THE EFFECTS OF LEPTOSPIRA POMONA HEMOLYSIN ON PREGNANT EWES, COWS AND SOWS

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ABSTRACT

THE EFFECTS OF LEPTOSPIRA POMONA

HEMOLYSIN ON PREGNANT EWES, COWS AND SOWS

By Stuart Duane Sleight

A series of experiments was conducted using pregnant ewes, cows and sows to study the effects of intravenous administration of partially purified and concentrated Leptospira pomona hemolysin. The hematological changes, clinical symptoms and gross and microscopic pathological alterations were recorded.

Pregnant ewes developed a severe hemolytic anemia evidenced by hemoglobinuria, hematuria and icterus. Average hemoglobin levels decreased to 57 per cent of normal within 48 hours. The histopathological alterations were characterized by hepatic centrolobular necrosis, splenic congestion and renal glomerular extrusion of blood with some tubular necrosis. Death of 3 of 8 fetuses appeared to be due to pathological disturbances of the maternal and fetal relationship rather than to a hemolytic effect upon fetal blood cells. Ewes receiving hemolysin and having live fetuses in utero at necropsy exhibited progressive placental damage. The maternal-crypt syncytial cells appeared to be the first to become necrotic and disrupted. These changes

were followed by necrosis and vacuolization of fetal villi. A complete disintegration of the maternal-fetal relationship was noted in the cotyledons of dead fetuses.

Two cows died during severe hemolytic response. Gross and microscopic pathological changes were similar to those observed in the ewes. Six other cows underwent less severe hemolysis. Maximum hemoglobin loss varied from 26 to 62 per cent in individual animals. Two of 6 fetuses were aborted, one 3 days and the other 8 days after hemolysin administration to the dams. The histopathological lesions in the maternal placenta were typified by maternal-crypt epithelial necrosis and connective tissue proliferation. Extensive necrosis of villi was noted in sections of the placenta from the aborted fetus. In the 6 cows, renal, hepatic and splenic changes were minimal.

With the exception of one bovine fetus, the ovine and bovine fetal hemoglobin values were within the normal range. Thus, there is little evidence that L. pomona hemolysin crosses the placental barrier. It is postulated that the role of hemolysin in the abortion syndrome in cattle and sheep is related to placental metabolic disturbances elicited by lowering of the oxygen carrying capacity of the blood.

Ewes and cows with high serum titers against L. pomona were resistant to the effects of hemolysin. One cow which

recovered without having a detectable titer was resistant to a second injection. This may indicate possible antigenicity of L. pomona hemolysin.

Sows or their fetuses were not affected by L. pomona hemolysin. This verified the in vitro observations of porcine erythrocyte resistance to hemolysin and indirectly indicated that hemolysin per se was responsible for disturbances in the placentomes of cattle and sheep.

Erythrocytes obtained from different cows showed variations in susceptibility to the same hemolysin as demonstrated by in vitro assays.

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By

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Dedicated

to

Gerry

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INTRODUCTION

Hemolytic manifestations have long been associated with bovine and ovine leptospirosis. Such manifestations led to the supposition that an agent or agents produced by the leptospiral organism was responsible for the lysis of erythrocytes. Russell (1954) was the first to demonstrate a hemolytic factor associated with Leptospira pomona. The procedures developed by Bauer et al. (1961) enable one to concentrate from the supernatant fluids of L. pomona cultures a preparation of high hemolytic activity. This has made it possible to investigate the in vivo effects of a possible virulence factor apart from actual infection of experimental animals by the organism.

Abortion is a common sequel of L. pomona infection and the largest single contributor to the great economic loss caused by leptospirosis. There has been a great deal of interest in the specific action of the organism responsible for the death of the fetus. That a toxin, such as hemolysin, might be a cause of fetal death was first postulated by Ferguson et al. (1957).

In view of the aforementioned developments and postulates, it appeared that the logical approach in the determination of the role of leptospiral hemolysin would be to set up a series of controlled experiments in pregnant ewes, cows and sows.

An accurate record of hematological changes, clinical symptoms and gross and microscopic alterations following hemolysin administration was the basis of the following described work.

REVIEW OF THE LITERATURE

The characteristics of a hemolytic anemia have long been associated with leptospirosis in ruminants. Stewart (1934) of Australia noted that in calves housed near piggeries, hemoglobinuria was a common finding. Michin and Azenov (1935) associated icterus of cattle with a spirochete. After Jungherr (1944) first diagnosed bovine leptospirosis many workers in the United States associated icterus, anemia and hemoglobinuria with leptospiral infection. Marsh (1945) reported an ictero-hemoglobinuria in cattle and demonstrated what appeared to be leptospirae in silver-stained sections. Sutherland and Morrill (1948) recovered Leptospira pomona from cattle by guinea pig inoculation. They reported that the disease was characterized by icterus, anemia, hemoglobinuria, centrolobular hepatic necrosis and interstitial nephritis. Baker and Little (1948) reported that the agent they had isolated 2 years earlier from cows suffering from a febrile disease characterized by bloody and thickened milk was Leptospira sp. Cordy and Jasper (1952) described the pathological changes in cattle dying from a severe hemolytic anemia due to leptospirosis. They noted icterus, friable livers and reddish-brown spots on the kidney. Microscopically, centrolobular hepatic necrosis was observed. Blood pigment granules were present in hepatic cells, but there was little accumulation of pigment in the spleen.

Stoenner (1954) emphasized that most infected cattle experience some destruction of erythrocytes and anemia is a prominent symptom in cases which succumb during the acute phase of the disease. In severely affected animals, anemia persists and normal blood levels may not be restored for several months. Gochenour (1954) observed that, in general, young calves and feeder stock often have a hemoglobinuria, while mature cattle more often show abortion, agalactia and mastitis with hemolytic evidences less common. Veterinary practitioners (Morse et al., 1955a) and other workers who have observed field outbreaks of bovine leptospirosis (Bryan, 1955; Sippel et al., 1952; Sutherland, 1949) noted the following symptoms: fever, mastitis, icterus, hemoglobinuria and abortion. In a review of the literature by Reinhard (1955) he noted that while the ictero-hemoglobinuric picture captured the attention of early investigators, it is presently known that leptospirosis is of considerable importance as an abortifacient disease. Morse (1955) and the United States Department of Agriculture (1954) analyzed the economic importance of bovine leptospirosis and emphasized losses due to abortion.

Field cases of ovine L. pomona infection have been reported to show hemolytic anemia and abortion. Hartley (1952) reported an outbreak of ovine leptospirosis in which many animals had marked icterus and thin-watery blood. Upon histopathological

examination he observed extensive hepatic centrolobular necrosis and nephritic indications of a recent severe hemoglobinuria. Beamer et al. (1953) reported leptospirosis in a flock of Illinois sheep. Abortions occurred accompanied by marked depression and death of many ewes. Postmortem examinations revealed icterus, mild fatty changes in the liver and hematuria. Observations by Bokori et al. (1958) of a field outbreak of L. pomona infection in lambs led them to conclude that L. pomona infection should be the first consideration in any disease in sheep manifesting itself by hemoglobinuria associated with icterus.

Experimental bovine and ovine L. pomona infection, in general, corroborates observations on naturally occurring cases. Reinhard (1951) in 3 of 6 experimentally infected calves observed fever, transitory anemia and hemoglobinuria. Ferguson et al. (1957) in 12 experimentally infected cattle reported death in 1 animal with severe hemoglobinuria the prominent symptom. Three heifers aborted fetuses 19, 20 and 47 days following exposure. Fennestad et al. (1956a) did not report hemoglobinuria or anemia in experimental leptospirosis in pregnant heifers but apparently did not record hemoglobin or erythrocyte values. Two of 21 heifers aborted but each of these were infected with a serotype other than L. pomona. Morse and McNutt (1956) in following the course of infection

in 7 experimentally infected pregnant heifers observed abortion in 1 animal and no hemoglobinuria or icterus. Blood values were not reported.

Morse et al. (1957) in describing experimental ovine leptospirosis due to L. pomona reported hemoglobinuria in 5 of 8 lambs. Icterus was observed in 1 animal. He postulated that there may be a relationship between the appearance of antibody and hemolysis of erythrocytes and hemolysis might be due to a hemolytic endotoxin released upon the lysis of the organism by the specific antibody. Lindquist et al. (1958) observed only a mild and transient hemolytic response in experimentally infected ewes. Lambings occurred normally and leptospirae were not demonstrated in fetal organs, blood or amnioallantoic fluid. Leptospiral antibody was not present in either fetal blood or the blood of lambs at birth. Smith et al. (1960a, b) and Reynolds et al. (1960) in a series of reports on infection in pregnant ewes found a considerable difference in pathologic response depending upon the inoculum used. Infected hamster blood as an inoculum produced acute leptospirosis with hemolytic anemia in 3 of 14 ewes. The ewes given organisms grown in Schuffner's medium developed only a febrile response. No abortions were produced and fetal blood values remained normal.

Clinical reports on the symptoms noted in naturally occurring outbreaks of leptospirosis in young swine vary greatly. Many infections are asymptomatic (Bohl, Powers and Ferguson, 1954; Sippel, 1954). Bryan (1955) reported the following symptoms noted by veterinarians: fever, anorexia, hemoglobinuria, icterus and anemia. Symptoms of a hemolytic anemia have not been observed in experimentally L. pomona-infected young swine and, other than fever and anorexia, no clinically recognizable symptoms develop (Burnstein and Baker, 1950; Morse et al., 1958; Sippel, 1954; Sleight et al., 1960). These workers reported no significant changes in erythrocyte, packed cell volume or hemoglobin values.

The most important symptoms of L. pomona infection in sows are abortion and the birth of weak or unthrifty pigs (Bohl et al., 1954; DeLay et al., 1955; Gambrel et al., 1956; Morse et al., 1955a; Powers et al., 1956). Abortion appears to depend on the stage of gestation at time of exposure, occurring mainly during the last 3 weeks of pregnancy (Powers et al., 1956). Several authors have reported the isolation of L. pomona from aborted fetuses or from weak, full-term pigs (Bohl et al., 1954; Bryan et al., 1953; Preston and Morter, 1960). Preston and Morter recommended routine dark-field examination of kidney tissue smears or body fluids of aborted

swine fetuses. They reported that the organism is usually in sufficient numbers to enable a rapid laboratory confirmation.

The pathogenesis of bovine leptospiral abortion is still undetermined (Fennestad et al., 1960). Te Punga and Bishop (1953) postulated 3 possible causes for leptospiral abortions: (1) pyrexia and systemic reaction, (2) interrupted transfer of metabolites, due to localized lesions in the maternal fetal cotyledonary junction, (3) actual invasion of the fetus by the leptospirae. Ferguson et al. (1957) proposed as a possible cause the release of a toxic material at the time the leptospirae are lysed by antibody which would be able to cross the placental barrier. The toxin would have a hemolytic action on fetal erythrocytes with resultant anoxia and death. However, Lundberg (1960) reported no significant alterations in erythrocyte and hemoglobin values of fetuses from L. pomona-infected heifers. Morter et al. (1958) suggested that alterations in the epithelium of the maternal crypts might result in hormonal imbalance and abortion. They found a series of histopathological changes in the cotyledons which they concluded could interfere with fetal development and result in death and abortion.

Fennestad and Borg-Petersen (1958) supported the hypothesis of fetal infection and death and believed any hypothetical cause of fetal death other than fetal infection was superfluous.

As evidence they cited the demonstration of leptospirae by silver-impregnation in tissues of aborted bovine fetuses (Bridges, 1958; Fennestad and Borg-Petersen, 1956, 1958b, 1960; Mathews, 1946; Ringen, 1955; Te Punga and Bishop, 1953) and the cultural isolations of L. pomona from aborted fetuses (Podgwaite et al., 1955; Dacres and Kiesel, 1958). Ferguson et al. (1957) in a discussion of the reported isolations by Podgwaite et al. (1955) concluded they were not dealing with typical leptospirosis since 2 of 3 cows were serologically negative at the time of abortion. An isolation of L. pomona from the liver of 1 of the cows in the chronic phase of the disease was, according to Ferguson, contrary to all published reports.

Several workers have failed to make cultural isolations from fetuses of experimentally infected cows. Morse and McNutt (1956) could not isolate L. pomona from an aborted fetus or from 6 viable fetuses. Ferguson et al. (1957) reported a similar failure to isolate the organism from 9 fetuses, 3 of which had been aborted. Morter (1960) made no isolations from fetuses of 10 experimentally infected heifers. Fennestad et al. (1956) could not obtain cultural isolations from fetuses.

There have been no reports of cultural isolation of L. pomona from aborted ovine fetuses. Smith et al. (1960)

reported isolations of L. pomona from fetal umbilical blood, caruncles, cotyledons, and amniotic fluid. These isolations were made 3 days after inoculation of the ewes. Isolations were not made from fetal organs or from any of the before mentioned tissues later than 3 days after inoculation. Lindqvist et al. (1958) were not able to isolate L. pomona from organs of fetuses from experimentally infected ewes.

Russell (1954) reported a hemolytic factor associated with L. pomona. Stock cultures inoculated on blood agar plates showed zones of hemolysis. It was further shown that hemolysis could be demonstrated using 5 per cent suspensions of sheep erythrocytes.

The production of an hemolysin by some leptospiral serotypes was reported by Alexander et al. (1956). Hemolysin concentrations were found to be highest 1 to 3 days after maximum leptospiral growth had occurred and the hemolysin was in the supernatant portions of the cultures. The hemolysin was reported to be heat labile, oxygen stable, nondialyzable and exhibited greatest activity against goat, cow and sheep erythrocytes. Alexander et al. (1956) could detect no evidence of antigenicity. Russell (1956) reported that the hemolysin was inhibited by leptospiral antiserum.

Titrations of various serotypes for their hemolytic activity have been reported by Alexander et al. (1956); Dozsa et al.

(1960); Russell (1956) and Van Riel (1959). L. pomona, L. grippotyphosa and L. canicola produced hemolysin but none or very little hemolysin was produced by either L. icterohaemorrhagiae or L. hyos. Dozsa et al. (1960) determined the hemolytic activity of leptospiral hemolysin against the erythrocytes of 44 domesticated and wild animal species. Only the Pecora sp. (roe deer, red deer, black buck, Mongolian gazelle, mouflon, Barbary sheep, goat, Alpine ibex, cattle, buffalo and zebu cattle) had erythrocytes susceptible to the hemolysin.

The effect of hemolysin on lambs was investigated by Kemenes (1958). A single intravenous injection of 150 to 200 ml. of the supernatant fluid of L. pomona cultures or repeated injections of smaller amounts resulted in anemia. Bauer et al. (1961) using concentrated and partially purified L. pomona hemolysin in lambs, produced hemolytic anemia evidenced by icterus and hemoglobinuria. Microscopic lesions consisted of centrolobular hepatic necrosis with disruption of the hepatic cords in the peripheral portions. Spleens were markedly congested. Renal tubules of lambs contained a homogeneous red-staining material.

