ABSTRACT

PATHOLOGY OF EXPERIMENTAL LEPTOSPIRA POMONA INFECTION IN HAMSTERS

by Mohamed-Tewfik Fawi Abdu

A series of experiments using 91 hamsters was conducted to study experimental Leptospira pomona (strain Ohio) infection. In addition to gross and microscopic pathological studies; serological, bacteriological, hematological and clinical observations were made.

Marked central nervous system disturbances were noticed as early as day 6 (post-inoculation) in hamsters which received the high dose (approximately 2, 500 organisms), and on day 8 in animals inoculated with the low dose (approximately 100 organisms).

The first microscopic renal lesions were observed in the renal cortices of hamsters inoculated with the high dose by day 2. These lesions consisted of albuminous degeneration, vacuolization and some necrosis as evidenced by pyknosis of the epithelial cells in the proximal and distal convoluted tubules with a few intertubular and peri-corpuscular lymphocytic foci. By day 6, in addition to these lesions, hyaline casts were seen in the lumens of the renal tubules. There was also partial disappearance of tubular cells with areas of intertubular hemorrhage. The liver had areas of albuminous degeneration and hepatic cell individualization.

Demonstrable microscopic renal lesions were present by day 6 in animals inoculated with the low dose. These lesions consisted of a few intertubular and pericorpuscular foci of lymphocytic infiltration. By day 7, in addition to the lymphocytic foci, the renal tubular epithelium had marked albuminous degeneration with vacuolization, and some of the Bowman's capsules appeared to be slightly thickened. There were also numerous hyaline casts in the lumens of the tubules with a few areas of perivascular lymphocytic infiltration. The liver had areas of hepatic cell individualization with perivascular lymphocytic infiltration in the areas of the portal triads. By day 9, early proliferation of fibroblasts and leukocytic infiltration were present in the portal triad regions.

Gross renal lesions consisting of greyish-white foci approximately 0. 5 mm. in diameter on the cortical surface were observed in hamsters inoculated with the high dose starting on day 5. Similar lesions were seen in hamsters inoculated with the low dose starting on day 7.

No significant microscopic lesions were seen in the brains of infected hamsters, even in those showing marked central nervous system disturbances.

The concentration of hemoglobin and the packed-cell volume decreased to levels below normal on day 7 in hamsters inoculated with the high dose. On post-inoculation day 8, the hemoglobin concentration

and packed-cell volume values reached a level considerably higher than normal which was indicative of hemoconcentration. Only terminal hemoconcentration was noticed on days 8 and 9 in animals which received the low dose.

Blood nonprotein-nitrogen determinations showed that there was a progressive rise starting on day 7. Terminal values of 424 mg./100 ml. or approximately ten times the normal levels of 40 mg./100 ml. were recorded. The dramatic rise in blood nonprotein nitrogen was believed due to acute renal failure. Central nervous system disturbances were postulated to be a result of the severe uremia.

All blood cultures from infected animals were positive including cultures made from infected animals showing significant antibody titers.

The first detectable antibody production was on day 5 in the case of hamsters inoculated with the high dose, and on day 8 in hamsters inoculated with the low dose.

Leptospira pomona was found to be present in the kidneys of hamsters inoculated with the high dose starting on day 4, and in the brain starting on day 6. In the case of hamsters inoculated with the low dose L. pomona was present in the kidney tissue starting on day 6 and in the brain starting on day 8.

PATHOLOGY OF EXPERIMENTAL

LEPTOSPIRA POMONA INFECTION IN HAMSTERS

By

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Dedicated to

My Father and Mother.

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INTRODUCTION

Leptospirosis, caused by Leptospira pomona, is a disease of considerable economic and public health importance (25, 44). This disease in domestic animals is important not only because of the effects on the animals (30, 32), but also because infected hosts may serve as reservoirs of infection for man (6).

The possibility of using the Syrian hamster as the animal of choice for isolation of leptospirae has been investigated by several workers (24, 33, 34). Trier (42) observed that central nervous system disturbances were produced in hamsters after inoculation with L. pomona (strain Ohio).

The purpose of this experiment was to study the pathological manifestations of L. pomona (strain Ohio) in hamsters with particular reference to central nervous system disturbances.

LITERATURE REVIEW

Leptospira pomona was first isolated from a dairy farmer in Queensland, Australia, by Clayton et al., in 1936 (12).

Natural infections produced by L. pomona have been reported in man (8), cattle (1), swine (5, 17), horses (36), sheep (20, 37) and dogs (45).

Serological surveys have shown that L. pomona infections are distributed in all states of the United States (5, 6, 9, 18, 30, 35, 37).

It has been estimated that the loss from leptospirosis in farm animals is greater than 100 million dollars per year (44). The greatest share of this loss is due to bovine leptospirosis. Although mortality may not be high, the financial losses may be considerable due to abortions, loss of milk yield in dairy cattle and weight loss in beef cattle (27).

Within the last 10 years there has been a remarkable change in opinion regarding the importance of leptospirosis in human and animal health. It is now considered a major public health problem. Many wild and domestic animals living in close association with man are capable of transmitting the infection either by direct or indirect contact (25).

Swine are apparently the main natural host of L. pomona (46). The majority of cases in swine are subclinical, with the exception of

abortion and death of newborn pigs (7). Experimentally, it has been shown that swine can transmit L. pomona infection to other pigs, cattle, sheep and goats (11, 31, 32).

Leptospira pomona infection in cattle is characterized by abortions, decreased milk yield, anorexia, general depression and hemoglobinuria (30). A naturally occurring case of bovine leptospiral meningitis with clinical symptoms was described by Hoag and Bell (21).

The disease in sheep results in inappetence, fever, hemoglobinuria and abortion (31, 37).

Langham et al. (23), in their study of experimental ovine leptospirosis, found that the principal macroscopic changes in L. pomona infected lambs were greyish-white circumscribed foci and streaks in the renal cortex. Some of these lesions extended through the cortex into the medulla. Microscopically the lesions consisted of focal infiltrations of lymphocytes, some plasma cells and macrophages. These lesions were usually peritubular, perivascular, or pericorpuscular. The central nervous system of some of these lambs showed one or more of the following: scattered areas of increased oligodendroglial and microglial cells in the cerebrum, meningitis with lymphocytic infiltration, and perivascular hemorrhages.

