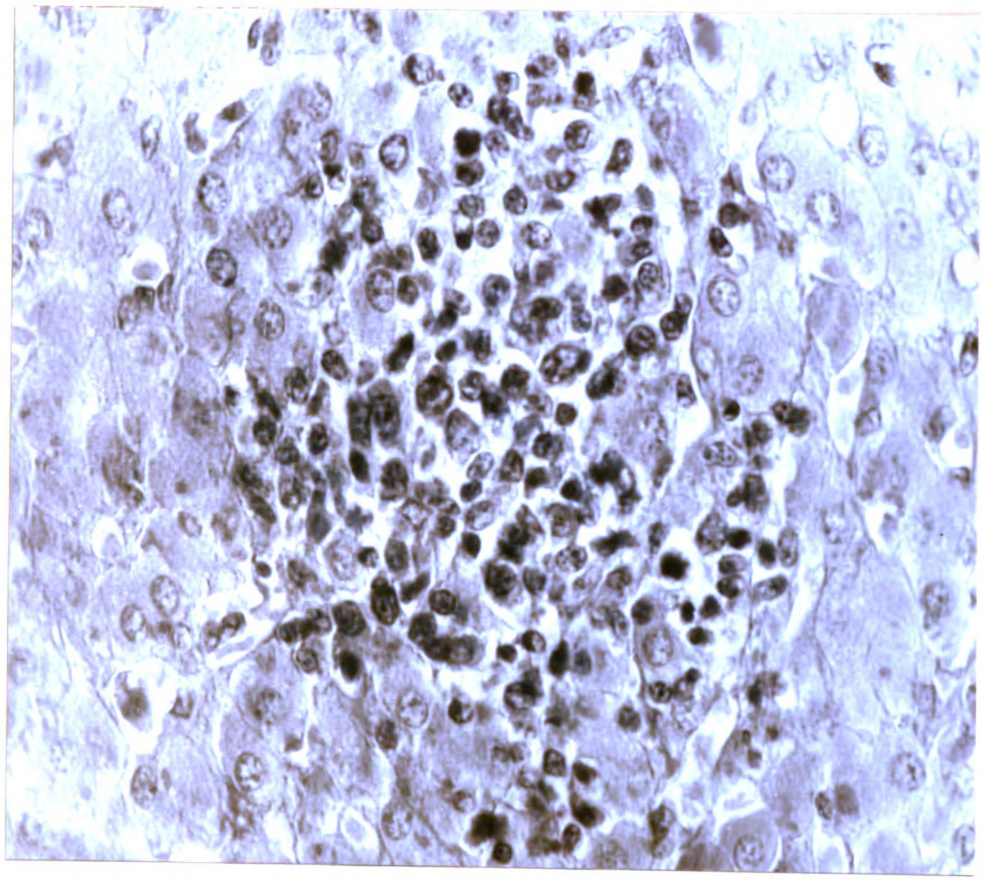

FIGURE 11. Adrenal gland at day 12 to show lymphocytic focus in zona fasciculata. x600.