In lambs with serum agglutinin titers against L. pomona, there was little alteration in hemoglobin levels. It was further noted that leptospiral antiserum, in vitro, inhibited hemolysis if added before the addition of erythrocytes.

Addition of antiserum with or after the erythrocytes resulted in decreased inhibition.

Bauer et al. (1961) also studied the in vivo hemolytic process following infection with washed L. pomona cells. This study indicated that hemolysin was associated with the appearance of leptospirae in the circulation and hemolysis continued up to 2 days in the presence of agglutinins. These observations suggested that the hemolytic process was irreversibly established before antibody was produced. In vitro experiments indicated the hemolysin was adsorbed non-specifically to erythrocytes and once adsorbtion had occurred, the inhibitory effect of antibody was diminished. Bauer concluded that, therefore, in vivo hemolysis could occur by production of hemolysin during leptospiral multiplication and subsequent adsorbtion to erythrocytes. The production of agglutinins would destroy leptospirae but have relatively little effect upon adsorbed hemolysin. Such a sequence of events would be in accord with the findings of Morse et al. (1957).

Rogols et al. (1959) suggested that the hemolysin might be similar to a phospholipase on the basis of the inhibitory effect of certain phospholipids such as lecithin. Bauer et al. (1961) could find no action of the hemolysin on lecithovitellin and suggested the inhibitory activity described by Rogols et al.

might be non-specific.

Bertók and Kemenes (1960) demonstrated that leptospiral lipase activity is present in non-pathogenic and pathogenic strains. This enzyme had different properties than hemolysin. Lipase was more heat stable than hemolysin and appeared earlier and persisted longer in cultures. Like hemolysin, it could not be dialyzed. Lipase, specifically tributyrinase, activity was present in virulent serotypes, e.g., L. icterohaemorrhagiae, L. canicola, L. pomona, etc. Of interest was the observation that non-pathogenic leptospirae also could split triglycerides but had much less activity on animal fats than the pathogenic leptospirae.

Kemenes and Lovrekovich (1959) demonstrated a thermostable "fatty acid" in cultures of leptospirae which was capable of producing hemolysis in all animal species. Pentek-Juhász (1960) described a heat labile hemolysin produced by strains of L. biflexa active against mouse but not ruminant erythrocytes.

Hemolysin production by cultures of L. pomona was shown by Bauer et al. (1958) to have no correlation with virulence in hamsters. Bertók and Kemenes (1960) emphasized that hemolysin and lipase production, of themselves, cannot be associated with virulence. Some strains will not produce infection but filtrates from cultures will be fatal if injected into sheep or deer. Alexander (1960) mentioned that there is a

great difference in hemolytic activity of different strains of L. pomona, an observation confirmed in our laboratory. A strain of L. pomona called Johnson, widely used as an antigen, apparently has no hemolytic activity.

MATERIALS AND METHODS

Ewe Experiment

Strain LW, previously described by Bauer et al. (1961) was employed in the production of hemolysin. A non-hemolysin producing strain (J) was used as a control. Hemolysin was prepared by growing strain LW in Stuart's medium (Difco) containing 10 per cent rabbit serum for approximately 16 days, or, 1-2 days after maximum turbidity was reached. Leptospirae were removed by passage through a St-1 Seitz filter pad. The hemolysin was precipitated from the culture filtrates by 36 per cent saturation with ammonium sulfate at 4°C. for 4 - 6 hours. The precipitate was collected by cold centrifugation for 15 minutes at 10,000 r.p.m. and resuspended in .15 molar phosphate buffered saline (pH 7.1) to 5 per cent of the original volume of filtrates. The suspensions were dialyzed in the cold against buffered saline until no sulfate ion could be detected in the dialysate. Any insoluble material remaining after dialysis was removed by centrifugation and the solution was assayed for hemolytic activity as described below.

The procedure for hemolysin assay (Bauer et al. 1961) was as follows. A series of hemolysin dilutions was made in saline to a total volume of 2 ml. Eight to 10 dilutions between 1:10 and 1:200 were usually employed. To each dilution was added

an equal volume of a 1 per cent suspension of sheep erythrocytes. All tubes were incubated for 30 minutes at 37°C followed by 60 minutes at 4°C. To sediment the erythrocytes, tubes were centrifuged at 1500 r.p.m. for 5 minutes. The optical density of the supernatant fluid was determined in a Bausch and Lomb Spectronic 20 colorimeter at 540 mμ. In each test a tube containing saline instead of hemolysin was included and the supernatant fluid served as a negative control. The positive control consisted of 2 ml. of a 1:1000 saponin solution and 2 ml. of the erythrocyte suspension. The optical density of the supernatant fluid from the positive control was assigned a value of 100 per cent hemolysis. The optical density readings of the hemolysin dilutions were converted to per cent hemolysis. The reciprocal of the hemolysin dilution producing 50 per cent hemolysis was designated as the number of hemolytic units (HU) per ml. of hemolysin.

Twelve pregnant and 1 non-pregnant ewes served as experimental animals. Pre-injection serum antibody titers of each animal were determined using the agglutination-lysis (AL) test (Morse et al. (1955b). Titers are expressed as the logarithm of the reciprocal of the highest serum dilution in which 50 per cent or more of the antigen (L. pomona, strain J) was agglutinated and/or lysed. Ewes 12 and 809 had titers of 10^5 and 10^2, respectively. The remaining animals were negative.

Each of 8 ewes (1, 3, 5, 11, 12, 36, 809 and 880) in their last month of pregnancy were intravenously injected with 112-118 HU of hemolysin per kilogram of body weight. Ewe 975, in her third month of pregnancy, was similarly injected. Ewe 21 was given 100 ml. of strain J preparation. Ewes 770, 4 and 6 and non-pregnant ewe 888 were each given 100 ml. of saline intravenously.

Erythrocyte, packed volume and hemoglobin values (Coffin, 1953) were determined for each animal before injection and from 1 to 4 times daily after injection. Hemoglobin determinations were made from each fetus alive at time of necropsy of the ewes.

Necropsies were performed on pregnant ewes 1, 5, 880, 36, 11 and 809 at 24, 36, 48, 72, 96 and 132 hours, respectively, after hemolysin injections. Non-pregnant ewe 888 and control ewes 4 and 6 were also sacrificed. The remaining 4 animals were allowed to recover.

Tissues from the kidneys, liver, spleen, heart, adrenals and cotyledons of each ewe and from the kidneys, liver and spleen of each fetus were placed in one or more of the following fixatives: Zenker's fluid, Carnoy's fluid and 10 per cent buffered formalin solution. Appropriate sections were stained with hematoxylin and eosin for general characteristics, Sudan IV for fat, Best's carmine for glycogen and Prussian-blue for iron-containing pigments.

Cow Experiment

The procedures for hemolysin preparation and assay were similar to that outlined previously with the following exceptions. Leptospirae were removed by filtration through a 02 Selas filter after Seitz filtration. Hemolysin was precipitated at an ammonium sulfate concentration of 45 per cent. In 7 of 11 cows given hemolysin, the assays were made using homologous erythrocytes.

Thirteen cows in the last third of their gestations were used as experimental animals. Twelve were negative to the AL test and the remaining animals had a serum antibody titer of 10^4 with L. pomona antigen. Eleven animals, including cow 18 with a titer, were each given hemolysin intravenously. Amounts of hemolysin given to 8 cows ranged from 10 - 30 HU per kilogram of body weight. In cows 12, 1 and 11 the dosage was 85, 200 and 500 ml., respectively. Due to a lack of standardization of the assay procedure the exact dosage expressed as HU per kilogram of body weight was not known. Control cow 21 was given 50 ml. of strain J preparation and control cow 15 was uninjected.

Erythrocyte, packed cell volume and hemoglobin values were determined before injection and thereafter 1 - 2 times daily. Hemoglobin determinations were made from each fetus alive at the time of necropsy.

Necropsies were performed on 10 cows at the following intervals after hemolysin injection: 2 each at 12 hours, 8 days and 9 days and 1 each at 32 hours, 44 hours, 16 days and 30 days. Cows 3, 4, and 18 were allowed to recover.

Terminal blood samples from each cow were tested for serum antibody using strain J antigen. If the terminal samples were positive, the remaining samples were tested to determine the initial reaction. Fetal blood was also tested for serum antibody.

Tissues were saved, fixed and stained as before described for the ewes.

Sow Experiment

Six sows in the last month of pregnancy served as experimental animals. Hemolysin preparation and assay were similar to that described for the ewes. Each of 3 sows was given 200 ml. of hemolysin intravenously. One sow was given 200 ml. of strain J preparation and another 100 ml. of strain J preparation previously passed through both Seitz and Selas filters. The remaining sow was given 200 ml. of saline intravenously.

Erythrocyte, packed cell volume and hemoglobin determinations were made daily. Agglutination-lysis tests were performed on all blood samples including composite fetal samples.

Necropsies were performed on 5 sows at the following

intervals after hemolysin administration: 5 days, 7 days, and 3 at 8 days. The sixth sow farrowed 24 hours after receiving hemolysin and was not killed.

Tissues were saved, fixed and stained as described previously.

Other Procedures

Erythrocytes from 3 cows were used to determine *in vitro* variations in susceptibility to the same preparation. A second hemolysin preparation was assayed using erythrocytes from 2 cows and a third was assayed using erythrocytes from 3 cows.

To determine *in vitro* susceptibility of porcine erythrocytes to hemolysin, erythrocytes from each of 7 sows were used in a hemolysin assay. Comparative susceptibility of porcine and bovine erythrocytes were ascertained in the same assay.

Cow 13 recovered from hemolysin administration without having a detectable titer. The following procedure was used to determine whether serum from cow 13 would inhibit *in vitro* hemolysis to a greater or less degree than sera from 3 cows which had not received hemolysin. Erythrocyte suspensions from each of the 4 cows were added to each of 5 tubes of a 1:50 dilution of hemolysin. Approximately .06 ml. of serum from each cow was added to its own erythrocyte suspension and to each of the other 3. Control dilutions of hemolysin and

erythrocytes had no serum added. At the end of the incubation period, the per cent hemolysis was determined.

Cow 13, which had no detectable AL titer, was given a second injection of hemolysin 4 months later. A control cow was similarly injected. The second hemolysin was prepared from LW cultures grown in Stuart's medium with 10 per cent bovine serum to lessen the danger of anaphylaxis. Assay procedures were used to insure that similar amounts of hemolysin were given each time on the basis of *in vitro* activity. Hemoglobin, packed cell volume and erythrocyte determinations were made daily.

RESULTS

Ewe Experiment

Hematological data for the 7 AL-negative ewes given L. pomona hemolysin are summarized in Table 1. Hemolytic effects were apparent in 6 of 7 within 12 hours after hemolysin administration. Hemoglobin, packed cell volume and erythrocyte values declined progressively until necropsy. Average hemoglobin values of ewes 1, 5, 11, 36, 880 and 995 decreased to approximately 57 per cent of normal within 48 hours. In ewes 11 and 975 the values were 34 per cent of normal by 96 hours. Ewe 975 was allowed to recover and its hemoglobin gradually returned to normal. Ewe 3 was apparently resistant and no significant alterations occurred. The hematological data are summarized in Table 2 for the AL positive ewes 12 and 809, control ewe 21 given strain J preparation and control ewe 770 given normal saline. No significant changes occurred in the two control ewes or ewe 12 with the serum antibody titer of 10^5. Ewe 809 with a titer of 10^2 had a decrease of 30 per cent in hemoglobin values over a 120-hour period. This hemolytic response was less than half that of the more susceptible ewes.

Symptomatically, the susceptible ewes displayed hemoglobinuria, hematuria, icterus, mild depression and slight anorexia. Ewes 1, 5, 36 and 809 sacrificed at 24, 36, 72 and

132 hours respectively had live fetuses. Ewe 880 killed at 48 hours had 1 live and 1 dead fetus. Ewe 11 sacrificed at 96 hours was in labor and 2 dead fetuses were in the uterus. All viable fetuses had normal hemoglobin values and these are correlated with those of their respective ewes in Table 3.

Ewe 21, given the strain J preparation, the apparently resistant ewe 3, ewe 975, given hemolysin during early pregnancy, and the control animal 770 lambed normally. Ewe 12 with a titer of 10^5 delivered a dead lamb with manual assistance, 8 days after receiving hemolysin. Dystocia was thought to be the cause of the lamb's death.

Grossly, in addition to icterus, the pathological alterations consisted of friable and copper-colored livers and congested spleens in the severely affected animals. Numerous reddish-brown spots 1 to 2 mm. in size were observed on the renal cortical surfaces of ewe 1 sacrificed 24 hours after receiving hemolysin. Ewe 880, killed at 48 hours, had a large area of detached placental membranes in the portion which had served the dead fetus. The maternal caruncles and fetal cotyledons showed marked autolysis in ewe 11, sacrificed at 96 hours.

Microscopically, the liver lesions consisted primarily of centrolobular necrosis with mild fatty changes in the periphery of the lobules. The lesions in the liver of the 48

hour ewe typified the earliest hepatic manifestations (Fig. 1). Characteristically, there were several pyknotic cells near the central vein with marked disruption of the hepatic cords. By 96 hours the necrosis was extensive with the nuclei showing pyknosis and karyorrhexis, Mild fatty changes were present in the periphery of the lobules (Fig. 2). The fatty nature of the material represented by vacuoles was confirmed by the Sudan IV stain.

In contrast to normal spleen sections (Fig. 3), those from the severely affected ewes revealed marked congestion (Fig. 4). Prussian-blue stained sections for iron pigments were negative.

Many renal corpuscles had hemolyzed blood which had passed through the glomerular tufts (Fig. 5). Some areas in the renal cortices showed changes in the epithelium of the proximal convoluted tubules consisting of pyknosis, karyolysis and a partial disappearance of tubular cells. Many of the tubules were filled with erythrocytes or red staining casts (Figs. 5 and 6). The medulla had many blood-filled collecting tubules (Fig. 7).

Figures 8 and 9 are representative of the placentomes of the control ewes. Continuity of the maternal-crypt syncytial cells was well preserved and the chorionic villi had the appearance of a functional unit. There was considerable fixation separation of the opposing surfaces. In most sections,

including the normals, areas of hemorrhage could be found in the superficial portions of the placentomes (Fig. 10). The trophoblastic cells in these areas showed evidence of erythrocyte ingestion.

