THE PATHOGENESIS OF LEPTOSPIRAL INFECTIONS (*Leptospira pomona*) DURING PREGNANCY IN CATTLE AND SWINE

By

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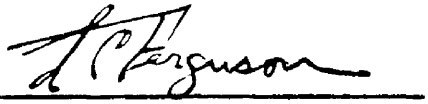
ABSTRACT

All of ten pregnant heifers exposed to Leptospira pomona developed either clinical or serological manifestations of leptospirosis. The heifers were killed 10 to 60 days after exposure. None of the fetuses developed leptospirosis. Leptospirae were not demonstrable in the fetal fluids or tissues and with one exception the placental tissues.

The histopathological lesions of the heifers closely resembled those observed in other reported experimental L. pomona infections of cattle. The presence of a cytopathogenic agent produced by the leptospirae is suggested. This proposed agent is probably responsible for alterations in the maternal-fetal placental relationship that interfere with fetal development and may cause fetal death.

Two fetuses were infected with L. pomona by direct inoculation in utero. The fetal leptospirosis was similar to the postnatal infections. Leptospiremia was observed in one fetus on postexposure day 7 and by day 14 the leptospirae were demonstrable in the kidney but no other tissues. The histopathological lesions of the placenta resembled those associated with maternal infections.

Five sows had been experimentally infected with L. pomona 10 to 14 months previously. The sows were re-exposed during the last one-half of the gestation period. The immunity produced by the active infection was sufficient to protect the pregnant sows against challenge with a virulent strain of L. pomona.

Approved 

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INTRODUCTION

The ability of the female to maintain pregnancy and give birth to normal young is dependent on a complex of interrelated factors. Obviously the genital tract must be anatomically normal. The dam should be free of disease, in particular those diseases which are known to have specific effects on the female genital tract. Abortions are a manifestation of such diseases. It is frequently possible to recover the etiological agent from the aborted fetus and often from the female genital tract. Infectious diseases can alter the physiological blance or placental relationships and cause abortion without actual infection of the fetus or the genital tract. The endocrine system of the dam must maintain a delicate hormonal balance to meet requirements through all stages of gestation. The nutritional status of the dam must be adequate to meet the added metabolic requirements.

Leptospirosis (Leptospira pomona) frequently has been associated with abortions in cattle and swine. Leptospirae have been regularly isolated from aborted swine fetuses. Attempts to recover leptospirae from aborted bovine fetuses have failed rather consistently. Invasion of the fetus by leptospirae is an important cause of porcine abortions. Bovine abortions probably result from other causes, but the conditions incompatible with fetal life have not been well defined.

It was the purpose of this work to ascertain the effects on the bovine fetus of: 1) experimental L. pomona infections of the dam, and 2) experimental L. pomona infection of the fetus in utero.

Further, pregnant sows were re-exposed to L. pomona to determine if the maternal leptospiral antibodies were capable of protecting the fetuses against infection.*

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LITERATURE REVIEW

Jungherr (18) demonstrated leptospirae in kidney sections of diseased cattle, however leptospirae were not isolated from these cases and the serotype remained unknown. Earlier reports of icterohemoglobinuria and idiopathic hemoglobinuria may be considered bovine leptospirosis (22,40). The symptoms of icterus, hemoglobinuria and abortions are similar to those associated with L. pomona infections of cattle.

In 1946 Mathews (23) demonstrated leptospirae in tissue sections and was able to transmit the disease from cattle to calves and guinea pigs. Blood taken during the febrile stage of the disease was used for passage.

The first cultural demonstration of L. pomona from cattle in the United States was accomplished by Baker and Little (1). Abortion, usually occurring during the last trimester of pregnancy, has been reported as a common manifestation of bovine leptospirosis (6,9,11,13, 14,15,17,28,31,34,42,43).

Only two reports of isolation of L. pomona from the aborted bovine fetus have been found (9,34). The report of Podgwaite et al. (34) has been reviewed by Ferguson et al. (15) and possible inconsistencies in the report were discussed. Dacres and Kiesel (9) recovered leptospirae from an aborted bovine fetus by inoculating chinchillas with thoracic fluid and subsequent blood culture in Chang's semisolid medium. The inability to isolate leptospirae from the aborted bovine fetus, fetal fluids or placentae (13,14,15,28,31, 35) suggests that other mechanisms may play a more important role than

invasion of the fetus in leptospiral abortions. Te Punga and Bishop (43) suggest three causes for bovine abortions: 1) pyrexia and systemic reaction resulting in abortion; 2) interrupted transfer of metabolites, due to localized lesions in the maternal-fetal cotyledonary junction, with subsequent fetal death; and 3) actual invasion of the fetus by the leptospirae, with death and expulsion following active fetal infection.

Ferguson et al. (15) found an apparent lack of erythrocytes in the circulatory system of aborted bovine fetuses. A suggested mechanism for this phenomenon was the release of a toxic material from the leptospirae which was able to cross the placental barrier and lyse the fetal erythrocytes.

Leptospirae were not demonstrated in fetal or placental material from experimental or field cases by Borg-Petersen and Fennestad (13,14) but the authors considered the findings compatible with the hypothesis that leptospiral abortion is most often due to the fetal leptospirosis of the fetus. The failure to isolate leptospirae was thought due to the inability of the organisms to survive in the environment of the autolyzing fetus. Fetal infection probably results from invasion of the fetus at the time of the maternal leptospiremia and acute infection of the fetus follows an incubation period of a week or more (13).

Leptospira-like bodies have been demonstrated in fetal and placental tissues by silver impregnation techniques (6,35,43). None of these observations was substantiated by bacteriological findings. Determining the presence of leptospirae in the placenta and fetal tissues by silver impregnation cannot be considered a definitive

diagnosis unless such tissues are found to be bacteriologically positive (30).

Examination of aborted fetuses in experimental studies failed to reveal leptospirae by cultural or animal inoculation techniques (28, 35). Experimentally infected heifers were slaughtered 10-14 and 25-33 days after exposure. All fetuses were alive but leptospirae were not demonstrated (28,31). Leptospiral antibodies were not found in the fetal sera by the agglutination-lysis test, indicating that maternal antibodies had not been transferred to the fetus and fetal antibodies had not been produced in response to invasion by leptospirae.

Morter (29) reported a series of histopathological changes in the cotyledons which might interfere with the development of the fetus and result in fetal death and abortion.

Turner *et al.* (44) isolated *Leptospira canicola* from the urine of a newborn calf. It is presumed that the infection was contracted *in utero* as the calf was only two days old at the time the urine sample was collected. Morter *et al.* (30) suggested that serotypes other than *L. pomona* might be involved in bovine abortions.

Fennestad and Borg-Petersen (11) have reported experimental *in utero* infection of the fetuses of six immune dams by performing a laparotomy and intraplacental inoculation of viable cultures of leptospirae. They concluded that leptospiral abortion in cattle is usually the result of fetal death following invasion of the fetus by leptospirae during leptospiremia of the dam. Further, the interval between fetal death and expulsion of the fetus exceeds the survival time of the leptospirae in the dead fetus.

Occasionally the bovine fetus may be capable of producing antibodies *in utero* (12). Two fetuses infected with *Leptospira saxkoebing* produced specific antibodies *in utero* and survived intraplacental inoculation.

L. pomona infections have been demonstrated by experimental and clinical studies to be an important cause of porcine abortions (5,7,8,10). Leptospirae have been recovered from the aborted swine fetus (5,7,8).

It has been inferred that swine previously infected would be refractive to subsequent exposure and able to complete a normal gestation period (7).

Morter (29a) reported an apparent incidence of leptospirosis of 10 percent in Iowa swine and that abortions were the most marked clinical manifestation of the disease.

Leptospiral abortions occur in other species. Leptospiral abortions in sheep in Illinois have been reported, but the etiological agent was not identified (3,4). Experimental *L. pomona* infections in pregnant ewes failed to produce any abortions but leptospirae were recovered from the cotyledon of one ewe (20,21).

Smith *et al.* (41) recovered leptospirae from the fetal tissues or fluids of 3 of 17 ewes experimentally infected. The isolations were accomplished by hamster inoculation with fetal tissues collected at necropsy on postinoculation day 3.

Van der Hoeden (45) described an outbreak of caprine leptospirosis in Israel cause by *Leptospira grippotyphosa*. A majority of the pregnant goats aborted.

A mare aborted during an outbreak of *L. pomona* infection in horses (37).

MATERIAL AND METHODS

Fifteen apparently normal purebred Holstein-Friesian heifers 24 to 30 months old were used as experimental animals for the study of experimental leptospirosis in pregnant cattle. The animals were procured at 8 to 12 months of age from the State Prison of Southern Michigan. They were maintained free of contact with other cattle during the period preceding the experiments. No leptospiral antibodies were observed in the serum of any heifer. The modified agglutination-lysis test (27) was employed using L. pomona (strain Johnson), L. canicola and L. icterohemorrhagiae(AB) living antigens. End point titers were interpreted as the greatest tenfold dilution in which at least 50 percent agglutination or lysis occurred. This serological method was used throughout all phases of the work. All heifers were official Brucella abortus strain 19 vaccinates. The heifers were bred artificially with semen furnished by the Michigan Artificial Breeders Cooperative. All heifers were in the 4th to 8th month of pregnancy (table 2) when experimentally infected.

The heifers used in experiment one were divided into three groups. The distribution is set forth in table 1. They were inoculated subcutaneously. Heifers 5, 1, 8 and 9 were assigned to group 1 (table 1). Heifer 9 served as a control. The heifers were exposed to L. pomona strain Wickard (24) which had been maintained in continuous guinea pig passage since its original isolation. The inoculum consisted of 5 ml of heparinized guinea pig blood harvested at the peak of the febrile response (104.5-106.0 F). Heifer 8 received 5 ml of normal cavian blood.

Group 2 was composed of heifers 07, 08, 88 and heifer 3 serving as control (table 1). The inoculum was 5 ml of calves' blood per animal taken during pyrexia. Three serial blood passages in guinea pigs had preceded the inoculation of the calves. The control received 5 ml of normal calf blood.

Group 3 composed of heifers 4, 7, 10, 11 and 6 as control, was exposed to a hamster-lethal variant of L. pomona (strain Wickard). This variant has been designated as "Wickard L" by Bauer (2). Four serial blood passages in hamsters were made. The inoculum was prepared from 10 ml of heparinized blood and 30 ml of a 10 percent emulsion in sterile 0.85 percent saline of kidney, liver and spleen harvested from moribund hamsters (table 1).

Animals 13 and 23 were used for inoculation directly into the fetus. The heifers were given 8 ml of Diquel^{R} (Jen-Sal) intravenously. A T-block of the left paralumbar fossa was accomplished by local injection of xylocaine. The laparotomy site was clipped and scrubbed. The animals were restrained in lateral recumbency on the right side with legs mechanically extended. The site was draped with sterile towels and an incision was made in the posterior part of the paralumbar fossa extending from the level of the tuber coxae to the fold of the flank. The uterus was exteriorized and manually fixed to present the muscle mass of the fetal thigh in suitable position for inoculation. A sterile 16 gauge needle $2\frac{1}{2}$ inches long served as a cannula and was passed through the wall of the uterus. Sterile 2 by 2 gauze sponges saturated with bovine antileptospiral (L. pomona) serum were packed about the cannula. One and one-half ml of a 7-day culture of L. pomona (strain GLD) was inoculated into the fetus by passing a 6-inch

needle through the cannula into the fetal thigh. This strain of L. pomona was originally isolated from dogs by Morter et al. (32) and was in the fifth passage in culture. As the syringe and its attached needle were withdrawn sponges saturated with antileptospiral serum were used to prevent contamination of the surface of the uterus with the inoculum. The exposed area of the uterus was flooded with antileptospiral serum. The cannulating needle was withdrawn and the uterus returned to its normal position. The incision was closed in three layers: the peritoneum, the muscle layers, and the skin. A sterile dressing was applied.