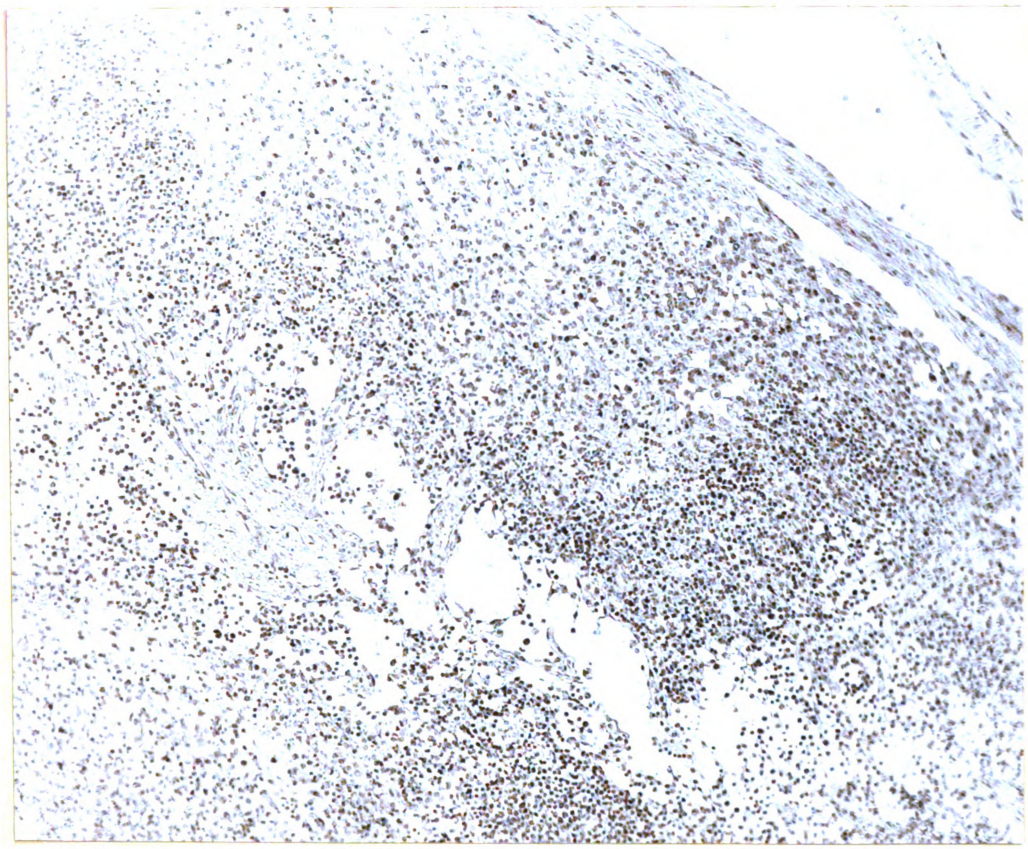

FIGURE 12. Edema of renal lymph node at day 11. x75.

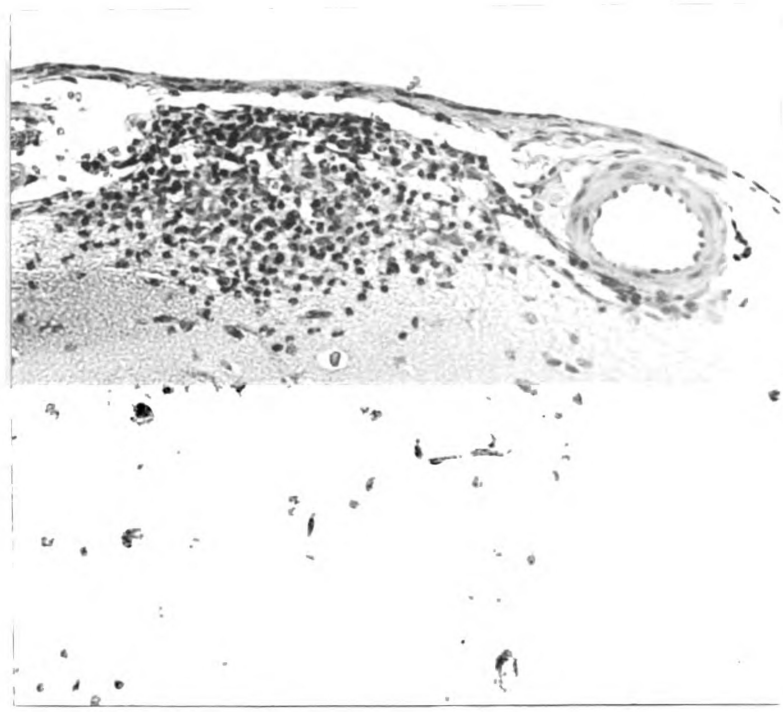

FIGURE 13. Lymphocytic infiltration of meninges and cortex of brain at day 11. x150.