Morse et al. (29), in their study of experimental caprine leptospirosis, found that the gross pathological alterations in goats were

confined to the kidneys as greyish-white foci extending throughout the depth of the cortex. Microscopically these foci consisted of accumulations of lymphocytes, plasma cells and a few macrophages. Some goats in the experiment showed microscopic brain lesions. The cerebellum, cerebrum and medulla showed perivascular cuffing with lymphocytes, plasma cells, macrophages and edema. In some brain sections there was an increase of oligodendroglia, while others showed lesions in the medulla oblongata with numerous gitter cells.

Sleight et al. (40), in their study of the early pathogenesis of L. pomona infection in young swine, observed macroscopic lesions in the kidneys similar to those previously described. Microscopically, the majority of these lesions were rather discrete, consisting mainly of infiltrations of small numbers of lymphocytes between tubules. Other lesions included hyaline casts, hydropic degeneration and pyknosis of tubular cells and an increase in collagenous fibers. Some animals showed brain lesions which were located principally in the cerebrum and consisted of meningoencephalitis characterized by perivascular lymphocytic infiltrations.

Sleight and Lundberg (41) isolated L. pomona from brain tissues of experimentally infected swine as long as 18 days after inoculation. They found that leptospirae were present in the kidneys from post-inoculation day 4 until the experiment was terminated at day 45.

Morton (33) in 1942 was the first one to report on the susceptibility of Syrian hamsters to leptospirosis. He showed that subcutaneous injections of L. icterohaemorrhagiae into 3-to 5-week old hamsters resulted in death of the animals 5 to 8 days after inoculation. Subcutaneous inoculation of L. canicola into 3-to 5-week old hamsters failed to bring death, but cultures made from heart blood were positive for leptospirae up to 96 hours after inoculation.

Randall and Cooper (34) in 1944 reported that the Syrian hamster was the animal of choice for the isolation of leptospirae. Both L. canicola and L. icterohaemorrhagiae killed hamsters in 5 to 10 days.

Larson (24) in his investigations of experimental leptospirosis in hamsters, found that intraperitoneal inoculation of a 10% emulsion of liver and kidney tissue from a guinea pig infected with L. canicola produced death of the animals within a week after inoculation. At necropsy, hemorrhages were found in the lungs. Examination of the peritoneal fluid under dark-field illumination showed many active leptospirae. Inoculation of a 10% emulsion of liver and kidney tissue from a mouse injected with L. icterohaemorrhagiae into hamsters caused death of the animals 5 to 8 days after inoculation. At necropsy, leptospirae were detected in the tissues by dark-field illumination. The tissues were icteric and hemorrhages were present in the abdominal muscles, lungs and kidneys.

Uhlenhuth (43) infected Syrian hamsters with aspirated peritoneal fluid of a guinea pig inoculated with L. canicola. The hamsters became sick and died in 7 to 12 days. Icterus occurred shortly before death. At necropsy, hemorrhages in the lungs, kidneys and liver were observed.

Doljansky (14) reported that Syrian hamsters did not show any clinical signs nor lesions when inoculated with certain strains of L. grippotyphosa. However, a serological examination showed a rise in agglutinin titer and the hamsters were found immune to infection against pathogenic strains of L. grippotyphosa.

Hamdy and Ferguson (19) studied the effect of serial passages on the virulence of L. pomona (Hardacre strain) for hamsters. The animals recovered from early passages but their kidneys were grossly involved. As passages progressed, animals died within 4 days after inoculation. At necropsy there were hemorrhages in the lungs, and the liver and the kidneys were congested. Microscopically, pulmonary hemorrhages and infiltration with lymphocytes and monocytes occurred, accompanied by broken alveolar walls. There was necrosis of hepatic cells. The kidneys showed hemorrhages in the tubules with some hyalinization of the glomeruli. In the chronic phase of the disease only the kidneys were involved. There was an increase of interstitial connective tissue and degeneration of the epithelium of the tubules. Some glomeruli were shrunken while others were edematous.

Bauer (2) reported that hamsters were susceptible to infection with L. pomona (strain Ohio). He found that there was an inverse relationship between the size of the inoculum and the duration of survival of infected hamsters. He also observed that 4-, 8- and 15-week-old hamsters were nearly equally susceptible to L. pomona.

Bauer (3) later observed hematological changes when hamsters were inoculated intraperitoneally with L. pomona (Strain L W). The blood changes occurred 72 hours after inoculation. There was an increase in the urea values and in the bilirubin levels, while a decrease in hemoglobin and hematocrit readings was observed. Differential leukocyte counts indicated a relative neutrophilia and a corresponding relative lymphocytopenia.

Fizette (15) studied the relationship between the leptospirae and the animal host using golden hamsters infected with a highly virulent strain of L. pomona. Blood taken from the diseased hamsters in the terminal stages of the infection showed a slight increase in blood clotting time. The administration of a wide range of dosages of different forms of vitamin K failed to bring about the decrease of hemorrhages in the infected animals.

According to Trier (42), central nervous disturbances were produced in hamsters as early as the fifth day after inoculation with L. pomona (strain Ohio). The head was held back, the legs stiffened

in extensor rigidity and paralysis occurred. In some cases the animals trembled violently. There was considerable hematological change. Minimal levels of 9.3 gm./100 ml. of hemoglobin and 4,910,000 erythrocytes /cu. mm. were observed. A leukocytosis occurred but the author was of the opinion that this might have been the result of hemocentration. L. pomona (Ohio strain) was shown to be lethal by day 7 after inoculation.

Burk (10) reported that strains of leptospirae highly virulent for hamsters gave rise to a fatal acute leptospirosis. Strains of moderate virulence produced a chronic leptospirosis with kidney localization and positive serological tests after 21 days.

Lewis and Gray (26) established an acute fatal leptospirosis in gerbils when 1000 or more virulent L. pomona organisms were given. Animals inoculated with less than 1000 organisms did not develop a lethal infection but a chronic infection was established.

MATERIALS AND METHODS

The strain of L. pomona used in these experiments was first isolated from porcine urine in Ohio (39). Lately it has been maintained in continual guinea pig passage to assure retention of its virulence.