For the re-exposure of pregnant sows, a susceptible boar, a susceptible barrow and five pregnant sows served as experimental animals. The sows had been experimentally infected with L. pomona 10 and 14 months previously. Urinary shedding had not been demonstrated for 8 to 10 months (26). Breeding was by natural service 75 to 95 days prior to re-exposure. Serum antibodies for L. pomona were demonstrated in the sows at the end point titers of 10^{-3} and 10^{-4}.

The challenge strain of L. pomona, of porcine origin, had been maintained in continuous guinea pig passage in this laboratory. A single passage in hamsters was made. The inoculum consisted of heparinized hamster blood and 10 percent suspension of hamster kidney and liver tissue in 0.85 percent sterile sodium chloride solution. Four of the sows, the boar and the barrow each received 7.5 ml of the mixture of blood and tissue suspension. Sow 4928 served as a control and received 7.5 ml of normal hamster blood and tissues.

The inoculum for each of the experimental groups was titrated in hamsters. The highest dilution which caused death of the hamsters or stimulated antibody response was considered the end point. Less than 10 organisms are known to be infective for hamsters (2). The number of organisms per ml of inoculum varied from 10^4 to more than 10^6 when prepared from blood or tissues (table 1). The culture used to inoculate the two fetuses *in utero* was found to contain $>10^7$ organisms per ml.

All animals were observed for clinical manifestations of disease. Rectal temperatures were taken twice daily (table 2) until agglutination-lysis end point titers of 10^{-2} or greater were observed or the animals were necropsied.

Animal inoculation techniques were employed to determine the presence of leptospirae in tissues or fluids. Tissues were processed in tissue grinders with sterile 0.85 percent saline to give a final tissue concentration of approximately 10 percent. The material was injected intraperitoneally into young guinea pigs weighing approximately 250 grams or 4- to 5-week-old hamsters. Guinea pigs received 2 ml of the inoculum and hamsters 1 ml. Undiluted amnio-allantoic fluids, fetal stomach contents, urine and blood were also injected intraperitoneally in the same amounts into hamsters or guinea pigs. Approximately three weeks postinoculation the animals were killed and sera checked for agglutination-lysis reactions. The presence of leptospiral antibodies was considered indicative of leptospirae in the original materials.

Hemocultures were prepared with bovine blood in fluid media on postexposure days 7 and 8 or later at the time of a temperature

rise and with swine blood on days 5 to 8 postexposure. Blood was collected aseptically from the jugular vein of the cattle or the precaval venous well of swine. Each of five tubes of Chang's fluid medium (24) or Stuart's fluid medium (Difco) was inoculated with five drops of blood and incubated at 29 C. Each tube was examined microscopically, utilizing darkfield illumination (600x), at ten-day intervals for the presence of leptospirae. Each of two guinea pigs or hamsters was inoculated with 0.5 ml of blood from the heifers at the same time media were inoculated. Positive agglutination-lysis titers in the laboratory animals 18 to 24 days postinoculation was considered indicative of leptospirae in the inoculum, i.e. heifers were in the leptospiremic phase of the disease.

To procure arterial blood samples from the heifers for comparative evaluation of fetal and maternal hematological determinations, the carotid artery was surgically fixed to the skin of the midcervical region. Surgery was performed under local anesthesia accomplished by injection of 2 percent xylocaine into the area. The site was clipped and prepared for surgery. The skin was incised dorsal and parallel to the jugular furrow. The skin was reflected dorsally, the cervical muscles separated exposing the carotid which was dissected free of the surrounding connective tissue. A loop of the carotid was positioned beneath the skin. The muscles were sutured together below the carotid and the loop of the vessel was fixed in the subcutaneous area by nonabsorbable sutures. The skin incision was closed by interrupted mattress sutures. A minimal recovery period of two weeks intervened between the surgical fixation of the carotid and experimental exposure to L. pomona.

To obtain fetal blood samples a hysterotomy was performed under local anesthetic and tranquilizer as previously described. The surgical procedures to expose the uterus were the same as those for the *in utero* inoculation of the fetus. Arterial and venous blood samples were obtained from the umbilical vessels. The heifers were then exsanguinated.

Heifers were killed from postinoculation days 10 to 60 in an attempt to delineate the pathogenesis of bovine leptospirosis during pregnancy. The maternal tissues collected at necropsy were: kidney, adrenal, liver, spleen, lung, uterus, cotyledon, mammary gland and brain. Fetal tissues included: thymus, lung, heart, liver, spleen, kidney, adrenal and brain. The swine were anesthetized by intravenous administration of sodium pentobarbital, exsanguinated and postmortem examinations conducted. Fetal and maternal kidney, liver, brain, adrenal, heart and placenta were collected. Tissues were collected for both animal inoculation procedures and histopathological evaluation. One or more fixatives were used: Zenker's, 10 percent buffered formalin, or Carnoy's. Tissues were embedded in paraffin and sectioned at 7 to 8 u. Hematoxylin and eosin, analine blue and Sudan IV were the staining procedures employed.

RESULTS

1. Exposure of Pregnant Heifers.

The clinical manifestations of leptospirosis observed from postinoculation days 1 through 10 included polypnea, anorexia and increased body temperature. Maximal rectal temperatures varied from 102.5 to 106.3 F, with seven heifers having temperatures greater than 104 F (table 2). Other symptoms were variable and not distinctive. The temperature rise was accompanied by a transient anorexia, a slight increase in respiratory rate, some constipation and a dulling of the hair coat.

Leptospirae were isolated from five heifers by direct culture or animal inoculation or by both procedures during leptospiremia. No isolations were made from five of the exposed heifers or from the three controls. The agent was recovered from the kidneys or urine of four heifers and from the cotyledons of one. The agent was not isolated from any of the fetal tissues or fluids nor the kidneys or urine of the control heifers (table 3).

The initial agglutination-lysis reactions were observed from days 5 to 14. All 10 of the infected heifers developed serum titers in a dilution of 10^{-2} or greater. The controls remained serologically negative. No agglutination-lysis reactions were demonstrable in any of the fetal sera, body fluids or amnio-allantoic fluids. The serological results are summarized in table 4.

Each animal in group 1 except the control was given 5 ml of cavian blood that contained 10^4 organisms per ml. Heifer 5 had a rectal temperature of 104.4 F on postexposure day 8; the agent was isolated culturally from blood on day 8; and from the kidney by

animal inoculation on day 10. The highest rectal temperature recorded for heifer 1 was 102.5F on postexposure day 8. Leptospirae were recovered by hemoculture and animal inoculation on day 10 and from the kidneys on day 23. The agent was not recovered from the blood or kidneys of heifer 8 although a rectal temperature of 104.5 F was recorded on postinoculation day 3. Hemocultures were not made until day five.

The first demonstrable titers for the heifers in group 1 were: heifer 5, 10^{-2} on day 9; heifer 1, 10^{-3} on day 13; and 10^{-2} for heifer 8 on day 14. The titers persisted until the animals were killed (table 4).

Heifers 07, 08 and 88 of group 2 each were inoculated with 5 ml of calves' blood which contained more than 10^4 organisms per ml. Hemocultures prepared on postexposure days 8 or 9 were positive for all three of the infected animals. Heifer 07 died on day 33 as the result of an embolus that detached from the intima of the carotid. A severe chronic necrotic endarteritis was found at the point of subcutaneous fixation of the carotid. Death was attributed to the embolus lodging in blood vessels of the brain. Isolations of leptospirae were not attempted from fetal or maternal tissues of this animal.

Guinea pigs inoculated with kidney tissue from heifer 88 on day 40 developed a positive serum titer for L. pomona. Leptospirae were not recovered from the kidneys of heifer 08. The three heifers developed significant agglutination-lysis reactions (table 4).

The heifers in group 3 inoculated with the hamster blood and tissue (table 1) were observed to have the maximal rectal temperatures on days 1 through 5 with temperatures from 103.0 to 106.3 F recorded.

Attempted isolations from the blood stream all gave negative results. No hemocultures were made before the appearance of agglutinins to L. pomona in the sera of all four exposed heifers (table 4). The agent was demonstrated in the kidneys of heifer 4 on day 30 but not in the kidneys of heifers 7, 10 or 11 (table 3).

The significant gross lesions observed at necropsy were limited to the maternal kidneys and were extremely variable according to size and number. Some kidneys had only two or three grayish-white foci one to two mm in diameter. In other kidneys, multiple lesions up to 8 mm in diameter were observed. On cross section the less marked lesions were superficial, i.e. involving one to two mm of the cortex. The more pronounced lesions invaded the medullary portion of the kidney. No gross lesions were found in any of the fetuses.

Microscopically the foci in the kidneys consisted primarily of lymphocytes with a few plasma cells and macrophages. Within the areas of dense lymphocytic infiltration much of the parenchyma had undergone degeneration. Many of the tubules had atrophied. The epithelium of some tubules was granular, vesicular or desquamated, and pyknotic nuclei were observed in many of the tubular epithelial cells. In heifer 08 the central portions of the lymphocytic foci were less dense than the perimeter of the lesions. The nuclei of the lymphocytes in the central portion were less dense than normal. Repair by regeneration of renal parenchymatous cells of fibroblast proliferation was not apparent (figure 1). The cortex was contracted at the sites of the foci of lymphocytic infiltration in heifer 7. Connective tissue proliferation was marked at the cortico-medullary junction but decreased in amount

toward the capsule. In the subcapsular portion of the cortex the concentration of lymphocytes remained practically unchanged from an early lesion. There was a marked depression in the cortical surface at the site of the foci (figure 2).

Small foci of lymphocytes had infiltrated the areas adjacent to interlobular bile ducts (figure 3). In heifers 5 and 7 the hepatic perivascular spaces were infiltrated with eosinophils and an occasional polymorphonuclear granulocyte (figure 4). No microscopic lesions were found in the kidney or liver of any of the control animals.

The microscopic lesions in the cotyledons were most pronounced in those heifers killed during the first thirty days postexposure. On day 10 in heifer 5, an acute focal placentitis was characterized by necrotic chorionic villi and maternal crypts filled with tissue debris and blood clots. Alterations in the relationship between the fetal and maternal placentae consisted of changes in the respective epithelial surfaces. Some maternal crypts were devoid of epithelium. The chorionic epithelium was hydropic and contained pyknotic nuclei. Pyknosis was most apparent in the diplokaryocytes (figure 5). Necrotic fetal placental tissue had lost its normal structure by day 23. Some of the fetal cells were in close proximity to the maternal epithelium. The maternal epithelium no longer formed a well defined demarcation between itself and the necrotic fetal villi (figure 6). In figure 7 the pyknosis of the maternal epithelium was marked. One crypt was devoid of epithelium. Increased amounts of fibrous connective tissue surrounded the maternal crypts (figure 7). By postexposure day 45 the presence of large cells, some with hyperchromatic nuclei and a loss of the normal appearance of the maternal epithelium, suggested

regenerative hyperplasia. The fetal placenta showed no apparent damage (figure 8). The other heifers in this group showed similar changes in cotyledons with the most marked changes in those killed within thirty days after inoculation. Comparable changes were not observed on the control sections studied.