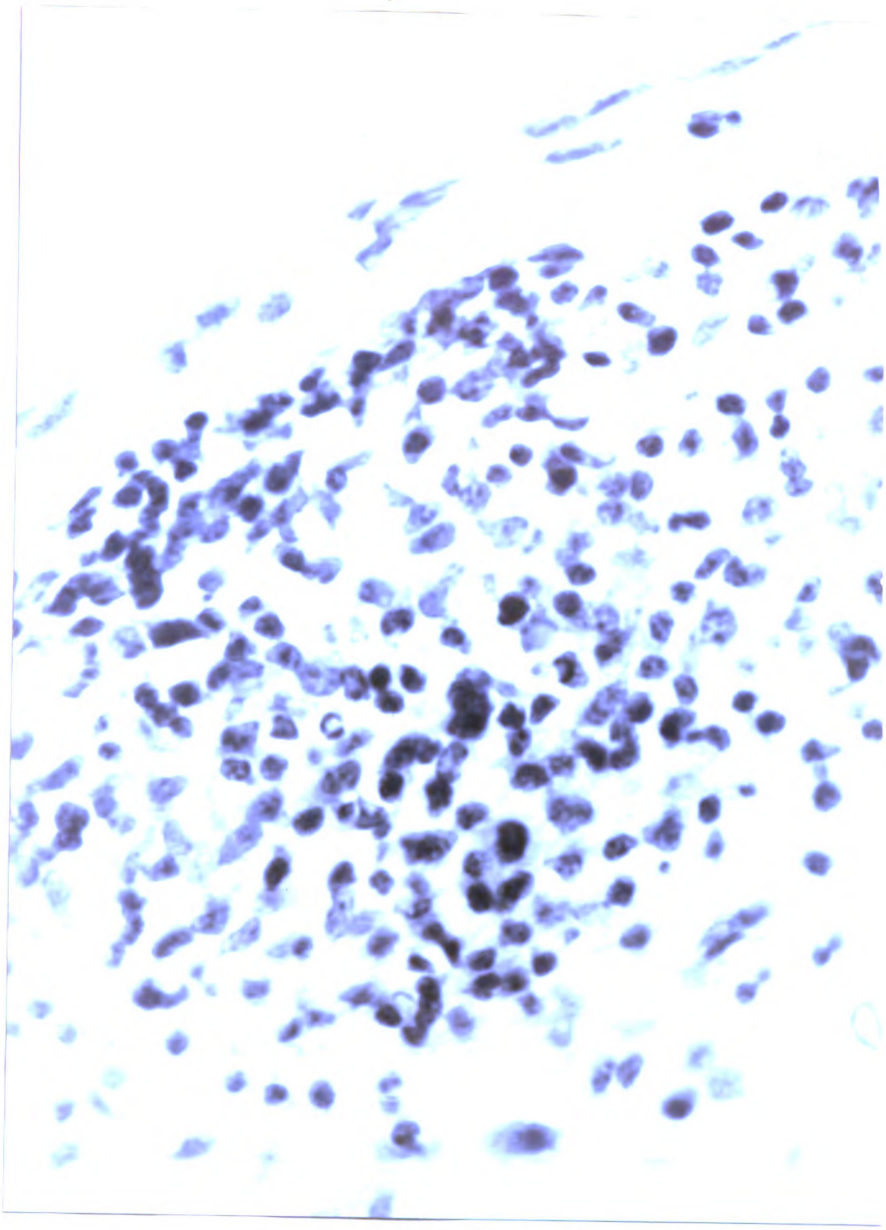

FIGURE 14. Higher power of Figure 13. x650.

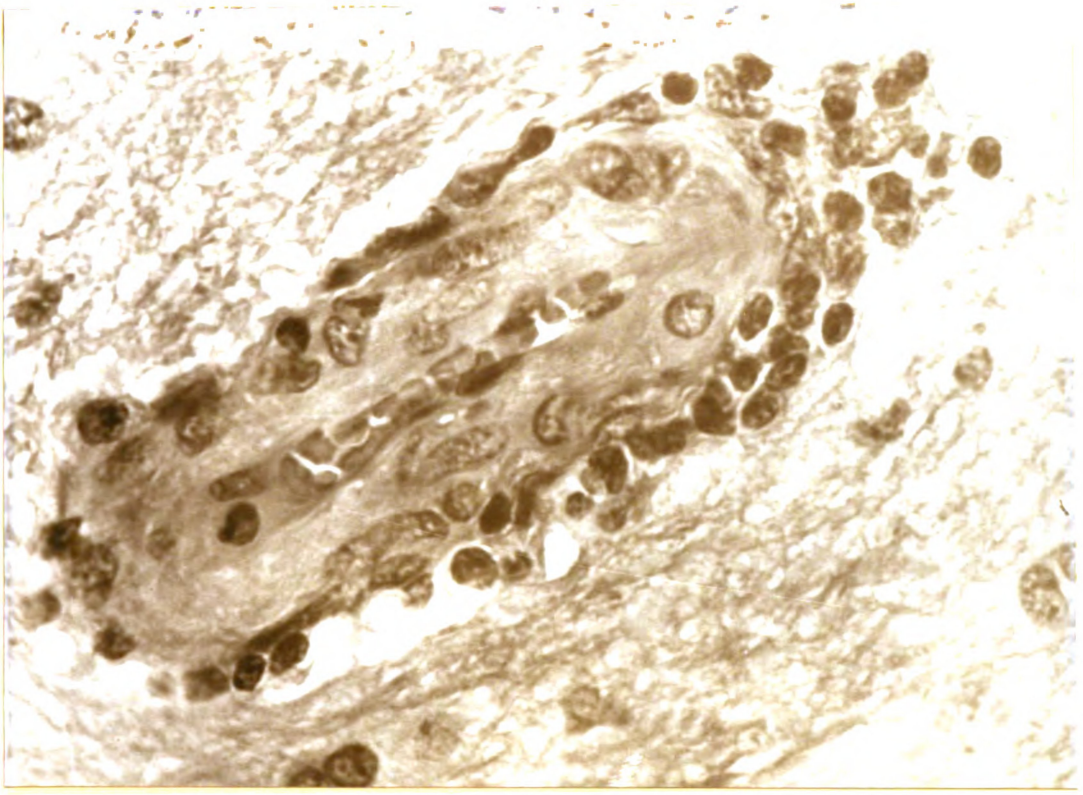

FIGURE 15. Perivascular infiltration of lymphocytes around small arteriole of cerebrum at day 12. x900.