In the first experiment 20 hamsters 4 to 6 weeks old were used. Sixteen animals were each inoculated intraperitoneally with an inoculum which consisted of 0.5 ml. of a 1:1 saline dilution of L. pomona infected guinea pig blood collected at the peak of the febrile response. Due to the shortage of guinea pigs, titration to determine the number of organisms present per ml. of guinea pig infected blood at the height of pyrexia was not done. However, previous studies in this laboratory have shown that approximately 10^4 organisms per ml. are present in the infected blood of guinea pigs at the climax of pyrexial response (105° to 106° F.) (42). Therefore, it is calculated that each hamster received approximately 2,500 organisms. These hamsters are designated from here on as high-dose animals. The remaining 4 hamsters were used as controls and inoculated with 0.5 ml. each of a 1:1 saline dilution of normal guinea pig blood. Each animal was put in an individual cage, with controls being separated from the infected ones.

The killing date for each animal was predetermined by random number assignment, 3 infected hamsters and one control being killed on alternate days starting on day 2. (Here and hereafter, the day referred to is the post-inoculation day.) Some animals having become moribund, it was necessary to kill them before their assigned day. By day 8 the remaining infected animals died.

In the second experiment, 24 hamsters 4 to 6 weeks old were used. Nineteen of these animals were inoculated and the remaining 5 served as controls. The inoculum was similar to that used in the first experiment and these animals are included in the high-dose group. Isolation procedures were as previously described. Three infected animals and one control were killed on alternate days starting with day 3. Animals were killed before their assigned day as soon as they were observed to be moribund. By day 8 the remaining infected animals had been killed.

In the third experiment, 18 hamsters 4 to 6 weeks old were inoculated intraperitoneally with L. pomona (Ohio strain). The inoculum consisted of 1 ml. of a 1:100 saline dilution of infected cavian blood for each hamster. By calculation, each hamster thus received approximately 100 organisms. These hamsters are designated from here on as the low-dose animals. Four hamsters, serving as controls in this experiment, were each inoculated intraperitoneally with 1 ml. of

sterile saline solution. Isolation procedures were as previously described. The sacrificing date for each hamster in this group was predetermined by random number assignment; three infected animals and one control were to be killed on days 9, 12, 15, 18, 24 and 30. Animals were killed before their assigned day as soon as they were recognized as moribund. The first of the infected hamsters died on day 8. By day 9, the remaining infected animals died or were killed.

In the fourth experiment, 25 hamsters 4 to 6 weeks old were used. Twenty of these animals were artificially infected and the remaining five were used as controls. The inoculum was similar to that used in the third experiment and these animals are included in the low-dose group. Isolation procedures were as previously described. The killing date for each hamster in this group was predetermined. Starting on day 3, 2 infected animals and 1 control were killed daily, except that no control was killed on days 5 or 7. Moribund animals were killed before their assigned day as soon as they were observed to be in that state.

The animals in the four experiments were anesthetized with ether and bled aseptically by cardiac puncture using heparinized syringes and needles. A few drops of the collected blood were used in inoculation of Stuart's medium (Difco) enriched with 10% rabbit serum, using 2 tubes of medium for each animal. The cultures were incubated at 30° C.

for a period varying up to 4 weeks and examined for the presence of leptospirae employing the dark-field microscope. The remaining blood was used for serological and hematological studies. After bleeding, the animals were sacrificed by administering an overdose of ether and were necropsied.

Serological examinations were conducted by the modified microscopic agglutination-lysis test (16, 29), using L. pomona (strain Johnson) live antigen.

Hematological studies were done on hamsters of the last three experiments to establish hemoglobin values, packed cell volumes, and differential leukocyte counts (4, 13). In addition, blood nonprotein nitrogen (4, 13) and total leukocyte counts were run on some hamsters of the third experiment and all hamsters of the fourth experiment. When volumes of individual blood samples were not sufficient for determining nonprotein nitrogen, pooled blood samples were used.

Tissues were saved from the kidneys, liver, spleen, lung, skeletal muscle, brain and spinal cord of each animal. These tissues were placed in one or more of the following fixatives: Zenkers fluid, 10% neutral formalin solution and Carnoy's fluid.

The sections were stained by one or more of the following staining procedures: hematoxylin and eosin for general characteristics, oil red O for fat, Best's carmine stain for glycogen, Warthin-Starry method

for spirochetes and cresyl echt violet for cellular details of brain substance (28).

Samples of kidney and brain tissue were saved for guinea pig inoculation from every group of animals killed, including the controls. The tissues were homogenized in 0.85% sterile sodium chloride solution to give approximately a 10% tissue suspension. One guinea pig was inoculated intraperitoneally with 1 ml. kidney suspension, and another was injected intraperitoneally with 1 ml. brain suspension from each group killed, including the controls. Blood from these guinea pigs was obtained for serological study 14 days after inoculation. The inocula were considered to contain leptospirae if the sera of the inoculated guinea pigs contained antibodies for L. pomona at a dilution of 10^2 or higher (29, 39).

EXPERIMENTAL RESULTS

Clinical

The only significant clinical symptoms observed were the marked central nervous system disturbances. These were noticed as early as the 6th day in hamsters which were inoculated with the high dose and on the 8th day in animals inoculated with the low dose. The animals showed hyperexcitability with dorsal head flexion, lordosis and tonoclonic spasms.

Hematological

Hematological data obtained during the course of the experiments are summarized in Table 1. In animals inoculated with the high dose, the hemoglobin concentrations and packed-cell volumes were normal up to day 6. On day 7 the hemoglobin and packed-cell volume values were markedly lowered, but on day 8 the hemoglobin concentrations and packed-cell volumes reached levels of 18.1 gms./100 ml. and 49.8 per cent respectively. Hamsters with hemoconcentration were moribund and were showing the central nervous system disturbances listed above.

In hamsters which were inoculated with the low dose, the hemoglobin concentrations, packed-cell volumes and total leukocyte counts were found to be normal up to the 7th day. On the 8th and 9th days

the values of the above-mentioned three constituents rose. These hamsters were also moribund and showed the central nervous system disturbances.

Blood nonprotein nitrogen determinations were done on blood samples obtained from animals inoculated with the low dose. The values of blood nonprotein nitrogen remained for the most part within the normal range (40 mg./100 ml.) up to the 6th day. On day 7 the blood nonprotein nitrogen rose to a value of 124 mg./100 ml. and reached a maximum of 424 mg./100 ml. by day 9.

