EXPERIMENTAL LEPTOSPIROSIS: PATHOLOGY OF LEPTOSPIRA POMONA INFECTION IN MALE CATTLE

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ABSTRACT

EXPERIMENTAL LEPTOSPIROSIS: PATHOLOGY OF

LEPTOSPIRA POMONA INFECTION

IN MALE CATTLE

by Osman Abd-Elaziz Atallah

An experiment using ten male cattle was conducted to study experimental Leptospira pomona infection. Inoculums in one or more animals consisted of leptospiremic guinea pig, hamster or cattle blood. In one animal bovine urine obtained during leptospiruria was used. The subcutaneous route of inoculation was used in all animals while two bulls were simultaneously inoculated intraperitoneally. In addition to gross and microscopic pathological studies; clinical, serological, hematological and bacteriological observations were recorded.

Clinically, fever was observed for periods ranging from two to seven days. Temporary anorexia was seen in only one animal during the thermal reaction. Leptospiremia in two animals was detected on the fourth, fifth and/or sixth day after inoculation. Leptospiruria in four animals started between the fourteenth and twenty-third day

after inoculation and continued until at least the thirty-eighth day after inoculation. Initial serological responses occurred between the fifth and the twelfth day after inoculation. Maximum titers observed ranged from 10^2 to 10^8 using the microscopic agglutination-lysis test. The hemoglobin, packed cell volume, erythrocyte and leukocyte values were slightly decreased in all animals except one. The relative lymphocytic and monocytic counts were slightly raised.

Grossly, the renal surface in three animals had variable numbers of small white foci. These foci extended into the renal cortex and medulla. The renal lymph nodes of one animal were enlarged and edematous.

Microscopic changes were demonstrated in particular locations of the genital tract (testes, rete testes, epididymides, seminal vesicles and prostate gland). These lesions were focal and diffuse interstitial cellular (mostly lymphocytes) infiltration of variable extent. Similar microscopic lesions were seen in the kidneys (particularly in the cortex), liver and heart. These involved areas showed vacuolar degeneration in the renal and seminiferous tubules, necrosis in the liver and an increased number of Anitschkow myocytes in the myocardium.

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INTRODUCTION

Leptospira pomona infection has been shown to be a major cause of abortion in pregnant female cattle (2, 6, 10, 16, 17, 21). Due to the gradual replacement of natural breeding with artificial insemination in the past several years and the great importance of the bull in this respect, leptospirosis in male cattle was selected for experimental study.

The purpose of this present study was to investigate the clinical, hematological, serological and pathological effects of *L. pomona* infection in bulls with particular emphasis on the pathological aspect.

REVIEW OF THE LITERATURE

Pathogenic leptospires were first seen in New Orleans by Stimson (55) in 1907 in sections of kidney from a human patient who was believed to have died of yellow fever. Michin and Ažinov (42) in 1935 in North Caucasus observed spirochetal forms in eight cultures made from the heart blood, liver and spleen of a calf which had died of icterohemoglobinemia. They injected this culture into guinea pigs and white mice. These animals died on the seventh or eighth day after injection. Clayton and Derrick (12) in 1936 isolated leptospires from a dairy farmer near Pomona in Australia. Jungherr (30) in 1944 described the pathologic changes in three fatal bovine cases in which he demonstrated leptospires in Levaditi-stained kidney sections. This was the first microscopic demonstration of the leptospiral organism in cattle in the United States. Marsh (35) in 1945 reported 25 fatal cases in calves six to ten weeks old. Leptospires were demonstrated in Levaditi-stained renal and hepatic sections. Baker and Little (2) in 1948 were the first to isolate the causative agent of leptospirosis in cattle in the United States. They injected abnormal milk into seven-day-old chick embryos, guinea pigs

and cultural media and obtained positive results.

Leptospirosis has been reported in most areas of the United States (2, 5, 8, 11, 25, 29, 35, 48, 52, 55, 59).

Many investigators reported that leptospiral infection may be transmitted through urine (2, 4, 20, 21, 28, 45, 47, 61), semen (53), blood (2, 3, 36, 45) and abnormal milk (2) from carrier animals. Contaminated water (22) and food (28) were believed to play a role in the transmission. Some arthropods (Ornithodoros turicala) may (2, 9) or may not (57) transmit the disease.

Many natural reservoirs for leptospires have been found, including the jackal (64), rat (28), opossum (49), brown rat (43), cottontail rabbit, marsh rabbit, red fox, gray fox, dog, raccoon, striped skunk, spotted skunk, otter, wildcat and house cat (38).

The route of infection was suggested to be the mucous membranes and/or the abraded skin (20, 62, 47). Experimental subcutaneous injection was more effective than intranasal inoculation while feeding did not transmit the disease (2, 3, 36, 45).

A wide variety of clinical symptoms was observed in leptospiral infected cattle with calves showing more severe symptoms than adults. Calves showed a rapid course in fatal cases,

a high mortality rate and evidences of hemolytic anemia such as icterohemoglobinemia, jaundice and hemoglobinuria (2, 3, 15, 28, 35, 42, 51, 63, 64). The thermal reaction was subnormal, normal or above normal (2, 10, 63). Several other nonspecific symptoms such as weakness, anorexia, stunted growth, dullness, bumping pulsation of the heart, labored respiration, depression, weight loss and difficulty in standing were reported in calves (6, 15, 28, 59, 64). Leptospiruria started during the second or third week after inoculation (19, 26) and continued for a few months (30).

The clinical symptoms in adult cattle were generally mild but some showed signs of hemolytic anemia such as hemoglobinuria (2, 34, 37, 50) and icterus (37). Abortion in the last trimester of pregnancy and lowered milk production caused major economic losses (2, 6, 10, 17, 16, 21, 26, 27, 37, 60, 41, 44, 50, 52). Leptospiremia on the fourth and fifth day after inoculation (10, 18, 19) and leptospiral antibodies in the blood serum on the sixth day after inoculation have been demonstrated (10, 42, 61, 64). Other symptoms reported included pale color of the teats (37), fever (10, 16, 19, 29, 34, 37, 41, 44, 61), normal

(52) or subnormal (29) temperature, anorexia (10, 16, 46), evidence of pulmonary congestion (29), depression, tachycardia (44), convulsions and rarely death (19, 37).

Some workers reported breeding difficulties (50) while others found no statistically significant change in the reproductive efficiency of cattle (33).

In one experimental group of heifers, hemoglobinuria, albuminuria or leptospiruria could not be demonstrated. The heifers showed retained placenta, photophobia, conjunctival hyperemia and mucopurulent discharge from the eyes (18).

Naturally infected steers showed fever (34), hemoglobinuria (34), enlarged superficial lymph nodes, diarrhea, anorexia, rough hair coat, high morbidity and low mortality (46). One steer was unable to stand and died (37). Ramirez et al. (46) showed that 88 percent of one group of steers had a positive serum titer against L. hardjo while only 7 percent reacted with L. pomona antigen. No leptospires were isolated or demonstrated from these steers.

Work with leptospirosis in bulls has not been reported frequently in the literature. Some experimental investigators in this field observed fever or subnormal temperature,

leptospiremia, weak rapid pulse, rapid respiration, hemoglobinuria and rarely death (19). In experimentally infected six-months to one-year-old bulls, symptoms included a slight fever on the day of inoculation, fever of three days duration after inoculation, leptospiremia on the first and fourth day and leptospiruria 21 days after inoculation. A maximum antibody titer of 1:6,400 was recorded on the 26th day after inoculation in a two-month-old calf (26).

Pathological findings associated with this disease in the bovine are of wide variation. Generally the lesions reported in the literature were mostly attributed to damages of the capillaries, small veins and parenchymatous tissues especially of the kidney and liver (15). Some of these findings were hydrothorax, hydroperitoneum, hydropericardium, subserous and subcutaneous hemorrhages, reddish tinted fibrin on the diaphragm, patches of congestion in the abomasum and duodenum and a thickened and congested wall of the gall bladder (17, 30, 50). There was evidence of jaundice in the aorta, tendons and connective tissue (1, 14, 15, 28). A marked yellow gelatinous edema was reported in the mediastinum just dorsal to the sternum (28).

The renal lesions have been considered to be highly significant in the pathology of leptospirosis. Grossly, the kidney was enlarged (15), turgid with indistinct lobulation (61), dark in color (14, 1) or pale with reddish-brown spots (26) or white spots (2, 15, 28) on the cortical surface. These spots extended into the cortex and occasionally reached the medulla (24, 29, 44, 46, 52, 61). Te Punga (61) described the renal cut surface as showing considerable exudate. The renal pelvis was either empty or contained yellowish sandy material and several brownish calculi in some of the renal calyces (24).

Microscopically, the kidneys were reported to have edema (15) and cellular infiltration (mostly lymphocytes and monocytes mixed with fewer neutrophils and eosinophils) in the interstitial tissue of the cortex, corticomedullary junction and medulla and in the lumens of the affected tubules (2, 3, 14, 15, 24, 52). The renal tubules were destroyed in the area of the cellular infiltration (1, 2, 14). There were hemorrhages around the arcuate and interlobular blood vessels and blood pigments in the superficial tubules (15). The renal epithelium of the proximal convoluted tubules appeared as solid masses with intracellular hyaline droplets (15) or swollen with finely vacuolated

cytoplasm which contained hemosiderin and no clear nucleus (24). In other instances, the renal epithelium showed hypertrophy (52), vacuolation (19, 29), atrophy (52, 51) or necrotic changes (19, 24, 29, 52). Tubular basement membranes were thickened (24, 52) or absent (19). A few renal epithelial cells were replaced by masses similar to giant cells of the Langhan's type (24, 61). There were degenerative changes (24, 46, 52) or discrete fibrosis (57). The tubular lumens contained protein precipitate (2), granular debris (14), strongly eosinophilic hyaline, hematin or cellular casts (4, 7, 15, 19, 24, 29, 61), leptospire-like structures (1, 14, 15), inflammatory cells and desquamated necrotic epithelium (24, 52). The tubular lumens were dilated and lined with a thin layer of epithelium (24, 61). The renal medulla was either normal (2, 3) or had alternate pale and dark striations (61), swollen tubular epithelium and/or calcium in the renal papillae and interstitial tissue (24). Glomerular changes, though not extreme, were reported. These included thrombi in the glomerular capillaries (15) and increased cellularity (24, 61) and congestion (29) of the capillary tufts to completely fill the corpuscular cavities (15). The glomerular tufts showed various stages of degeneration (19, 29).

Bowman's capsules were thickened (24, 29, 46, 50, 61). There was thickening and metaplasia of the corpuscular parietal cells (29, 61). Eosinophilic granular material was reported within Bowman's capsule (19,29). Hemoglobinuria was reported in some cases (1, 14, 15).

Macroscopically, the liver was enlarged (1, 14, 15, 61) with distinct lobulation (61), friable (1, 14) and reddish-brown, yellowish-tan or orange in color (14). The liver had necrotic areas especially in the caudate lobe (61).

Microscopically, the hepatic cells were distorted (15, 28), showing finely granular (24) or fragmented and lysed (15) cytoplasm which contained brown pigments (14) and leptospires (1, 14, 15). There was centrolobular necrosis (14, 19, 29) and sinusoids distended with blood in the necrotic areas (15, 24). The interlobular connective tissue and the portal tracts contained cellular infiltrates including lymphocytes (15, 28), histiocytes, plasma cells, a few neutrophils (14, 15, 24, 28) and excess fibrous tissue (29, 61). Hadlow et al. (25) reported a small thrombus in one interlobular vein, hydropic degeneration of the hepatic cells and a mild fatty change but no leptospires were seen in silver-impregnated sections. Bile stasis

was reported in the canaliculi and interlobular ducts (14).

The gall bladder was distended (1, 61) with bile that was usually thick, granular and of yellow color (14). It had a thickened congested wall (61).

The spleen showed engorged red pulp (14, 24, 32) and some hemorrhages (24, 32), scattered neutrophils (14, 24, 32), megakaryocytes (around the follicles) 24, 32), plasma cells, eosinophils (24, 29), and macrophages engulfing erythrocytes (14) and iron pigments (24, 29). The splenic follicles were inactive (14, 24, 29).

The lymph nodes were edematous (14), hyperemic (14), juicy and of pinkish or red color (1, 14). Their sinusoids contained erythrocytes, neutrophils (14) and leptospire-like structures especially in the mesenteric lymph nodes (1, 29). Jungherr (26) observed lymphoid necrobiosis.

The lungs have been described as partially collapsed (14), with atelectasis (24), subpleural hemorrhage (1), and pulmonary adhesions (29) on the apical lobes. Microscopically, there were alveloar emphysema (14) and edema (1, 14,) thickened alveolar walls (24), some fibrin (14, 29) and a few neutrophils (14), histiocytes and lymphocytes (24). Leptospires were found (1, 14) in the pulmonary tissues.

Lesions reported in the heart included a pale, friable and flabby myocardium (1), and petechial hemorrhages in the epicardium (14). Microscopically, the cardiac muscle showed degenerative changes (1, 61), diffuse areas of fatty infiltration (61), and leptospires in silver-stained sections (1).

Hadlow et al. (24) observed some macrophages loaded with hemosiderin in the wall of the uterus.

The fetal membranes from abortion cases were light brown (17, 16, 61) and translucent (16, 61) with uniformly edematous (17, 16, 40, 61) intercotyledonary areas. The cotyledons were leathery (61), harsh to touch, dry (61), or moist, atonic (17) and light fawn in color (60).

Morter et al. (44) reported the pathological changes in the placenta after various number of days following experimental L. pomona inoculation of pregnant heifers. Ten days after inoculation, there were hemorrhagic areas and hemolysis in the cotyledons. There were vascular and necrotic changes in the chorionic maternal junction 25-28 days after inoculation. By 47 days after inoculation, the fetal villi were absent and the normal relationship between the fetal and maternal tissue was absent.

Te Punga et al. (61) in 1953 reported a dry and leathery vaginal wall with concentric and longitudinal striations mostly at the cervical end. They also found an edematous wall of the urinary bladder with congested small blood vessels in the mucosal surface. Awrorow et al. (1) and Baker et al. (2) found red-colored urine in the urinary bladder.

In a thorough review of the literature only Awrorow (1) observed necrotic areas in the muzzle, gums, tongue and skin around the eye. He also reported catarrhal gastritis and edema in the subcutaneous and subserous tissues.

The pathological findings in the aborted fetuses were nearly the same as the general lesions mentioned above except for the fact that the fetuses were usually aborted 24 hours after death (16, 61). This fact altered the pathological picture due to advanced postmortem changes. The adrenal glands of the aborted fetuses were congested, enlarged and abnormally colored (61).

No lesions have been reported in the genital system of male cattle.

MATERIALS AND METHODS

Nine healthy bulls were inoculated with Leptospira pomona (strain Ohio) and one bull was used as a control. Three of the animals were not inoculated by the author, but histological sections and experimental data from these three animals were evaluated and included in the results of this work. The microscopic agglutination-lysis test (43) showed that all animals were serologically negative for leptospiral antibodies prior to inoculation. The procedures for inoculation and the necropsy schedule are outlined in Table 1.

Four methods were used for determining leptospiremia and leptospiruria in the experimental animals:

1. Guinea pig inoculation: Weanling guinea pigs were inoculated intraperitoneally with two ml. of diluted urine (one volume centrifuged urine added to one volume buffered saline) at three-day intervals starting from the second week after inoculation until the time of necropsy. At necropsy of each bull, pieces (about one cc.) of kidney, spleen and liver were pooled and homogenized in buffered normal saline to make a 10 percent final suspension. Two ml. of this suspension were inoculated intraperitoneally into each of four weanling guinea pigs. All guinea pigs

were checked twice (at two and three weeks after inoculation) by the microscopic agglutination-lysis test for antibody production against L. pomona. The bovine blood, urine or tissue suspension was considered to have contained leptospires if the serum from the corresponding guinea pigs contained antibody for L. pomona at a dilution of 10^2 or higher at two to three weeks after inoculation.

2. Dark-field examination: Daily examination of the urine and blood serum was done.

3. Medium inoculation: Four tubes of Stuart's (Difco) media enriched with 10 percent rabbit serum were each inoculated daily with three drops of blood from the experimental cattle starting with the first day of inoculation and continuing for 14 days. These tubes were incubated at 30° C. and checked weekly for leptospires by dark-field microscopy. They were not discarded until the sixth week.

4. Serological tests: The microscopic agglutination-lysis test was run on the bovine serum collected daily starting with the first day of inoculation. The antigen used was living L. pomona (strain Johnson) incubated at 30° C. for five to seven days in liquid Stuart's medium enriched with 10 percent rabbit serum.

Blood was collected daily from the jugular vein for at least a week before inoculation to establish the normal erythrocyte, leucocyte and differential leucocyte, hemoglobin and packed cell volume values (13). The nonprotein nitrogen values were determined once a week (31, 32, 63). The forementioned blood study was continued after inoculation until the necropsy of the cattle.

Urine was collected weekly to check for albumin, blood, sugar, bile pigment, specific gravity, pH., ketone bodies and sediment.