Pathological changes were observed in the placentomes of all the ewes which received hemolysin and underwent a hemolytic response. The earliest alterations appeared in the maternal crypts (Fig. 11). There was considerable disruption of the syncytial cells with many nuclei becoming pyknotic. The interstitial tissue of the maternal crypts appeared to be hyalinized with occasional vacuolization. The chorionic villi were nearly normal at this stage. In sections from the uterus of the 36-hour ewe, maternal-crypt changes were more pronounced (Fig. 12). There was considerable disintegration of the maternal syncytium and the maternal-fetal relationship was disorganized in many areas. Similar changes were observed in sections from that part of the placenta from the 48-hour ewe which served the live fetus (Fig. 13). There was more marked disruption and disintegration of both maternal-crypt cells and fetal villi in the portion of the uterus which served the dead fetus (Fig. 14). Less marked changes occurred in the uterus of the 72-hour ewe (Fig. 15) and in the ewe posted at 132 hours which had a titer of 10^2 (Fig. 16). The most extensive alterations occurred in the 96-hour ewe (Fig. 17).

The normal histological relationships were nearly obscurred and individual nuclei were pyknotic with some undergoing karyorrhexis.

No significant lesions were observed in brain, heart, and adrenal sections.

Examination of kidney, liver and spleen sections from the fetuses alive at necropsy revealed no abnormalities. No definitive ante-mortem lesions were observed in the fetuses dead at the time of necropsy. Grossly, there was an accumulation of dark amber to brownish, relatively clear fluid in the peritoneal and pleural cavities of the dead fetuses.

TABLE 1

SUMMARY OF HEMATOLOGICAL DATA

AL NEGATIVE EWES RECEIVING HEMOLYSIN(1)

Ewe No.		11	3	36	975	880	5	1
Normal values	Hgb	12.2	11.4	11.7	13.4	12.7	14.2	13.6
	Hct	38.5	38.0	38.0	37.0	36.0	40.0	40.0
	Rbc	12.85	9.28	9.18	10.06	8.34	10.90	12.60
12 Hours	Hgb	11.0	10.0	10.7	11.6	10.6	12.3	11.7
	Hct	35.0	35.0	32.0	37.0	30.0	35.0	37.0
	Rbc	8.35	8.13	6.32	8.74	5.28	7.62	8.80
24 Hours	Hgb	9.1	12.7	10.2	10.5	6.1	11.1	9.1
	Hct	25.0	42.0	-	30.5	17.0	31.5	22.0
	Rbc	4.82	10.51	5.73	7.22	3.35	6.80	*4.19
36 Hours	Hgb	7.6	12.1	7.8	8.8	6.6	*8.5	
	Hct	24.0	36.5	24.0	24.5	15.0	25.0	
	Rbc	4.13	9.63	4.89	5.92	2.40	3.62	
48 Hours	Hgb	6.2	11.7	8.8	7.8	*6.0		
	Hct	22.0	37.0	25.0	20.0	14.0		
	Rbc	4.65	8.35	4.21	5.27	3.41		
60 Hours	Hgb	5.6	12.7	6.2	6.9			
	Hct	20.0	39.0	17.5	20.5			
	Rbc	3.84	9.71	3.23	4.63			
72 Hours	Hgb	4.2	11.7	*6.2	7.0			
	Hct	18.0	38.0	19.0	19.0			
	Rbc	4.01	8.67	3.34	4.23			
84 Hours	Hgb	4.1	-		-			
	Hct	15.0	-		-			
	Rbc	3.74	-		-			
96 Hours	Hgb	*4.1	11.1		4.7			
	Hct	12.0	35.0		16.0			
	Rbc	3.37	8.48		3.91			

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

Hct = packed cell volume expressed as per cent.

Rbc = erythrocytes in millions per cmm.

* Indicates that ewe was sacrificed at this hour.

(1) Dosage of hemolysin was 112-118 HU/Kg. body weight.

TABLE 2

SUMMARY OF HEMATOLOGICAL DATA

CONTROL EWES AND AL-POSITIVE EWES RECEIVING HEMOLYSIN

Ewe No.		12 *10^5	809 *10^2	21 X	770 Y
Normal	Hgb	14.0	12.6	12.0	11.6
	Hct	42.0	38.0	35.0	37.0
	Rbc	13.53	9.08	8.04	8.51
24 Hours	Hgb	13.4	11.4	--	11.8
	Hct	40.5	35.0	--	36.0
	Rbc	11.87	9.80	--	8.14
48 Hours	Hgb	--	10.4	11.1	11.8
	Hct	--	30.0	34.0	38.0
	Rbc	--	8.89	7.71	8.81
72 Hours	Hgb	13.2	9.8	12.6	11.5
	Hct	40.0	30.0	34.0	--
	Rbc	11.11	7.90	8.94	8.27
96 Hours	Hgb	--	9.2	11.8	11.8
	Hct	--	30.0	33.0	36.0
	Rbc	--	7.21	9.90	8.47
120 Hours	Hgb	13.6	8.8	--	--
	Hct	41.0	24.5	--	--
	Rbc	11.40	6.55	--	--

*Indicates AL titer. The titers are expressed as the logarithm of the reciprocal of the highest serum dilutions showing 50 per cent or more agglutination-lysis.

X Ewe 21 received strain J preparation.

Y Ewe 770 received normal saline

Hgb = hemoglobin in grams per 100 ml. blood.

Hct = packed cell volume expressed as per cent.

Rbc = erythrocytes in millions per cmm.

TABLE 3

COMPARISON OF OVINE MATERNAL AND FETAL HEMOGLOBIN VALUES (Gm/100 ml.)

Sheep No.	Maternal	Fetal	
5	8.5	13.4	
809	9.1	*12.1	13.0
880	6.0	*15.0	Dead
36	6.2	12.6	
1	9.1	13.4	
11	4.1	*Dead	Dead

*Twin fetuses.

Cow Experiment

Cow 11, given 500 ml. of the hemolysin preparation, died within 10 hours. Diarrhea, dyspnea and depression were observed prior to death. The untoward response was attributed to the rabbit serum globulin or other foreign protein in the preparation. To lessen the likelihood of similar responses, all subsequent animals were given 20 ml. of antihistamine solution (dimethylmethylamino-ethoxy-methyl-benzyl-pyridine succinate)* intravenously before hemolysin injections. No further severe reactions occurred until the last 2 cows (18 and 20) used in this series were injected. These animals reacted similarly to cow 11. Cow 20 died within 12 hours. Cow 18 recovered but was markedly depressed, had a severe diarrhea and was in labor within 18 hours.

Cows 12 and 1 were given 85 and 200 ml. of hemolysin respectively. Due to an inaccurate assay, the dosages are not expressed in HU/kg. of body weight. The hematological data for cows 1 and 12 are summarized in Table 4. Both of these animals exhibited severe hemoglobinuria, icterus and

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*A-H Solution, Jensen-Salsbery Laboratories, Inc., Kansas City, Missouri.

marked depression within 24 hours. Cow 1 died at 32 hours and cow 12 at 44 hours.

The gross pathological changes, in addition to icterus and hemoglobinuria, consisted of splenic engorgement with blood, renal cortical petechiation and friability of the livers. The fetus of cow 1 appeared to have died at the time of the dam's death and no abnormalities of placental relationships or fetal fluids were noted. A fetal heart blood sample had a hemoglobin value of 9.1 grams per 100 ml. The fetus from cow 12 apparently died before the death of the dam. Postmortem decomposition was present in fetal tissues but not in tissues from the dam. The fetal peritoneal and pleural fluids were dark amber colored. There was a partial detachment of the fetal membranes.

Microscopically, a comparison of placentome sections from the two animals verified the gross observations (Figs. 18 and 19). For the most part, normal architectural relationships were evident in placental sections from cow 1, while cellular continuity was markedly disrupted in sections from cow 12.

Hemolyzed blood which had passed through the glomeruli was observed in sections from both animals (Figs. 20 and 21). Degeneration and necrosis of tubular cells and blood-filled tubules were present. In comparison with spleen sections from the control animals (Fig. 22), the spleens of cows 1 and

12 were engorged with erythrocytes (Figs. 23 and 24). The liver changes were not pronounced, but in contrast to a control (Fig. 25), there was mild fatty change with very little necrosis (Fig. 26). The fatty character was verified by the Sudan IV stain.

The hematological data for the 8 other cows receiving hemolysin are summarized in Table 5. The initial detectable hemolytic response occurred on post-injection (PI) day 2 in the 6 susceptible cows (2, 3, 4, 5, 13 and 16) given hemolysin. The blood values continued to fall and reached their lowest levels by PI days 6 through 9. By PI day 10 the values began to rise in the 4 cows which were not sacrificed until later (4, 5, 13 and 16). Percentages of maximum hemoglobin loss ranged from 26 to 62 per cent. Packed cell volume and erytyrocyte values varied in a corresponding manner. Cow 18 with a serum antibody titer of 10^4 did not show a significant hemolytic response nor did cow 21 given the strain J preparation.

Following administration of the hemolysin preparation, cows 2, 5, 13 and 18 exhibited varying degrees of anorexia and depression which lasted for approximately 24 hours. Diarrhea was evident in cows 13 and 18. The remaining animals showed little untoward effects from the injections. Hemoglobinemia was present in all 6 animals during the hemolytic

response and hemoglobinuria was noted in animals 2, 3, 5, 13 and 16. Icterus was observed in cow 3 at time of autopsy.

Cow 13 aborted on PI day 3 and cow 3 had a fetus in the cervix and vagina at time of necropsy on PI day 8. The degree of fetal decomposition indicated that death of both fetuses had occurred approximately 24 hours previous to abortion. Cow 18, was markedly depressed and had a severe diarrhea following hemolysin administration. It was in labor within 18 hours and required manual assistance to deliver a dead fetus. The fetus appeared to have died during the period of labor. Cow 4 was near term at time of hemolysin administration and delivered a live calf within 48 hours. The hemoglobin level of the fetus was 10.9 gm./100 ml. The remainder of the fetuses were alive at necropsy and a comparison of maternal and fetal blood values is given in Table 6.

Fetal hemoglobin values, with one exception, were the same or higher than values for the dams. The fetus of cow 5 had values considerably less than those of its dam.

At necropsy, cow 3 revealed a slight degree of icterus, a friable liver and a spleen enlarged to approximately twice the normal size. No detectable gross lesions were observed in the remaining cows or their fetuses.

Microscopically, placental lesions were present in cows 2, 3, 5 and 16. Sections from cow 21, given strain J

preparation (Fig. 27) and uninjected cow 15 (Fig. 28) were normal. The lesions noted in sections from cow 3 included marked connective tissue proliferation, atrophy and absence of maternal-crypt epithelium and necrotic villi (Figs. 29 and 30). Cows 2 and 5 showed a few areas in which the maternal-crypt epithelial cells were pyknotic and the continuity disrupted (Figs. 31 and 32).

Sections from the placentomes of cow 16 revealed a marked disintegration of the fetal villi with a disappearance of many maternal cells. Fetal trophoblasts were pyknotic (Figs. 33 and 34). Approximately one-third of the area in each placentome was similarly affected.

Kidney sections revealed an increase in glomerular extrusions of proteinaceous material and partial disintegration of the glomeruli (Fig. 35). There were areas in which tubular cells were disrupted and blood pigments had been phagocytized by cells of the proximal convoluted tubules (Fig. 36).

In contrast to cows 1 and 12 which died of hemolytic anemia, the remainder of the animals showed no significant hepatic or splenic lesions. No lesions were observed in brain, adrenal or heart section or in fetal tissues.

Table 7 gives the results of the AL tests of 6 cows. Three of 6 cows had no detectable antibody titers for L. pomona. No fetal samples were positive.

TABLE 4

HEMATOLOGICAL DATA FOR COWS 1 AND 12

Cow No.	1 (200 ml.)		12 (85 ml.)	
Normal	Hgb	13.8	Hgb	11.5
	Hct	45.0	Hct	44.0
	Rbc	9.44	Rbc	8.47
12 Hours	Hgb	12.0	Hgb	11.0
	Hct	40.0	Hct	42.0
	Rbc	6.75	Rbc	6.76
24 Hours	Hgb	6.3	Hgb	8.2
	Hct	5.0	Hct	26.0
	Rbc	.63	Rbc	--
30 Hours	Hgb	4.6*	Hgb	6.8
	Hct	3.0	Hct	17.0
	Rbc	.25	Rbc	3.01
36 Hours	Hgb		Hgb	5.2*
	Hct		Hct	12.0
	Rbc		Rbc	1.68

*Indicates animal died before next determination.

Hgb = hemoglobin in grams per 100 ml. blood.

Hct = packed cell volume expressed as per cent.

Rbc = erythrocytes in millions per cmm.

TABLE 5

SUMMARY OF HEMATOLOGICAL DATA FOR

COWS RECEIVING HEMOLYSIN

Day	Dose 1 / Cow No.	8000 HU 2[x]	9000 HU 3[x]	6500 HU 4[x]	9000 HU 5[x]	3250 HU 13	3250 HU 16[x]	6600 HU 18[x a]	50 ml. J 21
Nor-mal	Hgb	13.0	11.6	11.3	10.1	10.8	11.8	11.9	9.7
	Hct	36.0	40.0	30.0	33.0	43.0	40.0	35.0	28.5
	Rbc	8.41	10.16	7.73	7.50	7.44	8.80	8.35	6.20
1	Hgb	16.6	10.9	11.6	11.3	12.9	11.8	16.3	9.4
	Hct	42.0	38.0	32.0	38.5	41.5	40.0	44.0	26.5
	Rbc	8.70	8.93	7.14	8.30	10.20	7.82	8.91	5.77
2	Hbg	12.7	10.0	9.4	10.2	10.7	11.2	15.0	9.1
	Hct	36.0	33.0	25.0	37.0	37.5	38.0	41.0	26.5
	Rbc	7.66	7.80	5.90	7.64	7.12	7.25	8.54	5.41
3	Hgb	11.7	7.9	9.1	10.1	9.2	9.6	13.4	9.3
	Hct	31.0	24.0	24.0	33.0	31.5	34.5	37.0	27.5
	Rbc	6.54	5.27	5.68	6.97	6.22	6.35	7.04	5.94
4	Hgb	10.7	6.8	8.1	9.7	7.8	8.5	--	9.7
	Hct	27.5	18.0	21.0	27.5	26.5	28.0	--	26.0
	Rbc	5.60	4.72	4.97	6.17	5.59	5.91	--	5.90
5	Hgb	9.4	5.0	7.5	9.5	7.1	7.7	12.2	9.1
	Hct	22.0	13.5	21.0	26.0	24.5	25.5	35.0	24.0
	Rbc	5.22	3.24	4.62	5.50	4.76	5.14	7.72	5.83
6	Hgb	8.6	4.4	6.6	8.0	7.0	7.6	11.9	10.0
	Hct	24.0	12.0	20.0	22.5	25.0	25.0	33.0	28.0
	Rbc	4.93	2.88	4.34	5.53	5.02	4.90	6.94	6.11
7	Hgb	7.8	4.4	6.6	8.1	7.0	6.8	11.3	9.4*
	Hct	24.0	12.0	19.0	21.0	23.0	23.0	31.0	29.0
	Rbc	4.40	2.92	4.52	5.32	4.40	4.93	5.83	5.72
8	Hgb	6.6	*4.4	6.6	7.8	6.5	6.2	11.6	
	Hct	23.0	13.0	19.0	22.0	23.0	21.0	31.0	
	Rbc	4.62	2.90	3.73	5.25	4.93	3.64	7.25	

TABLE 5--Continued

Day	Dose / Cow No.	8000 HU 2[x]	9000 HU 3[x]	6500 HU 4[x]	9000 HU 5[x]	3250 HU 13	3250 HU 16[x]	6600 HU 18[x a]	50 ml[(1)] J 21
9	Hgb	*6.8		6.6	7.5	6.1	5.8	11.3	
	Hct	24.0		19.0	21.0	22.0	21.0	31.0	
	Rbc	4.65		3.42	4.04	3.97	4.21	5.64	
10	Hgb			7.2	7.5	6.8	6.1	11.3	
	Hct			20.0	23.0	22.0	20.0	31.0	
	Rbc			4.29	4.21	5.21	3.40	5.94	
11	Hgb			6.9	--	7.5	6.6	z10.9	
	Hct			20.0	--	22.0	18.0	31.0	
	Rbc			3.51	--	4.16	3.45	5.82	
12	Hgb			8.1	8.1	7.5	7.2		
	Hct			23.0	25.0	22.0	19.0		
	Rbc			4.68	4.70	4.01	3.60		
13	Hgb					7.5	6.6		
	Hct			y	y	y21.0	y19.0		
	Rbc					3.83	3.32		

(1) - Strain J had no hemolytic activity. Amount in ml. similar to other cows.

x - Indicates that animal's own erythrocytes were used in hemolysin titrations.