Results of differential leukocyte counts are given in Table 2. The high-dose animals had a relative neutrophilia and lymphocytopenia starting on day 7. The low-dose group showed a relative and absolute neutrophilia and lymphocytopenia starting on day 6.

Serological

Table 3 summarizes the agglutination-lysis test results both in the control and infected hamsters. The first detectable antibody production was on day 5 in the case of hamsters inoculated with the high dose and on day 8 in hamsters inoculated with the low dose. The maximum agglutination-lysis reactions were present in the 1:100 serum dilution on day 8 in the animals inoculated with the high dose.

Bacteriological

Leptospirae were demonstrated in tissues of the infected hamsters by inoculation of guinea pigs with homogenized hamster tissues (Table 4). These findings indicated that L. pomona was present in the kidneys of the hamsters inoculated with the high dose from day 4 until the animals died or were killed, and in the brain starting on day 6 and thereafter until the animals died or were sacrificed. In the case of hamsters inoculated with the low dose, the organisms were present in kidney tissue starting on day 6 and thereafter until the animals expired, and in the brain from day 8 until the animals died or were killed. All blood cultures from infected animals were positive including cultures made from infected animals showing significant antibody titers.

Pathological

A. Gross

A few greyish-white foci mainly located in the renal cortices were observed in hamsters inoculated with the high dose starting on day 5. These lesions were approximately 0.5 mm. in diameter. In hamsters inoculated with the low dose, a few scattered greyish-white foci principally in the renal cortices were first seen in hamsters sacrificed on day 7.

There were no other gross lesions observed which could be attributed to L. pomona infection. No significant gross lesions were observed in the control animals.

B. Microscopic

The first microscopic renal lesions were observed in the cortices of hamsters inoculated with the high dose killed on day 2. These lesions consisted of albuminous degeneration, vacuolization and some necrosis as evidenced by pyknosis of the epithelial cells in the proximal and distal convoluted tubules with a few foci of lymphocytes in the intertubular and pericorpuscular areas (Figure 1). On day 6, in addition to the lesions described above, hyaline casts were in the lumen of the renal tubules. There was also partial disappearance of tubular cells with areas of intertubular hemorrhage (Figures 2, 3, 4 and 5). The liver had areas of albuminous degeneration and hepatic cell individualization (Figure 6).

In hamsters inoculated with the low dose, the first microscopic lesions were present in the renal cortices of animals killed on day 6. The lesions consisted of a few foci of intertubular and pericorpuscular lymphocytic infiltration (Figure 7). On day 7, in addition to the lymphocytic foci, the renal tubular epithelium had marked albuminous degeneration with vacuolization. Some of the Bowman's capsules appeared to be slightly thickened. There were also numerous hyaline

casts in the lumens of the tubules with a few areas of perivascular lymphocytic infiltration (Figures 8, 9, 10 and 11). The liver had areas of albuminous degeneration, and hepatic cell individualization with perivascular lymphocytic infiltration in the areas of the portal triads (Figures 12 and 13). By day 9, early proliferation of fibroblasts and lymphocytic infiltration were in the portal triad regions (Figures 14 and 15).

No significant microscopic lesions were seen in the brains of the infected animals, neither in sections stained with hematoxylin and eosin nor in the sections stained with cresyl echt violet.

No significant microscopic lesions were seen in the control animals.

Leptospirae were found in the Warthin-Starry stained sections of the kidney and liver in hamsters inoculated with the low dose and killed on days 8 and 9 (Figures 16 and 17 respectively). No leptospirae were demonstrated in the remainder of the stained tissues.

DISCUSSION

The hamsters used in these experiments were found to be susceptible to infection with L. pomona (strain Ohio). This strain has previously been observed to be lethal for hamsters by several workers (2, 3, 42). In addition Trier (42) noted that severe central nervous disturbances were produced by this strain in hamsters but no attempt was made to study the pathological changes.

As previously stated, it was estimated that approximately 2,500 leptospirae were present in the inoculum given to the hamsters receiving the high dose and 100 organisms were in the inoculum given to those hamsters which received the low dose. It has been calculated that the I. D. 50 for hamsters using L. pomona (strain Wickard) is 3.5 organisms (3).

Bauer (2) reported that there was an inverse relationship between the size of the inoculum and the duration of survival of infected hamsters. In the present experiment, hamsters inoculated with the high dose were moribund and died earlier than those inoculated with the low dose. The shorter course of infection was evident not only in survival time but also in the earlier appearance of gross and microscopic lesions, in earlier positive blood cultures and in earlier detectable antibodies.

The strain of L. pomona used in these experiments did not induce a chronic infection in any of the experimental hamsters. By day 9 the

hamsters which received approximately 100 organisms died or were moribund. It would have been desirable to induce a chronic infection in order to possibly demonstrate brain lesions, and chronic kidney and liver lesions. Lewis and Grey (26) were able to induce a chronic infection in gerbils by giving fewer than 1,000 organisms. Gerbils receiving larger numbers of leptospirae died during the acute phase of the disease. By receiving smaller number of organisms the animals evidently were able to produce sufficient antibody before the leptospirae had reached a concentration which would have been lethal. The organisms present in the renal tubules survived and produced a chronic infection. This diversity in results between hamsters and gerbils may be due to the difference in host susceptibility and/or variations in the virulence of the infecting organisms.

On day 7, the concentration of hemoglobin and packed-cell volume decreased to levels below normal in hamsters inoculated with the high dose. This suggests that some hemolysis took place around the seventh day after infection. This reaction was probably caused by the release of leptospiral hemolysin in the blood. This observation has been reported by other investigators (2, 42).

Starting on day 8, increases in hemoglobin concentration and packed-cell volume were noticed. This was considered an indication of hemoconcentration which may have been a result of dehydration due to the effects of the disease.

The relative and absolute neutrophilia and lymphocytopenia which were observed starting day 7 (high dose) and day 6 (low dose) have been observed by Bauer (3).

The blood cultures were all positive through all days of the experiments which indicated that the agent was still present in the blood at the time of death or sacrifice of the animals. Although antibody production was detected by the agglutination-lysis test as early as the 5th day in animals receiving the high dose, and starting on day 8 in animals inoculated with the low dose, antibody production was evidently not in sufficient concentration to enable the hamsters to overcome the overwhelming leptospiremia.