Rectal temperatures were recorded daily.

The male cattle received 10-20 ml. chlorpromazine hyrochloride (25 mg. per cc. with benzyl alcohol 2 percent preservative), followed by 100-200 ml. pentobarbital-sodium (one grain per cc. in 10 percent propylene glycol and water) prior to necropsy.

At the time of necropsy, tissues were saved from the kidneys, liver, spleen, eyes, adrenal glands, renal lymph nodes, testes, epididymides, prostate gland, urethra, urinary bladder, cardiac muscle, skeletal muscle, brain, spinal cord, thyroid gland and pituitary gland from each bull. These collected tissues were immediately placed in one or more of the following fixatives: Zenker's fluid,

10 percent neutral formalin solution, Carnoy's fluid and Bouin's fluid (41).

The following staining methods were used: hematoxylin and eosin for general characteristics, Sudan IV and Oil-Red-O for fat, Best's carmine stain for glycogen and Warthin-Starry method for leptospires (41).

This experiment was also designed to study semen from male cattle infected with L. pomona. A Plectron electric ejaculator was used to collect semen from bulls number I and 298. Semen tests were run for volume, color, sperm concentration, motility and morphology, live-dead count and the presence of L. pomona. However, animals were very sensitive to the stimulation and often went down in spite of reasonable means of restraint. Many of the semen samples were highly contaminated with urine and there was considerable struggle to obtain even these contaminated samples. For these reasons, this phase of the investigation was discontinued.

EXPERIMENTAL RESULTS

The clinical, hematological, serological and pathological findings in the animals experimentally inoculated with L. pomona are reported with major emphasis on the pathological aspect.

Clinical: All the animals showed good appetite and condition during the course of the experiment. The average daily temperatures are recorded in Table II. The temperature range for the control animal was 100.1° to 102.0°F. Five animals had fevers of variable duration and with peaks ranging from 103.0° F. to 106.4° F. Two animals had normal temperatures (2647, I) while one had a subnormal temperature of 96.0° F. on intermittent days (3247) (Table II). The fever was generally observed between the fourth and ninth days after inoculation.

The urine analyses for albumin, blood, pH, sugar, specific gravity, bile pigment, ketone bodies and sediment showed no deviation from the normal values. Leptospires could be demonstrated by dark-field microscopy in four animals (3108, 3514, 3249, 3425) but were not seen in the others. Leptospiruria started between the 14th and the 23rd day after inoculation and continued until at least the 38th

day after inoculation in those animals in which it was observed. Urine from animals (3425, 3514) was tested for the presence of antibody against L. pomona and was found negative for 60 days after inoculation. Guinea pigs inoculated with the saline tissue suspension from the bovine kidney, spleen and liver (298,2647, 3247, 3268, 3249) failed to show any antibody response against L. pomona.

Serological: Serum-antibody response against L. pomona was recognized (in the infected animals) on the 5th to the 12th day after inoculation. Maximum titers against L. pomona ranged from 10^2 to 10^8 (Table III). Two animals' (I, II) sera were negative for leptospiral antibodies throughout the test period.

Hematological: Table IV shows the average ($\bar{x}$) and the standard deviation ($\hat{\sigma}$) of hemoglobin, packed-cell volume, erythrocytes, leukocytes and blood nonprotein nitrogen (NPN) values before and after inoculation. The postinoculation values for the hemoglobin, packed cell volume, erythrocytes and leucocytes showed a slight persistent fall in all the animals except bull 3247 which had a slight rise in the values for hemoglobin and packed cell volume while the erythrocyte values remained the same before and after inoculation. The blood nonprotein nitrogen showed no deviation from normality.

Table V represents the arithmetic mean ($\bar{x}$) and the standard deviation ($\hat{\sigma}$) of the differential leukocyte counts before and after inoculation in the bulls. The post-inoculation absolute lymphocyte counts were raised in four animals (2647, I, 3514, 3248). The lymphocyte count went down in three animals (298, 3247, 3108). The values for the segmented neutrophils were slightly decreased in five bulls (I, II, 3425, 3514, 3248) and slightly elevated in four. The post-inoculation monocyte counts were slightly raised in six animals while three animals had a slight fall (298, II, 3514). The post-inoculation eosinophil counts fell in seven animals and were slightly raised in the other two (2647, I).

Leptospiremia was demonstrated in two animals (298, 3108). It started on the fourth, fifth and/or sixth day after inoculation. Its duration ranged up to at least two days. No leptospires could be isolated from the blood of the other animals.

Pathological: The renal surface of the calf had innumerable white small foci. These foci extended through the renal cortex and into the medulla. Two bulls (3425, 3514) had numerous whitish lesions similar to those found in the calf. The renal lymph nodes of the calf were

enlarged and edematous. The myocardium appeared flabby in one animal (3249). No other significant gross lesions were observed.

Table VI summarizes the histopathological findings. Generally, the kidneys had interstitial cellular infiltration mostly with lymphocytes, some plasma cells and a few neutrophils, macrophages and fibroblasts (Figs. 1, 2, 3, 4, 5, 6, and 7). Neutrophils were also seen inside the lumen of some renal tubules in the calf (Fig. 4). The tubules were distended with neutrophils. A few segments of the renal tubules in the area of the cellular infiltration lost their normal architecture and resembled syncytial giant cells (Figs. 1 and 2). Some of the Bowman's capsules around and in the area of cellular infiltration were thickened (Fig. 3). The subcapsular spaces of the renal corpuscles contained several pinkish rounded bodies varying in diameter from 10-30 μ. Several renal tubules contained similar granular pinkish material. The tunica adventitia of the arcuate artery contained degenerated collagen which appeared as homogeneous dark-pinkish foci. The renal papillae of the calf had several dark-bluish foci resembling areas of calcification. A kidney section of the control bull had a focus of lymphocytes around the arcuate artery (Fig. 8).

The livers of all the experimental cattle, including the control, had a few lymphocytes and immature fibroblasts in the interlobular connective tissue and in the areas of the portal triads (Figs. 9 and 10). The livers of two animals (3247, 3425) had a few small degenerative and necrotic foci (Figs. 11 and 12) and in some regions a hypertrophy of Kupffer cells. Collagen breakdown was evident in the interlobular connective tissue of the liver (2647, 3247, 3425).

The gall bladder of one animal (3247) had follicles of lymphocytes (Fig. 13) in the lamina propria around the crypts.

The testicular interstitial tissue in four animals (3247, 3425, 3514, 3108) had several foci of predominantly lymphocytic infiltration (Figs. 14, 16, 17, 18 and 19). In these areas tubular cells were in some instances undergoing degeneration (Figs. 15 and 16). The rete testes in two animals (3514, 3108) had a few lymphocytes in the interstitial tissue.

The intertubular connective tissue of the epididymides in two animals (3514, 3108) had lymphocytic infiltration (Fig. 19). This lesion in the calf (3108) contained both lymphocytes and neutrophils.

The seminal vesicles in two animals (3247, 3425) had extensive lymphocytic follicles in the lamina propria (Figs. 20 and 21).

The connective tissue between the acini of the prostate gland of the calf had lymphocytes, eosinophils and collagen breakdown. Another animal (298) had only lymphocytic infiltration in the interstitial tissue of the prostate gland.

Microscopically the hearts of two animals (2647, 3249) had epicardial cellular infiltrations of mostly eosinophils and a few lymphocytes. Animals 3425 and 3249 had a few lymphocytes among the myocardial fibers. One animal (3247) had increased numbers of Anitschkow myocytes in the myocardium.

The spleen was congested in all the animals including the control. The splenic red pulp contained numerous macrophages (3247, 3249), plasma cells (3249), eosinophils (3248, 3247) a few megakaryocytes and excess brownish pigments (298, 3249) both free and within the macrophages. There was also some edema (3425).

The cerebrum in one animal (298) showed focal areas of malacia in the cortex.

The adrenal glands in two animals (2647, 3514) had a few lymphocytes and eosinophils in the zona glomerulosa and fasciculata (Figs. 22, 23, 24 and 25). There was early connective tissue proliferation in the capsule of the adrenal gland in one animal (3249).

The lymph nodes of five animals (3248, 2647, 3247, 3514, 3249) had eosinophilic infiltration both in the capsule and the pulp (Fig. 26). Edema and plasma cells were demonstrated in the lymph nodes of the calf (3108) (Fig. 27). The lymph nodes of only one animal (3247) had megakaryocytes (Fig. 28) and erythropoietic foci. Dark bluish foci resembling areas of calcification were in the lymph nodes of two animals (2647, 3108). Brownish pigments were seen in the lymph nodes of three animals (2647, 3247, 3249). Collagen breakdown, fibrin and neutrophils were observed in the lymph nodes of animal 2647.

No microscopic lesions were demonstrated in the vas deferens, bulbourethral glands, urethra, penis, urinary bladder, aorta, eyes, thalamus, brain stem, medulla oblongata, spinal cord, thyroid gland or pituitary gland.

Several incidental pathological findings were observed during this experiment. These findings included ciliated

protozoa in the pulmonary alveoli and bronchioles of two animals (3248, 3249) and plant material in the alveoli and bronchioles of one bull (3249) accompanied by excessive eosinophilic infiltration in the pulmonary tissue particularly around the small arterioles.

The cardiac muscle of two animals (2647, 3249) had several sarcocysts as did the cremaster muscle in one (3247).

DISCUSSION

In a thorough review of the literature, no references to pathological changes in the genital system of bulls could be found. Tammemagi *et al.* (60) could not find lesions in the testes of boars experimentally infected with *L. pomona*. Stoenner *et al.* (58) reported orchitis during the convalescent period in humans naturally-infected with *L. ballum*. The present work demonstrated that the testes, rete testes, epididymides, seminal vesicles and prostate gland had microscopic lesions due to *L. pomona* infection.

The testes of the calf had only a few lymphocytes infiltrating between the seminiferous tubules (Fig. 14). The testes of three adult animals (3247, 3425, 3514) had mild to extensive intertubular lymphocytic infiltrations (Fig. 17). Several seminiferous tubules, in the areas of lymphocytic infiltrations, were undergoing vacuolization (Fig. 16). In analyzing these lesions, it seemed that the testicular lesions of the adult animals were more extensive than those seen in the calf. No clear-cut conclusion could be established because of the number of the animals used in the experiment. However, the factors which differentiate the adult animal from the immature one could

have some relation to the extent of the pathological lesions seen in the testes. No leptospires could be demonstrated in silver-stained testicular sections but leptospires were isolated from the semen of two animals (3514, 3425). Leptospires were isolated from the semen beginning on the 15th to the 18th day after inoculation and continuing at least until the 38th day after inoculation. Leptospires isolated from the semen are most likely contaminants from the urine but the occurrence of testicular lesions suggested the possibility of the presence of leptospires without urine contamination. That the leptospires in the semen of shedding animals are virulent enough to produce the disease in cows served by these animals was shown by Sleight et al. (53). They demonstrated the possibility of spread of L. pomona among cattle by shedder bulls through either natural coitus or artificial insemination. The significance of the previously mentioned testicular lesions needs more study.

The importance of the epididymis in the bovine genital tract is well established. In two of the animals (3514, 3108), there were a few scattered foci of lymphocytes in the interstitial tissue as shown in Fig. 19.

It is interesting to note that the two animals which had this particular lesion were also the youngest (one year and eight weeks respectively) of all the experimental animals. The scope of this work did not cover the effect of these epididymal lesions on the semen.

The seminal vesicles of two animals (3247, 3425) had lymphocytes in an arrangement resembling a follicle in the lamina propria (Figs. 21 and 22). This lymphocytic tissue was not seen inside the crypts. Whether this lesion was due to infection with L. pomona or whether this was an aberrant lymph follicle could not be determined. The effect of this tissue on the physiology of the seminal vesicle is not known.

The prostate gland of two animals (298, 3514) had a few lymphocytes, eosinophils and collagen breakdown.

These lesions in both the bovine male genital tract and accessory sex organs point up the need for more work in this area.

No leptospires could be demonstrated in silver-impregnated sections from the genital system or in sections from the other tissues. This failure to demonstrate leptospires could be due to technical difficulties in the staining procedure.

The pathological changes seen in the other organs were, for the most part, as reported by previous workers.

Macroscopically, lesions similar to the small white foci on the renal surface have been described by several investigators (2, 15, 28).

Microscopically, intertubular and periglomerular lymphocytic infiltration was present in the renal cortex and sometimes in the medulla. This finding is in agreement with the results of many other workers (2, 3, 14, 15, 24, 52). Only the calf had neutrophils distending the renal tubules. Many neutrophils were mixed with lymphocytes and a few eosinophils between the renal tubules. This type of cellular response was earlier reported by several authors (2, 3, 14, 15, 24, 52). The neutrophilic response of the calf could be a manifestation of a more severe inflammatory response in younger animals. Renal tubules in the area of the extensive cellular infiltration had degenerative changes and atrophy similar to that reported by earlier workers (1, 2, 14). In the middle of the cellular infiltration, there were several syncytial giant cells (Figs. 1, 2) like those described by Hadlow et al. (24) and Te Pungâ et al. (61). These giant cells could be the remnants of destroyed renal tubules which coalesced to resemble

giant cells. Early fibrosis around the areas of cellular infiltration is in agreement with Ramirez et al. (46). Several renal tubules and Bowman's capsules contained granular pinkish material. This could be unabsorbed plasma protein in the glomerular filtrate (23). Similar granular material was reported by Baker et al. (2) inside the lumen of the renal tubules and Bowman's capsule. Leptospire-like structures in the renal tubules (1, 14, 15) could not be demonstrated in this present work. A few Bowman's capsules in the areas of cellular infiltration had hyalinized thickened walls (Fig. 3). This is in agreement with several reports (24, 30, 50, 51, 56). Several Bowman's capsules were indistinct while the glomeruli showed increased cellularity similar to the description by Hadlow et al. (24) and Te Pungâ (61). These changes made the glomeruli appear as collections of dark nuclei among the renal tubules. The renal papillae of the calf had a few dark-bluish focal areas. These are probably similar to areas of calcification reported by Dodd in leptospiral-infected calves (15). The kidney of the control animal had a focus of lymphocytic infiltration beside the arcuate artery (Fig. 8). Whether this was normal lymphocytic tissue or whether an incidental

infection may have caused this lesion could not be determined.

Macroscopically the liver was unchanged. Several investigators reported enlargement (1, 14, 15, 60), friability (1, 14), reddish-brown to distinct yellowish color (14) and necrotic areas particularly in the caudate lobes (59) related to acute leptospirosis.

Microscopically, the liver of all animals had a few lymphocytes and early connective tissue proliferation in the areas of the hepatic trinity and interlobular connective tissue (Figs. 9 and 10). Similar lesions were earlier reported in several publications (15, 28). The finding of this lesion in all bulls, including the control, tends to minimize the relationship of this lesion to the experimental infection. There were small foci of degeneration and necrosis in the hepatic parenchyma (Figs. 11 and 12). The liver in the present work did not show distorted, lysed or fragmented hepatic cells, leptospires inside the cells or sinusoids distended with blood in the necrotic areas as were reported by several investigators (15, 24, 28) who were dealing with acute cases of the disease.

Microscopically the gall bladder in one bull had lymphocytes in a follicular type arrangement in the lamina propria particularly around the crypts (Fig. 13). This finding could be normal according to Bailey (1958), who reported lymph nodules in the wall of the human gall bladder.

The spleen was enlarged in all the animals. This was most likely due to the anesthesia used at the time of necropsy (54) and less likely due to leptospirosis. Several reports on acute bovine leptospirosis described engorged red pulp of the spleen (14, 24, 29).

Microscopically, the spleen contained excessive numbers of macrophages, plasma cells and eosinophils plus a few megakaryocytes. Considerable brownish pigment was both free and within the macrophages. Similar lesions were observed by earlier workers (14, 24, 29).

The lymph nodes did not show definite gross lesions. Some previous reports indicated edema, hyperemia and pinkish or red color in cases of acute bovine leptospirosis (1, 14).

Microscopically, some of the lymph nodes had various numbers of eosinophils, plasma cells, macrophages, megakaryocytes and neutrophils. These microscopic findings

concur with the results in an earlier report (14). Several workers (11, 26, 29) observed leptospire-like structures and lymphoid necrobiosis in the lymph nodes that could not be seen in the present experiment. These lymph nodes were edematous and had bluish foci similar to areas of calcification. These bluish areas may have been artifacts since special stains for calcium were not run. Brownish pigments and a few erythropoietic centers were very likely related to hemolytic changes.

The lungs did not show any gross pathological alterations. A few earlier reports included partial collapse of the lung (14, 24), subpleural hemorrhage (1) and pulmonary adhesions on the apical lobe.

No specific microscopic lesions were present in lung sections examined. Other reports described alveolar emphysema (14), edema (1, 14), thickened alveolar walls (25), little fibrin (14, 29), some neutrophils (14), histiocytes, lymphocytes (24) and leptospires (1, 14) in the pulmonary tissue.