II. Direct Inoculation of the Fetus *in utero*.

Heifer 23 had an uneventful postsurgical recovery period. No clinical signs of leptospirosis were observed. It proved to be impossible to determine fetal viability following inoculation. The four-month fetus was not palpable, being located well below the pelvic brim. An enlarged edematous cervix was found by rectal palpation on post-inoculation day 8 and thereafter. On day 8 the heifer had a positive serum titer of 10^{-3} which rose to 10^{-5} on day 14. Leptospirae were not isolated in hemocultures prepared on postinoculation days 6, 7 or 8.

At necropsy, on day 14, the uterus was cyanotic, the cotyledons were pale and the maternal and fetal placentae separated easily. The fetus was dead and edematous. All body cavities and tissue spaces were filled with a serosanguineous fluid. The heart and large vessels were empty of blood. Microscopically, nucleated erythrocytes were found in the blood vessels of all fetal tissues examined (figure 9). No mature erythrocytes were observed. In tissue sections of a noninfected control fetus of comparable age no immature erythrocytes were observed.

The maternal crypts were devoid of fetal villi and were characterized by increased amounts of connective tissue and disappearance of the maternal epithelium (figure 10). Foci of lymphocytic infiltration were not observed in any fetal tissues. A few scattered foci of lymphocytes were observed in the maternal liver.

No fetal blood sample was obtained. Thoracic fluid had a detectable agglutination-lysis reaction, probably nonspecific. The amniotic fluids were negative.

Inoculation of guinea pigs with amniotic fluid, fetal liver, spleen, muscle, lung and kidney demonstrated the presence of leptospirae only in the fetal kidney (three of five guinea pigs). Of the maternal tissue samples only the kidney was found to contain leptospirae by the guinea pig inoculation technique. Routine cultural procedures did not yield any evidence of bacterial contamination of the fetus.

Heifer 13 was killed on postinoculation day 7. No gross lesions were found in any of the maternal tissues. The fetal placentae were not firmly attached to the maternal placentae, separating without difficulty. The uterus was normal in appearance. The cervix was normal and closed by a mucous plug. The 7-month fetus was viable but appeared weak and the heart ceased to beat shortly after opening the uterus. The fetus made no respiratory movements. No gross lesions were observed. Microscopically small foci of immature lymphocytes and plasma cells were observed in the cortex of the fetal kidney (figure 11) and adjacent to the large vessels of the liver. A diffuse placentitis involved the epithelium of the chorion and maternal placentae (figures 12 and 13). The remnants of the chorionic epithelium were hydropic and the nuclei of many diplokaryocytes were pkynotic. Necrosis of the endometrium was characterized by numerous pyknotic nuclei. Lymphocytes had infiltrated the minor calyces but were not present in the parenchyma of the maternal kidney (figure 14).

Antibodies were not demonstrated by the agglutination-lysis techniques in the amniotic fluid, fetal blood or fetal stomach contents. The heifer was serologically negative on both postinoculation days 6 and 7. The dilutions used were 10^{-1}, 10^{-2} and 10^{-3}.

Heifer 13 was in the leptospiremic phase of the disease at the time of necropsy. Leptospirae were isolated in hemocultures inoculated on day 7 and were demonstrated by the guinea pig inoculation technique in the maternal liver, kidney and cotyledons. All guinea pigs inoculated with fetal stomach contents, amniotic fluid, liver, spleen, lung, skeletal muscle and kidney were serologically positive to L. pomona.

III. Re-exposure of Pregnant Sows.

None of the pregnant sows, control or challenged, demonstrated any clinical signs of leptospirosis. The boar and the barrow had temperature rises to 104.6 F on postinfection days 5 and 6. Concurrent constipation and anorexia were noted.

Leptospirae were isolated from the blood of the boar and the barrow by cultural methods on postinfection days 5, 6 and 7. Isolations were not accomplished from the blood of pregnant sows. Leptospirae were demonstrated by guinea pig inoculation in the kidney and brain of the barrow on postinfection day 7, but not from liver or spleen. Leptospirae were present in the kidney of the boar on postinfection day 13; all other tissues including the testes were negative.

The control sow, 4928, was killed on day 3. Maternal kidney, liver and spleen, amnio-allantoic fluid, and fetal blood, kidney and liver were inoculated into guinea pigs, but leptospirae were not demonstrated. Seven 90-day fetuses appeared normal. The maternal serum gave an agglutination-lysis titer of 10^{-4}. Urine antibody was

demonstrated at a dilution 10^{-3}. The fetal sera and amnio-allantoic fluids were serologically negative.

Sow 4929 was killed on postchallenge day 6. Nine apparently normal fetuses at approximately the 95th day of gestation, were present. Fetal and maternal tissues did not contain leptospirae. The terminal agglutination-lysis titer of the maternal serum was 10^{-7} and maternal urine 10^{-3}. No antibody reaction was observed in the fetal sera.

At necropsy, 11 days after challenge, 10 apparently normal, 86-day fetuses were present in sow 4958. Leptospirae were not isolated from the fetuses or the sow. The end point titer of the maternal serum was 10^{-8}; the urine titer was 10^{-2}. Leptospiral antibodies were not observed in the fetal sera.

Seventeen days after challenge, sows 4923 and 4969 farrowed litters of 9 and 10 respectively, which developed normally. Agglutination-lysis reactions occurred in a 10^{-1} dilution of sera of newborn pigs three hours after birth. Samples procured from the pigs at birth were negative. Two pigs from each litter were killed when 48 hours old, and tissues were inoculated into guinea pigs. The results were negative.

Sow 4969 was killed on postchallenge day 41 and sow 4923 on day 62. Leptospirae were not isolated from the kidneys or urine.

At necropsy the gross changes (greyish-white foci, measuring 1 to 4 mm in diameter) were confined to the kidneys of all mature pigs, including the control sow. On section, a few lesions extended through the cortex into the medulla. The placenta and fetal kidneys appeared to be normal.

Microscopically, many of the foci in the kidneys of the challenged sows consisted of an infiltration of lymphocytes, a few plasma cells and some macrophages (figure 15). Some areas were characterized by active proliferation of fibroblasts, while others were primarily cicatrical tissues (figures 16, 17). In these foci, a tubule was occasionally atrophied. The renal corpuscles had some thickening of Bowman's capsules and the glomerular tufts due to connective tissue proliferation. The epithelium of the proximal convoluted tubules had and increase of fat (figure 18). The kidney lesions of the boar and the barrow were composed of small areas of predominantly lymphocytic infiltration similar to the type described by Langham et al. (19).

An active inflammatory process, typified by foci of lymphocytic infiltration, was an unexpected finding in the kidneys of the control sow. Some of the foci extended only 1 to 2 mm below the cortical surface, while others penetrated to depths of 8 to 9 mm, reaching the medulla. Connective tissue proliferation was most marked in the medulla. No evidence of leptospirae was observed in the silver-impregnated kidney sections.

DISCUSSION

Abortions due to leptospirosis (L. pomona) are a major cause of economic losses in cattle and swine. In beef cattle and swine, abortions automatically limit the potential income of the producer. In dairy cattle the loss of a calf has less importance but production throughout the ensuing lactation period may be seriously curtailed.

The apparent difference in the ability of leptospirae to invade the porcine fetus and not the bovine fetus may be attributed to the species differences in placentation. The sow has a diffuse placenta with the entire surface of the amnio-chorion forming a placenta with the endometrial surface in apposition to it. The cow has a cotyledonary type of placenta with 100 to 120 specific points of attachment. By classical histological definition the placenta of the sow is of the epitheliochorial type with six cell layers intervening between the maternal and fetal circulation. The maternal intraepithelial capillaries of the porcine placenta are frequently in direct contact with the chorion. Fetal capillaries extend between the cells of the chorion and are found in direct apposition to maternal capillaries. From the middle of the gestation period to term the porcine placenta can be classified as endotheliochorial or endothelioendothelial with as few as 2 cell layers intervening between the fetal and maternal systems. The bovine placenta is considered to be syndesmochorial lacking the maternal epithelium and five cell layers separating the fetal and maternal circulatory systems. This is not actually the case: the bovine placenta normally maintains three maternal and three fetal cell layers. Only in the intercotyledonary spaces does the surface of the endometrium become denuded and even then the relationship between the fetal

and maternal circulation is less intimate than in the sow. The more complex placental barrier in the cow may prevent invasion of the fetus by leptospirae. Other characteristics of the placenta, trophoblastic enzymes or membrane potential may differ enough to permit invasion of the swine fetus but not the bovine fetus.

The bovine fetus furnishes a suitable environment for parasitism by L. pomona as demonstrated by the establishment of the experimental in utero fetal infections which appeared to follow a course similar to postnatal infections. Fetal leptospiremia occurred on postinoculation day 7 (fetus 13) with leptospirae present in all tissues and fluids examined. Leptospirae were demonstrated in the kidney of fetus 23 on postinoculation day 14. No leptospirae were present in other tissues. The appearance of circulating antibodies is positively correlated with the cessation of the leptospiremia and establishment of the renal carrier state in typical cases of leptospirosis. Leptospiral antibodies were not present in the fetal serum. Survival of leptospirae in the kidney and no other tissue cannot be explained by the protection afforded the organisms against antibodies in the kidney tubules. Possibly the physiological requirements of the organism can be satisfied only in the kidney. In any event, the typical course of the disease, except for antibody production, was mimicked by the course observed in the fetus in utero. Fetus 23 was dead and resulting autolytic changes may not have been compatible with the survival of the leptospirae in other tissues. This would suggest some special type of protective environment furnished by the kidney permitting their survival.

Leptospirae crossed the placental barrier from the fetal to the maternal tissues following the leptospiral infection of the bovine fetus. Leptospirae were isolated from kidney of one heifer and from the blood, liver, kidney and cotyledons of the second dam. Positive hemocultures were obtained on postinoculation day 7 and positive serum titers on day 8. The organisms appeared to have traversed the placental barrier, movement from fetus to dam, rapidly and produced concurrent infections of the dam and the fetus. The maternal infections could have resulted from contamination of the peritoneal surface of the uterus: however, the precautionary measures employed minimized this possibility.

Conditions may exist that permit the leptospirae to traverse the placental barrier from the fetus to the dam that are different than those conditions that appear to prevent invasion of the fetus as a result of maternal infection. A difference in membrane potential could permit movement in one direction but prevent retrograde movement. The charge at the maternal surface might repel the organisms. The direct infection of the fetus could produce alterations rendering the placental barrier more permeable to the passage of leptospirae. The validity of the latter hypothesis is questionable as the serological and cultural findings indicate that the infection of the dam occurred at approximately the same time as that of the fetus, and before marked alterations in the placenta could occur.