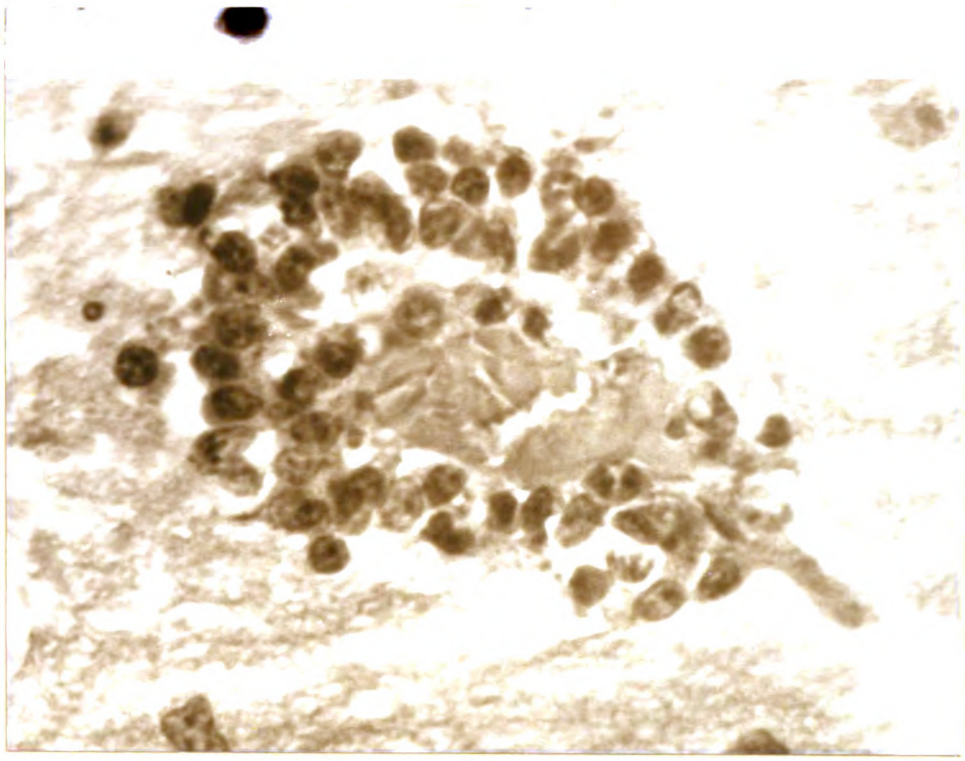

FIGURE 16. Same as Figure 15 only different pig at day 12. x900.