Grossly, the heart of one animal was flabby with no other macroscopic lesions in the other bulls. Previous work by other investigators demonstrated friable, pale and flabby myocardium (1) and petechial hemorrhages in

the epicardium (14). Microscopically the hearts of three animals (2647, 3514, 3249) had a few eosinophils and lymphocytes particularly in the epicardium. Other previous reports indicated myocardial degeneration, diffuse areas of fatty infiltration and leptospires in silver-stained sections of the myocardium (1, 60).

Two animals (I, II) did not become infected as indicated by the negative agglutination-lysis tests (Table III). Leptospires were isolated from the inoculum used in the attempt to transmit the disease to the bulls. The reason why these animals did not become infected could be due to one or more of the following factors:

1. As reported by Hamdy et al. (26) the bulls may have obtained immunity from their dams which were previously infected.

2. The bulls may have had a high natural resistance to the infection.

3. Unknown nutritional factors may have inhibited infection.

4. Some virulence factors of the organism for the host may have been absent at the time of inoculation.

These two bulls were not necropsied because they were uninfected.

Clinically, four infected animals (298, 3108, 3514, 3425) had fevers ranging from 102.5° F. to 106.4° F. and varying in duration from one day to four days. The start of this fever reaction varied from the third to the seventh day after inoculation. One animal (2047) did not show fever and animals 3247 had a subnormal temperature (96° F.) as shown in Table II. These variations in the degree of pyrexia, its day of start, duration and presence or absence may be related to the virulence of the infective organism and the resistance of the host. As reported previously (2, 3, 15, 62), immature animals seem to be more susceptible to infection. The calf (3108) had the highest temperature (106.6° F.) with a long duration (four days). Of interest was the apparent relationship of route of inoculation to response. Bulls 3514 and 3425 were inoculated by both the intraperitoneal and subcutaneous routes. These animals had higher fevers (103.0 and 105.0° F.) than bulls 2647 and 3247 which were inoculated subcutaneously only.

The appetite and general condition of the animals were generally good during the course of the experiment. One

animal (3108) had depressed appetite during pyrexia. These findings are in concurrence with the experimental work done by Hamdy and Ferguson (26).

Leptospiruria was recognized in four animals (3108, 3514, 3425, 3249). Leptospires were seen in the urine of the calf starting from the 23rd day after inoculation. This leptospiruria concurred with previous reports (19, 26) although, Fennestad et al. (18) could not demonstrate leptospires in the urine of 21 experimentally infected heifers. The inability to demonstrate leptospires in the urine of the infected bulls (298, 2647, 3247) could be due to the early appearance of leptospiral antibody in the urine but is more likely due to the failure of the organism to establish residence in the kidneys.

Leptospiremia was detected in two animals (298, 3108). In one animal (298) leptospires were isolated from the blood collected on the morning of the fourth day after inoculation. No leptospires could be isolated from a blood sample collected in the evening of the same day or thereafter. This was the first day of fever (103.2° F.) for this animal. Leptospires were isolated from the calf blood on the fifth and sixth days after inoculation. Several other workers observed leptospiremia on the fourth

to eighth day after inoculation (19, 19).

Sera from seven animals (Table III) had positive microscopic agglutination-lysis test results with titers ranging from 10^1 to 10^8. The antibody responses started between the 5th and 12th days after inoculation. Similar data on leptospiral antibody response have been reported previously (10, 41, 50).

The blood picture in general showed a slight decline in the hemoglobin concentration, packed-cell volume, erythrocytes, leukocytes (neutrophils and eosinophils) while both the lymphocytic and monocytic values were raised as shown in Table V. This rise in the mononuclears in the blood picture went hand in hand with the mononuclear infiltration in various organs. The decline in the numbers of erythrocytes could be associated with the excessive brownish pigments in the spleen and lymph nodes which indicated hemolysis. This hemolytic effect was demonstrated in lambs by Bauer et al. (1961).

SUMMARY AND CONCLUSIONS

Experimental work was done on six male cattle and histopathological sections were analyzed from four additional bulls to investigate the pathogenesis of L. pomona infection in these animals.

The following were considered the most important findings:

1. The testes, rete testes, epididymides, seminal vesicles and prostate gland had areas of interstitial lymphocytic infiltration. This had not previously been reported.

2. The kidneys of three animals had variable numbers of small white foci on the cortical surface.

3. Microscopically, the renal lesions were similar to those previously described including particularly intertubular and periglomerular lymphocytic infiltration (in addition to a few plasma cells, neutrophils and macrophages) primarily in the cortex.

4. There were a few areas of vacuolar degeneration in the renal tubules and glomeruli particularly in areas of cellular infiltration.

5. Clinical observations of the urine and nonprotein nitrogen indicated normal renal physiology.

6. Hematological studies indicated a slight drop in the hemoglobin, erythrocytes, total leukocyte count and packed cell volume with a rise in the relative values of lymphocytes and monocytes.

7. Leptospiremia (in two animals) was demonstrated on the fourth and fifth and/or sixth days after inoculation and leptospiruria was observed in four animals starting between the 14th and 23rd days after inoculation.

8. Serological and clinical findings were much as previously reported.

9. Generally the disease did not produce any definite clinical symptoms in the adult male cattle except temporary fever. Depressed appetite was observed in one animal (3108) during pyrexia.

Table I

Material and Methods of Inoculation and Day of Necropsy

Case No.	Breed	Age (Yrs.)	Inoculation	Route of Inoc.	Necropsy Day
G 298	HF	3	13 ml. GP blood (1)	S/C	PI 17
G 2647	HF	2	12 ml. calf blood (1)	S/C	PI 24
G 3247	H	1.5	15 ml. calf urine (2)	S/C	PI 27
G 3248	HF Control	1.0	3 ml. normal hamster blood. 12 ml. normal bovine blood (62 days later)	S/C	PI 90
I	HF	2.0	8 ml. GP blood (1) (AL was negative up until PI day 25)	S/C	not done
II	HF	1.5	3.0 ml hamster blood (AL was negative up to PI day 20)	S/C	not done
G 3108*	HF	0.15	1 ml. hamster blood (1)	S/C	PI 53
G 3249*	HF	1.5	5 ml. GP blood (1)	S/C	PI 400
F 3514*	J	1.0	10 ml. GP blood (1). 15 ml. GP blood (1)	S/C IP	PI 57
F 3425*	HF	3.0	3 ml. GP blood (1). 7 ml. GP blood (1)	IP S/C	PI 75

Table I (continued)

Key for Table I:

PI = postinoculation

S/C = subcutaneously

IP = intraperitoneally

AL = microscopic agglutination-lysis test

* = animals not infected by author, but tissues and data evaluated as part of this thesis

1 = taken during leptospiremia

2 = taken during leptospiruria

H = Hereford

HF = Holstein-Friesian

J = Jersey

GP = guinea pig

Table II

Average Daily Temperature of the Experimental Bulls

	Days	Case Numbers and Temperature								
		Control 3248	G 298	G2647	G3247	I	II	G3108	F3514	F3425
Preinoc.		101.4	101.0	100.6	100.3	101.6	101.0	---	---	---
Postinoculation	1	101.4	101.5	100.6	100.2	101.6	100.8	---	---	---
	2	100.6	102.1	100.6	100.4	101.7	101.0	102.0	---	---
	3	101.6	102.1	101.4	100.0	101.4	103.0	---	---	---
	4	101.8	103.2	100.6	98.0	101.0	101.2	---	---	---
	5	101.4	102.5	101.4	100.0	101.7	102.8	104.1	100.0	---
	6	101.2	103.0	100.4	96.0	101.5	102.8	106.2	102.0	---
	7	101.0	102.7	100.7	101.0	101.8	101.4	106.4	103.0	103.0
	8	102.0	102.5	100.4	101.0	101.6	101.4	103.2	103.0	104.0
	9	100.8	103.6	100.0	100.2	102.0	101.4	102.0	102.0	105.0
	10	101.2	102.6	101.0	100.3	101.4	100.0	---	101.5	100.0
	11	101.8	102.0	101.2	101.0	101.6	101.0	---	---	---
	12	100.8	102.1	100.0	100.0	101.8	101.0	---	---	---
	13	101.8	102.0	100.4	96.0	101.6	101.6	---	---	---
	14	102.0	102.0	100.4	100.0	101.6	100.0	---	---	---
	15	100.6	101.8	100.6	100.0	101.7	---	---	---	---
	16	101.4	102.8	100.4	100.4	101.2	---	---	---	---
	17	100.4	102.0	100.4	100.3	101.0	---	---	---	---
	18	100.8	102.0	100.5	100.0	101.3	---	---	---	---
	19	100.1	---	100.5	100.1	101.6	---	---	---	---

Table III

Antibody Titers* for L. pomona in Sera of Injected Bulls

Days After Inoc.	Case Number and Titer*								
	Control	G3248	G298	G2647	G3247	I	II	G3108	F3514	F3425
1	-	-	-	-	-	-	-			
2	-	-	-	-	-	-	-			
3	-	-	-	-	-	-	-			
4	-	-	-	-	-	-	-			
5	-	-	4	-	-	-	-			
6	-	-	6	-	-	-	-			
7	-	-	5	-	-	-	-			
8	-	-	6	-	-	-	-			
9	-	-	4	-	-	-	-	5		
10	-	3	5	1	-	-			2	
11	-	5	4	2	-	-	-			
12	-	6	5	1	-	-	3			
13	-	8	6		-	-		6		
14	-	7	8		-	-	3			
15	-	8	8		-	-				
16	-	8	7		-	-		6		
17	-		7		-	-		7	7	
23	-				-	-	7			

* The titers are expressed as the exponents of the highest 10-fold serial serum dilution showing 50 percent agglutination-lysis.

Table IV

Mean ($\bar{x}$) and Standard Deviation ($\hat{\sigma}$) of Hematological Values

Case No.		Hb	Hct.	RBC	WBC	NPN
G3248 (control)	AI	$\bar{x}$ = 13.85	$\bar{x}$ = 43.14	$\bar{x}$ = 10.07	$\bar{x}$ = 8.15	$\bar{x}$ = 29.85
		$\hat{\sigma}$ = ±2.19	$\hat{\sigma}$ = ±1.264	$\hat{\sigma}$ = ±1.272	$\hat{\sigma}$ = ±1.004	$\hat{\sigma}$ = ±4.012
	PI	$\bar{x}$ = 13.18	$\bar{x}$ = 39.73	$\bar{x}$ = 7.46	$\bar{x}$ = 5.99	$\bar{x}$ = 29.07
		$\hat{\sigma}$ = ±1.072	$\hat{\sigma}$ = ±2.429	$\hat{\sigma}$ = ±2.91	$\hat{\sigma}$ = ±1.241	$\hat{\sigma}$ = ±4.449
G298	AI	$\bar{x}$ = 14.70	$\bar{x}$ = 43.50	$\bar{x}$ = 9.5	$\bar{x}$ = 6.90	$\bar{x}$ = 32.00
		$\hat{\sigma}$ = ±0.10	$\hat{\sigma}$ = ±0.7	$\hat{\sigma}$ = ±0.27	$\hat{\sigma}$ = ±0.15	$\hat{\sigma}$ = ±0
	PI	$\bar{x}$ = 12.09	$\bar{x}$ = 35.22	$\bar{x}$ = 6.55	$\bar{x}$ = 6.54	$\bar{x}$ = 37.00
		$\hat{\sigma}$ = ±2.88	$\hat{\sigma}$ = ±3.728	$\hat{\sigma}$ = ±1.236	$\hat{\sigma}$ = ±1.326	$\hat{\sigma}$ = ±11.1
G2647	AI	$\bar{x}$ = 12.7	$\bar{x}$ = 35.00	$\bar{x}$ = 10.0	$\bar{x}$ = 10.0	$\bar{x}$ = 26.00
		$\hat{\sigma}$ = ±0	$\hat{\sigma}$ = ±0	$\hat{\sigma}$ = ±0	$\hat{\sigma}$ = ±0	$\hat{\sigma}$ = ±0
	PI	$\bar{x}$ = 11.61	$\bar{x}$ = 32.92	$\bar{x}$ = 6.97	$\bar{x}$ = 9.28	$\bar{x}$ = 28.50
		$\hat{\sigma}$ = ±1.28	$\hat{\sigma}$ = ±2.837	$\hat{\sigma}$ = ±1.241	$\hat{\sigma}$ = ±1.288	$\hat{\sigma}$ = ±1.118
G3247	AI	$\bar{x}$ = 9.9	$\bar{x}$ = 30.00	$\bar{x}$ = 5.3	$\bar{x}$ = 10.05	$\bar{x}$ = 32.0
		$\hat{\sigma}$ = ±0.35	$\hat{\sigma}$ = ±0	$\hat{\sigma}$ = ±0.82	$\hat{\sigma}$ = ±0.813	$\hat{\sigma}$ = ±0
	PI	$\bar{x}$ = 10.6	$\bar{x}$ = 30.79	$\bar{x}$ = 5.3	$\bar{x}$ = 9.0	$\bar{x}$ = 32.0
		$\hat{\sigma}$ = ±0.906	$\hat{\sigma}$ = ±1.296	$\hat{\sigma}$ = ±0.85	$\hat{\sigma}$ = ±0.643	$\hat{\sigma}$ = ±0
I	AI	$\bar{x}$ = 16.92	$\bar{x}$ = 47.25	$\bar{x}$ = 9.82	$\bar{x}$ = 8.07	$\bar{x}$ = 34.8
		$\hat{\sigma}$ = ±0.92	$\hat{\sigma}$ = ±2.628	$\hat{\sigma}$ = ±0.64	$\hat{\sigma}$ = ±1.445	$\hat{\sigma}$ = ±0
	PI	$\bar{x}$ = 15.42	$\bar{x}$ = 40.00	$\bar{x}$ = 8.09	$\bar{x}$ = 6.71	$\bar{x}$ = 28.35
		$\hat{\sigma}$ = ±1.378	$\hat{\sigma}$ = ±3.800	$\hat{\sigma}$ = ±0.745	$\hat{\sigma}$ = ±0.686	$\hat{\sigma}$ = ±5.949

Table IV (continued)

Case No.		HB	Hct.	RBC	WBC	NPN
II	AI	$\bar{x}$ = 12.98	$\bar{x}$ = 39.16	$\bar{x}$ = 7.8	$\bar{x}$ = 10.4	$\bar{x}$ = 24.16
		$\hat{\sigma}$ = ±2.010	$\hat{\sigma}$ = ±1.435	$\hat{\sigma}$ = ±0.350	$\hat{\sigma}$ = ±0.430	$\hat{\sigma}$ = ±1.944
	PI	$\bar{x}$ = 11.93	$\bar{x}$ = 36.23	$\bar{x}$ = 6.9	$\bar{x}$ = 7.7	$\bar{x}$ = 30.61
		$\hat{\sigma}$ = ±1.067	$\hat{\sigma}$ = ±1.609	$\hat{\sigma}$ = ±0.460	$\hat{\sigma}$ = ±0.770	$\hat{\sigma}$ = ±2.469
F3425	AI	$\bar{x}$ = 13.62	$\bar{x}$ = 40.75	$\bar{x}$ = 9.76	$\bar{x}$ = 13.26	
		$\hat{\sigma}$ = ±0.52	$\hat{\sigma}$ = ±1.66	$\hat{\sigma}$ = ±0.48	$\hat{\sigma}$ = ±2.42	
	PI	$\bar{x}$ = 13.11	$\bar{x}$ = 38.39	$\bar{x}$ = 9.09	$\bar{x}$ = 8.05	
		$\hat{\sigma}$ = ±1.039	$\hat{\sigma}$ = ±2.55	$\hat{\sigma}$ = ±0.63	$\hat{\sigma}$ = ±1.51	
F3514	AI	$\bar{x}$ = 12.4	$\bar{x}$ = 28.0	$\bar{x}$ = .73	$\bar{x}$ = 9.05	
		$\hat{\sigma}$ = ±1.13	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±0.48	$\hat{\sigma}$ = ±0.64	
	PI	$\bar{x}$ = 11.93	$\bar{x}$ = 35.37	$\bar{x}$ = 8.48	$\bar{x}$ = 8.75	
		$\hat{\sigma}$ = ±3.07	$\hat{\sigma}$ = ±2.22	$\hat{\sigma}$ = ±1.85	$\hat{\sigma}$ = ±2.21	
G3108	AI	$\bar{x}$ = 9.35	$\bar{x}$ = 28.25	$\bar{x}$ = 9.30	$\bar{x}$ = 11.47	
		$\hat{\sigma}$ = ±0.20	$\hat{\sigma}$ = ±0.35	$\hat{\sigma}$ = ±0.43	$\hat{\sigma}$ = ±0.81	
	PI	$\bar{x}$ = 8.62	$\bar{x}$ = 26.07	$\bar{x}$ = 8.76	$\bar{x}$ = 11.36	
		$\hat{\sigma}$ = ±1.61	$\hat{\sigma}$ = ±4.23	$\hat{\sigma}$ = ±1.68	$\hat{\sigma}$ = ±3.31	

Key for Table IV:

Hb. = hemoglobin in grams per 100 ml. blood.