Several hypotheses have been suggested to explain the inability of several workers to isolate leptospirae from the bovine fetus (13, 14,15,31,35): 1) inability of the organisms to survive in the dead fetus during the interval prior to expulsion, 2) invasion of the fetus

occurs during the leptospiremia of the dam and a secondary incubation period must follow before significant numbers of leptospirae are found in the fetus, 3) ability of the fetus to produce antibodies against the leptospirae with their elimination from the fetal system, 4) production of toxic materials in the maternal system by the leptospirae which traverse the placental barrier and cause fetal death, 5) cytotoxic effects upon the fetal-maternal placental junction which might interfere with the development of the fetus resulting in fetal death and abortion. Three of these hypotheses assume an actual infection of the fetus; two suggest that leptospirae do not invade the fetus.

If leptospirae invade the bovine fetus, proper application of cultural methods should result in recovery of the causal agent. Insufficient numbers of organisms in the material examined, interference by antibodies or lack of sensitivity of the techniques employed could contribute to the failure to recover leptospirae from the bovine fetus if fetal infection occurred. The ability of the animal inoculation technique to determine the presence of as few as five organisms (L. pomona) has been reported (2), and one virulent leptospira (L. pomona) may establish infection in guinea pigs or hamsters (46). Leptospirae were recovered from the experimentally infected fetuses by animal inoculation. Fetal tissues did not interfere with the results. Even if very few organisms were contained in the inoculum the animal inoculation technique would signify their presence.

Rudge (38) reported interference with demonstration of leptospirae in kidney tissue from a calf with leptospiruria by specific antibodies. The calf had a titer of 10^{-4} and the organisms were inactivated during the preparation of the tissue suspension for inoculation

into guinea pigs. No demonstrable agglutination-lysis reactions were observed with the fetal serums in these experiments. Interference by antibody could not have influenced the results of this work since the fetal sera in these experiments have uniformly been free of leptospiral antibodies.

The gross and microscopic lesions in the dead fetus (heifer 25) support the theory of fetal death due to the effects of hemolytic toxin (15). An overwhelming hemolytic anemia is suggested by the absence of blood in the heart and large vessels, pleural and peritoneal cavities filled with a serosanguineous fluid, and the predominance of immature, nucleated erythrocytes observed in tissue sections. It could be postulated that a leptospiral toxin produced severe hemolytic anemia. A compensatory release of immature erythrocytes from the hemopoietic centers occurred. Increased permeability of the vessel walls and a reduction in colloidal osmotic pressure resulted in the paucity of blood in the vascular system and the extravascular serosanguineous fluid.

The systemic reactions of the heifers did not affect fetal life although temperatures as high as 106.3 F were recorded. The hypothesis that maternal pyrexia and systemic reactions can cause fetal death lack proof. The reactions to experimental infection of the dam are usually mild and transient and seldom severe enough to produce an abortion. Further, the interval between the maternal leptospiremia and abortion suggests the two are not directly related.

Alterations in the chorionic-maternal placental relations reported by Morter (29) were progressive in nature. The early lesions were not extensive but increased in severity with time following

infection. To ascertain the effect of the experimental L. pomona infections of the pregnant heifers on the placental relationships selected tissues from the uterus and cotyledons of each heifer were examined microscopically. Placentitis was present in either the chorion or the maternal epithelium in all cotyledons examined from infected heifers. Similar changes were not observed in the 3 controls.

The placentitis was similar to that previously reported (30) but in no case as extensive as the changes observed following abortion. The most marked lesions were observed in those heifers killed approximately 30 days after inoculation. According to observations recorded in various published papers, most leptospiral abortions occur 3 to 4 weeks after infection in both naturally occurring and experimental infections (11,13,15,28,31). This is about the same time the most severe lesions were found. By day 45 the areas of placental damage were being repaired by regenerative hyperplasia. The placental lesions appeared to increase in severity until day 30 and then regress and in no case were severe enough to cause fetal death.

Leptospirae were isolated from the cotyledons of one heifer. Previous reports (20,28,41) indicated that sporadic invasion of the cotyledons of cattle and sheep occurs. In most cases the agent cannot be recovered from the placenta. The occurrence of a placentitis with no organisms demonstrable in these tissues suggests that a cytopathogenic factor may produce the changes. In bovine leptospirosis, infection of the placental tissue cannot be demonstrated routinely and is probably not important in the pathogenesis of placental lesions. If the tissue changes result from a cytopathogenic agent, the amount or activity of such a substance could result in great variation in damage

to the placenta. Death of the fetus would depend on the degree of interference with placental exchanges and, as in brucellosis, might not always occur. The placentitis produced by the experimental infection of the heifers was not severe enough to cause fetal death.

Serological or cultural evidence of leptospirosis was observed in all 10 heifers exposed experimentally to L. pomona but not in any of the three controls. No bacteriological, serological or histopathological evidence of infection was found in any of the fetuses from these 10 heifers. All of the fetuses were viable at the time of necropsy and consequently leptospirae could not have been destroyed by the autolytic products of a dead fetus. Fifty guinea pigs or hamsters were inoculated with fetal tissue emulsions or fluids from each fetus. The animal inoculation technique is sensitive to small numbers of leptospirae and the presence of organisms would have been detected had they been present in the fetuses. Under the conditions of this experiment organisms were unable to traverse the placental barrier and establish fetal infection. The heifers were necropsied from 10 to 60 days postexposure. If a secondary incubation period were required for the establishment of fetal infection such an event should have been determined by this procedure.

The ability of an organism to parasitize a host depends upon invasion of the host and establishment of an infection. This is usually followed by the production of lesions as a result of the infection. Bauer (2) reported a difference in virulence between strains of L. pomona. Two strains of L. pomona, Wickard (24) and Wickard L (2), known to differ in virulence, were employed experimentally to infect the heifers. Neither strain proved capable of invading the fetuses.

Virulence of an organism alters with continuous passage in media or adaptation to a particular host. Leptospirae maintained in fluid media become less virulent - nearly, if not completely, apathogenic. Strains of L. pomona maintained in serial passages of kidney tissue in guinea pigs retain a high degree of virulence for other animals (21,26, 27,28,31). Each group of heifers received an inoculum prepared in a different manner. The inocula were prepared in guinea pigs, hamsters and calves (table 1). The calf passage was used in an attempt to develop a bovine-adapted population of leptospirae, but the inoculum from calves elicited a response similar to that produced by the inoculum of guinea pig blood. The hamster lethal variant and the large number of organisms employed in inoculating group 3 were designed to create a more severe manifestation than produced in the other groups. The incubation period was shortened, antibodies were detectable sooner, maximal temperatures higher, but the other manifestations were no more marked than in other animals.

Those heifers receiving the material lethal to hamsters were given 600 times as many organisms as the groups receiving guinea pig or calf blood. Demonstrable agglutination-lysis reactions were observed in the serum of all four of these heifers by postinoculation day 6. The average incubation period in experimental L. pomona infections of cattle is 6 to 8 days, and antibodies are usually not detected before day 7 or 8. Apparently the large inoculum had shortened the incubation period. A similar phenomenon, i.e. leptospiremia on postinoculation days 1 to 4, has been reported in sheep and calves when large numbers of leptospirae were inoculated subcutaneously or intravenously (20,21,36,41).

Blood taken during pyrexia provides an inoculum of rapidly multiplying organisms. Such a population may differ widely in virulence from the population that localizes in the kidneys. Organisms shed in the urine provide the usual means for natural transmission of leptospirosis. The use of porcine or bovine urine from renal shedders as an inoculum might produce leptospirosis of a different character than that observed in these experiments.

The heifers were of similar genetic background, having been selected from a single herd of purebred Holstein-Friesian cattle. A degree of inherent resistance to leptospirosis could have minimized the effects of the experimental disease. A subsequent naturally occurring outbreak in the parent herd was very mild. Recognition was limited to serological diagnosis. The heifers were in excellent health, well housed and were receiving an adequate ration. Being primiparous, none of the heifers was lactating. Many of the factors thought to enhance susceptibility of animals naturally infected were minimized by the conditions of the experiment. Under different conditions of management, environment and nutrition, leptospirosis might be more severe and sporadic infection of the fetus occur.

The inability to recover leptospirae from the fetuses of eleven heifers experimentally infected with L. pomona has been previously reported (28,31). Alterations in the placental relationships that could interfere with fetal development were also found (30). In this series of experiments 10 pregnant heifers were infected with L. pomona and in no case was fetal infection produced. Direct infection of the fetus must be sporadic and may depend upon fortuitous conditions of exposure, nutrition, parasitism, lactation or nonspecific stress factors.

Leptospirae must be recovered from the aborted bovine fetus by accepted techniques to establish fetal leptospirosis as the cause of fetal death.

Maternal antibodies are unable to cross the placental barrier of the sow. It was postulated that leptospirae might escape the effect of the maternal antibodies, cross the placental barrier, and survive in the antibody-free fetal environment. The pathogenicity of the challenge strain used in these experiments was demonstrated by the course of the disease in susceptible swine. The demonstration of L. pomona in the brain of the barrow indicates an invasiveness of the organism for this tissue. Encephalitis or meningitis, a reported clinical manifestation of porcine leptospirosis (24,39), could result from direct infection of the central nervous system by leptospirae.

Since leptospirae were not demonstrable in fetal tissues, and fetal deaths or abortions did not occur, the maternal antibodies furnished adequate protection against challenge and uterine invasion apparently did not occur. Gestation and parturition were normal. Colostral antibodies were detected in the sera of newborn pigs three hours after birth. Protection against possible postnatal infections was afforded.

Marked increase in serum antibody levels was observed following challenge, with endpoint titers increasing by a factor of 10^4 in the immune sows. The response was transient; the serum titer of one sow decreased from 10^{-8} to 10^{-4} by postchallenge day 41.

The barrow had leptospiremia with concurrent serum titer of 10^{-4} on postinfection day 7. Leptospirae were isolated from the blood in the presence of homologous antibody. The early immune response may

produce an incomplete antibody, which is unable to react with all the leptospirae. Leptospirae can be successfully maintained through several passages in media containing homologous antibody (33). This phenomenon suggests a possible mechanism for *in vivo* selection or variance. Not all leptospirae are agglutinated by homologous antiserum *in vitro*. Morse *et al*. (27) suggested that agglutination is rarely complete in the agglutination-lysis test. Neutralization of the circulating antibody permits survival of some organisms.

The histopathological changes in the susceptible swine were comparable to the renal changes previously described (8,19). Langham, Morse and Morter (19) described microscopic lesions of repair and active inflammatory reaction 184 days after exposure. Similar lesions were observed in the kidneys of the re-exposed sows. Active renal inflammation could have been the result of challenge, i.e. the exposure of sensitized renal tissue to the leptospiral antigen. However, this would not explain the active inflammatory processes in the kidneys of the control sow. Since active inflammatory processes persisted for 10 to 14 months after initial infection, some antigenic principles of the leptospirae, capable of stimulating a cellular reaction, may be retained in localized areas after renal shedding is not detectable.

SUMMARY AND CONCLUSIONS

All of ten pregnant heifers exposed to Leptospira pomona developed either clinical or serological manifestations of leptospirosis. The heifers were killed 10 to 60 days after exposure. None of the fetuses developed leptospirosis. Leptospirae were not demonstrable in the fetal fluids or tissues and with one exception the placental tissues. Direct invasion of the fetus by L. pomona does not appear to play an important role in bovine leptospiral abortions.