* - Indicates necropsy performed on this day.

y - Indicates further values were obtained.

z - Final determination.

a - Cow 18 had serum antibody titer of 10^4.

Hgb - Hemoglobin in grams per 100 ml. blood.

Hct - Packed cell volume expressed as per cent.

Rbc - Erythrocytes in millions per cmm.

TABLE 6

COMPARISON OF BOVINE MATERNAL AND FETAL HEMOGLOBIN VALUES (GM./100 ml.)

Cow no.	Maternal	Fetal
2	6.8	10.0
21	9.4	9.4
11	4.6	9.2
16	7.5	7.5
5	10.3	5.9

TABLE 7

ANTIBODY TITERS* FOR L. POMONA IN SERA

OF INJECTED COWS

Cow No.	Day after Injection				
	5	8	11	16	30
2			All negative		
3			All negative		
5	2	2	2	2	1
13			All negative		
16	-		1	2	
21	-	3			

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

Sow Experiment

Hemoglobin values for 4 of 6 experimental sows are given in Table 8. Values were not obtained from 1 of the remaining 2 because of a hematocyst in the region of the anterior vena cava. The other sow farrowed within 24 hours after receiving strain J preparation and her blood was not sampled.

No significant alterations occurred in hemoglobin values. At time of autopsy, 37 of 39 fetuses from the sows given hemolysin were alive. The 2 dead fetuses were mummified. Hemoglobin values of 14 fetuses ranged from 9.0 to 10.6 gms./100 ml. Terminal agglutination-lysis tests were positive at a dilution of 1:1000 for the 4 sows given strain LW hemolysin and 1:100 for the sow given strain J preparation.

Fetal sera were negative when tested with *L. pomona* antigen.

Aside from depression and anorexia for about 12 hours following the intravenous administration of hemolysin, no untoward effects were observed.

No significant gross or microscopic lesions were observed in sows or their fetuses. Placental sections were normal in sows given hemolysin (Fig. 37).

TABLE 8

HEMOGLOBIN VALUES FOR SOWS (GM./100 ML.)

RECEIVING HEMOLYSIN

Day	Sow 2*	Sow 4[x]	Sow 5[x]	Sow 8[x]
Normal	14.0	11.4	11.0	11.4
1	14.0	13.0	12.7	11.4
2	14.5	12.9	11.8	9.4
3	13.6	13.0	13.6	10.4
4	12.3	--	--	12.0
5	13.4	12.2	13.4	12.3
6	13.2	--	--	
7	13.0	12.4	--	
8	12.4	14.3		

* Sow 2 received 200 ml. normal saline intravenously.

x Sows 4, 5 and 8 received 200 ml. of L. pomona hemolysin.

Results of Other Procedures

A summation of the data obtained in determining variations in bovine erythrocyte susceptibility is given in Table 9.

In two instances the erythrocytes of cow 15 were more resistant to hemolysis than those of cow 5 and in one instance were only 41 per cent as susceptible to hemolysis as were those of cow 3. With hemolysin A the erythrocytes of cow 5 were more resistant to hemolysis than were those of cow 3 while, with hemolysin C, the opposite was the case.

An indication of the individual resistance and variations in susceptibility of porcine erythrocytes and a comparison with bovine erythrocyte susceptibility to the same hemolysin is given in Table 10.

The *in vitro* resistance to hemolysin was much greater in porcine erythrocytes than in bovine erythrocytes. There was considerable individual variation in susceptibility in both the bovine and porcine erythrocytes.

The comparative effects of the addition of sera from 4 animals before incubation of hemolysin and erythrocytes is shown in Table 11. Cow 13 had been previously injected with hemolysin and undergone a hemolytic response.

Sera from all animals had an inhibitory effect upon hemolysis. Cow 13's serum was the least inhibitory and its cells were not resistant to hemolysis.

Cow 13, which failed to develop detectable agglutinins, was reinjected 4 months after the initial injection with L. pomona hemolysin prepared from cultures grown in bovine serum. A susceptible cow was injected similarly. Table 12 summarizes the results of this study.

Hemoglobin values following reinjection of cow 13 did not significantly change. The control heifer given hemolysin similarly prepared underwent a typical hemolytic response.

TABLE 9

INDIVIDUAL VARIATIONS IN BOVINE ERYTHROCYTE SUSCEPTIBILITY TO THE SAME HEMOLYSIN

Hemolysin	Cow 3	Cow 5	Cow 15
A	300*	180	125
B	--	100	65
C	60	95	--

* Hemolysin titer (HU/ml.).

TABLE 10

COMPARATIVE SUSCEPTIBILITY OF PORCINE AND BOVINE ERYTHROCYTES TO HEMOLYSIN

Animal No.	Hemolysin Titer (HU/ml.)
Sow 1	1
Sow 3	4
Sow 4	2
Sow 5	8
Sow 6	2
Sow 7	1
Cow 3	60
Cow 4	65
Cow 5	95

TABLE 11

EFFECT OF SERUM ADDITION BEFORE

HEMOLYSIN* INCUBATION

Cells	Cow 2	Cow 5	Cow 13**	Cow 21
Serum				
2	58[x]	15	37	28
5	38	20	22	14
13	78	65	75	65
21	50	13	30	25
None	95	100	100	90

* 1:50 dilution of hemolysin.

x Values in per cent hemolysis.

** Cow 13 received hemolysin and recovered without a detectable titer for L. pomona.

TABLE 12

RESISTANCE OF COW 13 TO HEMOLYSIN REINJECTION

	Initial Injection 3250 HU	2nd Injection** 6500 HU	Control 6500 HU
Normal	10.9[x]	12.2	11.4
Day 1	12.9	10.6	12.5
Day 2	10.7	12.2	10.3
Day 3	9.2	11.9	10.0
Day 4	7.8	10.9	9.7
Day 5	7.1	11.3	9.1
Day 6	7.0	11.6	8.4
Day 7	7.0	11.6	8.7
Day 8	6.5	10.9	8.7

x Hemoglobin in gms./100 ml.

** Second injection 4 months after initial injection.

DISCUSSION

While it was not in the scope of this work to investigate hemolysins of other serotypes, there appears to be a definite correlation between *in vitro* production of hemolysin and *in vivo* manifestations of hemolytic anemia. For example, *L. grippotyphosa*, a consistent hemolysin producer, commonly produces hemolytic anemia in sheep and goats (van der Hoeden, 1958). *Leptospira icterohaemorrhagiae* which produces little or no hemolysin has not been shown to cause hemolysis. Paltrinieri *et al.* (1959) in describing experimental *L. icterohaemorrhagiae* infection in sheep, observed no significant changes in erythrocyte or hemoglobin values. It is true that *L. icterohaemorrhagiae* commonly produces icterus in dogs and other animals (Low and Mather, 1960) but the icterus produced is believed to be hepatocellular in origin and not related to excessive red blood cell destruction. Field and Sellers (1950) described a "hemolytic jaundice" produced by *L. icterohaemorrhagiae* infection in calves, but no blood studies were reported and hemoglobinuria was not observed. One may conclude that they were either dealing with an atypical hemolysin-producing strain or the icterus was of the toxic type. The hepatic lesions described were typical of those seen in the toxic icterus in that generalized parenchymatous degeneration

of hepatic cells and areas of focal necrosis were described. Centrolubular necrosis is more typical of the type of hepatic destruction due to a hemolytic process. Experimental L. sejroe infection in cattle (Ristic et al., 1957) was not characterized by erythrocyte destruction. This serotype also has not been shown to produce hemolysin.

The clinical symptoms produced by the administration of L. pomona hemolysin to pregnant cows and ewes bear a striking similarity to those induced by the whole organism. Hemoglobinuria, icterus, anemia and abortion are commonly regarded as quite typical of L. pomona infection in sheep and cattle. All these symptoms were observed with varying degrees of severity in the experimental cows and ewes given hemolysin.

In order to properly assess the possible relationship of leptospiral abortions in cattle and sheep to the effects of hemolysin, a careful analysis of the views of various authors appears to be necessary.

Fennestad and Borg-Petersen (1960) strongly defend the hypothesis of bovine fetal leptospirosis followed by fetal death and abortion. While evidence indicates that L. pomona may occasionally cross the placental barrier in cattle (Podgwaite et al., 1955; Dacres et al., 1958) the, until now, universal failure of numerous investigators (Fennestad and Borg-Petersen, 1956 and 1960; Ferguson et al., 1957; Morse

and McNutt, 1956; Morter, 1961) to obtain cultural isolations from fetuses of experimentally infected cows raises serious doubts as to the organism's ability to routinely reach the fetus and establish infection. Autolysis can not be the basis of failure to isolate leptospirae from the experimental fetuses because most were alive at the time of their dam's necropsy. In the author's opinion, based on experience with cultural isolations from porcine tissue at various intervals after infection (Sleight and Lundberg, 1961), leptospirae would be relatively easy to isolate from fetal tissue.

Fennestad et al. (1960)cited the demonstration of leptospirae in aborted bovine fetuses by silver impregnation as further evidence of fetal infection. In fetuses from which cultural isolations cannot be obtained, these authors emphasized that silver-impregnated sections are reliable indicators of the presence of leptospirae if fetal autolysis is not advanced. These authors further emphasized that "investigators who make use of silver impregnation are able to find leptospirae in all examined fetuses where a diagnosis can be anticipated except in advanced autolysis." Contradictory evidence, which tends to refute this statement, is presented in this same paper by Fennestad et al. (1960). Four fetuses were infected by intraplacental inoculation. Following autopsy,

Levaditi-stained sections of fetal liver and kidney were positive in 3 out of 4 and each of these 3 had been dead longer than 24 hours. Leptospirae were isolated from 2 of the 3 by guinea pig inoculation. The fetus from which leptospirae could not be demonstrated by silver impregnation was one which was delivered after sacrifice of the dam and had been dead more than 5 but less than 24 hours. L. pomona was isolated in culture and by guinea pig inoculation from spleen, liver, kidney, peritoneal fluid and pleural fluid. In this case, presence of leptospirae was firmly established and their presence was anticipated but not demonstrated in silver-stained sections. The isolation of leptospirae from 2 of 3 fetuses which had been dead longer than 24 hours indicated that the organism can survive a considerable length of time after fetal death and this would further increase the chance of making cultural isolations.

It would seem that the basis of the fetal infection hypothesis rests largely on the reliability of silver impregnation methods. These procedures should be analyzed by a carefully controlled series of experiments in which known negative as well as known positive tissue is examined. The mere demonstration of argyrophyllic structures resembling leptospirae can not be

accepted as the cause of the death of the fetus *per se* but should be evaluated along with other factors which may cause fetal death. It is quite conceivable that fetal infection could occur, but that the actual cause of death would be impaired nutrition due to placental damage. The author, however, does not wish to discount the value of silver impregnation techniques when proper consideration is given to size, location, configuration and degree of argyrophilia.

Further evidence against fetal infection has been presented by Fennestad *et al.* (1957). These authors demonstrated the ability of fetuses to produce leptospiral agglutinins when the fetuses were intrauterinely infected with *L. saxboeing*. It would be logical to assume that fetuses naturally infected would have this same ability. Until now leptospiral antibodies have not been demonstrated in aborted fetuses or in fetuses from experimentally infected cows or ewes. One may logically conclude that fetuses without leptospiral antibody have not been exposed to the antigenic stimulus of the organism.

The isolations by Smith *et al.* (1960) of *L. pomona* from placenta and from fetal fluids and umbilical blood from 3 of 17 inoculated pregnant ewes would tend to support the hypothesis supported by Fennestad and Borg-Petersen (1960). However, a critical evaluation of Smith's data leaves many questions unanswered. These authors were able to make isolations from

the listed tissues 3 days after inoculation but no later. This was during maternal leptospiremia and any isolations from placental tissue would likely be no more than would be obtained from maternal blood cultures. Amniotic fluid and fetal umbilical blood would be more reliable but amniotic fluid could easily be contaminated by maternal blood during necropsy. The single demonstration of the presence of leptospirae in fetal umbilical blood indicated that it is possible for leptospirae to reach the fetal circulation. These authors were unable to obtain isolations from fetuses or to demonstrate leptospirae later than 3 days after inoculation of the ewes and were unable to demonstrate the organism's presence in fetal parenchymal organs at any time. This leaves room for considerable doubt as to the ability of L. pomona to establish itself in the ovine fetus. Lindqvist et al. (1958) were also unable to isolate leptospirae from fetuses of infected ewes.

Ferguson et al. (1957) postulated that a toxin, such as a hemolysin, could be released by lysis of leptospirae by antibody. This toxin, it was assumed, would cross the placental barrier and cause death of the fetus by destroying its erythrocytes. The work of Bauer et al. (1961) and observations of Smith et al. (1960) indicated that hemolysis takes place before the appearance of antibody. Therefore hemolysin appearance is not dependent upon lysis of leptospirae. Evidence of fetal

susceptibility to the effects of a hemolysin was provided by Bauer et al. (1961) when they demonstrated the low serum inhibition titers of fetuses. Thus fetal erythrocytes likely would be highly susceptible to hemolysin if hemolysin could cross the placental barrier and enter the circulation.