Hct. = packed cell volume expressed as percent.

RBC = erythrocytes in millions per cmm.

WBC = total leukocytes in thousands per cmm.

NPN = nonprotein nitrogen in mg. per 100 ml. blood.

AI = ante-inoculation.

PI = postinoculation.

Table V

Mean ($\bar{x}$) and Standard Deviation ($\hat{\sigma}$) of the Absolute Differential Leukocyte Count

Case No.		L	Neutrophils		E	M
			S	N		
G3248 (Control)	AI	$\bar{x}$ = 77.57	$\bar{x}$ = 16.0	$\bar{x}$ = 0.0	$\bar{x}$ = 5.71	$\bar{x}$ = 0.28
		$\hat{\sigma}$ = ±8.00	$\hat{\sigma}$ = ±6.928	$\hat{\sigma}$ = ±0.00	$\hat{\sigma}$ = ±2.289	$\hat{\sigma}$ = ±0.00
	PI	$\bar{x}$ = 81.46	$\bar{x}$ = 14.46	$\bar{x}$ = 0.69	$\bar{x}$ = 3.00	$\bar{x}$ = 0.53
		$\hat{\sigma}$ = ±11.34	$\hat{\sigma}$ = ±9.433	$\hat{\sigma}$ = ±1.315	$\hat{\sigma}$ = ±2.915	$\hat{\sigma}$ = ±0.871
G298	AI	$\bar{x}$ = 58.0	$\bar{x}$ = 25.0	$\bar{x}$ = 0.0	$\bar{x}$ = 9.0	$\bar{x}$ = 7.00
		$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±0.0
	PI	$\bar{x}$ = 51.5	$\bar{x}$ = 45.37	$\bar{x}$ = 0.0	$\bar{x}$ = 1.75	$\bar{x}$ = 0.0
		$\hat{\sigma}$ = ±3.714	$\hat{\sigma}$ = ±12.55	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±1.769	$\hat{\sigma}$ = ±1.38
G2647	AI	$\bar{x}$ = 59.00	$\bar{x}$ = 30.50	$\bar{x}$ = 0.0	$\bar{x}$ = 1.50	$\bar{x}$ = 0.0
		$\hat{\sigma}$ = ±5.056	$\hat{\sigma}$ = ±2.906	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±2.012	$\hat{\sigma}$ = ±0.0
	PI	$\bar{x}$ = 56.46	$\bar{x}$ = 34.75	$\bar{x}$ = 1.70	$\bar{x}$ = 11.55	$\bar{x}$ = 1.65
		$\hat{\sigma}$ = ±9.083	$\hat{\sigma}$ = ±12.00	$\hat{\sigma}$ = ±0.55	$\hat{\sigma}$ = ±6.300	$\hat{\sigma}$ = ±1.264
G3747	AI	$\bar{x}$ = 36.66	$\bar{x}$ = 15.00	$\bar{x}$ = 0.66	$\bar{x}$ = 46.66	$\bar{x}$ = 1.00
		$\hat{\sigma}$ = ±6.557	$\hat{\sigma}$ = ±1.0	$\hat{\sigma}$ = ±1.153	$\hat{\sigma}$ = ±3.834	$\hat{\sigma}$ = ±1.00
	PI	$\bar{x}$ = 59.83	$\bar{x}$ = 22.11	$\bar{x}$ = 3.00	$\bar{x}$ = 26.11	$\bar{x}$ = 2.11
		$\hat{\sigma}$ = ±7.733	$\hat{\sigma}$ = ±7.238	$\hat{\sigma}$ = ±2.061	$\hat{\sigma}$ = ±10.50	$\hat{\sigma}$ = ±1.378
I	AI	$\bar{x}$ = 37.5	$\bar{x}$ = 45.5	$\bar{x}$ = 0.0	$\bar{x}$ = 11.25	$\bar{x}$ = 2.75
		$\hat{\sigma}$ = ±8.584	$\hat{\sigma}$ = ±16.55	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±7.931	$\hat{\sigma}$ = ±12.60
	PI	$\bar{x}$ = 18.37	$\bar{x}$ = 29.12	$\bar{x}$ = 0.0	$\bar{x}$ = 11.37	$\bar{x}$ = 8.37
		$\hat{\sigma}$ = ±8.426	$\hat{\sigma}$ = ±7.669	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±5.282	$\hat{\sigma}$ = ±3.376

Table V (continued)

Case No.		L	Neutrophils		E	M
			S	N		
II	AI	$\bar{x}$ = 90.0	$\bar{x}$ = 7.28	$\bar{x}$ = 0.70	$\bar{x}$ = 1.42	$\bar{x}$ = 0.85
		$\hat{\sigma}$ = ±2.449	$\hat{\sigma}$ = ±2.868	$\hat{\sigma}$ = ±1.253	$\hat{\sigma}$ = ±0.9	$\hat{\sigma}$ = ±0.686
	PI	$\bar{x}$ = 91.38	$\bar{x}$ = 7.23	$\bar{x}$ = 0.76	$\bar{x}$ = 0.76	$\bar{x}$ = 0.07
		$\hat{\sigma}$ = ±5.357	$\hat{\sigma}$ = ±3.898	$\hat{\sigma}$ = ±1.476	$\hat{\sigma}$ = ±1.005	$\hat{\sigma}$ = ±0.00
F3425	AI	$\bar{x}$ = 44.7	$\bar{x}$ = 18.17	$\bar{x}$ = 1.0	$\bar{x}$ = 31.7	$\bar{x}$ = 1.41
		$\hat{\sigma}$ = ±14.2	$\hat{\sigma}$ = ±7.83	$\hat{\sigma}$ = ±1.11	$\hat{\sigma}$ = ±18.5	$\hat{\sigma}$ = ±1.00
	PI	$\bar{x}$ = 80.12	$\bar{x}$ = 11.37	$\bar{x}$ = 11.2	$\bar{x}$ = 5.0	$\bar{x}$ = 2.5
		$\hat{\sigma}$ = ±10.00	$\hat{\sigma}$ = ±5.06	$\hat{\sigma}$ = ±1.08	$\hat{\sigma}$ = ±3.11	$\hat{\sigma}$ = ±1.74
F3514	AI	$\bar{x}$ = 72.0	$\bar{x}$ = 13.5	$\bar{x}$ = 1.0	$\bar{x}$ = 11.0	$\bar{x}$ = 2.50
		$\hat{\sigma}$ = ±17.0	$\hat{\sigma}$ = ±77.3	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±6.29	$\hat{\sigma}$ = ±0.71
	PI	$\bar{x}$ = 81.78	$\bar{x}$ = 7.57	$\bar{x}$ = 1.0	$\bar{x}$ = 8.14	$\bar{x}$ = 1.42
		$\hat{\sigma}$ = ±9.62	$\hat{\sigma}$ = ±5.17	$\hat{\sigma}$ = ±0.78	$\hat{\sigma}$ = ±4.15	$\hat{\sigma}$ = ±1.99
G3108	AI	$\bar{x}$= 74.0	$\bar{x}$ = 24.0	$\bar{x}$ = 0.0	$\bar{x}$ = 2.0	$\bar{x}$ = 0.0
		$\hat{\sigma}$ = ±00.0	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±0.0	$\hat{\sigma}$ = ±0.0
	PI	$\bar{x}$ = 66.97	$\bar{x}$ = 78.84	$\bar{x}$ = 1.23	$\bar{x}$ = 1.92	$\bar{x}$ = 76
		$\hat{\sigma}$ = ±11.6	$\hat{\sigma}$ = ±11.99	$\hat{\sigma}$ = ±1.24	$\hat{\sigma}$ = ±2.059	$\hat{\sigma}$ = ±2.68

Key for Table V:

AI = ante-inoculation with leptospires.
PI = postinoculation with leptospires.
L = lymphocytes (number of cells per cmm.)
S = segmental neutrophils per cmm.
N = non segmented neutrophils per cmm.
E = eosinophils per cmm.
M = monocytes per cmm.

Table VI

Histopathological Findings in the Experimental Cattle

No.	Tissue and Lesion	Case Number and Finding							
		G3248	G298	G2647	G3247	F3425	F3514	G3108	G3249
1.	Kidney:								
	a. Neutrophils	-	-	-	-	-	-	+	-
	b. Lymphocytes	+	-	+	+	+	+	+	+
	c. Plasma cells	-	-	-	-	+	-	-	-
	d. Macrophages	-	-	-	-	+	-	-	-
	e. Thick. Bow. Caps.	-	-	-	+	+	+	-	+
	f. Collag. breakdown	-	-	+	+	-	-	+	-
	g. Degeneration	-	-	-	-	+	+	+	+
	h. Early Conn. tissue	-	-	-	-	+	-	+	+
	i. Calcification	-	-	-	-	-	-	+	-
2.	Liver:								
	a. Lymphocytes	+	+	+	+	+	+	+	
	b. Collag. breakdown	-	-	+	+	+	-	-	
	c. Degeneration	-	-	-	+	+	-	-	
	d. Enlarged Kupffer cells	-	-	-	+	+	-	-	
	e. Early conn. tissue	-	+	-	-	+	-	-	
	f. Erythropoiesis	-	-	-	+	+	-	-	
3.	Gall Bladder:								
	a. Lymphocytes	-			+				-
4.	Seminal Vesicles:								
	a. Lymphocytes	-	-	-	+	+			
5.	Epididymis:								
	a. Lymphocytes	-	-	-	-		+	+	-
	b. Neutrophils	-	-	-	-		-	+	-
6.	Testis:								
	a. Lymphocytes	-	-	-	+	+	+	+	-
	b. Deg. tubules	-	-	-	+	+	+	-	-
	c. Sync. giant cells	-	-	-	-	+	-	-	-
	d. Thick. basement membrane	-	-	-	-	-	+	-	-

Table VI (continued)

No.	Tissue and Lesion	Case Number and Finding							
		G3248	G298	G2647	G3247	F3425	F3514	G3108	G3249
7.	Prostate gland:								
	a. Lymphocytes	-	+	-	-	-	+	-	
	b. Eosinophils	-	-	-	-	-	+	-	
	c. Collag. breakdown	-	-	-	-	-	+	-	
8.	Cremaster muscle:								
	Sarcosporidiosis				+		-		
9.	Rete testis:								
	a. Lymphocytes					+	+		
10.	Cardiac muscle:								
	a. Lymphocytes	-	-	+	-	+	-	-	+
	b. Eosinophils	-	-	+	-	-	-	-	+
	c. Anitch. myocytes	-	-	-	+	-	-	-	-
	d. Sarcosporidiosis	-	-	+	-	-	-	-	+
11.	Lung:								
	a. Excess lymphocytes	-	-	-	-	-	-		+
	b. Eosinophils	-	-	-	-	-	-		+
	c. Ciliated protozoa	+	-	-	-	-	-		+
	d. Plant material	-	-	-	-	-	-		+
	e. Giant cells and macrophages	-	-	-	-	-	-		+
12.	Spleen:								
	a. Macrophages	-	-	-	+	-	-		+
	b. Congestion	+	+	+	+	+	+		+
	c. Plasma cells	-	-	-	-	-	-		+
	d. Eosinophils	+	-	-	+	-	-		-
	e. Edema	-	-	-	-	+	-		-
	f. Excess pigment	-	+	-	-	-	-		+
13.	Cerebrum:								
	a. Focal malacia	-	+		-	-	-	-	

Table VI (continued)

No.	Tissue and Lesion	Case Number and Finding							
		G3248	G298	G2647σ	G3247	F3425	F3514	G3108	G3249
13.	Adrenal Gland:								
	a. Lymphocytes	-	-	+	-		-	-	-
	b. Eosinophils	-	-	-	-		+	-	-
	c. Early conn. tissue in the capsule	-	-	-	-		-	-	+
15.	Lymph Node:								
	a. Edema	-	-	-	-	-	-	+	-
	b. Eosinophils	+	-	+	+	-	+	-	+
	c. Plasma cells	-	-	-	-	-	-	-	+
	d. Macrophages	-	-	+	-	+	-	+	+
	e. Megakaryocytes	-	-	-	+	-	-	-	-
	f. Neutrophils	-	-	+	-	-	-	-	-
	g. Fibrin	-	-	+	-	-	-	-	-
	h. Collag. breakdown	-	-	+	-	-	-	-	-
	i. Calcification	-	-	+	-	-	-	+	-
	j. Erythropoiesis	-	-	-	+	-	-	-	-
	k. Pigments	-	-	+	+	-	-	-	+

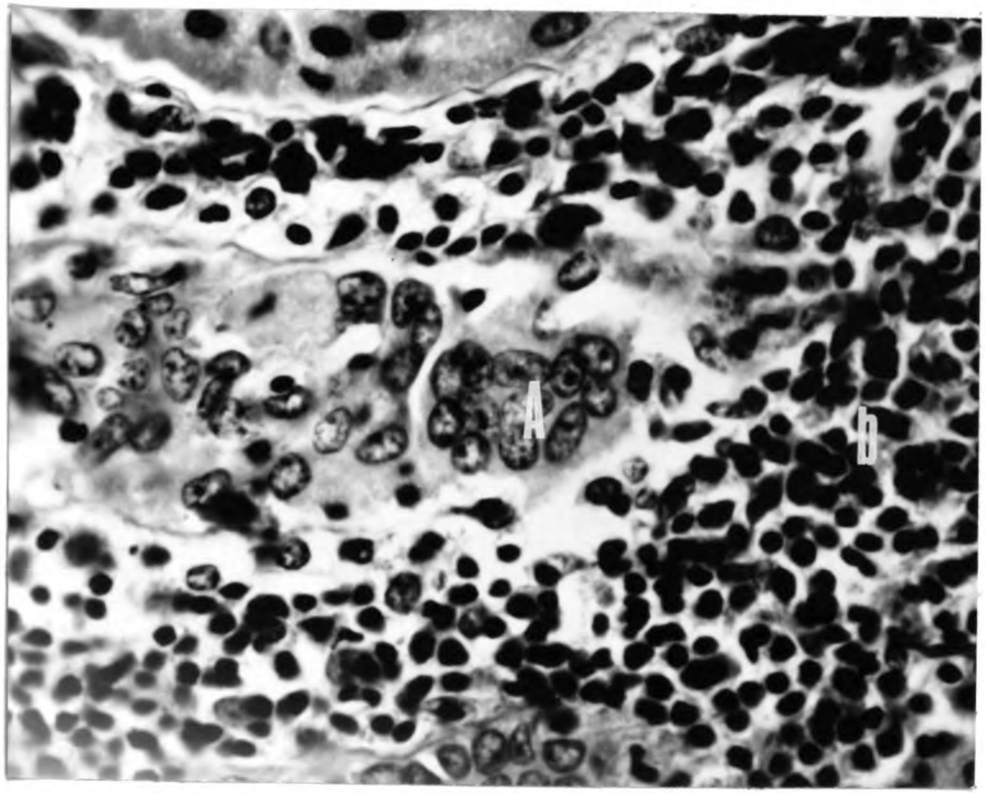

Fig. 1. Section through the kidney. A. Destruction of the renal tubules. B. Area of lymphocytic infiltration. H. and E. X680

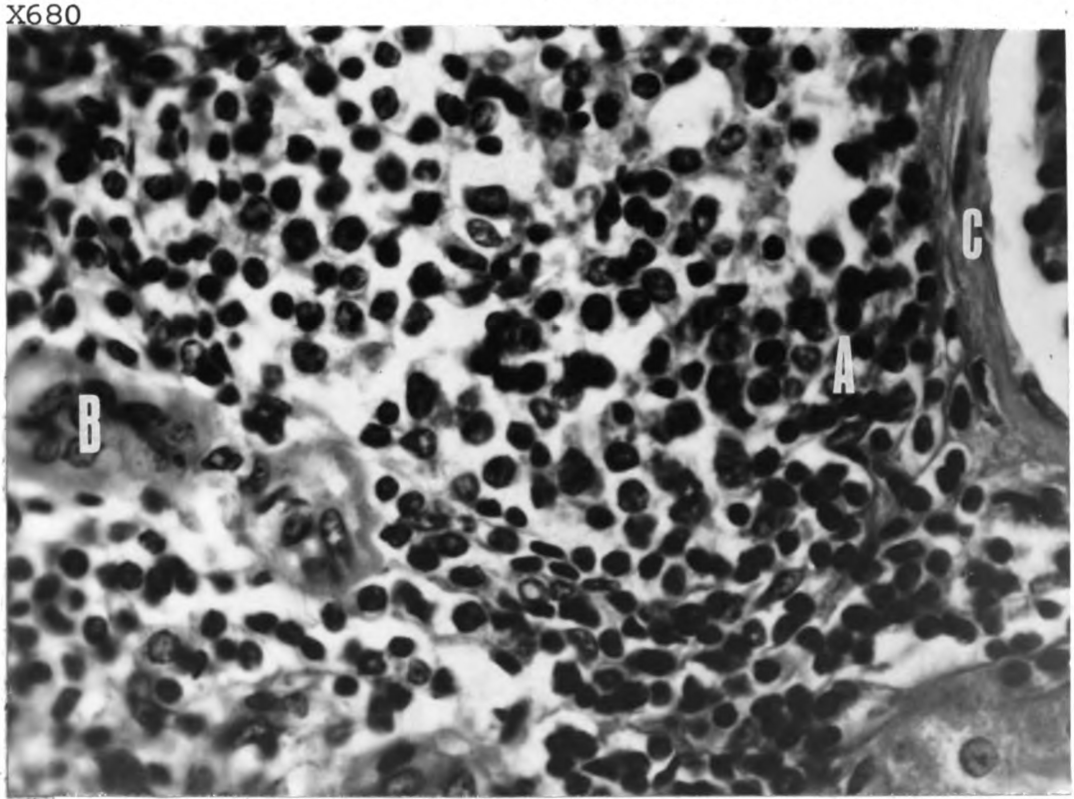

Fig. 2 Section through the kidney. A. Lymphocytic infiltration. B. Syncytial giant cells. C. Thickened Bowman's capsule. H. and E. X680