The histopathological lesions of the heifers closely resembled those observed in other reported experimental L. pomona infections of cattle. The presence of a cytopathogenic agent produced by the leptospirae is suggested. This proposed agent is probably responsible for alterations in the maternal-fetal placental relationship that interfere with fetal development and may cause fetal death. The placental lesions were the most marked at about thirty days after exposure. Fifteen days later regenerative hyperplasia of the maternal placental epithelium indicated that the lesions were undergoing repair.

Sporadic infections of the bovine fetus by L. pomona occur but probably are influenced by special conditions of virulence and susceptibility. The preponderance of the experimental evidence indicates that fetal leptospirosis is not the major cause of abortions.

Two fetuses were infected with L. pomona by direct inoculation in utero. The fetal leptospirosis was similar to the postnatal infections. Leptospiremia was observed in one fetus on postexposure day 7 and by day 14 the leptospirae were demonstrable in the kidney but no other tissues. The histopathological lesions of the placenta resembled those associated with maternal infections.

Five sows had been experimentally infected with L. pomona 10 to 14 months previously. The sows were re-exposed during the last one-half of the gestation period. The immunity produced by the active infection was sufficient to protect the pregnant sows against challenge with a virulent strain of L. pomona.

Active inflammatory processes persisted in the porcine kidney for 10 to 14 months after initial infection and 8 to 10 months after leptospirae were demonstrated in the urine.

Although direct invasion of the porcine fetus has been considered the cause of porcine leptospiral abortions the exact mechanisms of action on the fetus have not been determined. L. pomona infections in swine are very mild and frequently almost asymptomatic except for abortion by the pregnant sow. Further elucidation of the toxic principles and the pathogenesis of the porcine abortions is indicated. The chemical entities which are proposed as active in bovine abortions may have similar effects on the porcine fetus. Infection of the porcine fetus could be a manifestation of species susceptibility to the invasiveness of the organism and the toxic materials produced either in the maternal or fetal system.

TABLE 1

Source and Type of Leptospira pomona Used in Exposure of Heifers

Heifer Number	Source	Material	Passage*	Amount ml	Number ϕ per ml
GROUP 1 - Wickard strain					
Infected					
5,1,8	guinea pig	blood	5 GP	5	10^4
Control 9	guinea pig	blood		5	0
GROUP 2 - Wickard strain					
Infected					
07,08,88	calves	blood	3 GP & 1 calf	5	10^4
Control 3	calf	blood		5	0
GROUP 3 - Wickard L strain					
Infected					
4,7,10,11	hamster	blood & tissue	4 hamster	3 ϟ	10^7
Control 6	hamster	blood		3	0

* Serial passages of blood at pyrexia.

ϕ Titration in hamsters, $\log_{10}$; less than 10 organisms infective dose.

ϟ 2 ml hamster blood subcutaneously 24 hours after original inoculum.

TABLE 2

Observations on Febrile Response and Stage of Gestation

Group number	Heifer number	Maximum body temperatures Postinoculation day	Degrees F.	Month of gestation
1 Infected	5	8	104.4	4½
	1	8	102.5	8
	8	3	104.5	4
Control	9	5	100.9	8½
2 Infected	07	8	103.1	5
	08	8	104.4	7
	88	9	104.0	6
Control	3	6	100.6	8
3 Infected	4	3	103.0	7
	7	3	106.3	4
	10	5	105.1	5
	11	1	105.5	7½
Control	6	2	101.2	4

TABLE 3

Isolation of Leptospirae from Heifers and Fetuses

Heifer number	Postinoculation day		Isolations of Leptospirae: Blood* Cultural	Blood* Animal inoculation	Tissues Day of necropsy	Tissues Maternal	Tissues Fetal
GROUP 1							
Infected	5	8	+	-	10	+	-
	1	10	+	+	23	+	-
	8	-	-	-	34	-	-
Control	9	-	-	-	5/	-	-
GROUP 2							
Infected	07	10	+	-	33ϕ	not attempted	
	08	8	+	+	37	-	-
	88	9	-	+	40	+	-
Control	3	-	-	-	17	-	-
GROUP 3							
Infected	4	7-10	-	-	30	+	-
	7	7-10	-	-	45	-	-
	10	7-10	-	-	60	-	-
	11	7-10	-	-	42	calved normally	
Control	6	-	-	-	10	-	-

* Culturally in media; guinea pig or hamster inoculation. First hemocultures on postinoculation day 5.

/ Hysterotomy to obtain fetal and placental specimens.

ϕ Died of unrelated causes.

TABLE 4

Antibody Titers for Leptospira pomona

Heifer number		Initial Day	Initial Titer*	Final Day	Final Titer*	Fetal sera and fluids
GROUP 1						
Infected	5	9	2	10	2	0
	1	13	3	23	7	0
	8	14	2	34	5	0
Control	9		0	5	0	0
GROUP 2						
Infected	07	13	1	35	3	0
	08	10	3	37	5	0
	88	12	5	40	5	0
Control	3		0	17	0	0
GROUP 3						
Infected	4	6	1	30	5	0
	7	5	1	45	5	0
	10	6	2	60	4	0
	11	5	2	42	6	0
Control	6		0	10	0	0

* Reciprocal of the logarithm of the greatest serum dilution in which at least 50 percent of the organisms were agglutinated or lysed.

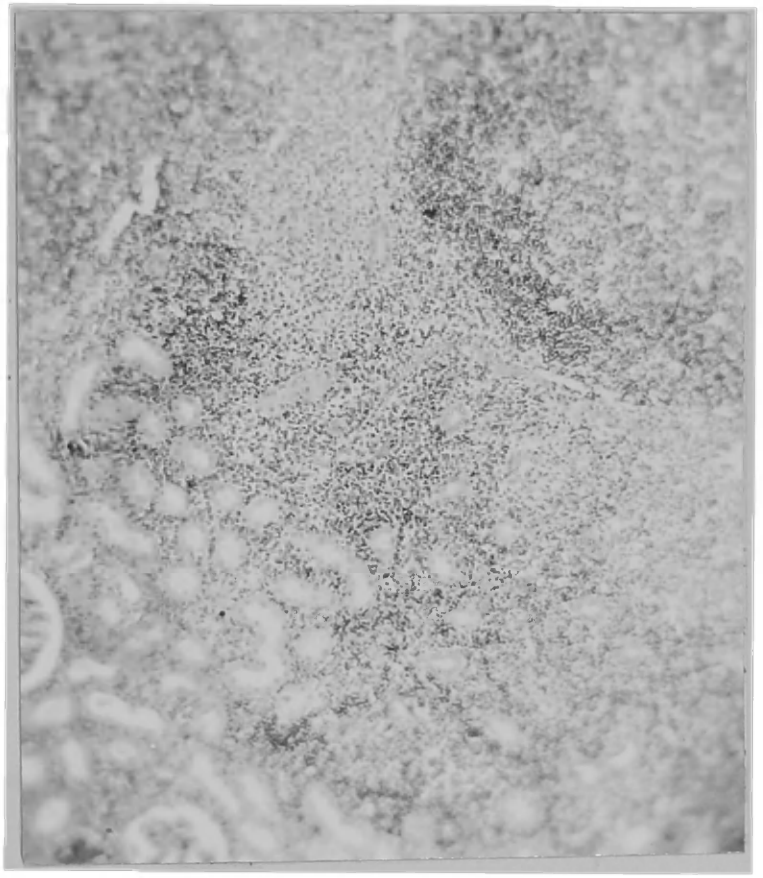

FIGURE 1. Bovine kidney from heifer experimentally infected with L. pomona at day 37 with marked lymphocytic infiltration of periphery of focal lesion and less dense infiltration in center. x100.

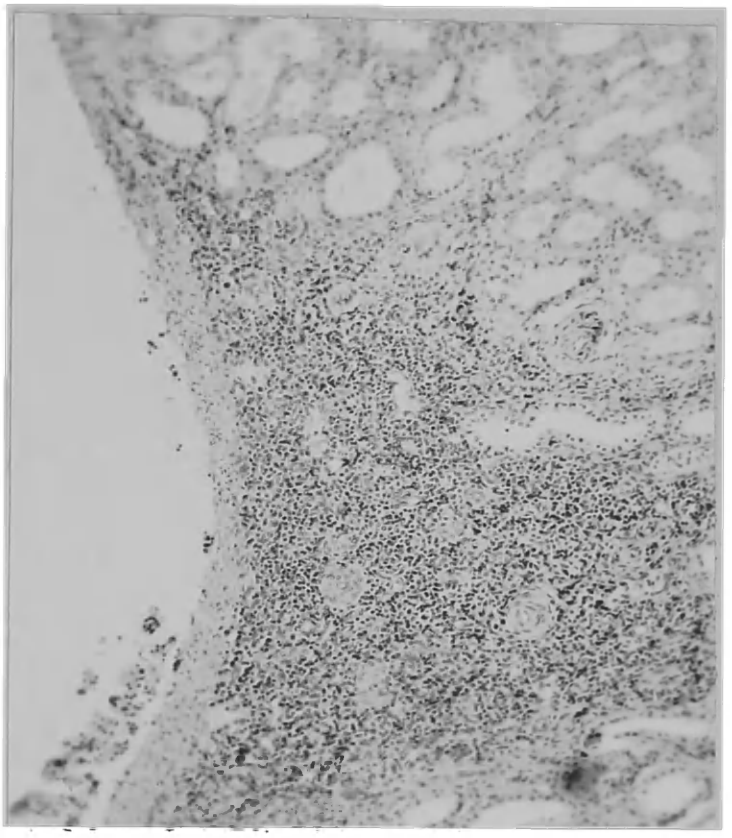

FIGURE 2. Bovine kidney from heifer experimentally infected with L. pomona at day 45. Contraction of the cortex at site of foci of lymphocytic infiltration. x100.

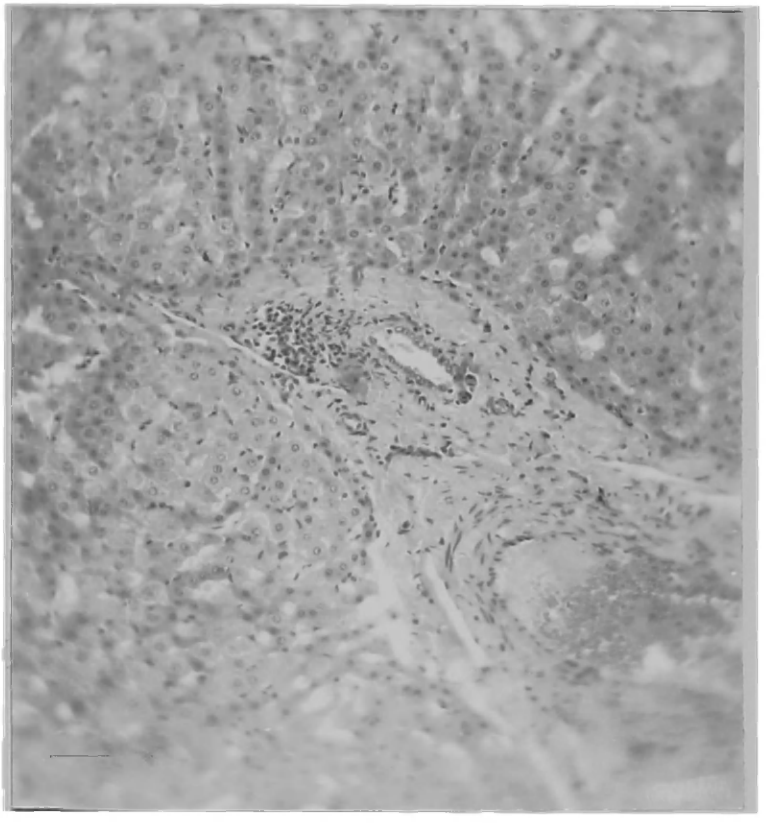

FIGURE 3. Bovine liver from heifer experimentally infected with L. pomona at day 37. Small focus of lymphocytes adjacent to the interlobular bile duct. x140.