A further indication of fetal susceptibility was shown by Morter (1961) when it was demonstrated that intrauterine inoculation of fetuses produces a marked loss of hemoglobin. However, fetuses from infected dams have not shown significant hemoglobin alterations (Lundberg, 1960).

A gross pathological alteration which might be interpreted as an indication that hemolysin can cross the placental barrier is the appearance of dark red transudates in the body cavities of aborted fetuses. Such transudates were observed in the dead ovine fetuses in the present work and to a lesser degree in the aborted bovine fetuses. It is the author's opinion that such transudates are indications of post-mortem hemolysis and not due to ante-mortem erythrocyte destruction by hemolysin. No dark red transudates were observed in live fetuses including the live fetus of the 48-hour ewe which had a live and a dead fetus in the uterus at the time of necropsy.

There was little evidence that the hemolysin used in the present experiment passed into the fetus. No alterations were shown to have taken place in fetal hemoglobin values even when

the dams had undergone a severe hemolytic disturbance. In one bovine fetus the hemoglobin value was less than that of the dam, but fetuses from normal non-infected dams have been shown by Lundberg (1960) to occasionally have similar values.

Morter et al. (1958), in a discussion of histopatholigical changes occurring in cotyledons of L. pomona-infected heifers, concluded that alterations in the intimate relationships between fetal and maternal systems could interfere with the transfer of essential materials across the placental barrier and result in fetal inanition, death and abortion. The progressive nature of the development of these lesions would explain the usual time interval of 2 - 4 weeks between infection and abortion. Fennestad et al. (1960), in analyzing the data reported by Morter et al., likened the changes described by them to the picture described by Björkman (1954) in normal bovine placentomes. However, a careful review of Björkman's work by the author disclosed no references to necrosis of the maternal-crypt epithelium, to marked increases in connective tissue, to vacuolization of fetal villi or to losses in normal placental architecture as phenomena accompanying normal pregnancy. All these changes were described by Morter et al. (1958). In addition, Fennestad and Borg-Petersen (1960) stated that the necrosis of the fetal villi, described by Morter et al. in one placenta retained for 24

hours after abortion, most likely occurred after death of the fetus. While this could be true, the marked connective tissue proliferation could not be satisfactorily explained on this basis.

The present series of experiments in which death of fetuses and/or pathological placental alterations followed intravenous hemolysin injections gives supportive evidence to Morter and his co-workers' observations and provides a mechanism to explain their findings. As far as the author knows, no one has yet associated hemolytic anemia with abortion in L. pomona-infected animals. The author, therefore, proposes the following as a supplement to the observations of Morter et al. and as a likely explanation of the pathogenesis of abortion. In susceptible pregnant cows and ewes infected with L. pomona, the organism produces hemolysin in sufficient concentration to produce severe destruction of erythrocytes. Due to the resultant decrease in oxygen carrying capacity of the blood, placental cellular metabolism is disrupted. Necrosis follows, first of the maternal, and later of the fetal cells. The fetus, unable to attain essential nutrients, dies and is expelled by the dam.

The above appeared to be the sequence of events in the experimental ewes and cows given hemolysin. In the ewes, the effects of the administered hemolysin were apparent within 12

hours and by 48 hours approximately 50 per cent of the hemoglobin had been lost. This rapid loss adversely affected the placental cells causing extensive placental alterations and necrosis and subsequent death of 3 of 8 fetuses. In the ewes having live fetuses, clear-cut evidence of placental pathological alterations were present but the changes were evidently not extensive enough to cause fetal death. Studies of the lesions indicated that the maternal cells were the first affected.

The results in the cows were complicated somewhat by adverse foreign protein reactions in some heifers apparently sensitive to the rabbit serum globulin or other foreign proteins. The ewes and sows did not exhibit a similar reaction. The untoward effects of administration were especially severe in cows 18 and 21. Cow 21 died within 12 hours and heifer 18 was markedly depressed, had a severe diarrhea and due to excessive smooth muscle stimulation was in labor within 18 hours. A dead fetus was manually delivered. Although the blood of cow 13 was undergoing extensive hemolysis, one cannot discount the possibility of a relation between foreign protein reaction and abortion because of the short period (72 hours) between hemolysin administration and abortion.

Aside from these 3 animals (13, 18 and 21), results and response in the cows were similar to the ewes. There was a

longer period after administration before hemolysis could be detected in cows and the effects of the hemolysin were more prolonged. Ewes allowed to recover had an increase in hemoglobin levels by post-injection day 5 while post-injection day 10 passed before injected cows had a rise in hemoglobin values. Cow 3 had a severe hemolytic response and was aborting on post-injection day 8. The placental changes were strikingly similar to those described by Morter et al. (1958) in experimental infection. The maternal epithelium was replaced by connective tissue and fetal villi were necrotic. Cow 16, sacrificed 16 days after hemolysin injection, had marked placental alterations similar to those described by Morter et al., 1958; Morter, 1960) in heifers infected but not aborting. Approximately one-third of the placentome was affected. Connective tissue was increased, maternal epithelium was absent in many crypts and fetal trophoblasts were pyknotic. Cow 12, which died as a result of hemolytic anemia, showed placental cellular disruptions but the shortness of time between hemolysin injections and death make these changes difficult to evaluate. The placental lesions in the remaining experimental cows were not severe and were confined principally to the maternal-crypt epithelium. The significance of these minimal lesions is increased by the lack of similar alterations in the strain J injected and normal uninjected animals.

The hemorrhages observed in the control and injected ewes are described by Wimsatt (1950) and appear to be a normal characteristic of the superficial portion of the placentome in the latter part of pregnancy. In these areas, it would seem to be possible for an organism to gain entrance to fetal circulation by passing through only the three fetal layers.

Critics of the proposed relationship between anemia and abortion may contend that abortions occur in animals in which the disease has apparently run an asymptomatic course. In natural outbreaks this criticism would lack a sound basis because the exact time of infection cannot be determined and blood studies are not recorded from exposure until abortion. Judging from the clinical appearance of hemolysin-injected cows, an animal can undergo considerable hemolysis with minimal outward manifestations. Hemoglobinuria may be present only a short period of time and not be observed, the appetite may remain good and, unless hemolysis is rapid, icterus may not be present. At the time of abortion the animal may already be recovering from hemolytic anemia so that the relationship of anemia to abortion would be obscure.

It is unfortunate that daily erythrocyte and hemoglobin determinations have not been reported in the few cases in which cows have been experimentally infected with L. pomona

and have aborted (Fennestad et al., 1956; Ferguson et al., 1957; Morse and McNutt, 1956). Until a highly virulent, consistently abortifacient strain of L. pomona can be used experimentally in pregnant cattle and sheep, the relationship of hemolytic anemia to abortion will be difficult to evaluate. Babudieri (1959) mentions the fact that experimental infection of pregnant cattle produces abortion much more rarely than many authors believe.

The principal liver lesion observed in the ewes and to a lesser extent in the cows was centrolubular necrosis with hepatic cord disruption and fatty infiltration. The hypoxia resulting from the lowered oxygen carrying capacity of blood would be manifested first in the central area of the lobule due to the character of the lobular circulation. The central area is the last to be oxygenated by the blood and thus the first to suffer from an anemic state. The similarity of the liver lesions described in this experiment to those resulting from an actual infection by L. pomona makes it appear logical to assume that the hepatic histopathological changes in L. pomona infection result from hemolytic anemia.

The congested and hemorrhagic spleens reported as lesions of L. pomona infection also are related to the hemolytic anemia. Such alterations were observed in this series of experiments.

The extent of the histopathological changes in the kidneys of cows and ewes given hemolysin was related to the severity of the hemolytic reaction. Cows 1 and 12, both of which died within 48 hours, and the susceptible ewes given hemolysin experienced similar severe renal pathological manifestations. Passage of blood through glomeruli and tubular cell destruction showed that filtration and absorption can be disturbed by rapid hemolysis. The phagocytic ability of renal tubular cells was shown in animals which were recovering from the hemolytic anemia. Renal lymphocytic infiltration, which is characteristic of L. pomona infection, was not observed in sections from hemolysin-injected animals. Apparently this response is elicited by the organism and not by any factor in the hemolysin.

The ability of animals with a titer to withstand the effects of hemolysin was shown in cow 18 and ewe 12. This supported the in vitro and in vivo observations of Bauer et al. (1961). The ewe with the lower titer (10^2) had a delayed and lessened response but was not entirely protected. Evidence that other factors besides agglutinins protect against L. pomona hemolysin was presented by ewe 3. This animal had no detectable titer and yet showed no hemolytic response.

The antigenicity of leptospiral hemolysin is as yet undetermined. It is the author's opinion that agglutinins induced by hemolysin administration resulted from impurities in the

preparation, probably fragments of killed organisms which had passed through the filters. Some evidence for this supposition was obtained from a separate experiment with swine (Sleight, 1961). Seitz-filtered hemolysin induced agglutinins whereas preparations passed through both Seitz and Selas filters did not. These animals were injected intraperitoneally and subcutaneously.

Some evidence that hemolysin administration may protect an animal against a second injection was obtained with cow 13. This animal underwent a hemolytic anemia and recovered without having a detectable titer. A second administration of similar amounts of hemolysin produced no hemolytic response. With only a single animal it is impossible to draw a sound conclusion from this observation, but it does indicate that this problem deserves further study.

An in vitro study performed during the course of the experiment indicated that the serum from an animal which has recovered following hemolysin administration would inhibit hemolysis no more than normal serum. As serum normally possesses hemolysin inhibitors, a much better approach to this problem would be daily measurements of serum inhibition titers following hemolysin administration to determine whether these titers would increase. Purified preparations would be desirable because of the inhibitors present in rabbit serum globulin.

While the hemolysin titration procedures developed by Bauer et al. (1961) were generally satisfactory for this series of experiments, titration results obtained by the author further confirm that there is a considerable margin of error in the procedures. The in vitro studies indicated considerable variation in erythrocyte susceptibility of different animals of the same species. When species susceptibility is being determined, it would be desirable to use pooled erythrocytes to minimize the error. A further observation (Sleight, 1961) made when the studies were nearly completed may explain some discrepancies in titration results. It was found that freshly obtained erythrocytes were more susceptible to in vitro hemolysis by L. pomona hemolysin than were erythrocytes stored several days in Alsever's solution.

The absence of hemolytic effects in sows given hemolysin supported the in vitro observations of resistance of porcine erythrocytes to L. pomona hemolysin. In this species there is no doubt of the ability of leptospirae to invade the fetus. Their presence in fetuses or in newborn pigs in numbers sufficient to be visualized by dark-field microscopy is a common finding (Preston and Morter, 1960). The negative effects of hemolysin on sows or their fetuses would seem to eliminate hemolysin as a factor in porcine abortion or in the other manifestations of the disease in swine. Furthermore,

the negative results indicated that in swine there was no other factor in the partially purified preparations of hemolysin used in this experiment which adversely affect the placental tissues or the fetuses. This was indirect evidence that the effects on the placentomes in ewes and cows were due to hemolysin *per se* and not to some other factor in the preparation. This supposition must await further proof because of the impurity of the preparations used and the scientific unsoundness of transposing results from one species to another.

With the recent developments of many satisfactory procedures for protein fractionation and characterization, it would be desirable for hemolysin work to proceed along this line. Until the substance is purified many questions regarding its chemical structure, antigenicity and mode of action will remain unanswered. The results of this experiment emphasize the importance of the effects of hemolysin and should encourage workers to further investigate its properties.

SUMMARY AND CONCLUSIONS

A series of experiments was conducted using pregnant ewes, cows and sows to study the effects of intravenous administration of partially purified and concentrated L. pomona hemolysin. The hematological changes, clinical symptoms and gross and microscopic pathological alterations were recorded.

Pregnant ewes developed a severe hemolytic anemia evidenced by hemoglobinuria, hematuria and icterus. Average hemoglobin levels decreased to 57 per cent of normal within 48 hours. The histopathological alterations were characterized by hepatic centrolobular necrosis, splenic congestion and renal glomerular extrusion of blood with some tubular necrosis. Death of 3 of 8 fetuses appeared to be due to pathological disturbances of the maternal and fetal relationship rather than to a hemolytic effect upon fetal blood cells. Ewes receiving hemolysin and having live fetuses in utero at necropsy exhibited progressive placental damage. The maternal-crypt syncytial cells appeared to be the first to become necrotic and disrupted. These changes were followed by necrosis and vacuolization of fetal villi. A complete disintegration of the maternal-fetal relationship was noted in the cotyledons of dead fetuses.

Two cows died during severe hemolytic response. Gross and microscopic pathological changes were similar to those

observed in the ewes. Six other cows underwent less severe hemolysis. Maximum hemoglobin loss varied from 26 to 62 per cent in individual animals. Two of 6 fetuses were aborted, one 3 days and the other 8 days after hemolysin administration to the dams. The histopathological lesions in the maternal placenta were typified by maternal-crypt epithelial necrosis and connective tissue proliferation. Extensive necrosis of villi was noted in sections of the placenta from the aborted fetus. In the 6 cows, renal, hepatic and splenic changes were minimal.

With the exception of one bovine fetus, the ovine and bovine fetal hemoglobin values were within the normal range. Thus, there is little evidence that L. pomona hemolysin crosses the placental barrier. It is postulated that the role of hemolysin in the abortion syndrome in cattle and sheep is related to placental metabolic disturbances elicited by lowering of the oxygen carrying capacity of the blood.

Ewes and cows with high serum titers against L. pomona were resistant to the effects of hemolysin. One cow which recovered without having a detectable titer was resistant to a second injection. This may indicate possible antigenicity of L. pomona hemolysin.

Sows or their fetuses were not affected by L. pomona hemolysin. This verified the in vitro observations of porcine

erythrocyte resistance to hemolysin and indirectly indicated that hemolysin *per se* was responsible for disturbances in the placentomes of cattle and sheep.

Erythrocytes obtained from different cows showed variations in susceptibility to the same hemolysin as demonstrated by *in vitro* assays.

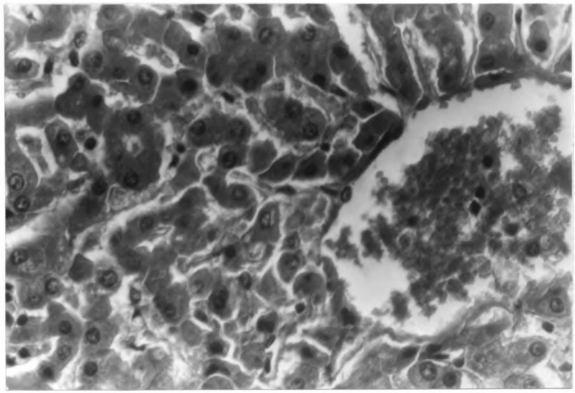

Fig. 1.--Liver of 48-hour ewe (48 hours after receiving L. pomona hemolysin) showing pyknotic cells near the central vein and disruption of hepatic cords. x 600

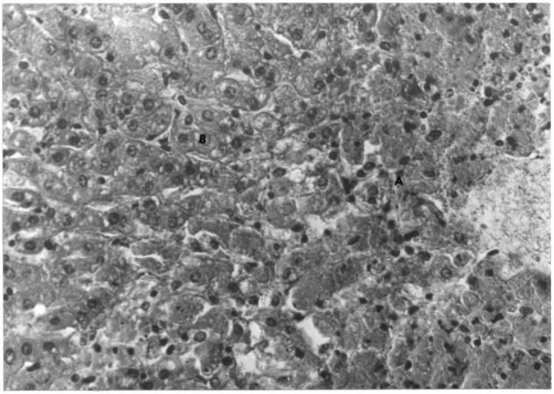

Fig. 2.--Liver of 96-hour ewe. A. Centrolobular necrosis. B. Fatty infiltration. x450.