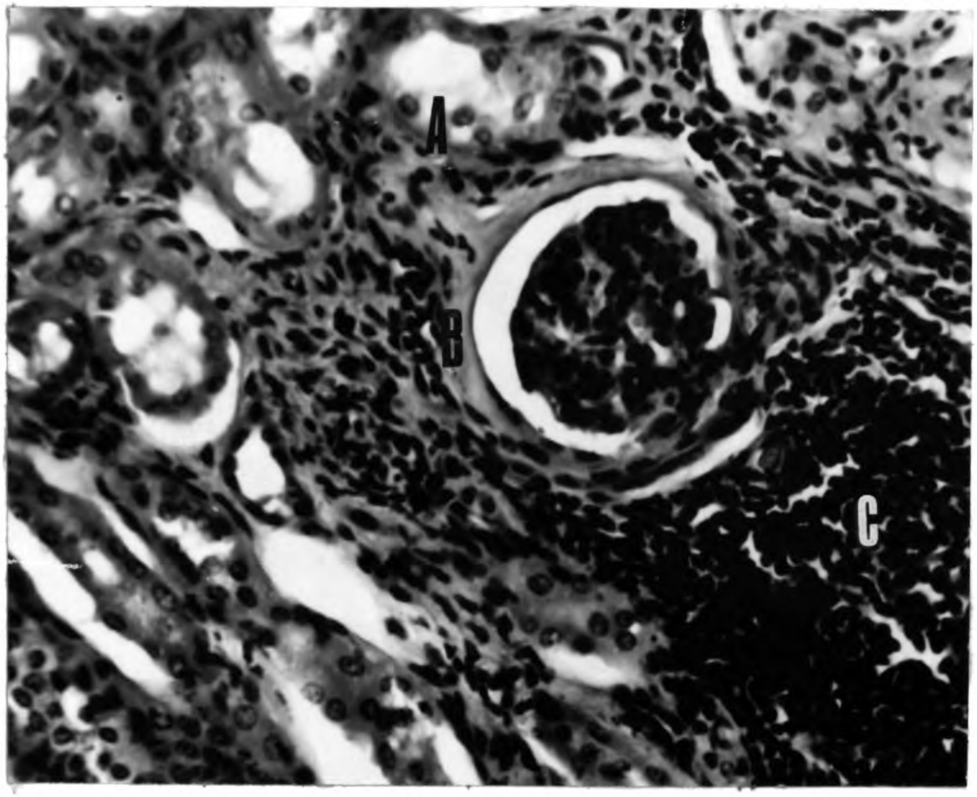

Fig. 3. Section through the renal cortex. A. Vacuolar degeneration in tubules. B. Thickened Bowman's capsule. C. Lymphocytic infiltration. H. and E. X411

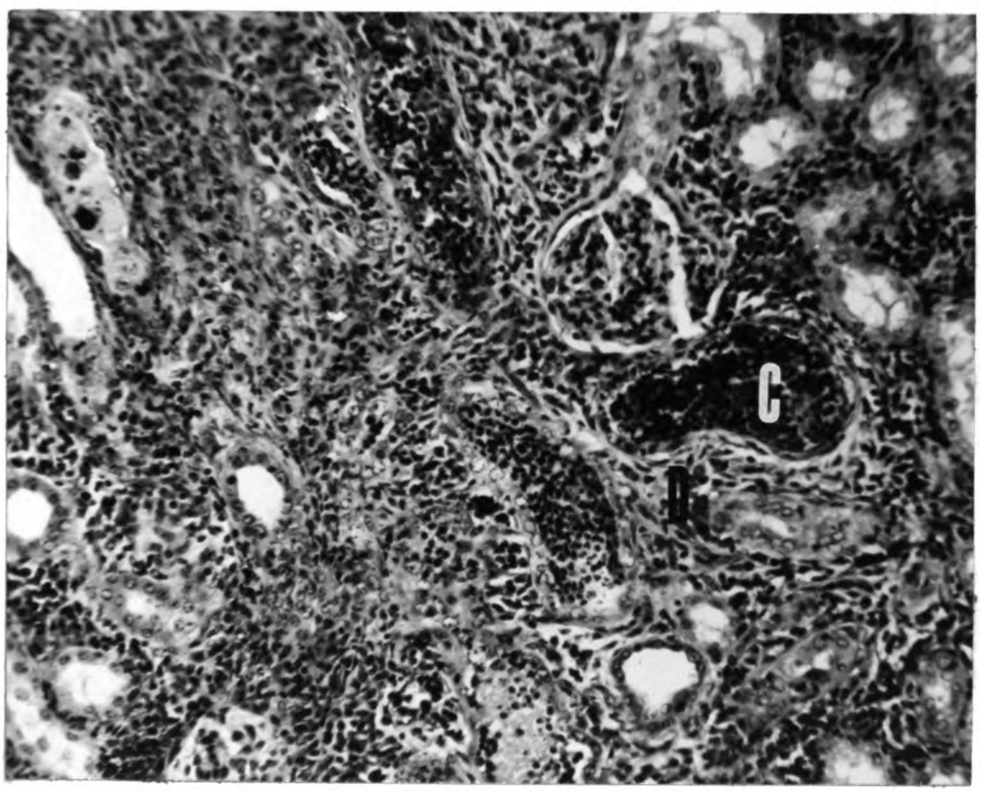

Fig. 4. Section through kidney of calf. C. Distended tubules with neutrophils. D. Connective tissue proliferation. H. and E. X160

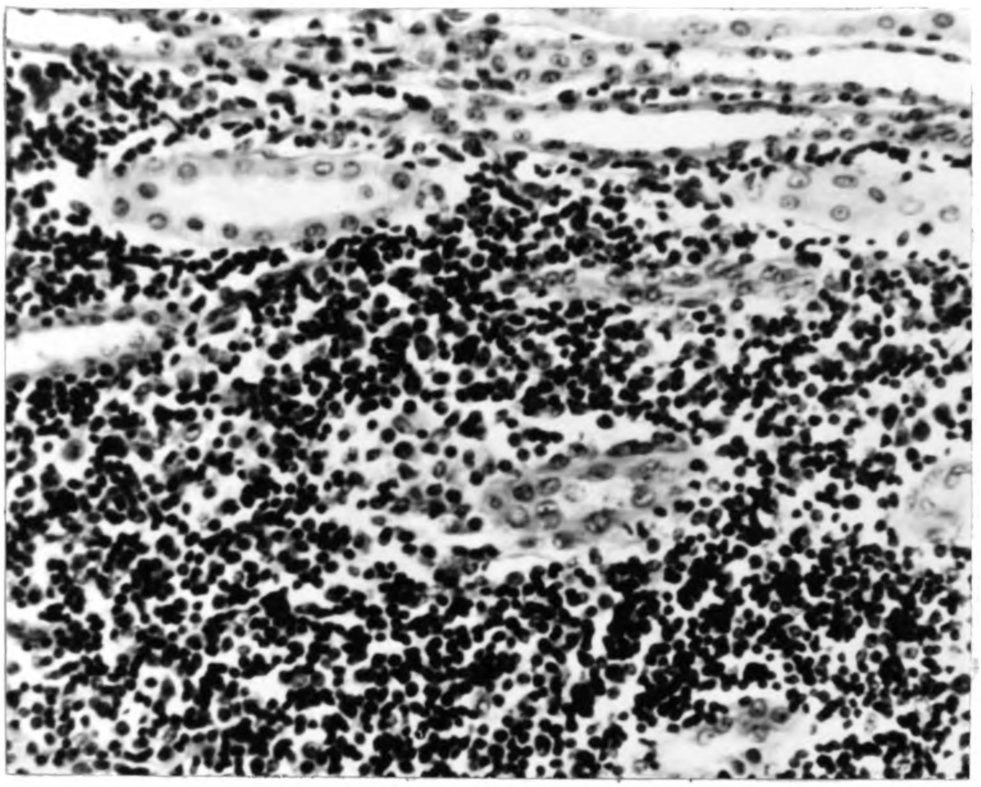

Fig. 5. Section through the kidney to show lymphocytic infiltration in medulla. H. and E. X411

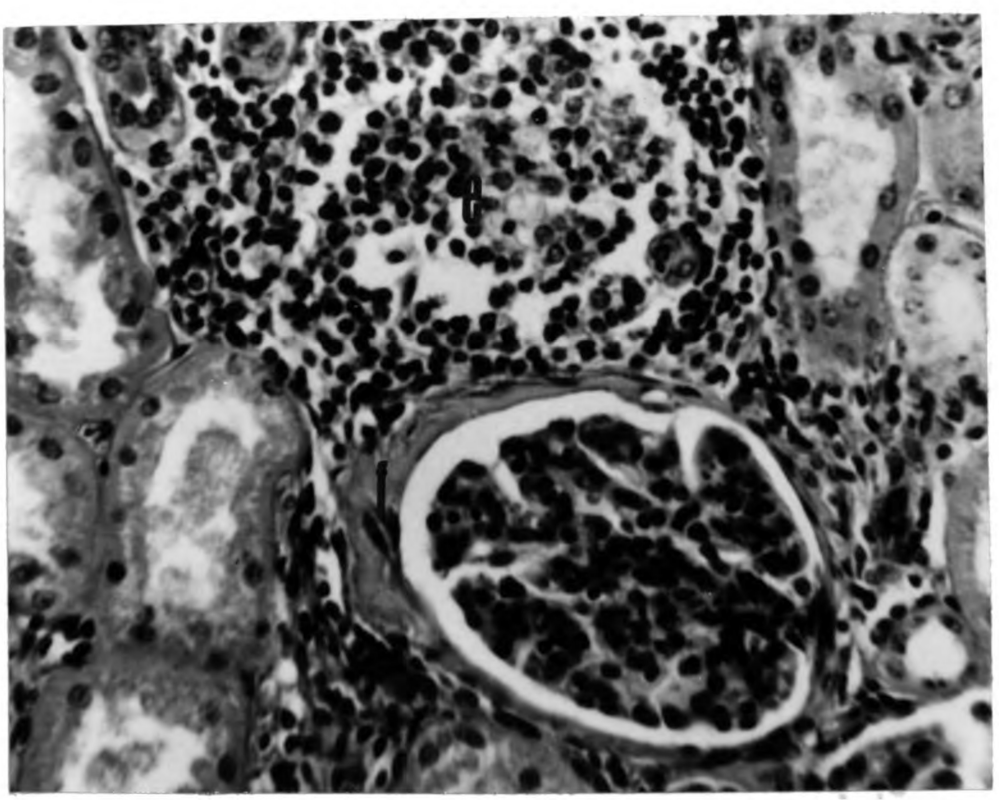

Fig. 6. Section through calf kidney. E. Lymphocytes and neutrophils in interstitial tissue. F. Thickened Bowman's capsule. H. and E. X680.

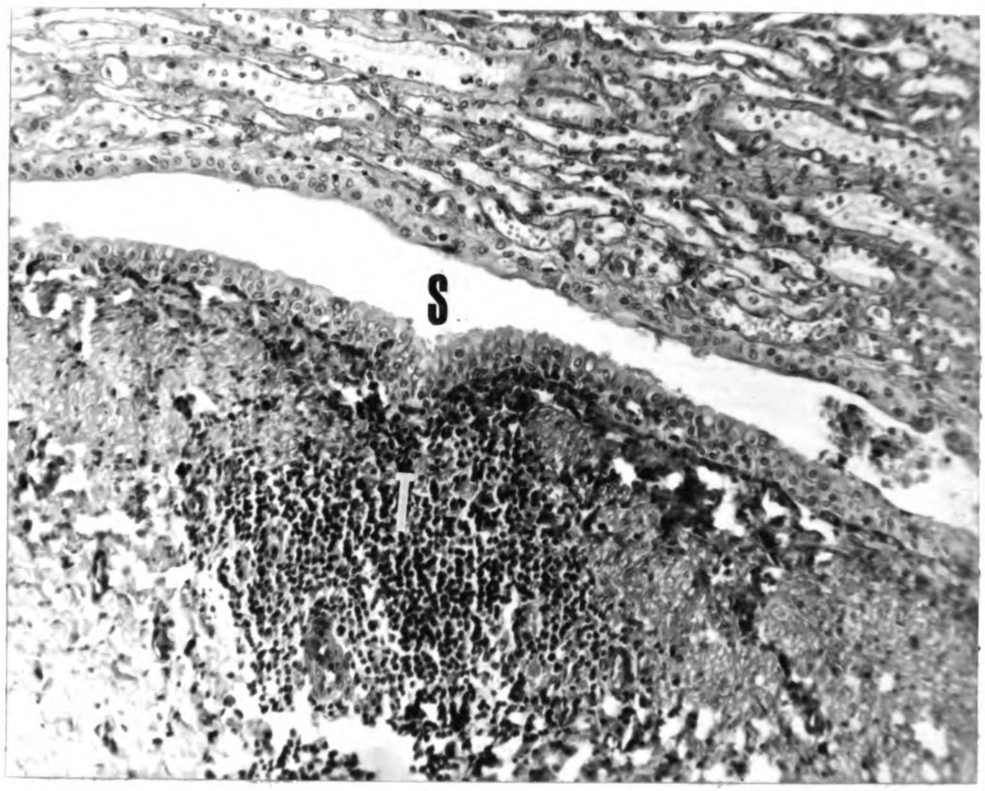

Fig. 7. Section through renal papillae and minor calyx. S. Minor calyx. T. Focus of lymphocytic infiltration. H. and E. X160

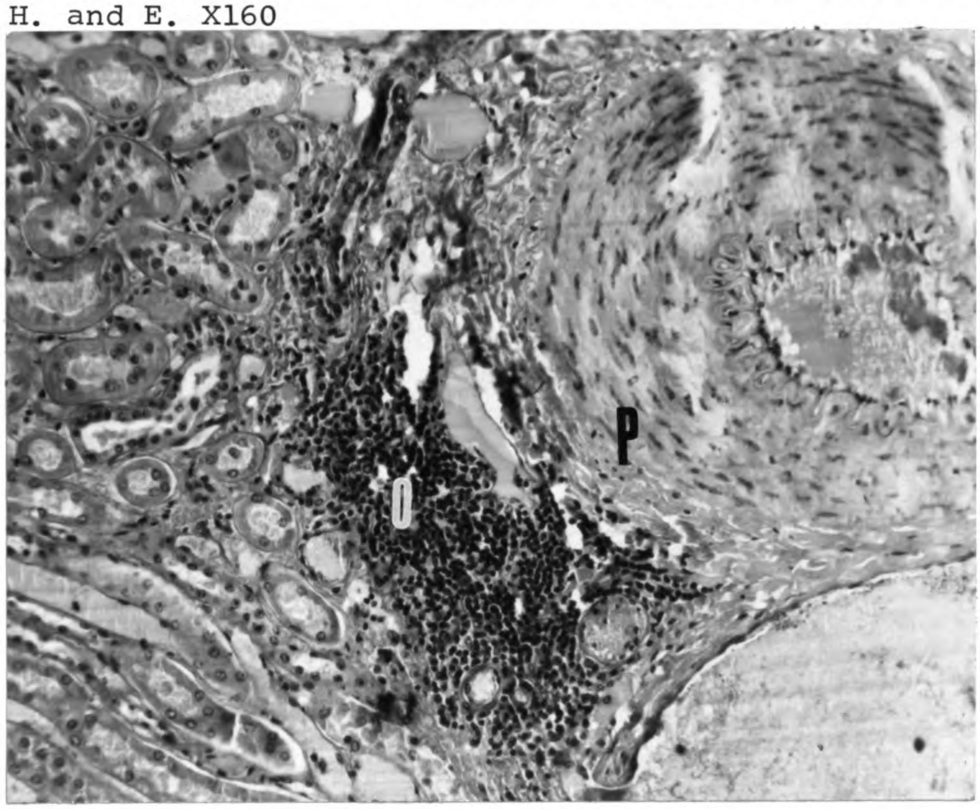

Fig. 8. Section through kidney of control bull. O. Focus of lymphocytic infiltration. P. Arcuate artery. H. and E. X160