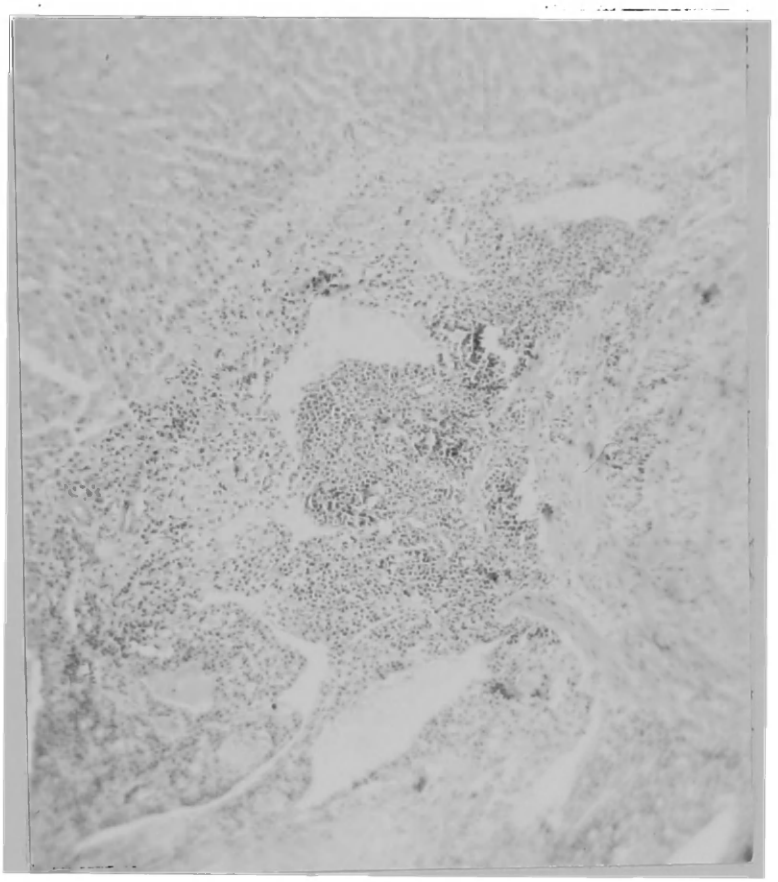

FIGURE 4. Eosinophilic infiltration of hepatic perivascular spaces of bovine liver. x100.

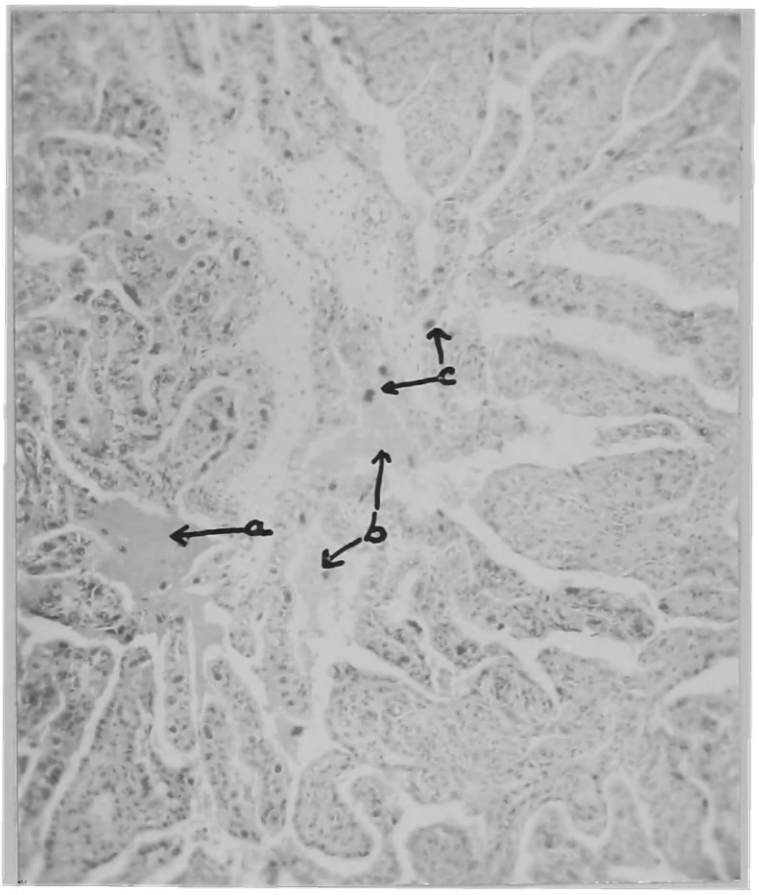

FIGURE 5. Cotyledon at day 10. Separation of maternal and fetal tissues: a) hemorrhage, b) tissue debris, and c) pyknotic nuclei of diplokaryocytes. x100.

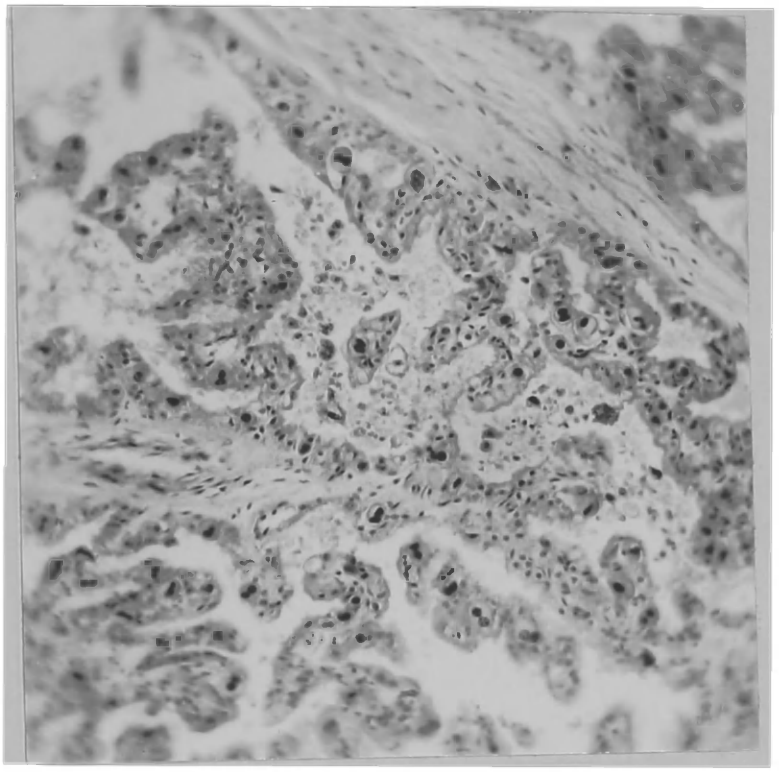

FIGURE 6. Cotyledon at day 23. Necrotic fetal tissue with some fetal cells adhering to the maternal epithelium. x140.

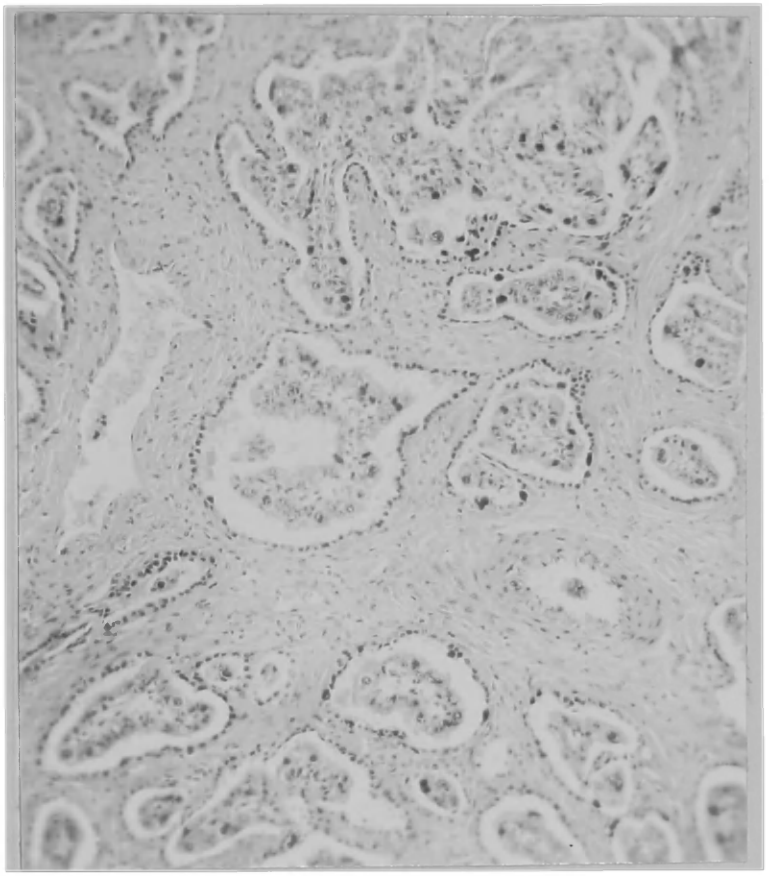

FIGURE 7. Cotyledon at 23 days. Marked pyknosis of the maternal epithelium and proliferation of fibrous connective tissue. x100.

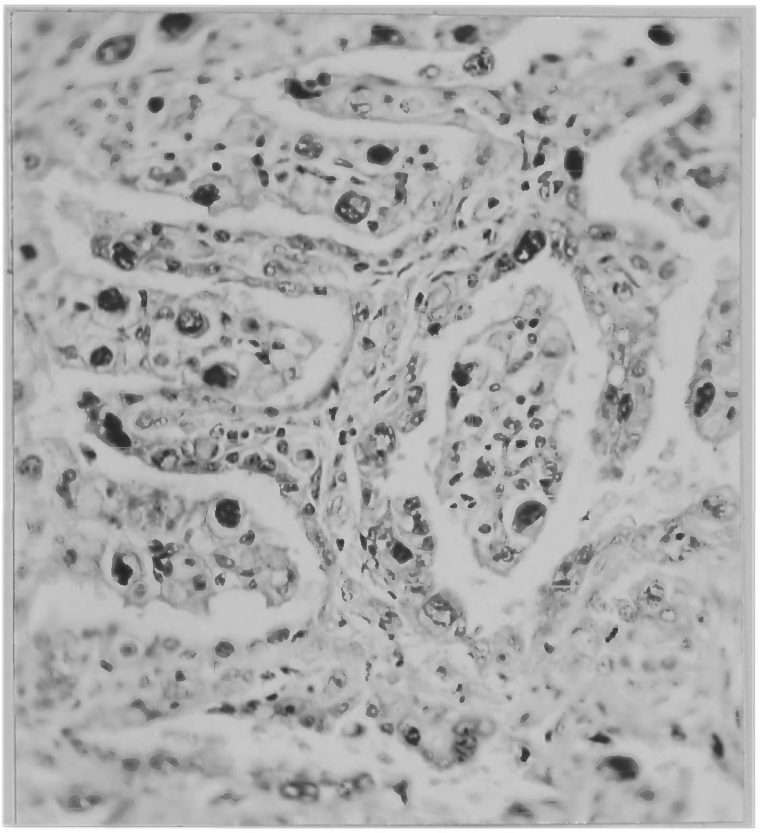

FIGURE 8. Regenerative hyperplasia of maternal epithelium. Bovine cotyledon day 45. x280.