Although renal lesions were seen as early as day 2 in hamsters inoculated with the high dose, no leptospirae could be demonstrated by guinea pig inoculation from these hamsters. The inability to demonstrate organisms by guinea-pig inoculation may be due to the possibility that these lesions are a sequel to the leptospiremia. The failure to isolate leptospirae from renal tissue may also be due to the possibility that an infective dose of the organisms was not present in the inoculum.

No gross or microscopic lesions were seen in lungs of infected animals in the experiments. This finding contradicts the observation by some workers (19, 24, 43) who found gross and/or microscopic lesions in lungs of infected hamsters.

The greyish-white foci in the kidneys which were seen as early as day 5 (high dose) and starting on day 7 (low dose) have been described in other species (11, 23, 38).

The microscopic appearance of the kidney sections demonstrated extensive degenerative changes; some necrosis, many hyaline casts and lymphocytic infiltration. However, the lymphocytic infiltration was not as extensive as has been reported in other species (23, 31).

The renal changes were the most logical explanation for the dramatic rise in blood nonprotein nitrogen. Therefore the term uremia to characterize the terminal phase of the syndrome could be justified.

The lesions in the livers of the infected animals consisted of albuminous degeneration with hepatic cell individualization in animals infected with both dosage levels. However, proliferation of fibroblasts with lymphocytic infiltration were found in hamsters infected with the low dose and which were killed on day 9. This may be an indication of the passing of the lesion into the subacute stage.

Brain lesions in leptospirosis have been described in man (22) and in domestic animals (31). These lesions have been found to occur in subacute or chronic infections with L. pomona. The nervous symptoms accompanying L. pomona infection in humans and other domesticated animals are due to encephalomeningitis characterized by perivascular lymphocytic infiltrations. The absence of brain or

spinal cord lesions in hamsters may be due to the overwhelming leptospiremia which terminated in death before giving the animals a chance to go into the subacute or the chronic stage of infection. The nervous symptoms in infected hamsters with L. pomona were likely due to uremia resulting from acute renal failure.

When these experiments were started it was hoped that some relationship could be established between the nervous signs and histopathologic changes shown by hamsters with leptospirosis and those shown in humans and other domestic animals suffering from this disease. The nervous symptoms in infected hamsters with L. pomona were thought to be due to uremia resulting from acute renal failure and animals died before lesions of meningoencephalitis could be seen. In man and other domestic animals the nervous symptoms are associated with inflammatory changes in the brain and meninges.

SUMMARY AND CONCLUSIONS

A series of experiments using 91 hamsters was conducted to study experimental Leptospira pomona (strain Ohio) infection. In addition to gross and microscopic pathological studies; serological, bacteriological, hematological and clinical observations were made.

Marked central nervous system disturbances were noticed as early as day 6 in hamsters which received the high dose (approximately 2, 500 organisms) and on day 8 in animals inoculated with the low dose (approximately 100 organisms).

The first microscopic renal lesions were observed in the renal cortices of hamsters inoculated with the high dose by day 2. These lesions consisted of albuminous degeneration, vacuolization and some necrosis as evidenced by pyknosis of the epithelial cells in the proximal and distal convoluted tubules with a few intertubular and pericorpuscular lymphocytic foci. By day 6, in addition to these lesions, hyaline casts were seen in the lumens of the renal tubules. There was also partial disappearance of tubular cells with areas of intertubular hemorrhage. The liver had areas of albuminous degeneration and hepatic cell individualization.

Demonstrable microscopic renal lesions were present by day 6 in animals inoculated with the low dose. These lesions consisted of a few intertubular and pericorpuscular foci of lymphocytic infiltration.

By day 7, in addition to the lymphocytic foci, the renal tubular epithelium had marked albuminous degeneration with vacuolization. Some of the Bowman's capsules appeared to be slightly thickened. There were also numerous hyaline casts in the lumens of the tubules with a few areas of perivascular lymphocytic infiltration. The liver had areas of albuminous degeneration and hepatic cell individualization with perivascular lymphocytic infiltration in the areas of the portal triads. By day 9, early proliferation of fibroblasts and leukocytic infiltration were present in the portal triad regions.

Gross renal lesions consisting of grayish-white foci approximately 0.5 mm. in diameter on the cortical surface were observed in hamsters inoculated with the high dose starting on day 5. Similar lesions were seen in hamsters inoculated with the low dose starting on day 7.

No significant microscopic lesions were seen in the brains of infected hamsters, even in those showing marked central nervous system disturbances.

The concentration of hemoglobin and the packed-cell volume decreased to levels below normal on day 7 in hamsters inoculated with the high dose. On post-inoculation day 8, the hemoglobin concentration and packed-cell volume values reached a level considerably higher than normal, indicating hemoconcentration. Only terminal hemoconcentration was noticed on days 8 and 9 in animals receiving the low dose.

Blood nonprotein-nitrogen determinations showed that there was a progressive rise starting on day 7. Terminal values of 424 mg./100 ml., approximately ten times the normal levels of 40 mg./100 ml., were recorded. The dramatic rise in blood nonprotein nitrogen was believed to be due to acute renal failure. Central nervous system disturbances were postulated to be a result of the severe uremia.

All blood cultures from infected animals were positive, including cultures made from infected animals showing significant antibody titers.

The first detectable antibody production was on day 5 in hamsters inoculated with the high dose, and on day 8 in hamsters inoculated with the low dose.

Leptospira pomona was found to be present in the kidneys of hamsters inoculated with the high dose starting on day 4, and in the brain starting on day 6. In the case of hamsters inoculated with the low dose, L. pomona was present in the kidney tissue starting on day 6 and in the brain starting on day 8.

From this investigation it was concluded that: (1) acute renal failure appeared to be the primary cause of death; (2) insufficient antibody production to overcome leptospiremia was postulated to be a contributory factor in the death of hamsters; (3) there were no detectable brain lesions which could be correlated with the clinical nervous system disturbances; (4) the central nervous system

disturbances were postulated to be a result of uremia; (5) no chronic infection could be induced with L. pomona (strain Ohio) due to the lethal effects of this strain.