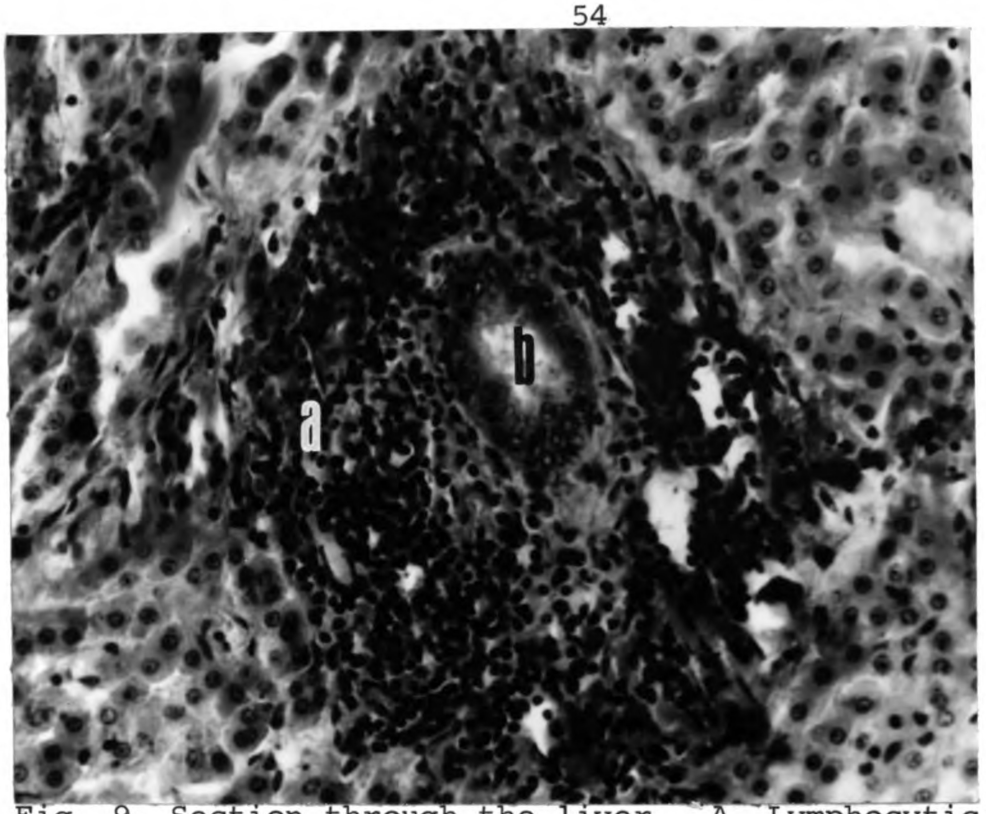

Fig. 9. Section through the liver. A. Lymphocytic infiltration in hepatic trinity. B. Bile duct. H. and E. X411

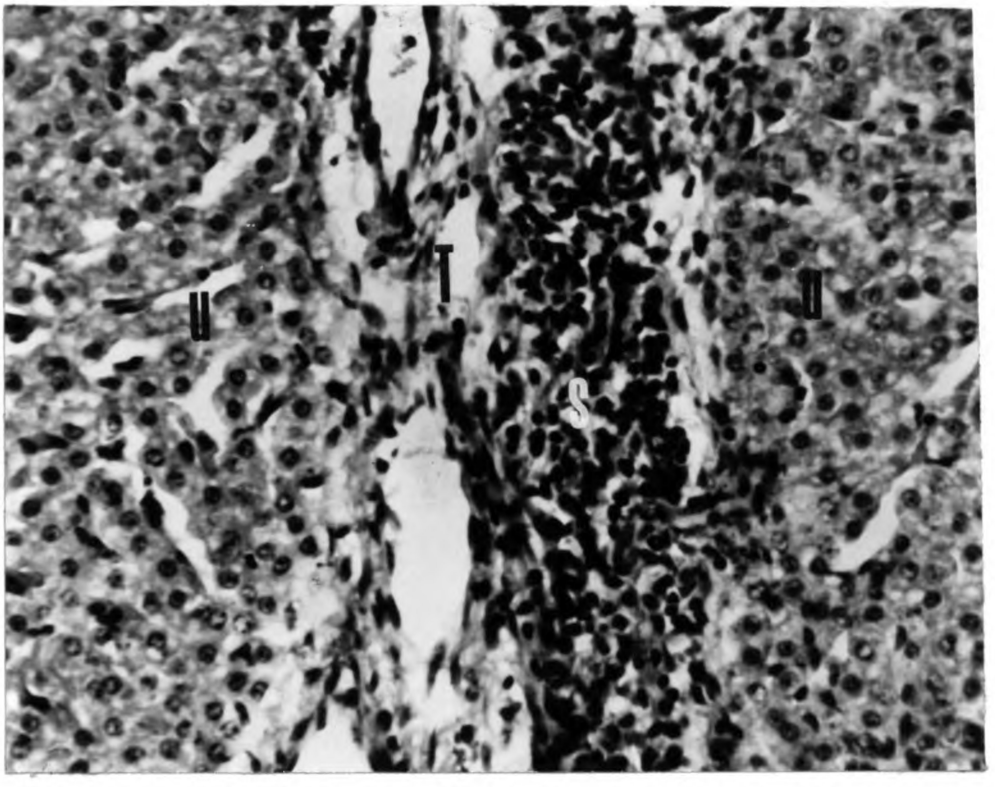

Fig. 10. Section through liver. S. Lymphocytic infiltration. T. Slight edema. U. Hepatic lobules. H. and E. X411

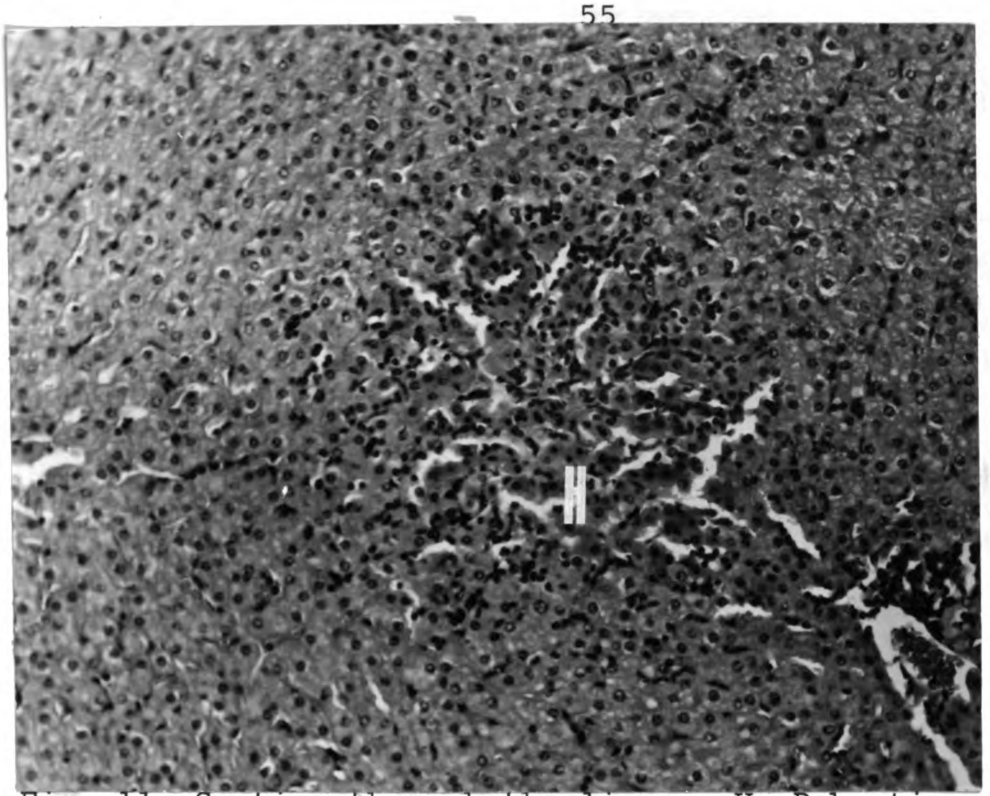

Fig. 11 Section through the liver. H. Pyknotic nuclei of hepatic cells. H. and E. X160

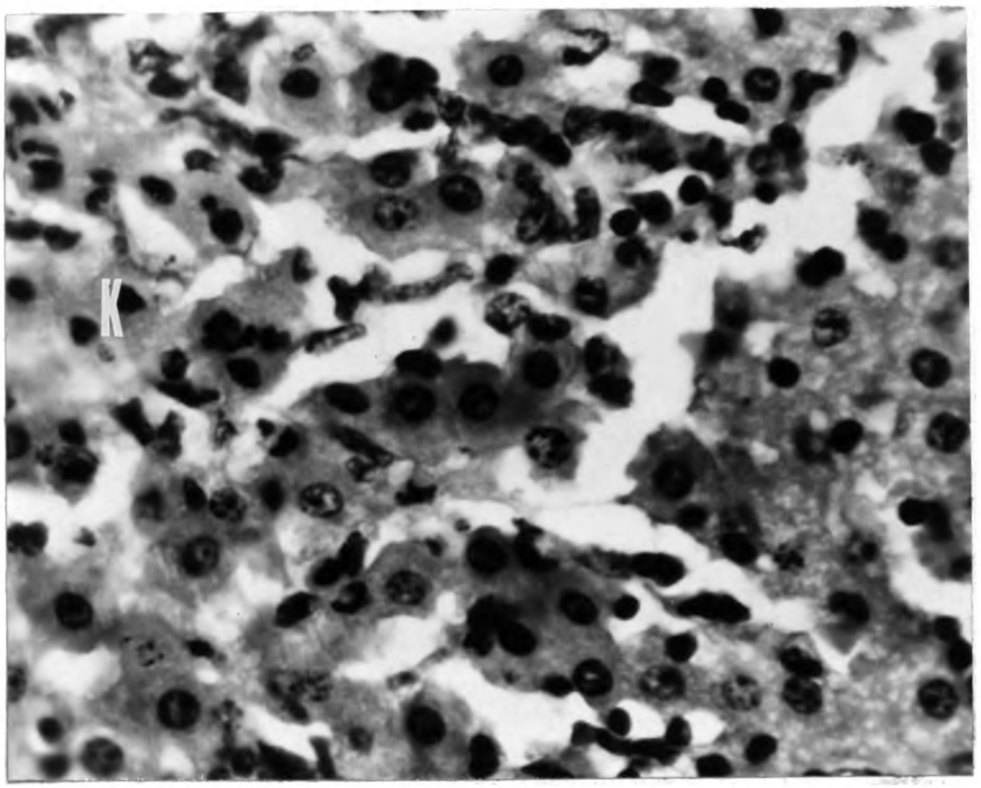

Fig. 12. Higher power of Fig. 11. K. Pyknotic nuclei of hepatic cells. H. and E. X680

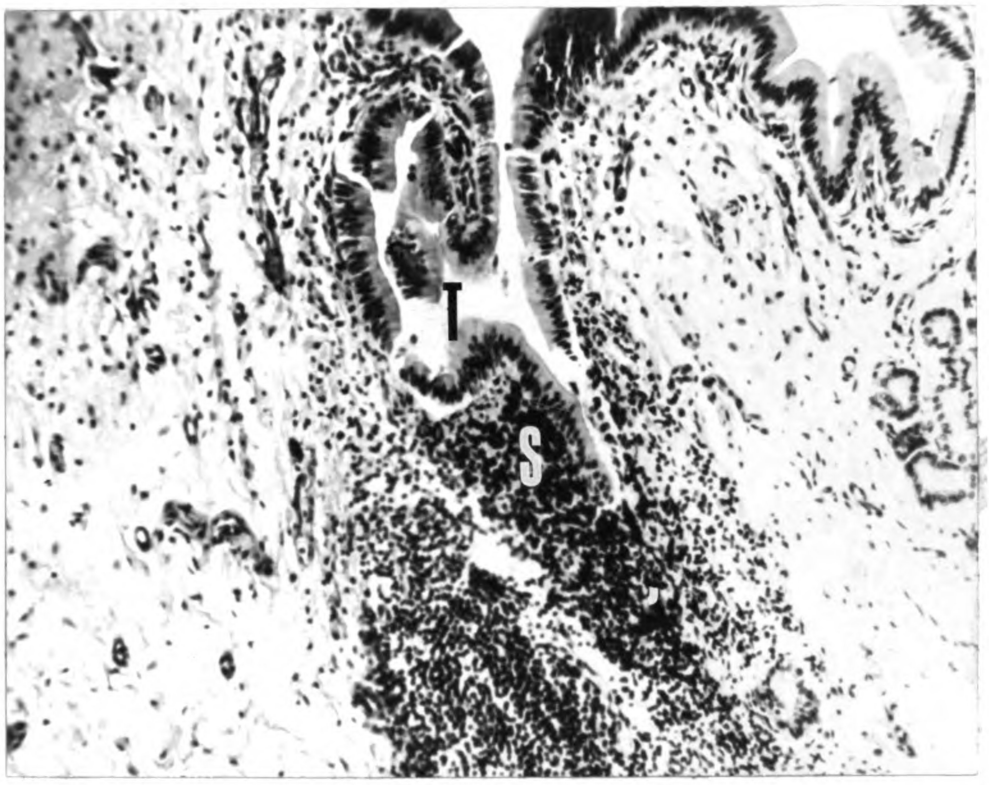

Fig. 13. Section through the wall of the gall bladder. S. Lymphocytic infiltration in lamina propria. T. Crypts. H. and E. X160

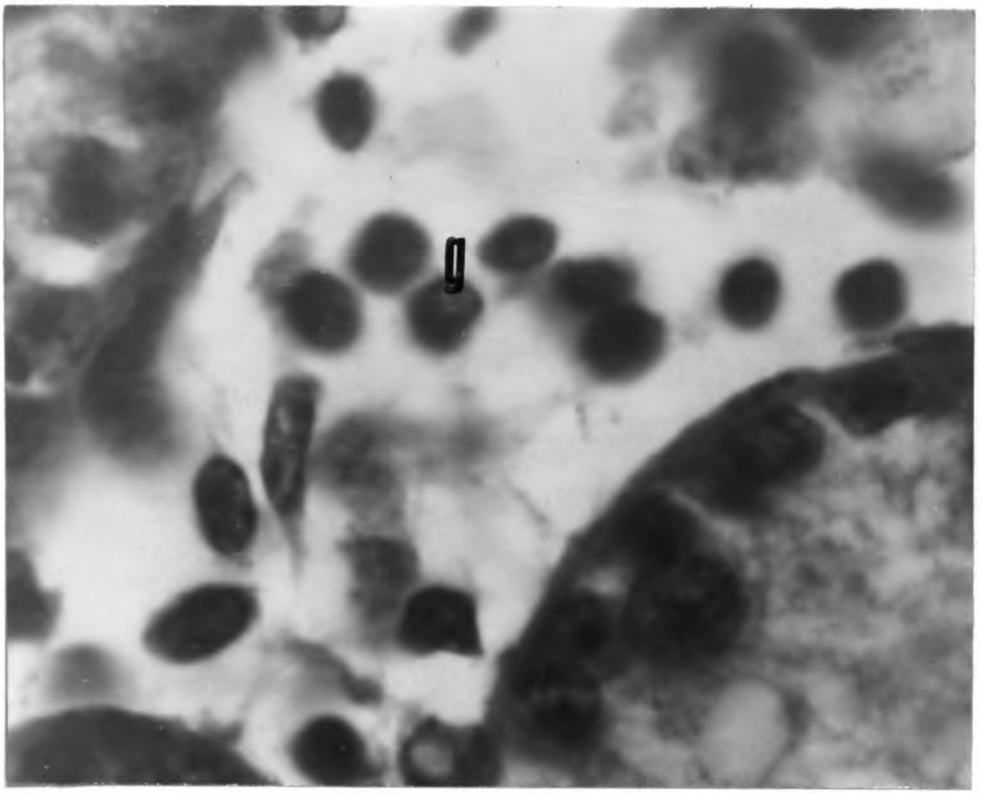

Fig. 14. Section through the testis of the calf. G. Scattered lymphocytes among seminiferous tubules. H. and E. X1700