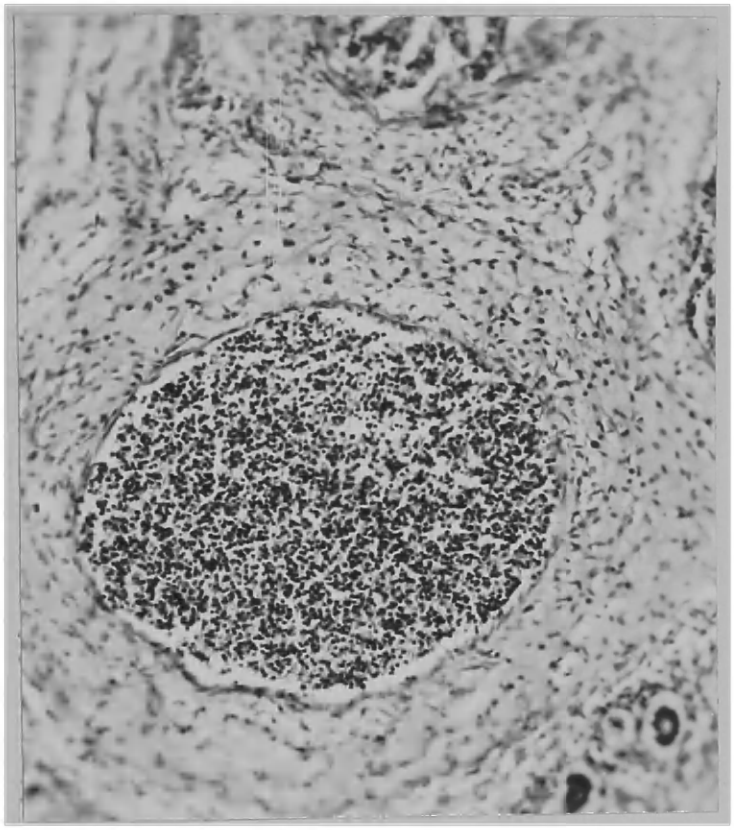

FIGURE 9. Nucleated erythrocytes in vessel of bovine fetus. x140.

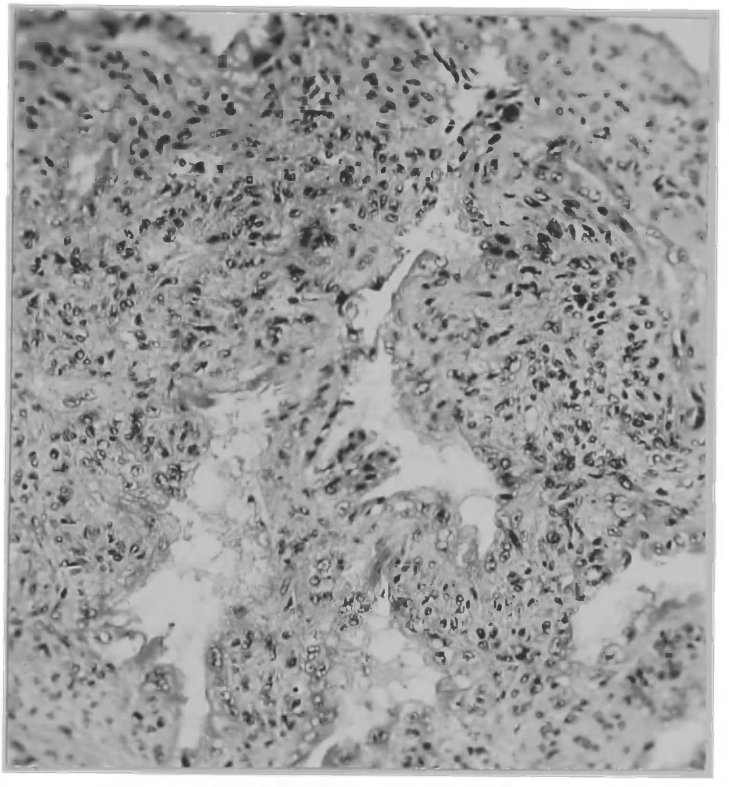

FIGURE 10. In utero infection, cotyledon at day 14. Absence of fetal villi and maternal epithelium with increased amount of connective tissue. x70.

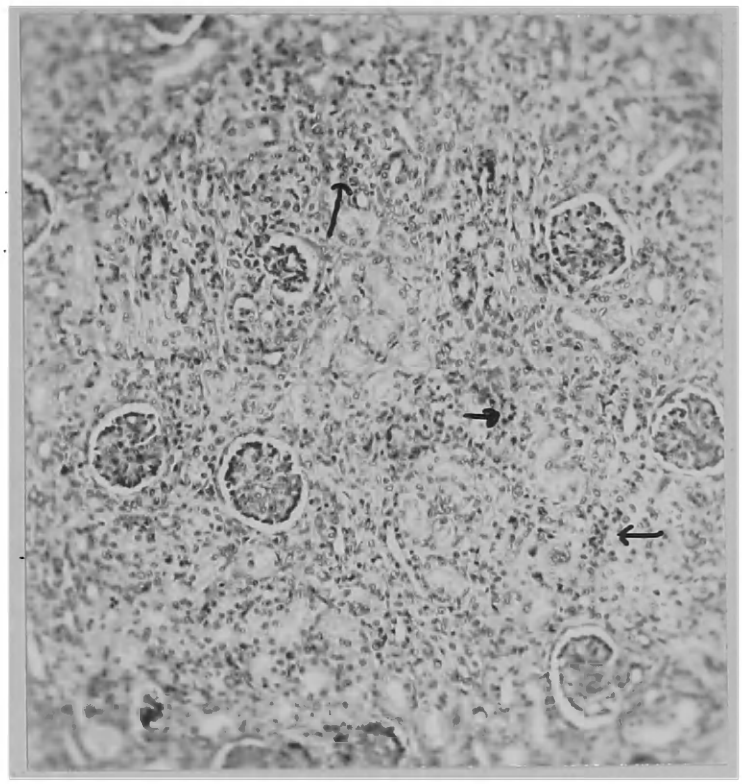

FIGURE 11. Kidney of bovine fetus at day 7. Small foci of immature lymphocytes and plasma cells. x140.

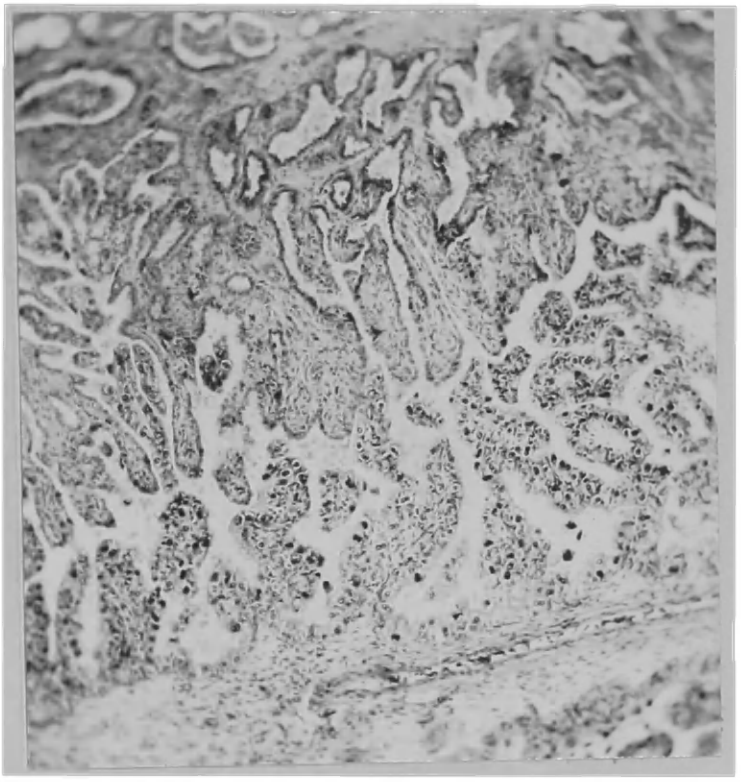

FIGURE 12. Cotyledon at day 7 postinoculation of bovine fetus *in utero*. Diffuse placentitis. x70.

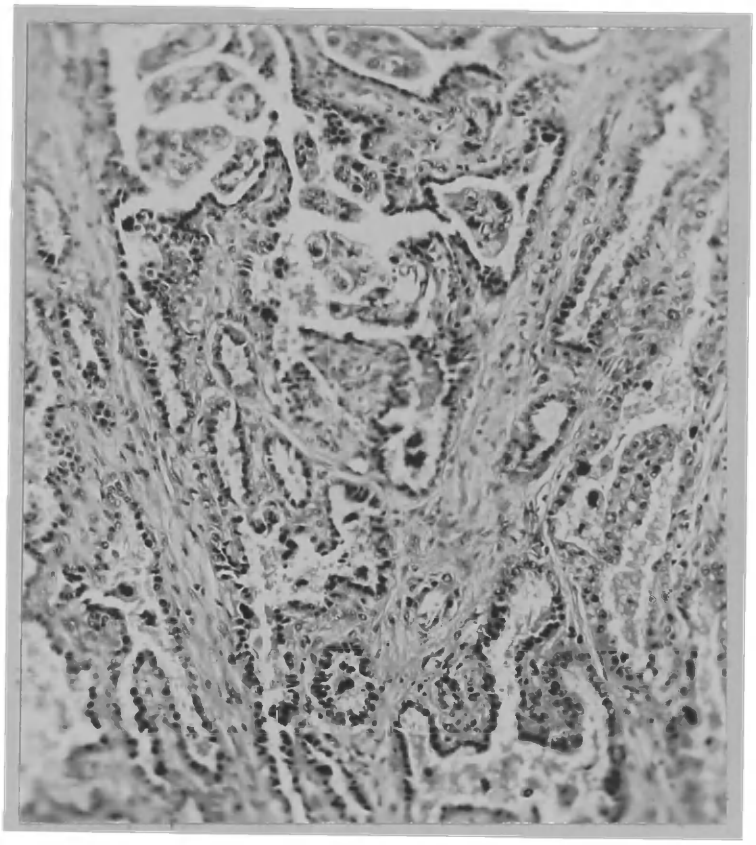

FIGURE 13. Another area of diffuse placentitis. Cotyledon at day 7. x140.