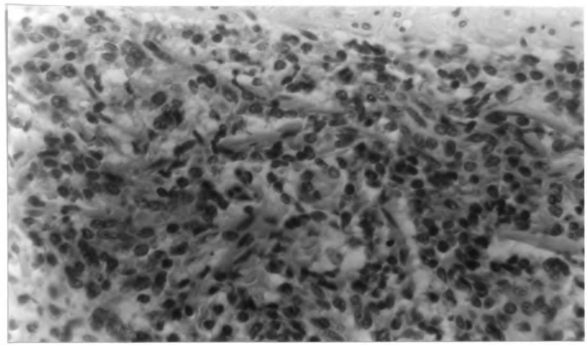

Fig. 3.--Normal ovine spleen, sub-capsular area. x600.

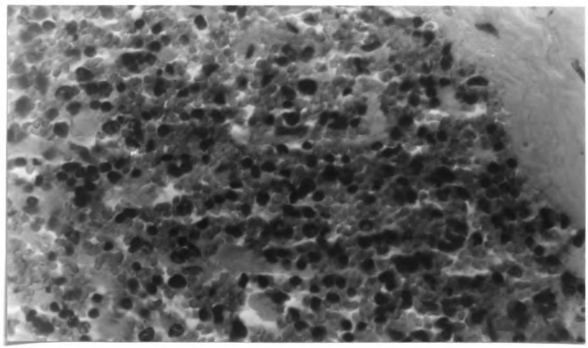

Fig. 4.--Spleen of 48-hour ewe showing congestion in subcapsular area. x600.

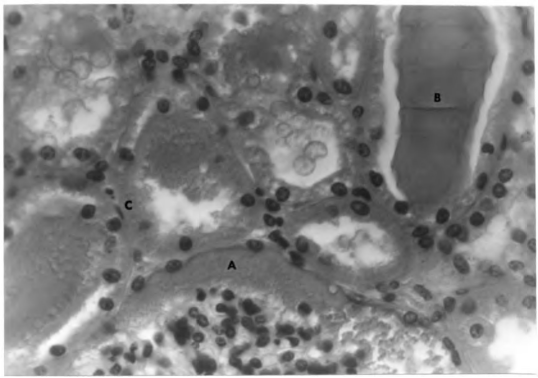

Fig. 5.--Kidney of 24-hour ewe. A. Hemorrhage in subcapsular space of renal corpuscle. B. Hemolyzed blood in tubules. C. Pyknosis and karyolysis of tubular epithelium. x600.

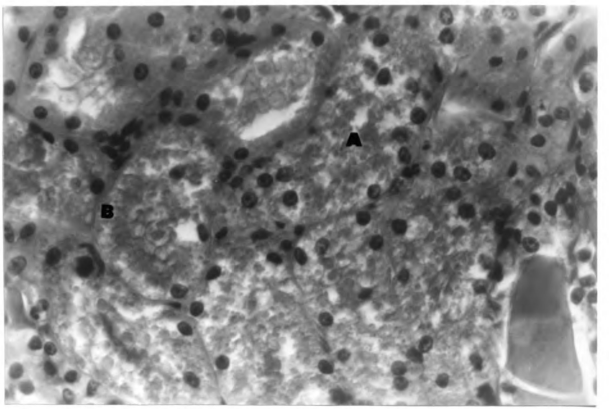

Fig. 6.--Kidney of 48-hour ewe. A. Blood in proximal convoluted tubules. B. Partial disappearance of tubular epithelium. x600.

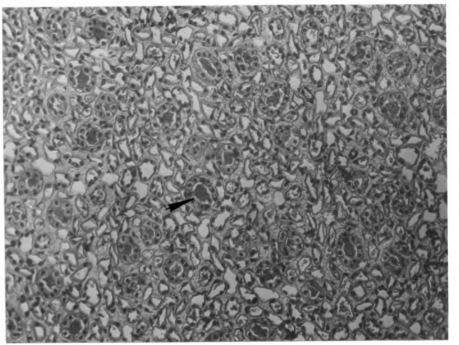

Fig. 7.--Medulla of kidney of 48-hour ewe. Note blood in collecting tubules. x225.

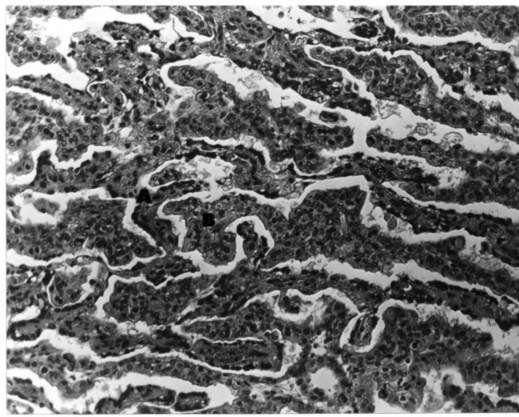

Fig. 8.--Cotyledon of control ewe given strain J preparation. A. Note relative continuity of maternal-crypt syncytium. B. Observe integrity of fetal villi. Separation of fetal and maternal tissues is an artifact. x225.

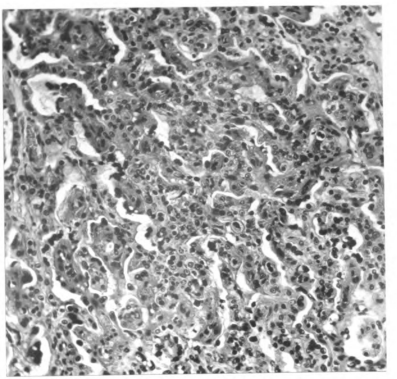

Fig. 9.--Cotyledon of uninjected control ewe. x225.

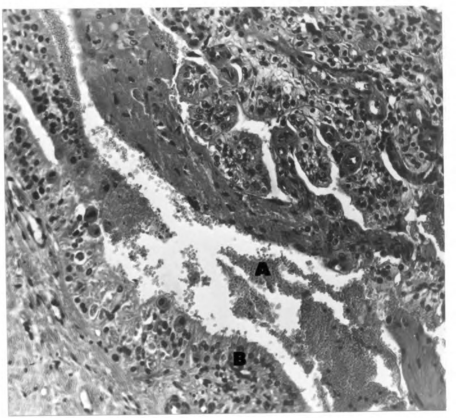

Fig. 10.--Hemorrhage in cotyledon of control ewe. A. Hemorrhage. B. Apparent phagocytosis of erythrocytes by epithelial cells. x225.

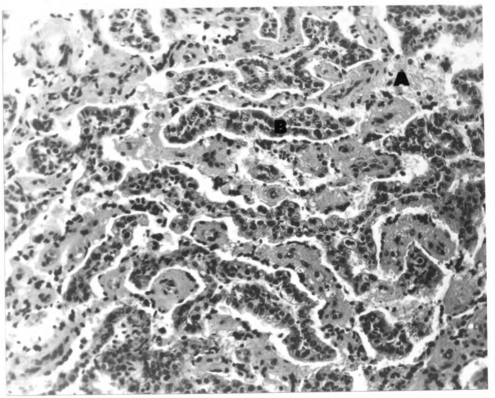

Fig. 11.--Cotyledon of 24-hour ewe. A. Note degeneration of maternal syncytium. B. Observe relatively normal appearance of fetal villi. C. Compare with appearance of maternal interstitial tissue in Fig. 9. x225.

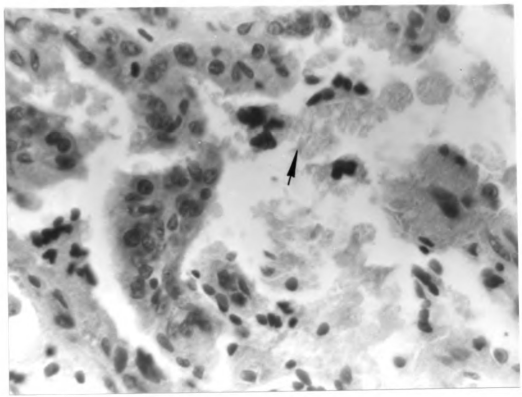

Fig. 12.--Cotyledon of 36-hour ewe demonstrating disintegration of maternal syncytium and disorganization of maternal-fetal relationship. x600.

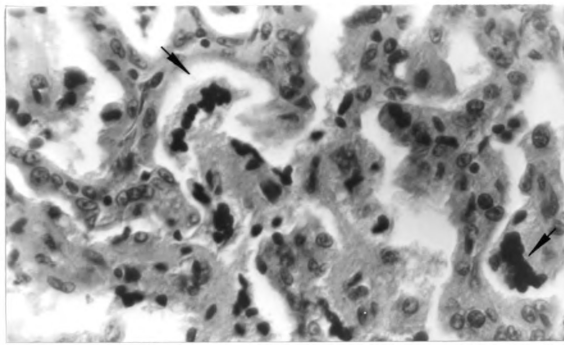

Fig. 13.--Cotyledon of 48-hour ewe from section serving live fetus demonstrating pyknotic maternal-crypt cells. Compare with Fig. 15 taken from same case. x600.

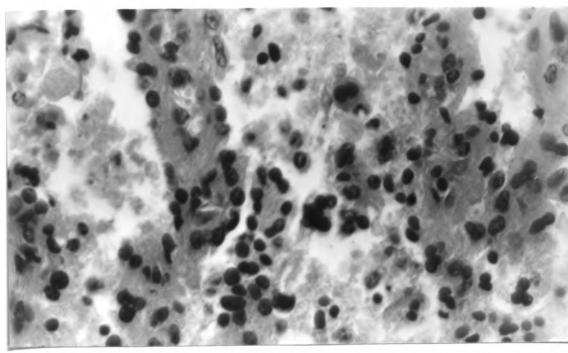

Fig. 14.--Cotyledon of 48-hour ewe from section serving dead fetus to show loss of normal relationships and disruption of maternal crypts and fetal villi. x600.

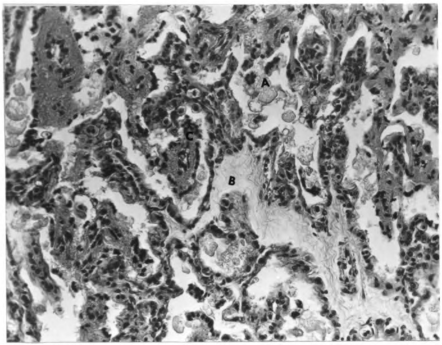

Fig. 15.--Cotyledon of 72-hour ewe. A. Vacuolization of maternal crypts. B. Edema in fetal villus. C. Pyknotic fetal and maternal cells. x225.

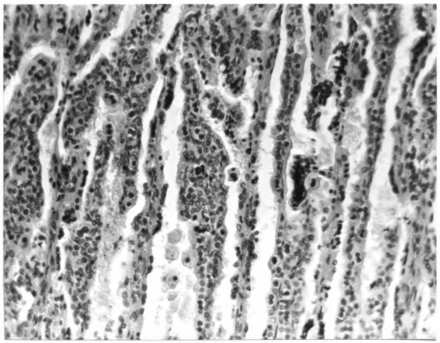

Fig. 16.--Cotyledon of ewe with serum antibody titer of 10^2 autopsied at 132 hours. Note relatively normal appearance of maternal-fetal relationship. x225.

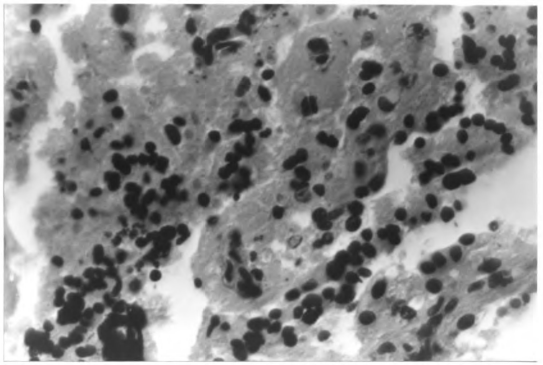

Fig. 17.--Cotyledon of 96-hour ewe showing maternal and fetal necrosis. Note pyknotic appearance of nuclei. x600.

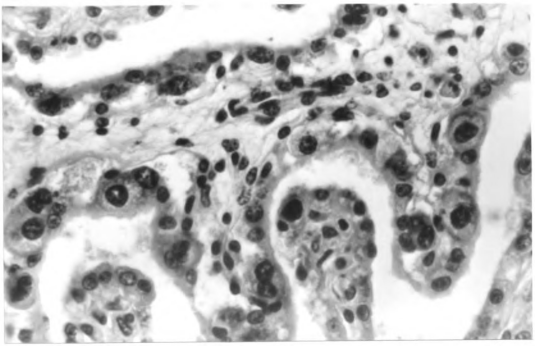

Fig. 18.--Cotyledon from cow 1 which died 32 hours after receiving L. pomona hemolysin. Fetal-maternal relationship intact. x600.

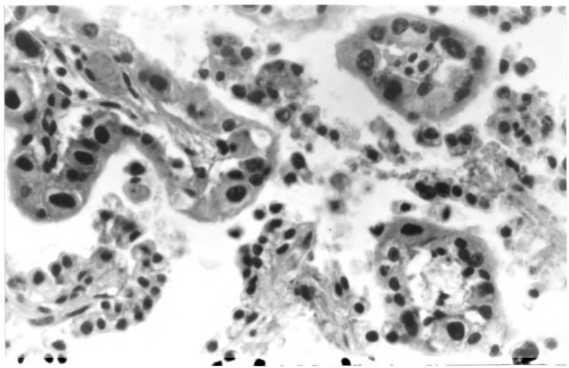

Fig. 19.--Cotyledon from cow 12 which died 44 hours after receiving hemolysin demonstrating necrosis of maternal-crypt epithelium and lack of normal maternal-fetal relationship. x600.