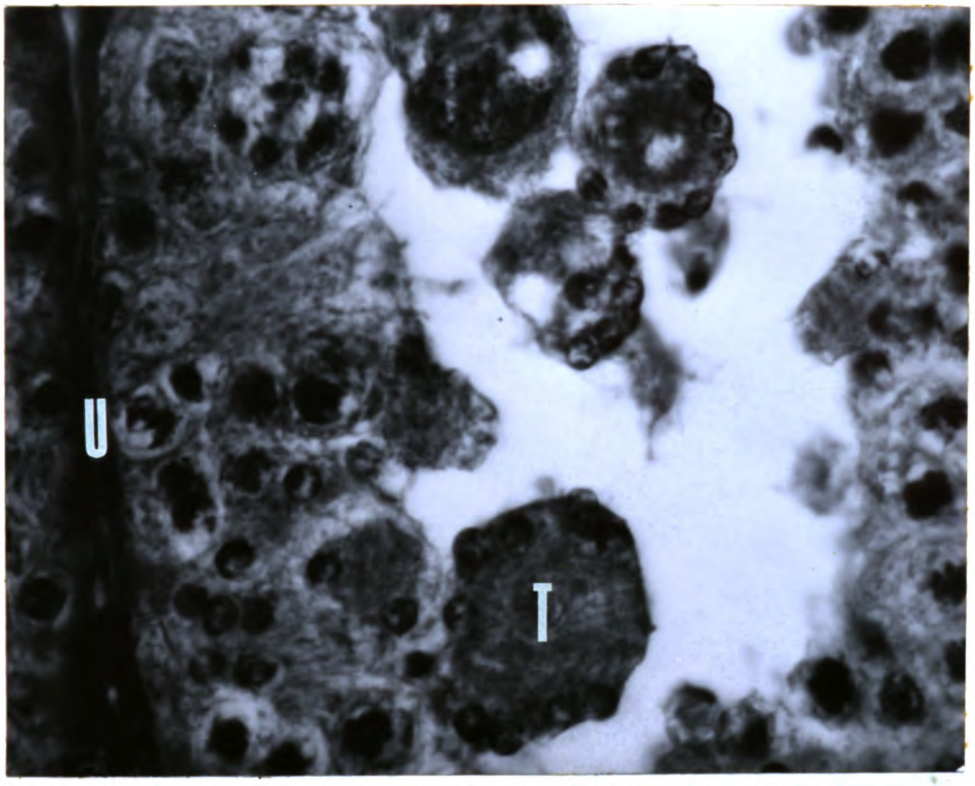

Fig. 15. Section through seminiferous tubule. T. Syncytial giant cells in lumen of tubule. U. Thickened tubular basement membrane. H. and E. X680

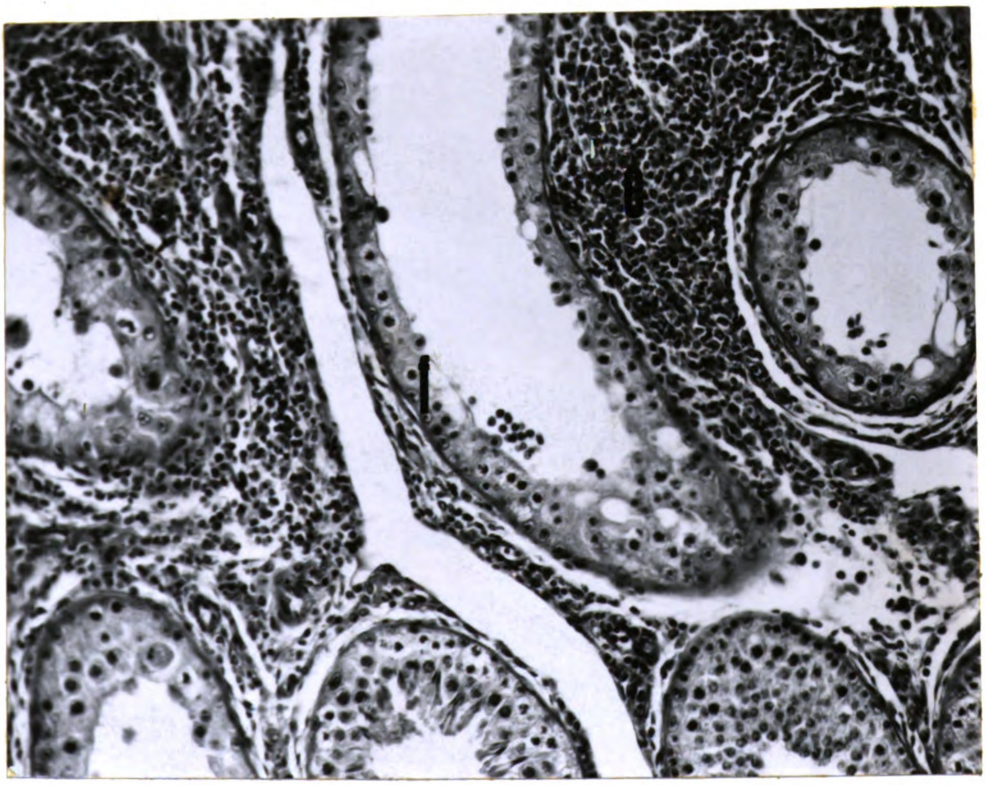

Fig. 16. Section through the testis. E. Lymphocytic infiltration among seminiferous tubules. F. Tubules with vacuolar degeneration. H. and E. X160

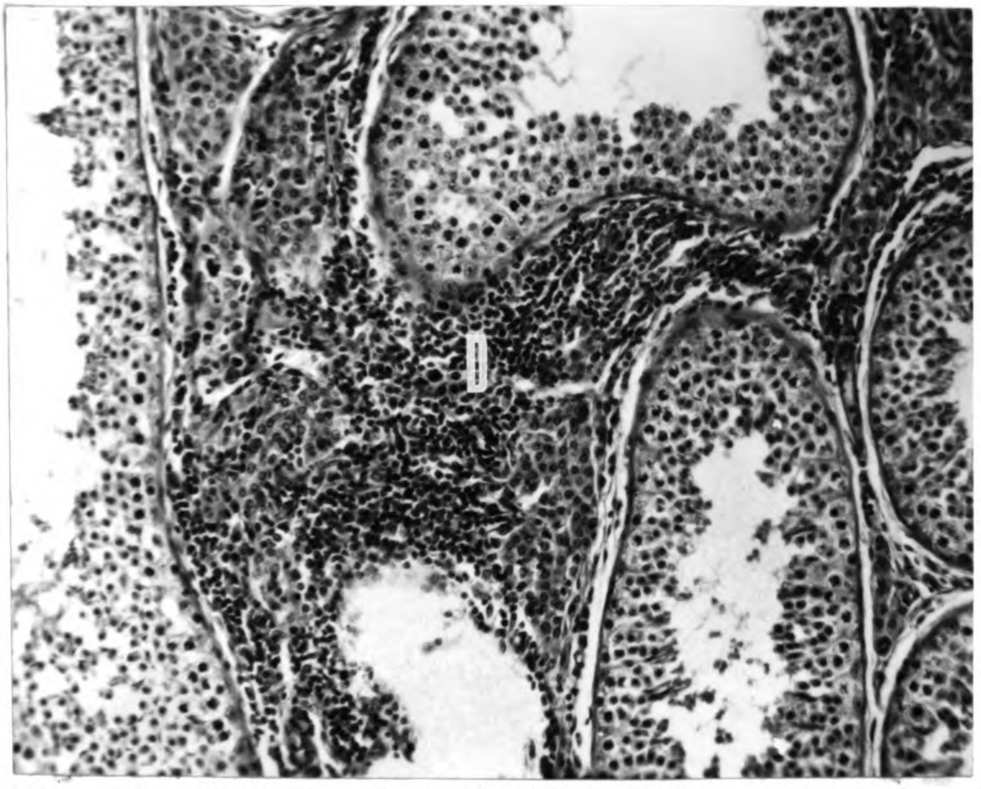

Fig. 17. Section through the testis. D. Lymphocytic infiltration among seminiferous tubules not showing vacuolar degeneration. H. and E. X160

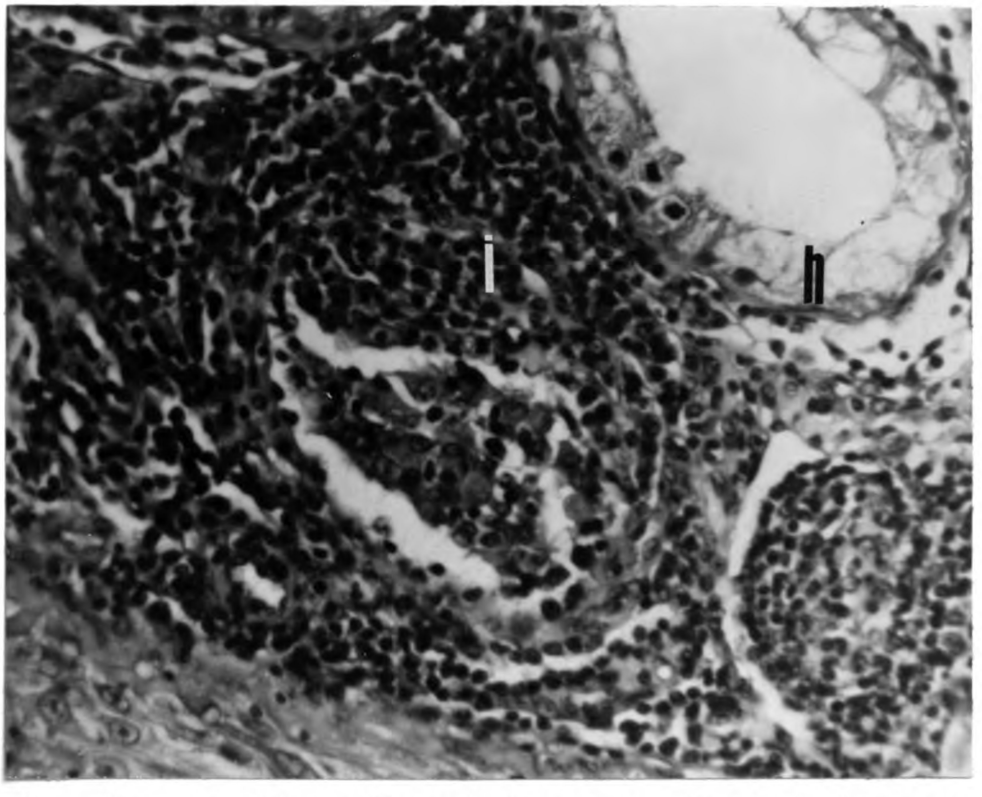

Fig. 18. Section through testis. H. Seminiferous tubules showing vacuolar degeneration. I. Lymphocytic infiltration. H. and E. X411

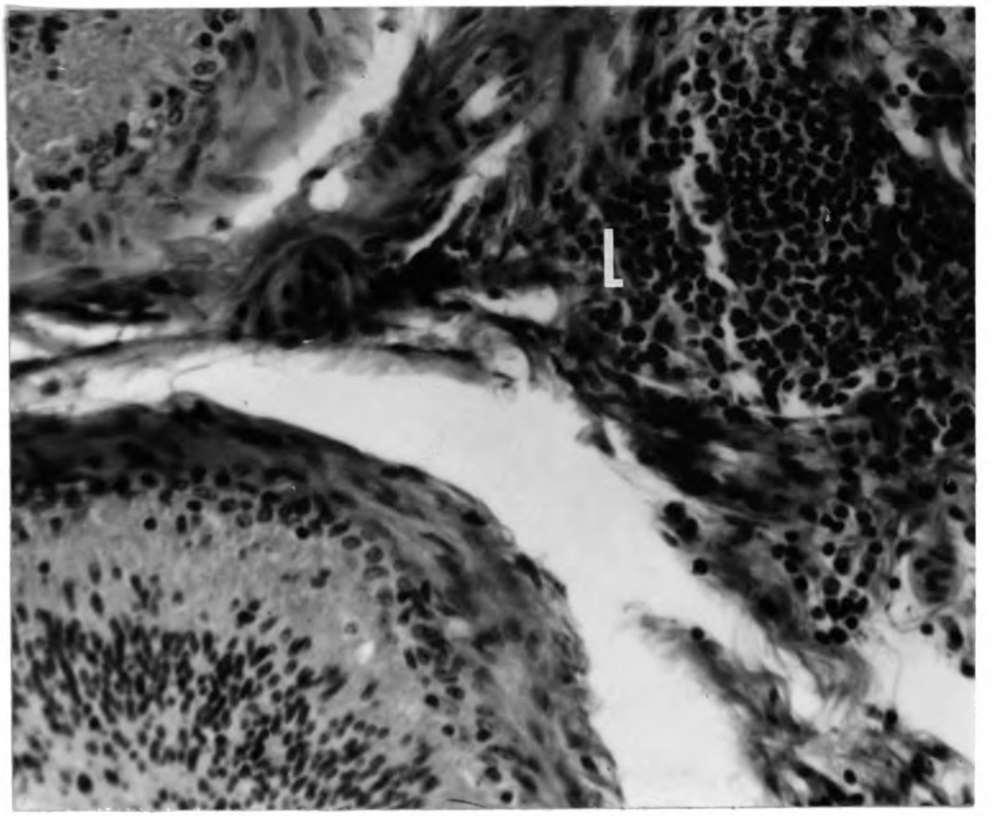

Fig. 19. Section through epididymis. L. Lymphocytic infiltration. H. and E. X411

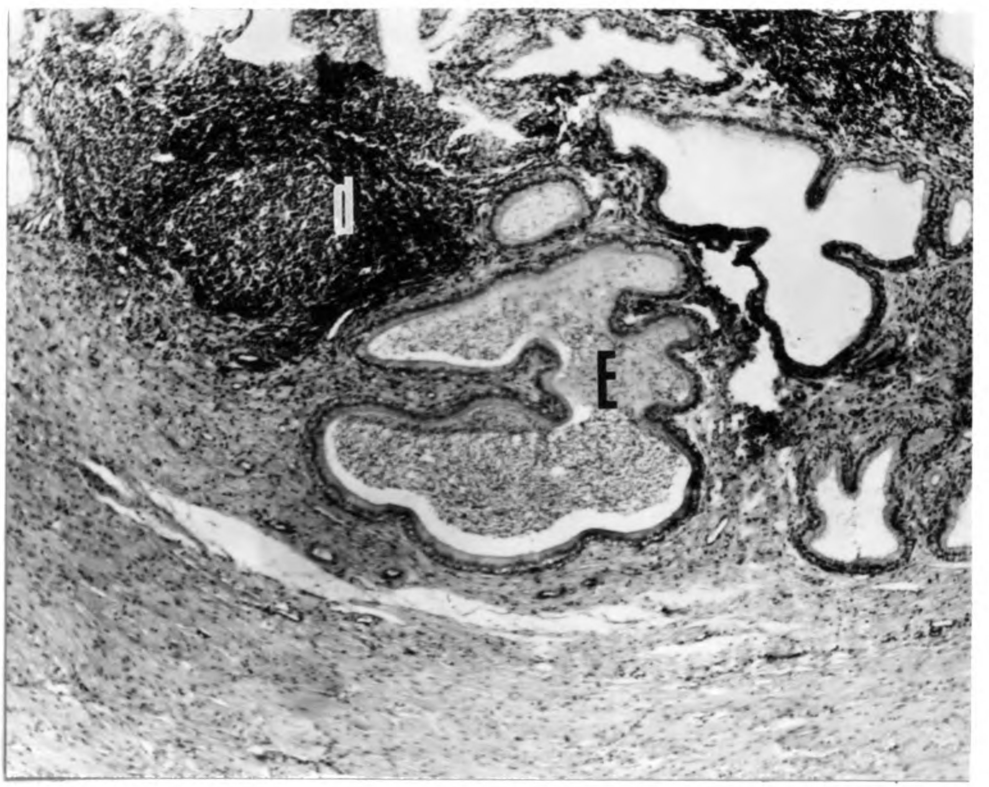

Fig. 20. Section through seminal vesicle. D. Follicular type lymphocytic infiltration. E. Crypt. H. and E. X56

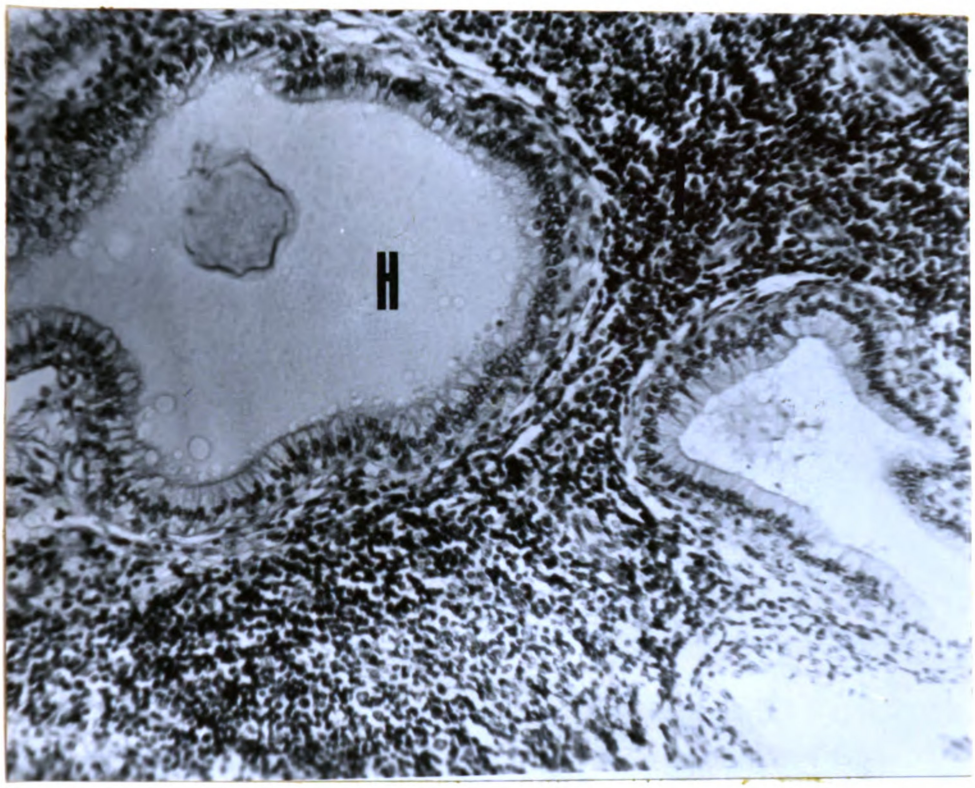

Fig. 21. High power for Fig. 20. H. Crypt of seminal vesicle. I. Lymphocytic infiltration. H. and E. X160