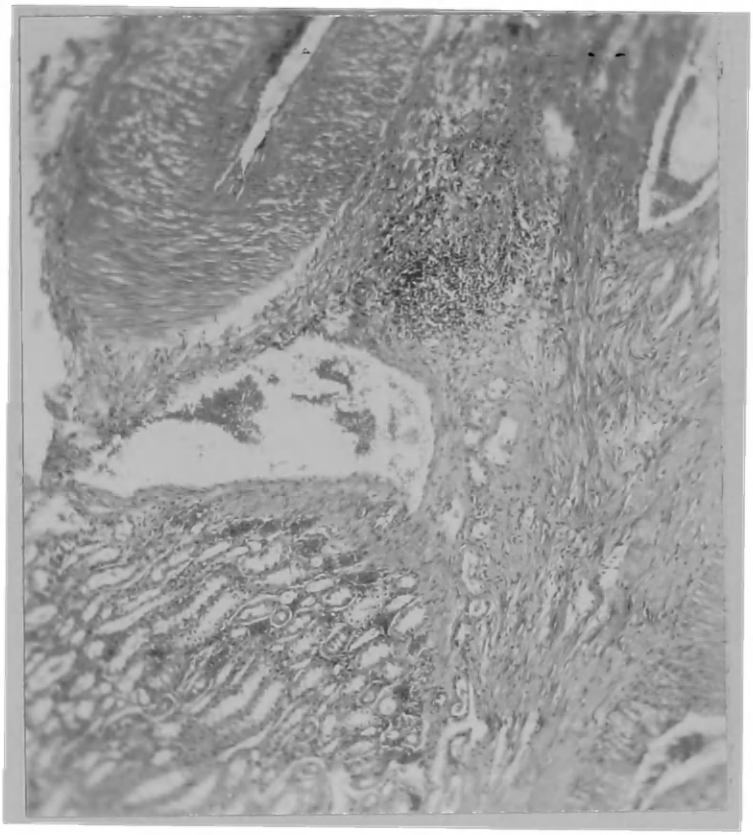

FIGURE 14. Maternal bovine kidney at day 7 of <u>in utero</u> infection. Lymphocytic infiltration of the minor calyces. x70.

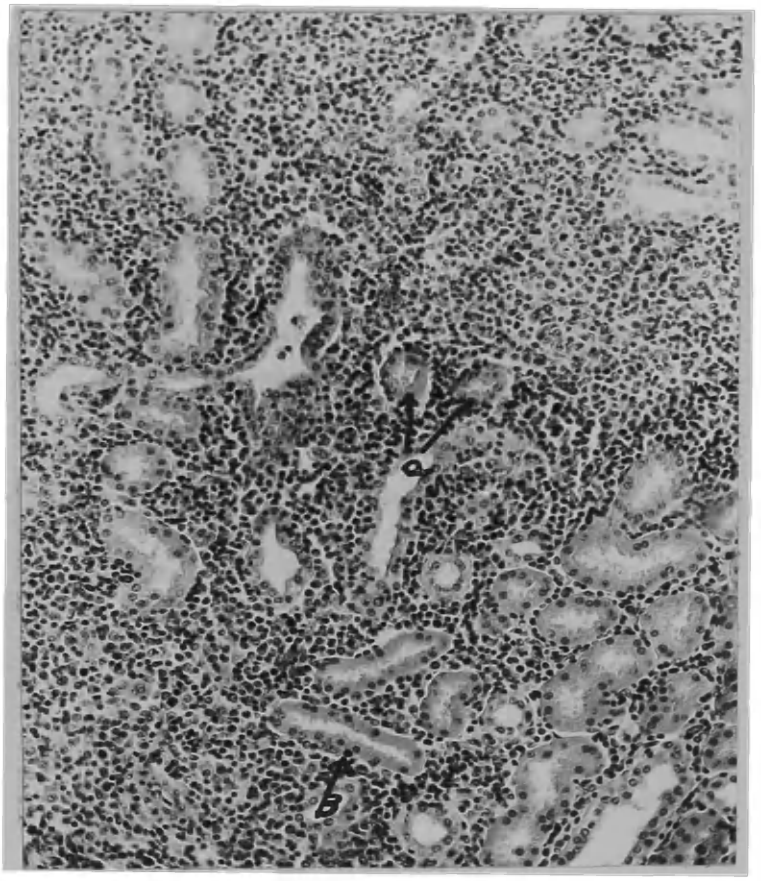

FIGURE 15. Marked infiltration of lymphocytes in cortex of porcine kidney. a) Note atrophy and disappearance of tubules. b) Pyknosis of nuclei of tubular epithelium. x130.

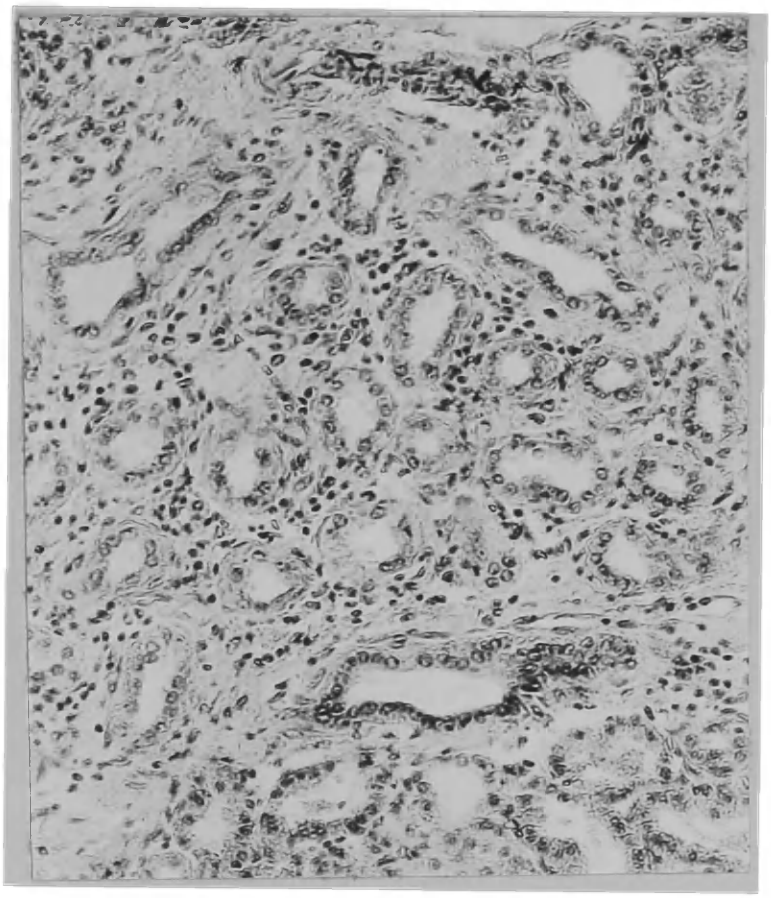

FIGURE 16. Extensive increase in fibrous connective tissue and some lymphocytes between tubules of medulla of porcine kidney. x130.

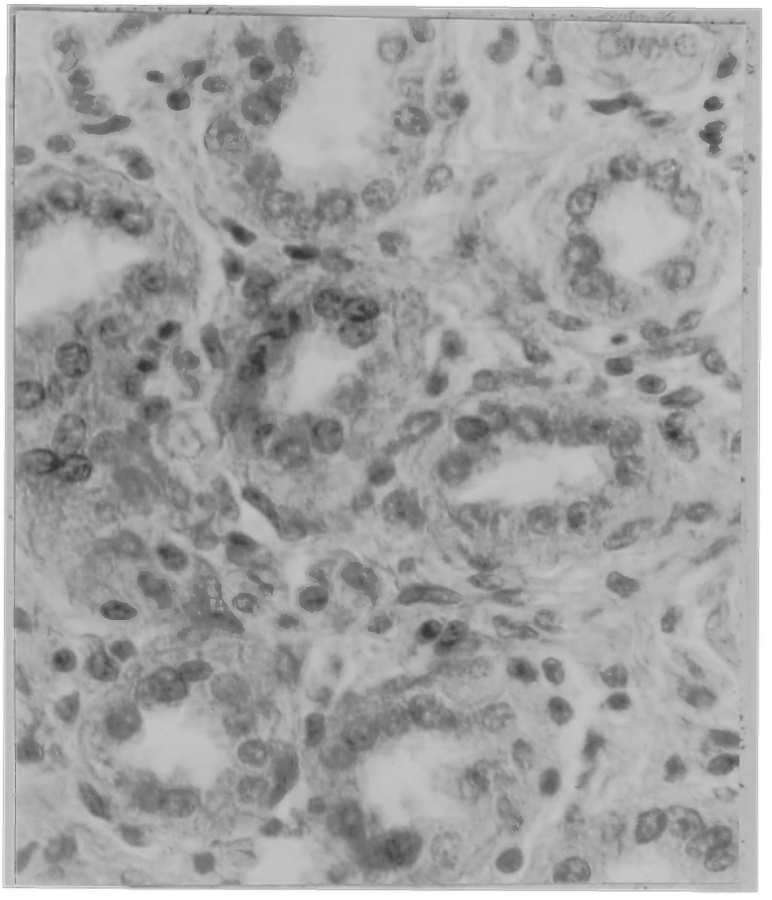

FIGURE 17. Higher magnification of figure 16. x360.

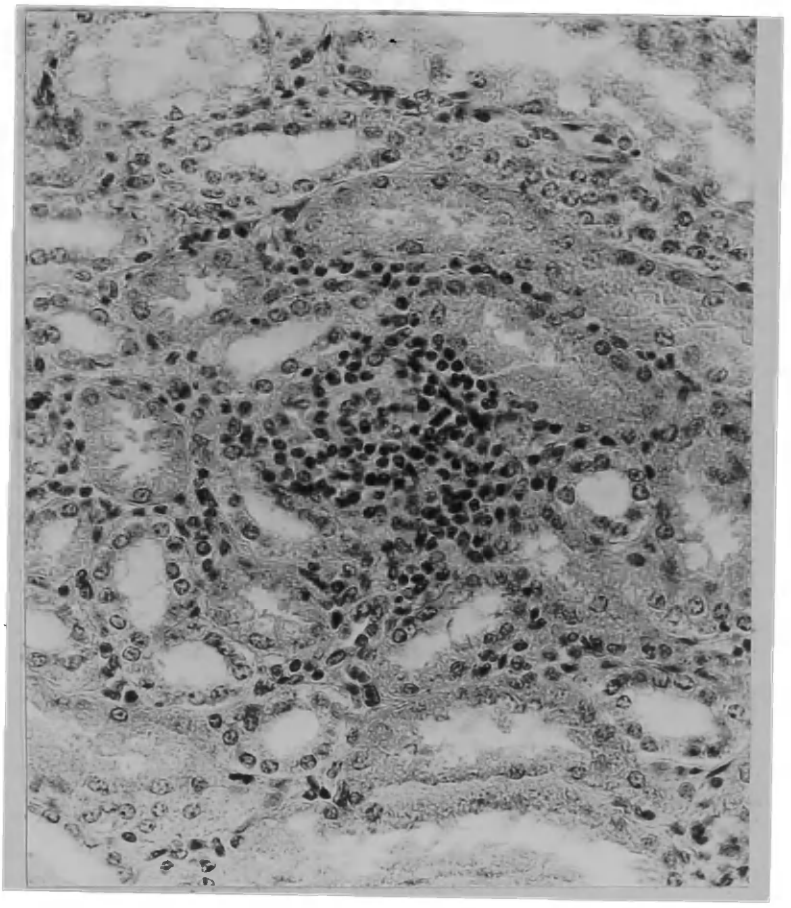

FIGURE 18. Medulla of porcine kidney to show fat vacuoles in terminal portions of the proximal convoluted tubules and some intertubular infiltration with lymphocytes. x130.

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