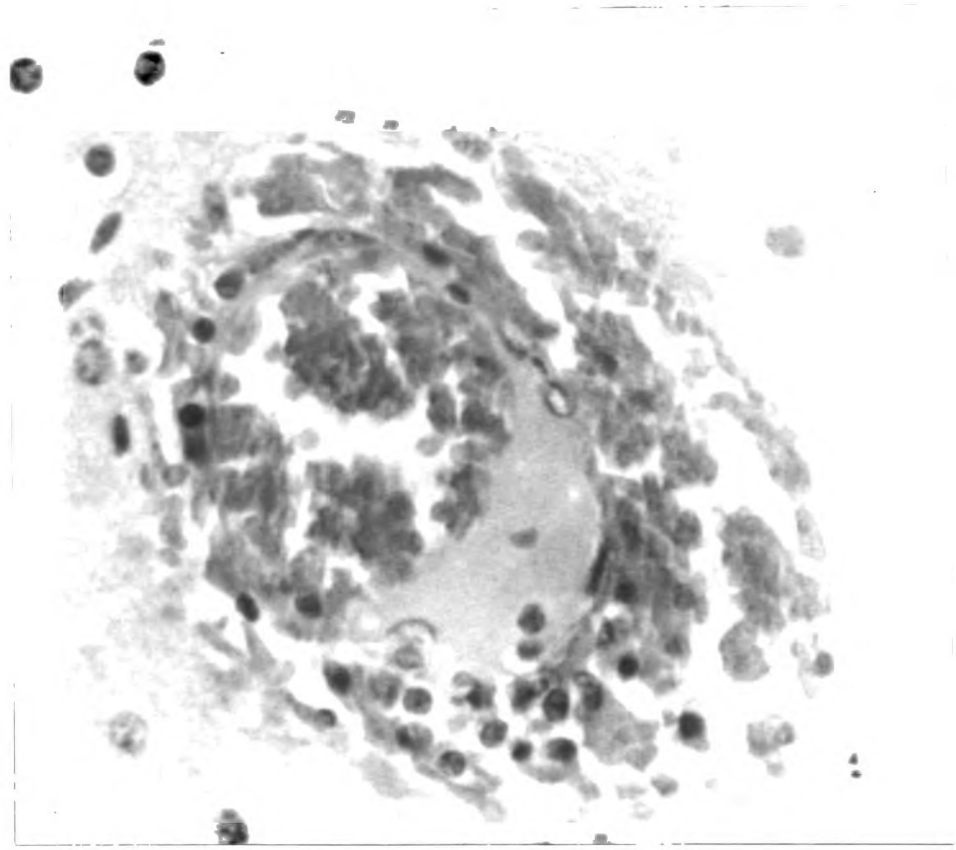

FIGURE 17. Perivascular hemorrhage and lymphocytic infiltration around vein in cerebrum at day 11. x600.

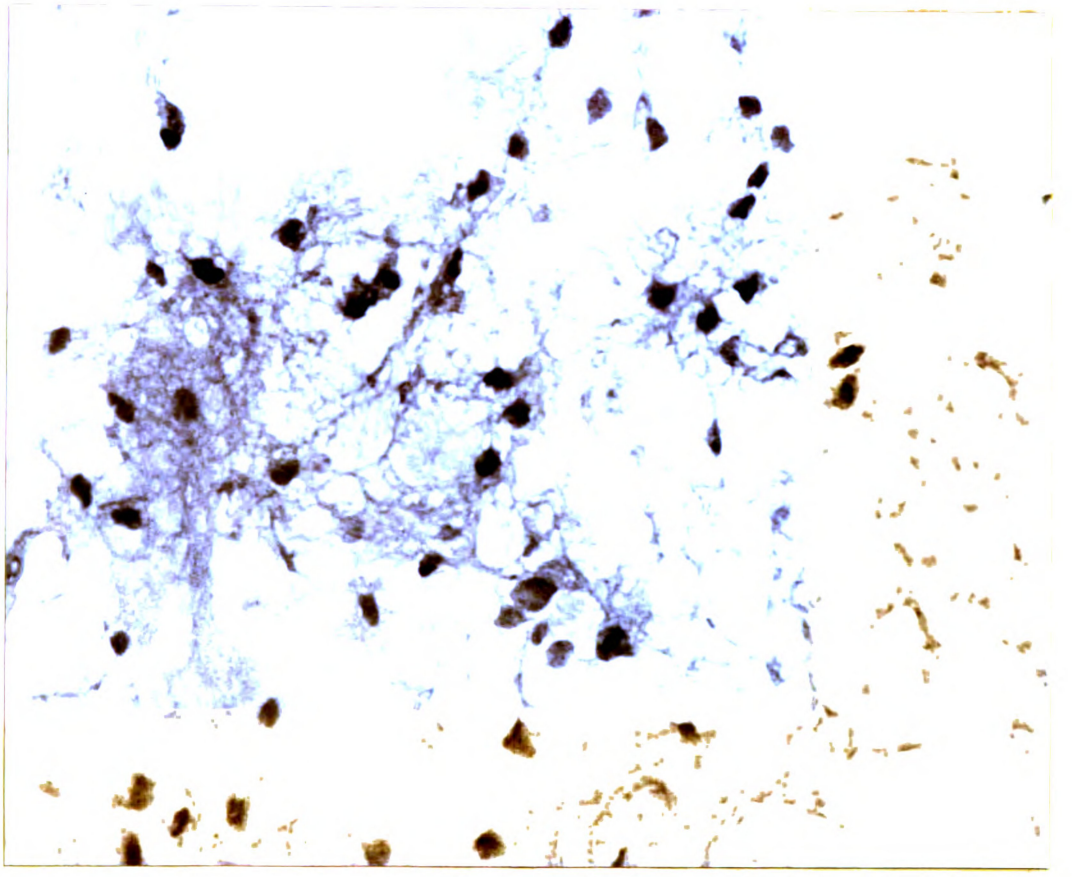

FIGURE 18. Small areas of encephalomalacia in cerebrum at day 12. (Same pig as Figure 15). x600.

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