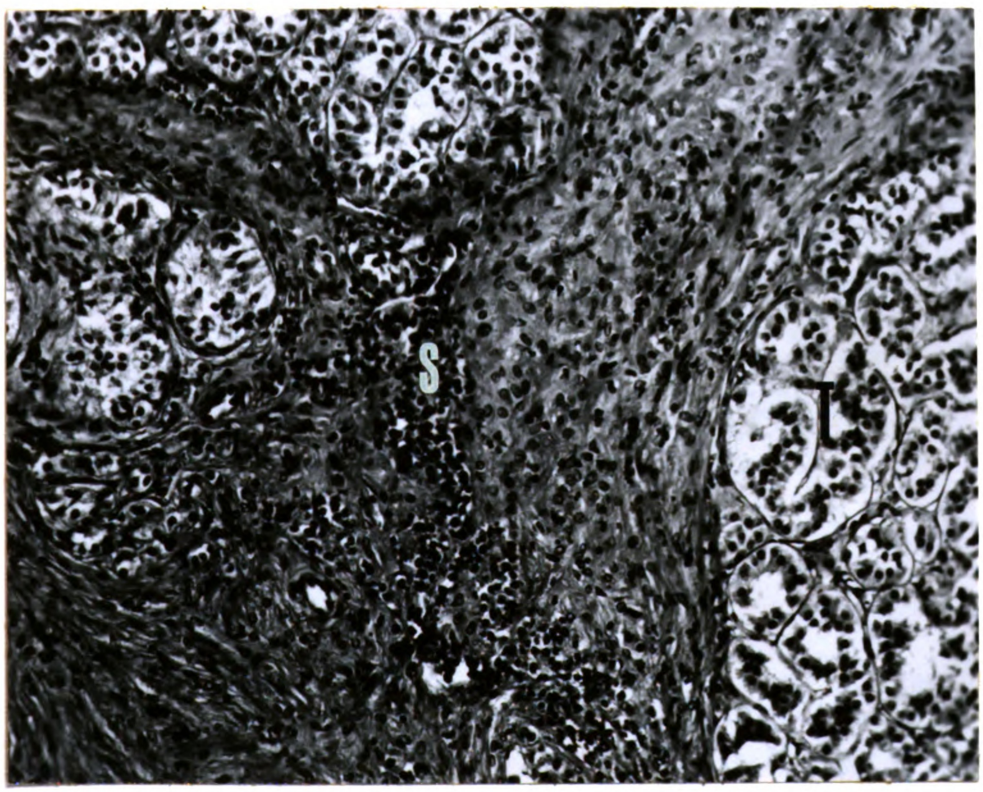

Fig. 22. Section through adrenal gland. S. Lymphocytic and eosinophilic infiltration. T. Zona glomerulosa. H. and E. X160

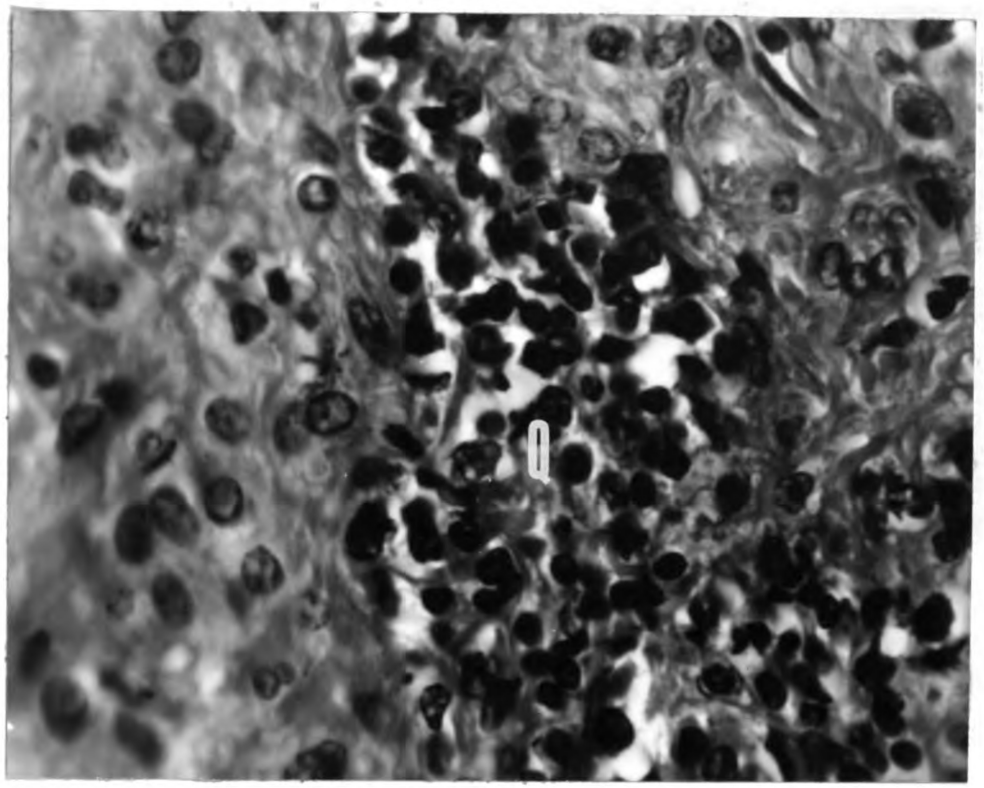

Fig. 23. Higher power for Fig. 22. Q. Lymphocytes and eosinophils. H. and E. X680

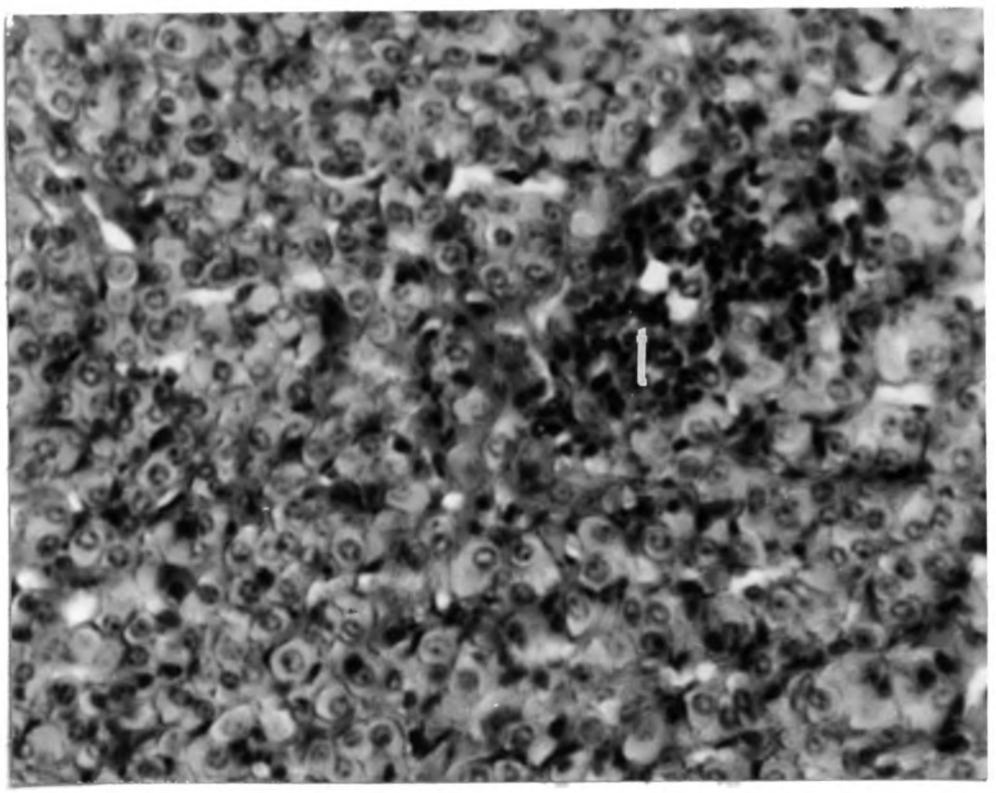

Fig. 24. Section through adrenal gland. T. Small focus of lymphocytic infiltration in zona fasciculata. H. and E. X411

Fig. 25. Higher power for Fig. 24. V. Lymphocytes and eosinophils. W. Zona fasciculata. H. and E. X680

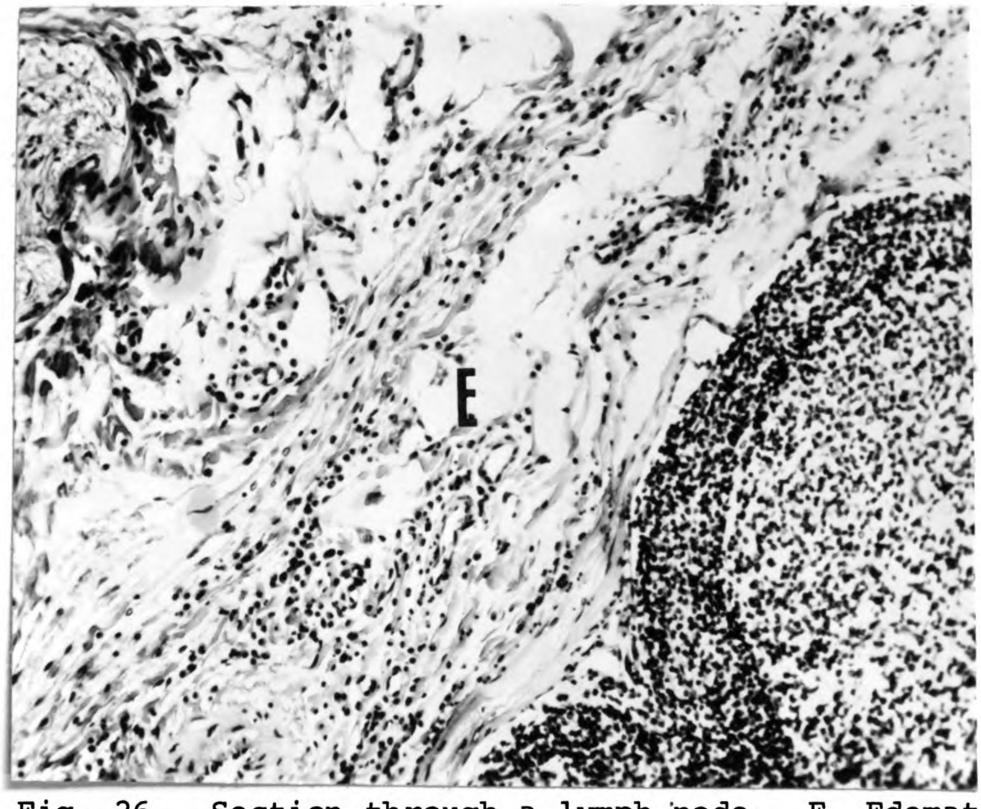

Fig. 26. Section through a lymph node. E. Edematous capsule with eosinophilic infiltration. H. and E. X160

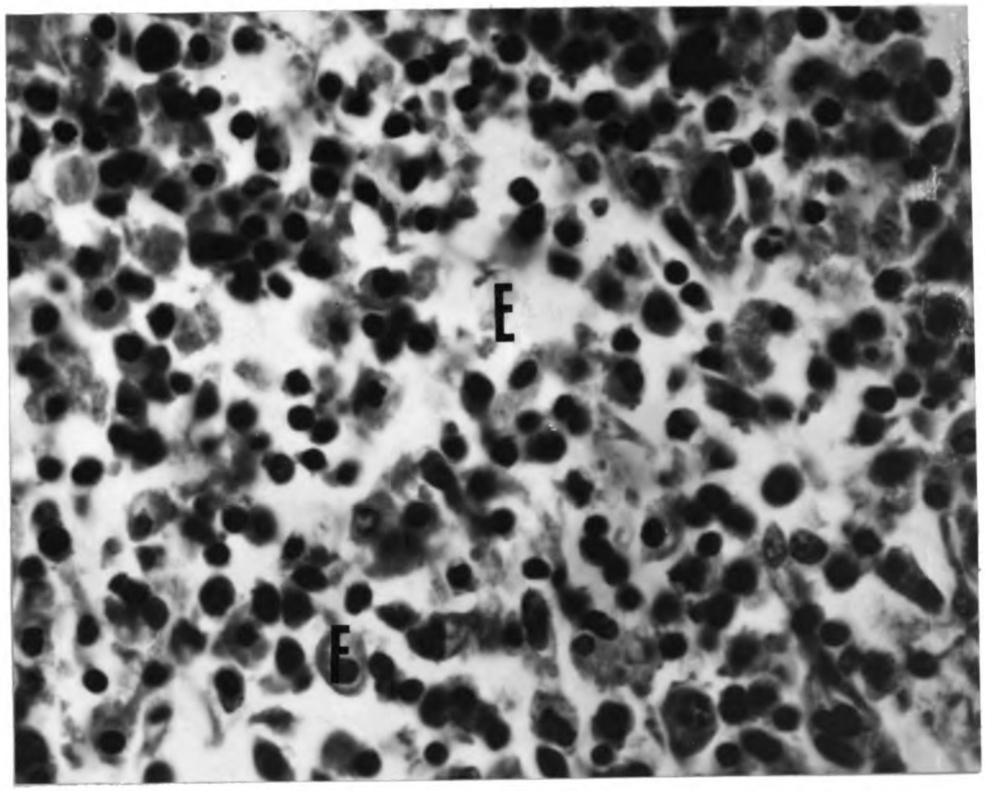

Fig. 27. Section through lymph node. E. Edema. F. Plasma cells. H. and E. X680

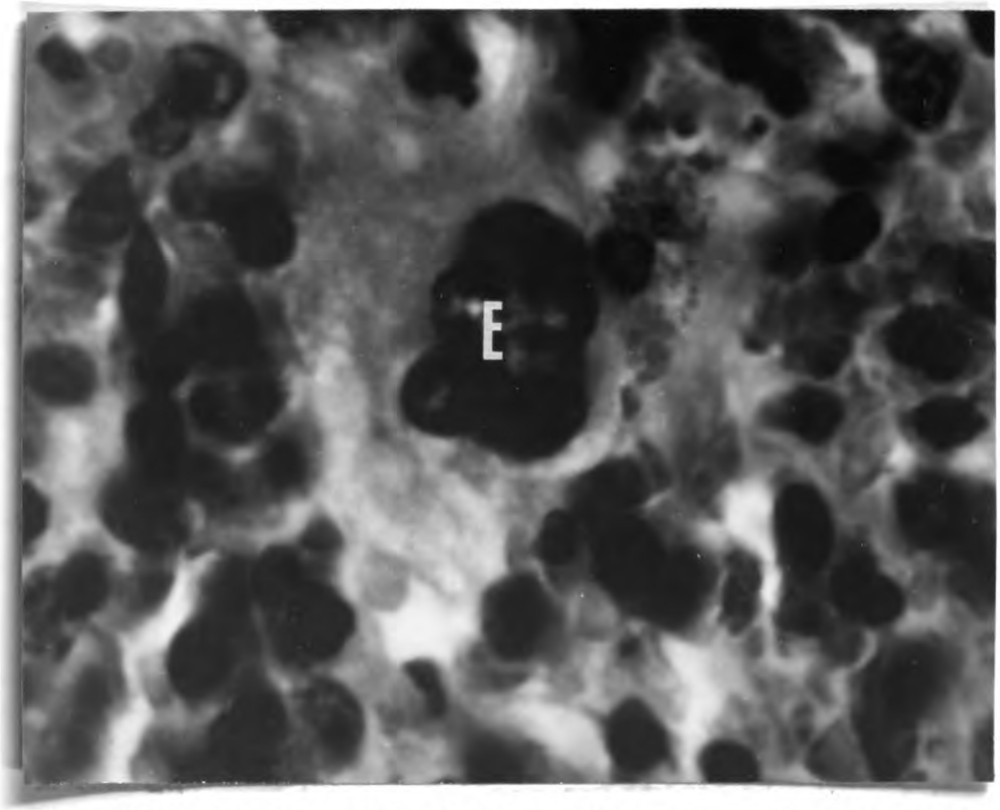

Fig. 28. Section through lymph node. E. Megakaryocyte. H. and E. X1700

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