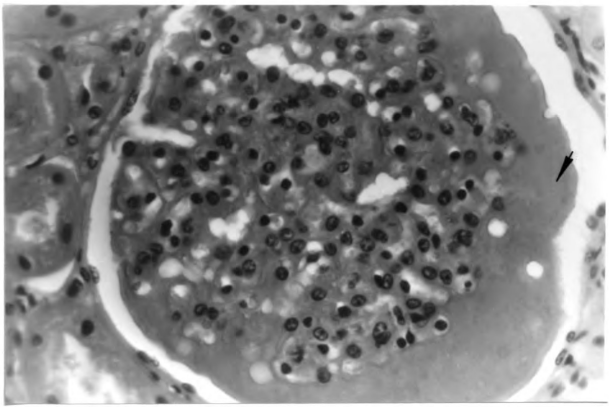

Fig. 20.--Kidney of cow 1 showing hemolyzed blood in subcapsular space of renal corpuscle. x600.

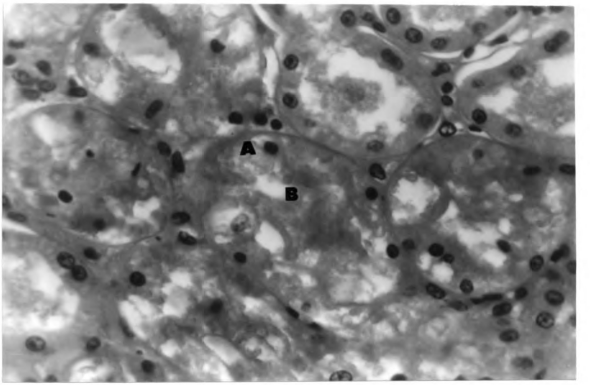

Fig. 21.--Kidney of cow 12. A. Degeneration and necrosis of tubular cells. B. Blood in lumen of tubules. x600.

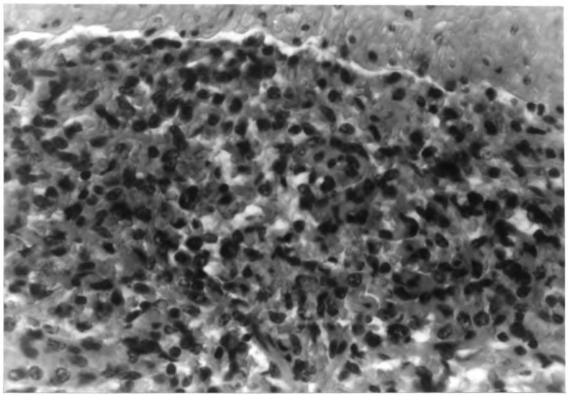

Fig. 22.--Spleen of control cow. x600.

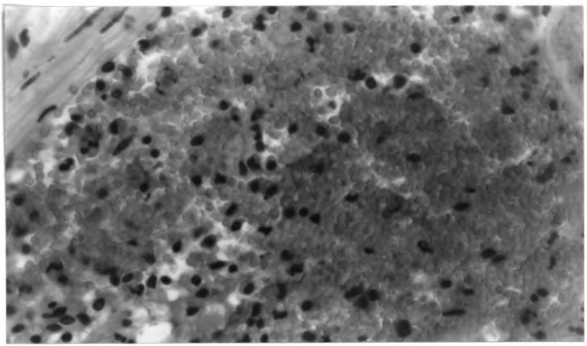

Fig. 23.--Spleen from cow 1 showing congestion in the sub-capsular area. x600.

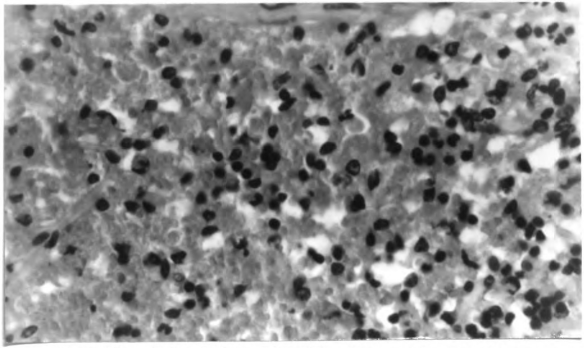

Fig. 24.--Spleen from cow 12. Note sub-capsular accumulations of erythrocytes. x600.

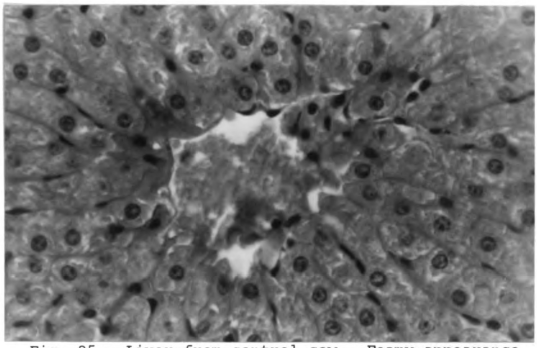

Fig. 25.--Liver from control cow. Foamy appearance of hepatic cells due to glycogen. x600.

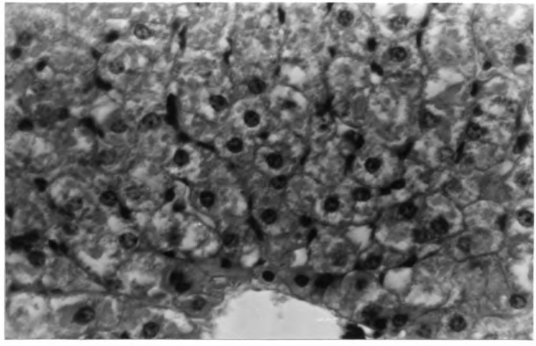

Fig. 26.--Liver from cow 12 demonstrating mild vacuolization due to fatty change. x600.

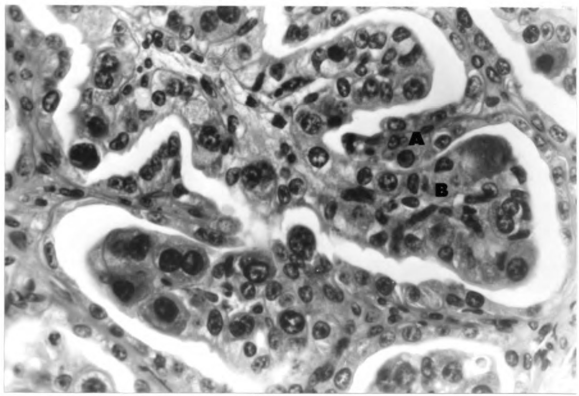

Fig. 27.--Cotyledon from strain J injected cow. A. Maternal crypt. B. Fetal villus. x600.

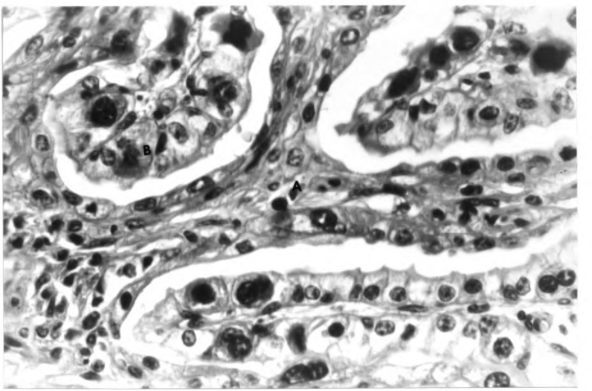

Fig. 28.--Cotyledon of uninjected cow. A. Maternal crypt. B. Fetal villus. x600.

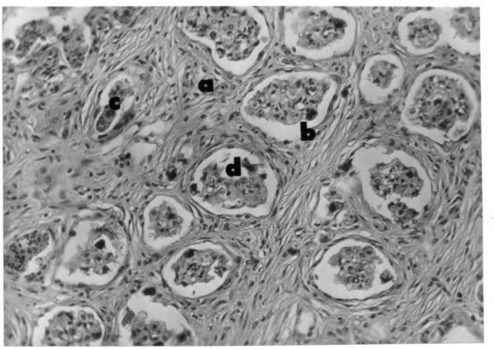

Fig. 29.--Cotyledon of cow 3 which aborted 8 days after receiving hemolysin. A. Connective tissue proliferation. B. Atrophy and absence of maternal-crypt epithelium. C. Necrotic villus. D. Villus with relatively normal appearing cells. x225.

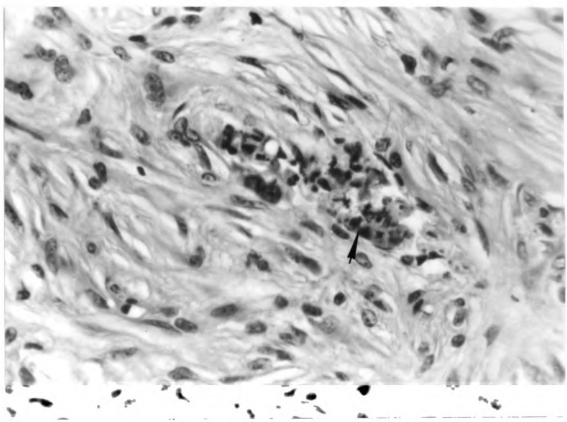

Fig. 30.--Cotyledon of cow 3 showing connective tissue replacing necrotic villus. x600.

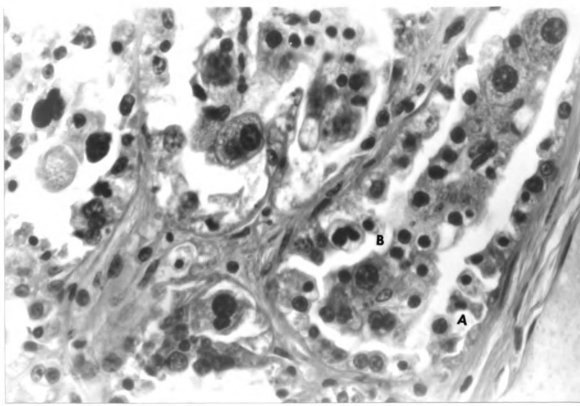

Fig. 31.--Cotyledon of cow 2 on day 9. A. Discontinuity and pyknosis of maternal-crypt epithelium. B. Pyknotic cells in fetal villus. x600.

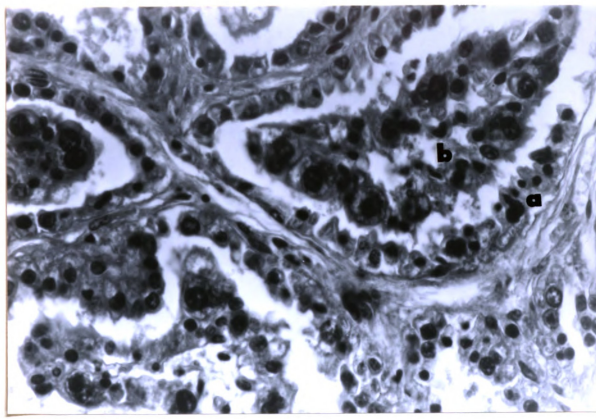

Fig. 32.--Cotyledon of cow 5. A. Pyknotic maternal cells. B. Pyknotic fetal cells. x600.

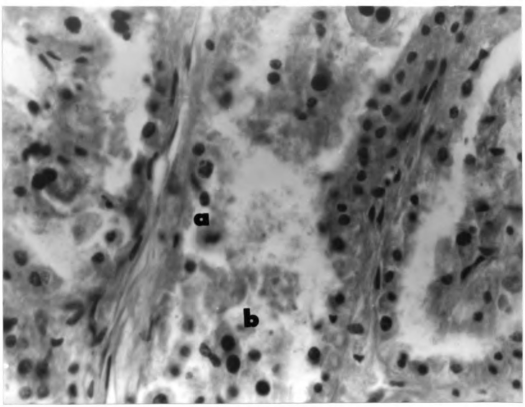

Fig. 33.--Cotyledon of cow 16 killed on day 16. A. Disruption of maternal epithelium. B. Necrosis of fetal villi. x600.

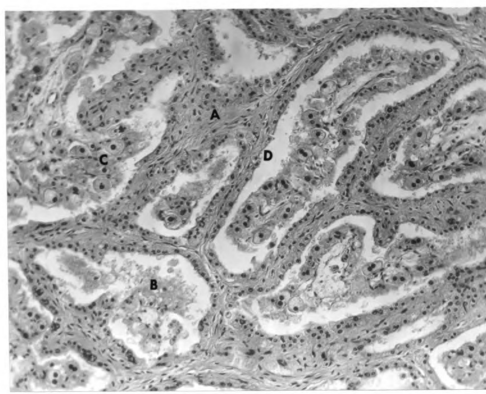

Fig. 34.--Cotyledon of cow 16. A. Connective tissue proliferation. B. Vacuolization in fetal villus. C. Pyknotic trophoblasts. D. Atrophy of maternal epithelium. x225.

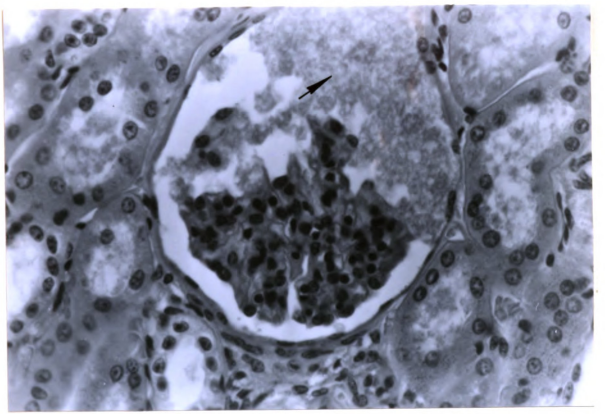

Fig. 35.--Kidney of cow 16 showing proteinaceous material in renal corpuscle. x600.

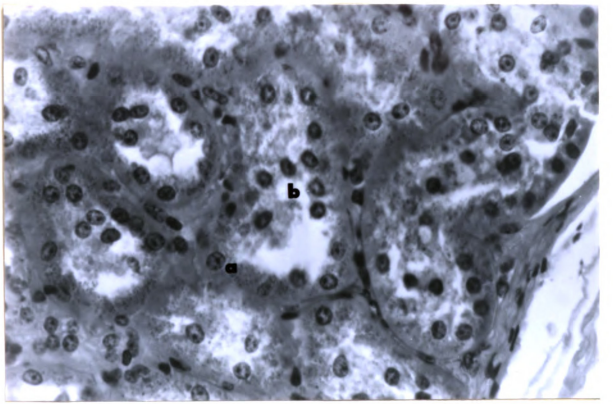

Fig. 36.--Kidney of cow 5. A. Blood pigment phagocytized by cells of proximal convoluted tubules. B. Tubular cell disruption. x600.

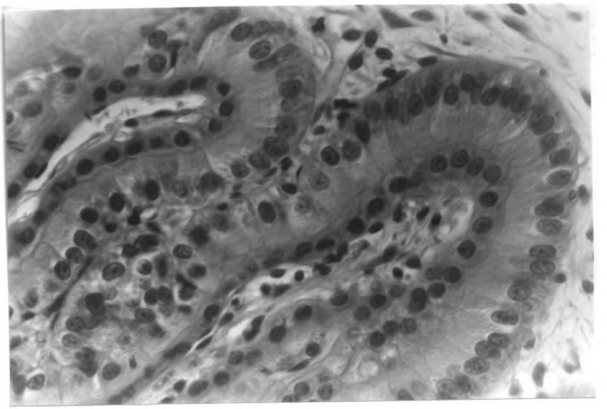

Fig. 37.--Porcine uterine section from sow given hemolysin demonstrating normal appearance. x600.