TABLE 1

SUMMARY OF AVERAGE HEMATOLOGICAL VALUES

	Hgb	Pcv	Wbc	Npn	Number of animals included
Controls	14.5	46.1	7,210	43	7
High Dose					
PI day 3	14.1	45.3	*	*	2
PI day 5	13.6	43.8	*	*	3
PI day 7	9.5	36.3	*	*	3
PI day 8	18.1	49.8	*	*	2
Low Dose					
PI day 3	14.0	45.4	6,810	52	2
PI day 4	13.4	43.4	7,650	80	3
PI day 5	13.2	43.5	7,000	50	3
PI day 6	13.5	43.7	7,150	42	3
PI day 7	13.7	43.9	6,000	124	3
PI day 8	19.5	51.6	13,210	410	3
PI day 9	18.6	50.9	11,910	424	3

* Not attempted

Hgb = hemoglobin in grams per 100 ml. of blood
Pcv = packed-cell volume expressed as per cent
Wbc = leukocytes per cmm.
Npn = non protein nitrogen in mg. per 100 ml. of blood
PI = post-inoculation

High Dose = 0.5 ml. of a 1:1 dilution of infected guinea pig blood

Low Dose = 1 ml. of a 1:100 dilution of infected guinea pig blood

TABLE 2

DIFFERENTIAL LEUKOCYTE COUNT -- AVERAGE VALUES

	Baso-phil	Eosino-phil	Nonseg-mented hetero-phil	Segmented heterophil		Lymphocyte		Monocyte
				Relative	Absolute	Relative	Absolute	
Controls	0	0	1	36	2596	60	4326	3
Infected hamsters (high dose)								
PI day 3	0	0	3	34	*	62	*	1
PI day 5	0	0	5	42	*	50	*	3
PI day 7	0	0	2	65	*	30	*	3
PI day 8	0	0	3	75	*	20	*	2
Infected hamsters (low dose)								
PI day 3	0	0	1	40	2724	54	3677	5
PI day 4	0	0	2	45	3442	51	3901	2
PI day 5	0	0	3	48	3360	45	3150	4
PI day 6	0	1	2	64	4576	32	2288	1
PI day 7	0	0	1	63	3780	35	1800	1
PI day 8	0	0	3	68	8983	27	3566	2
PI day 9	0	0	4	74	8813	19	2262	3

* Total leukocyte counts were not done.

TABLE 3

ANTIBODY TITERS FOR L. POMONA IN SERA OF INFECTED HAMSTERS

PI Day[a]	Dose used	Agglutinin titer[b]
2	High dose	-
3	"	-
4	"	-
5	"	10^{1+}
6	"	10^{2+}
7	"	10^{2++}
8	"	10^{2+++}
3	Low dose	-
4	"	-
5	"	-
6	"	-
7	"	-
8	"	10^{1+}
9	"	10^{2+}

a PI = post-inoculation

b The titer is expressed as the logarithm of the reciprocal of the highest dilution of serum in which leptospirae were agglutinated or lysed.

\+ = 25 per cent agglutination or lysis

++ = 50 per cent agglutination or lysis

+++ = 75 per cent agglutination or lysis

TABLE 4a

SUMMARY OF GUINEA PIG INOCULATIONS WITH HOMOGENIZED TISSUES FROM HAMSTERS INOCULATED WITH THE HIGH DOSE

Day after infection	Brain	Kidney
2	-	-
3	-	-
4	-	+
5	-	+
6	+	+
7	+	+
8	+	+

TABLE 4b

SUMMARY OF GUINEA PIG INOCULATIONS WITH HOMOGENIZED TISSUES FROM HAMSTERS INOCULATED WITH THE LOW DOSE

Day after infection	Brain	Kidney
3	-	-
4	-	-
5	-	-
6	-	+
7	-	+
8	+	+
9	+	+

\+ indicates that the serum of the guinea pigs contained antibodies for L. pomona at a dilution of 10^{-2} or higher two to three weeks after inoculation with the respective tissue suspensions of the infected hamsters.

\- indicates that the serum of guinea pigs was negative.

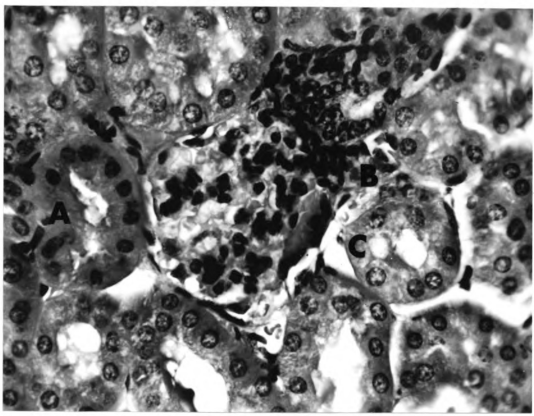

Fig. 1.--Kidney at day 2. A. Pyknotic nuclei in tubular cells. B. Lymphocytes in the intertubular and pericorpuscular areas. C. Vacuolization of tubular cells. x720.

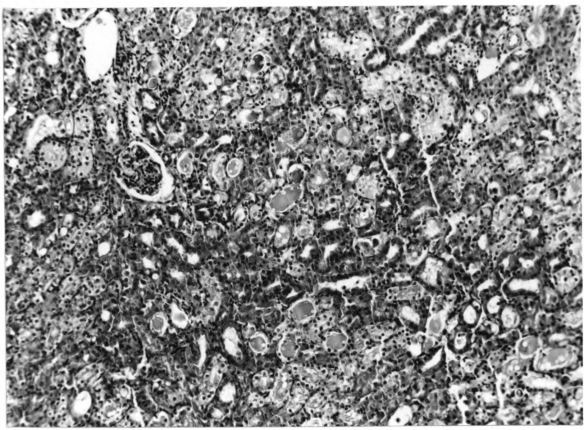

Fig. 2. --Kidney at day 6 demonstrating hyaline casts in lumen of tubules. x250.

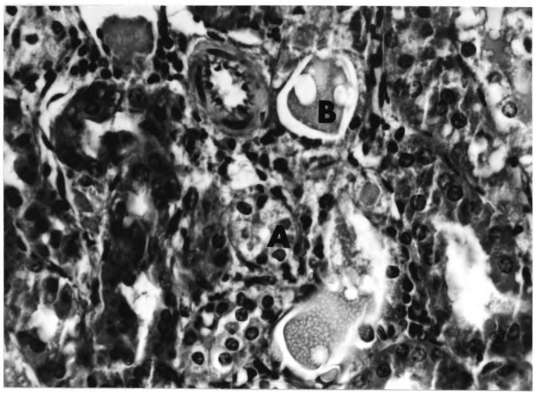

Fig. 3. --Kidney at day 6. A. Vacuolization of tubular cells. B. Hyaline casts in lumen of tubules. x720.