REFERENCES

Alexander, A. D. 1960. Personal communication.

Alexander, A. D., Smith, O. H., Hiatt, C. W., and Gleiser, C. A. 1956. Presence of hemolysin in cultures of pathogenic leptospires. Proc. Soc. Exptl. Biol. and Med., 91:205-211.

Babudieri, B. 1958. Animal reservoirs of leptospires. Annals of the New York Academy of Science, 70:393-413.

Baker, J. A. and Little, R. B. 1948. Leptospirosis in cattle. Jour. Exp. Med., 88:295-307.

Bauer, D. C. and Morse, E. V. 1958. Variation and hemolysin production in relation to virulence of Leptospira pomona. Proc. Soc. Exp. Biol. and Med., 98:505-508.

Bauer, D. C., Eames, L. N., Sleight, S. D., and Ferguson, L. C. 1961. The significance of leptospiral hemolysin in the pathogenesis of Leptospira pomona infections. J. Inf. Dis., 108:229-236.

Beamer, P. D., Hardenbrook, H. and Morrill, C. C. 1953. Studies on leptospirosis in domestic animals. I. Leptospirosis in sheep. Vet. Med., 48:365-366.

Bertók, L., and Kemenes, F. 1960. Studies on the lipase-system of leptospirae. I. Tributyrinase activity. Acta Microbiologica, 7:251-259.

Björkman, N. 1954. Morphological and histochemical studies on the bovine placenta. Acta Anatomica, Suppl. 22-2 ad Vol. 22:1-91.

Bohl, E. H., Powers, T. E., and Ferguson, L. C. 1954a. Abortion in swine associated with leptospirosis. J.A.V.M.A., 124:262-268.

Bohl, E. H., Powers, T. E. and Ferguson, L. C. 1954b. Leptospirosis in swine. Proceedings, Fifty-eighth Annual Meeting of the U. S. Livestock Sanitary Association, 48:365-367.

Bokori, J., Kemenes, F., Szemerede, G., and Szeky, A. 1960. Ovine leptospirosis caused by Leptospira pomona in Hungary. Acta Veterinaria, 10:101-109.

Bridges, C. H. 1958. Bovine fetal leptospirosis. Southwestern Veterinarian, 11:271-274.

Bryan, H. S. 1955. Leptospirosis in cattle and swine. Circular 750, University of Illinois.

Bryan, H. S., Rhoades, H. E. and Willigan, D. A. 1953. Studies on leptospirosis in domestic animals: II. Isolation of Leptospira pomona from aborted swine fetuses. Vet. Med., 48:438-439.

Burnstein, T. and Baker, J. A. 1954. Leptospirosis in swine caused by Leptospira pomona. J. Inf. Dis., 94: 53-64.

Coffin, D. L. 1953. Manual of veterinary clinical pathology: Comstock Publishing Co., Inc.

Cordy, D. R. and Jasper, D. E. 1952. The pathology of an acute hemolytic anemia of cattle in California associated with leptospira. J.A.V.M.A., 120:175-178.

Dacres, W. G. and Kiesel, G. K. 1958. Isolation of Leptospira pomona from a bovine fetus. J.A.V.M.A., 132:525-526.

De Lay, P. D., Daley, R. and Callahan, C. 1955. Leptospirosis in relation to porcine abortion. Bull. Dept. of Agr., Calif., 44:135.

Dozsa, I., Kemenes, F. and Szent-Ivanyi, T. 1960. Susceptibility of red blood cells of ruminants with a 4-chambered stomach (Pecora) to the haemotoxin of pathogenic leptospiras. Acta Veterinaria, 10:35-44.

Fennestad, K. L. and Borg-Petersen, C. 1956a. Studies on bovine leptospirosis and abortion. II, Experimental leptospirosis in pregnant heifers. Nord. Vet. Med., 8:815-833.

Fennestad, K. L. and Borg-Petersen, C. 1956b. Studies on bovine leptospirosis and abortion. III. Attempts to demonstrate leptospira in cotyledons from aborting cattle. Nord. Vet. Med., 8:882-886.

Fennestad, K. L. and Borg-Petersen, C. 1957. Leptospira antibody production by bovine fetuses. Nature, 180: 1210-1211.

Fennestad, K. L. and Borg-Petersen, C. 1958a. Studies on bovine leptospirosis and abortion. IV. Demonstration of leptospira in foetuses from field cases of abortion in cattle. Nord. Vet. Med., 10:302-308.

Fennestad, K. L. and Borg-Petersen, C. 1958b. Fetal leptospirosis and abortion in cattle. J. Inf. Dis., 102: 227-236.

Fennestad, K. L. and Borg-Petersen, C. 1960. Pathogenesis of leptospiral bovine abortion. Symposium of leptospirae and leptospirosis in man and animals, Lublin, 5-8 December, 1958. "Ossolineum," Warsaw, 1960.

Ferguson, L. C. and Powers, T. E. 1956. Experimental leptospirosis in pregnant swine. Am. J. Vet. Res., 17: 471-477.

Ferguson, L. C., Ramge, J. C. and Sanger, V. L. 1957. Experimental bovine leptospirosis. Am. J. Vet. Res., 18:43-49.

Field, H. I. and Sellers, K. C. 1950. Leptospira ictero-haemorrhagiae infection in the calf. Vet. Rec., 62: 311-312.

Field, H. I. and Sellers, K. C. 1951. Leptospira ictero-haemorrhagiae infection in piglets. Vet. Rec., 63: 78-81.

Gambrel, P. T., Gambrel, E. T. and Gambrel, L. E. 1956. An outbreak of porcine leptospirosis. No. Am. Vet., 37: 19-21.

Gochenour, W. S., Jr., Johnston, R. V., Yager, R. H. and Gochenour, W. S. 1952. Porcine leptospirosis. Am. J. Vet. Res., 13:158-160.

Gochenour, W. S., Jr. and Yager, R. H. 1953. Manifestations of bovine leptospirosis. Vet. Med., 48:218-221.

Hartley, W. J. 1952. Ovine leptospirosis. Australian Vet. J., 28:169-170.

Jungherr, E. 1944. Bovine leptospirosis. J.A.V.M.A., 105:276-281.

Kemenes, F. 1958. Die durch pathogene leptospiren verursachte hämolyse and ihre pathologische bedeutung. Acta Veterinaria, 8:143-150.

Kemenes, F. and Lovrekovich, F. 1959. Uber den fettabbau durch pathogene leptospiren. Acta Veterinaria, 9:235-241.

Langham. R. F., Morse, E. V. and Morter, R. L. 1958. Experimental leptospirosis. V. Pathology of Leptospira pomona infection in swine. Am. J. Vet. Res., 19:388-394.

Lindqvist, K. J., Morse, E. V. and Lundberg, A.M. 1958. Experimental Leptospira pomona infection in pregnant ewes. Cornell Vet., 48:277-290.

Low, D. G. and Mather, G. W. 1960. Canine leptospirosis. Allied Vet., 31:76-78.

Lundberg, A. M. 1960. The effects of ante partum Leptospira pomona infection on bovine fetal and maternal blood values. M. S. Thesis, Michigan State University.

Marsh, H. 1945. Leptospira in bovine icterohemoglobinuria. J.A.V.M.A., 107:119-121.

Mathews, F. P. 1946. A contagious disease of cattle associated with Leptospira. Am. J. Vet. Res., 7:78-80.

Michin, N. A. and Azinov, S. A. 1935. Spirochaetal jaundice of cattle in North Causasus. Sovyet. Vet., 10:23. Abst. 1937. Vet. Bul., 7:419.

Morse, E. V. 1955. The economic significance of leptospirosis in domestic animals. Proceedings, Ninety-second Annual Meeting of the American Veterinary Medical Association, Minneapolis, August 15-18.

Morse, E. V., Allen, V., Krohn, A. F. and Hall, R. 1955a. Leptospirosis in Wisconsin. I. Epizootiology and clinical features. J.A.V.M.A., 127:417-421.

Morse, E. V., Allen, V., Krohn, A. F. and Hall. R. 1955b. Leptospirosis in Wisconsin. II. Serological studies. J.A.V.M.A., 127:424-426.

Morse, E. V. and McNutt, S. H. 1956. Experimental leptospirosis. I. The course of Leptospira pomona infection in pregnant heifers. J.A.V.M.A., 128:225-229.

Morse, E. V., Bauer, D. C., Langham, R. F., Lang, R. W. and Ullrey, D. E. 1958. Experimental leptospirosis. IV. Pathogenesis of porcine Leptospira pomona infections. Am. J. Vet. Res., 19:388-394.

Morse, E. V. and Langham, R. F. 1958. Experimental leptospirosis. III. Caprine Leptospira pomona infections. Am. J. Vet. Res., 19:139-144.

Morse. E. V., Morter, R. L., Langham, R. F., Lundberg, A. M. and Ullrey, D. E. 1957. Experimental ovine leptospirosis, Leptospira pomona infection. J. Inf. Dis., 101:129-136.

Morter, R. L. 1960. The pathogenesis of leptospiral infections Leptospira pomona during pregnancy in cattle and swine. Ph. D. Thesis, Michigan State University.

Morter, R. L., Langham, R. F. and Morse, E. V. 1958. Experimental leptospirosis. VI. Histopathology of the bovine placenta in Leptospira pomona infections. Am. J. Vet. Res., 19:785-791.

Paltrinieri, S., Farina, R., Salutini, E., Sessi, P., Buonaccorsi, P. and Pierotti, P. 1959. Leptospirosi delle pecore infezione sperimentale da L. icterohaemorrhagiae. Annali della Facoltá di Medicina Veterinaria, 12:1-47.

Pentek, Juhász, M. 1960. Untersuchungen über die hamolytische eigenschaft von Leptospira biflexa stammen. Acta Veterinaria, 10:233-237.

Podgewaite, G. D., Tourtellotte, M. E., Jacobs, R. E., Helmboldt, C. F., Easterbrooks, H. L., Williams, L. F., Jungherr, E. L. and Plastridge, W. N. 1955. Isolation of Leptospira pomona from three aborted fetuses. Vet. Med., 50:164-165.

Powers, T. E., Bohl, E. H., and Ferguson, L. C. 1956. Clinical studies on leptospirosis as a cause of abortion in swine. J.A.V.M.A., 129:568-572.

Preston, K. S. and Morter, R. L. 1960. Rapid laboratory confirmation of a clinical diagnosis of Leptospira pomona infection in pregnant sows. Allied Vet., 31:104-106.

Reinhard, K. R. 1951. A clinical pathological study of experimental leptospirosis in calves. Am. J. of Vet. Res., 12:282-291.

Reinhard, K. R. 1955. Michigan State University Centannial Symposium on Reproduction and Infertility. 12-19.

Reinhard, K. R. and Hadlow, W. J. 1954. Experimental bovine leptospirosis: pathological, hematological, bacteriological and serological studies. J.A.V.M.A. Proc. Ninety-first Annual Meeting. 203-216.

Reynolds, I. M., Sakai, T., and Smith, R. E. 1960. Experimental leptospirosis in pregnant ewes. II. Hematological features of ewes and fetuses. Cornell Vet., 50:89-114.

Ringen, L. M., Bracken, F. K., Kenzy, S. G. and Gillespie, R. H. W. 1955. Studies on bovine leptospirosis. I. Some effects of dihydrostreptomycin and terramycin on the carrier condition in bovine leptospirosis. J.A.V.M.A., 126:272-276.

Ristic. M., Galton, M. M., McRae, L., Sanders, D. A. and Steele, J. H. 1957. Experimental leptospirosis in bovines. I. Establishment of infection with Leptospira sejroe. J. Inf. Dis., 100:228-240.

Rogols, S., Fizette, W. B. and Bohl, E. H. 1959. Studies on the relationships of a phospholipase to the hemolysin of Leptospira pomona. Am. J. Vet. Res., 20:592-596.

Russell, C. M. 1954. Hemolytic factor associated with Leptospira pomona. Fed. Proc., 13:510.

Russell, C. M. 1956. A "hemolysin" associated with leptospirae. J. Immunology, 77:405-409.

Sippel, W. L. 1954. Porcine leptospirosis. Iowa Vet., 25:18-34.

Sippel, W. L., Boyer, C. I. and Chambers, E. E. 1952. Bovine leptospirosis in Georgia. J.A.V.M.A., 120:278-282.

Sleight, S. D. 1961. Unpublished data.

Sleight, S. D. and Lundberg, A. M. 1961. The persistence of Leptospira pomona in various porcine tissues. J.A.V.M.A. In press.

Sleight, S. D., Langham, R. F. and Morter, R. L. 1960. Experimental leptospirosis: The early pathogenesis of Leptospira pomona infection in young swine. J. Inf. Dis., 106:262-269.

Smith, R. E., Reynolds, I. M. and Sakai, T. 1960a. Experimental leptospirosis in pregnant ewes. I. Clinical bacteriological and serological features. Cornell Vet., 50:34-46.

Stewart, J. R. 1934. "The right way": practical veterinary treatment of cattle, horses and pigs. Biological Institute of Australia, Sydney. Cited by Sutherland, A. K., Simmons, G. C. and Kenny, G. C. 1949. Bovine leptospirosis: three outbreaks in calves in Queensland. Austr. Vet. J., 25: 197-200.

Stoenner, H. G. 1954. The management of herds affected by bovine leptospirosis. J.A.V.M.A. Proc. Ninety-first Annual Meeting. 83-87.

Sutherland, A. K. and Morrill, C. C. 1943. An outbreak of leptospirosis in cattle. J.A.V.M.A., 113:468-471.

Sutherland, A. K., Simmons, G. C. and Kenny, G. C. 1949. Bovine leptospirosis: three outbreaks in calves in Queensland. Austr. Vet. J., 25:197-200.

Te Punga, A. W. and Bishop, W. S. 1953. Bovine abortion caused by infection with Leptospira pomona. New Zealand Vet. J., 1:143-149.

U.S. Department of Agriculture. 1954. Losses in agriculture. A preliminary appraisal for review. A.R.S., U.S.D.A., 127-159.

Van der Hoeden, J. 1958. Leptospires et leptospiroses animales en Israel. Bulletin de l'Office International des Epizooties, 50:274-283.

Van Riel, J. 1959. Les hémolysines leptospirennes. Annals de la Societe Belge de Médicine Tropicale, 39:331-342.

Wimsatt, W. A. 1950. New histological observations on the placenta of the sheep. Am. J. of Anat., 87:391-457.