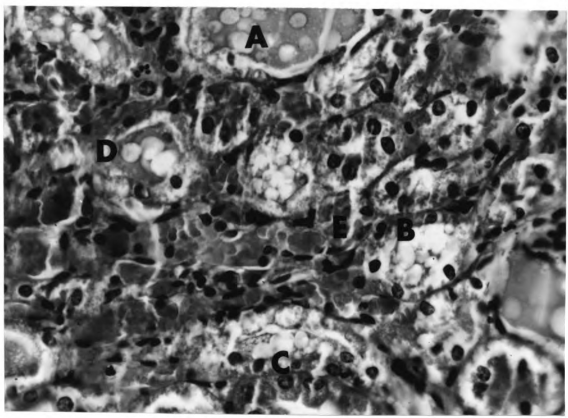

Fig. 4. --Kidney at day 6. A. Hyaline casts in lumen of tubules. B. Pyknotic nuclei in tubular cells. C. Vacuolization of tubular cells. D. Partial disappearance of tubular epithelium. E. Intertubular hemorrhage. x720.

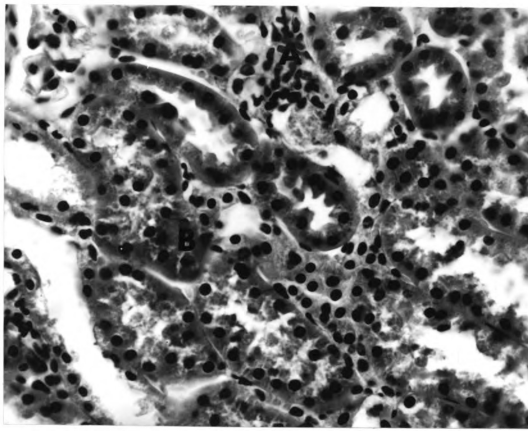

Fig. 5. --Kidney at day 6. A. Lymphocytes in intertubular area. B. Pyknotic nuclei in tubular cells. x720.

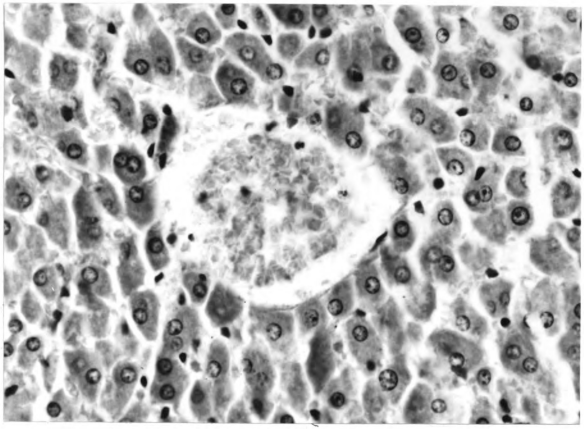

Fig. 6.--Liver at day 6 demonstrating hepatic cell individualization (high dose). x720.

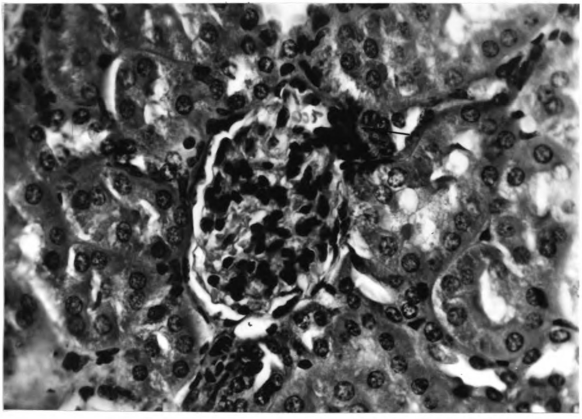

Fig. 7. --Kidney at day 6 demonstrating lymphocytes in pericorpuscular area. x720.

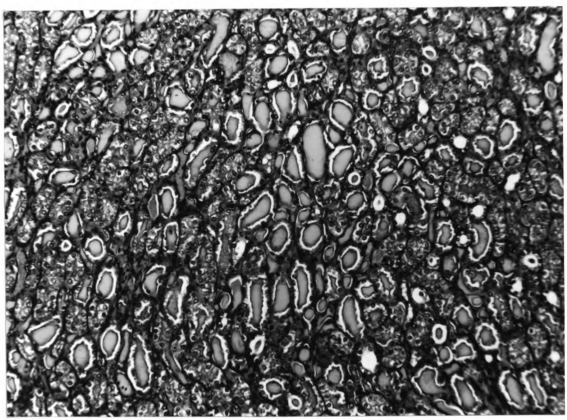

Fig. 8. --Kidney at day 7 demonstrating numerous hyaline casts in lumen of tubules. x250.

Fig. 9. --Kidney at day 7 showing marked vacuolization of tubular cells. x720.

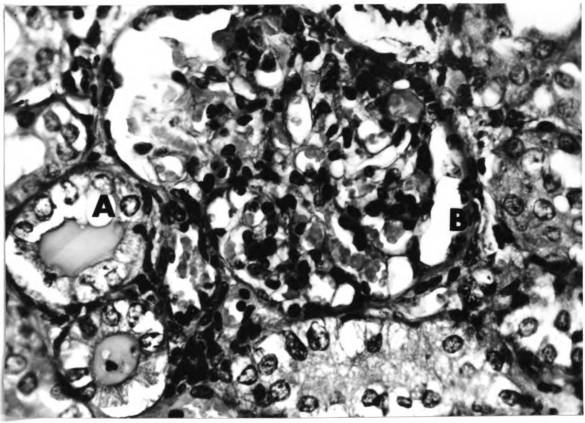

Fig. 10.--Kidney at day 7. A. Vacuolization of tubular cells. B. Slight thickening of Bowman's capsule. x720.

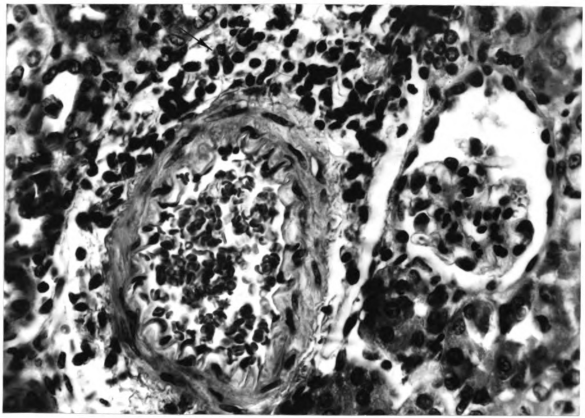

Fig. 11. --Kidney at day 7 demonstrating perivascular lymphocytic infiltration. x720.

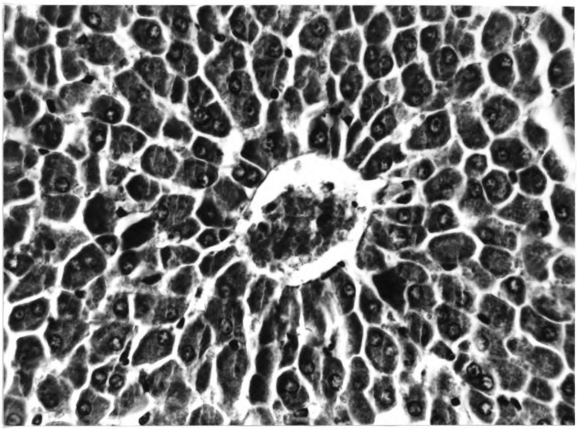

Fig. 12. --Liver at day 7 demonstrating hepatic cell individualization (low dose). x720.

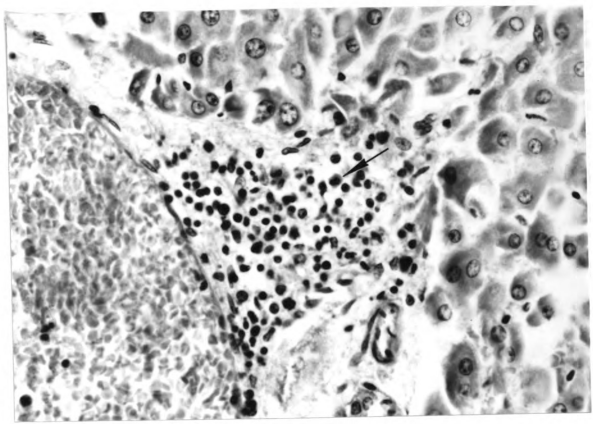

Fig. 13. --Liver at day 7 demonstrating perivascular lymphocytic infiltration in the area of portal triads. x720.

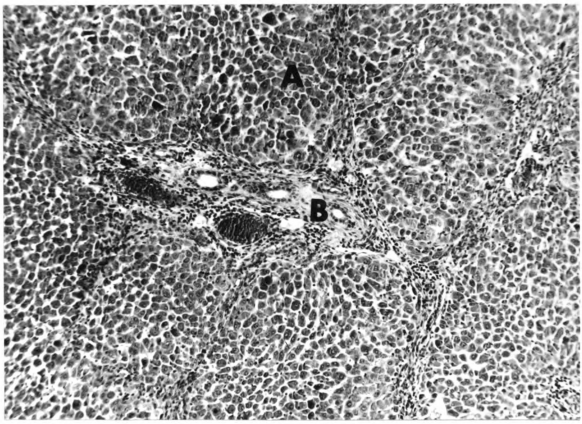

Fig. 14.--Liver at day 9. A. Hepatic cell individualization. B. Proliferation of connective tissue in areas of portal triads. x250.

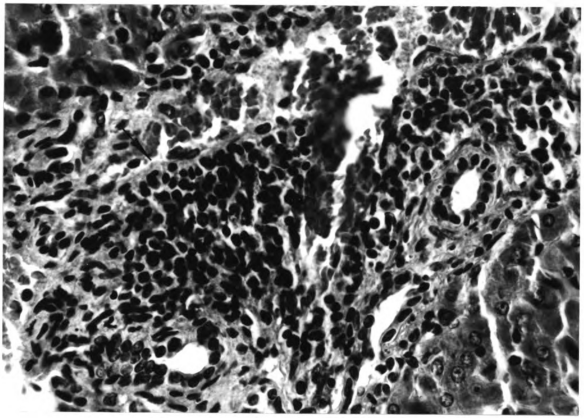

Fig. 15. --Liver at day 9 demonstrating lymphocytic infiltration in area of portal triad. x720.

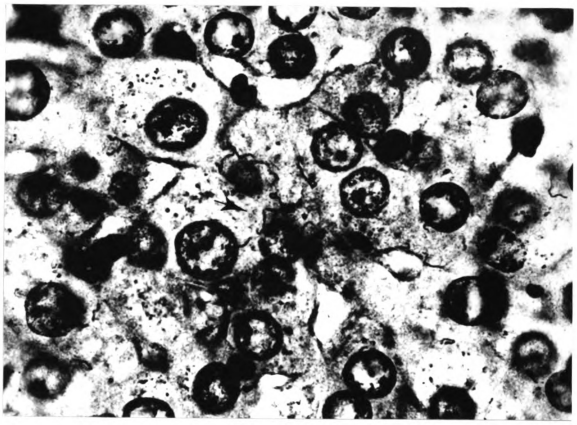

Fig. 16.--Liver at day 8 demonstrating leptospirae. x1, 150.

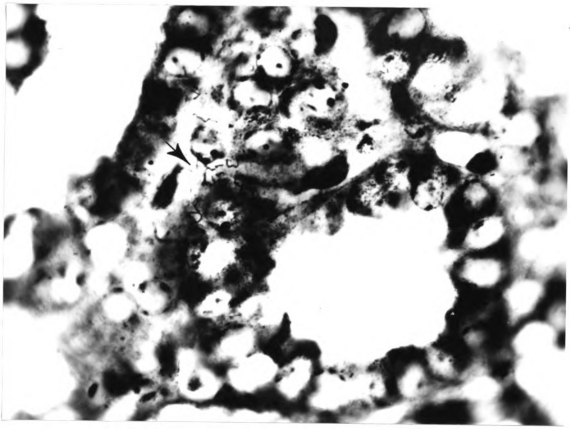

Fig. 17.--Kidney at day 9 demonstrating leptospirae. x1,150.

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