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PATHOLOGY AND BACTERIOLOGY OF ABORTION AND PERINATAL  
DEATH OF YOUNG IN RABBITS, SHEEP AND GOATS  
INDUCED BY LISTERIA MONOCYTOGENES

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## TABLE OF CONTENTS

|                                                                         | PAGE |
|-------------------------------------------------------------------------|------|
| I. INTRODUCTION                                                         | 1    |
| II. HISTORICAL BACKGROUND                                               | 3    |
| III. REVIEW OF LITERATURE                                               | 9    |
| IV. EXPERIMENTS                                                         | 22   |
| A. Rabbit Experiments                                                   | 22   |
| 1. Conjunctival Exposure                                                | 22   |
| a. Materials and Methods                                                | 22   |
| b. Results: Bacteriology                                                | 25   |
| c. Results: Pathology                                                   | 30   |
| 2. Oral Exposure                                                        | 52   |
| a. Materials and Methods                                                | 52   |
| b. Results: Bacteriology                                                | 55   |
| c. Results: Pathology                                                   | 56   |
| 3. Conjunctival Exposure of Rabbits Surviving<br>Previous Oral Exposure | 78   |
| a. Materials and Methods                                                | 78   |
| b. Results: Bacteriology                                                | 78   |
| c. Results: Pathology                                                   | 80   |
| 4. Oral Exposure of Rabbits Surviving<br>Previous Conjunctival Exposure | 80   |
| a. Materials and Methods                                                | 80   |
| b. Results: Bacteriology                                                | 81   |
| c. Results: Pathology                                                   | 82   |
| 5. Rebreeding of Infected Rabbits Without<br>Any Further Exposure       | 84   |
| a. Materials and Methods                                                | 84   |
| b. Results: Bacteriology                                                | 84   |
| c. Results: Pathology                                                   | 85   |
| B. Sheep Experiments                                                    | 90   |
| 1. Oral Exposure                                                        | 90   |
| a. Materials and Methods                                                | 90   |
| b. Results: Bacteriology                                                | 90   |
| c. Results: Pathology                                                   | 91   |

2

11

## TABLE OF CONTENTS (Cont.)

|                          | Page |
|--------------------------|------|
| C. Goat Experiments      | 97   |
| 1. Conjunctival Exposure | 97   |
| a. Materials and Methods | 97   |
| b. Results: Bacteriology | 97   |
| c. Results: Pathology    | 99   |
| 2. Oral Exposure         | 103  |
| a. Materials and Methods | 103  |
| b. Results: Bacteriology | 103  |
| c. Results: Pathology    | 104  |
| V. DISCUSSION            | 112  |
| VI. CONCLUSIONS          | 123  |
| VII. SUMMARY             | 127  |
| BIBLIOGRAPHY             | 132  |

N

11

# LIST OF TABLES

| TABLE                                                                                                                                    | PAGE |
|------------------------------------------------------------------------------------------------------------------------------------------|------|
| I. Reports of Isolations of <u>L. monocytogenes</u><br>from Abortions in Cattle . . . . .                                                | 10   |
| II. Reports of Isolations of <u>L. monocytogenes</u><br>from Abortions in Sheep and Goats . . . . .                                      | 11   |
| III. Reports of Isolations of <u>L. monocytogenes</u><br>from Perinatal Death of Young Animals (Calves,<br>Lambs, Foals, Pigs) . . . . . | 12   |
| IV. Reports of Isolations of <u>L. monocytogenes</u> from<br>Perinatal Death (Human Infants) . . . . .                                   | 13   |
| V. Isolations of <u>L. monocytogenes</u> from Aborted<br>Fetuses Following Conjunctival Exposure of<br>Rabbits . . . . .                 | 26   |
| VI. Isolations of <u>L. monocytogenes</u> from Rabbits<br>(2-3 Weeks Pregnant) Following Conjunctival<br>Exposure . . . . .              | 27   |
| VII. Isolations of <u>L. monocytogenes</u> from Perinatal<br>Death of Young Following Conjunctival Exposure<br>of Rabbits . . . . .      | 28   |
| VIII. Isolations of <u>L. monocytogenes</u> from Non-Pregnant<br>Rabbits Following Conjunctival Exposure . . . . .                       | 29   |
| IX. Isolations of <u>L. monocytogenes</u> from Aborted<br>Fetuses Following Oral Exposure of Rabbits . . . . .                           | 57   |

# LIST OF TABLES (Cont.)

| TABLE                                                                                                                                                                | Page |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| X. Isolations of <u>L. monocytogenes</u> from Rabbits<br>(2-3 Weeks Pregnant) Following Oral Exposure .                                                              | 58   |
| XI. Isolations of <u>L. monocytogenes</u> from Perinatal<br>Death of Young Following Oral Exposure of<br>Rabbits . . . . .                                           | 59   |
| XII. Isolations of <u>L. monocytogenes</u> from Young Rabbits<br>(Perinatal Death) Following Conjunctival Exposure<br>of the Dam Subsequent to Previous Exposure . . | 79   |

2

11



## I. INTRODUCTION

The isolations of Listeria monocytogenes from various clinical syndromes (meningo-encephalitis, septicemia, keratoconjunctivitis, abortions and perinatal death of young) in 30 species of animals including man have been reported from 31 countries all over the world. Attention has been drawn particularly to recent epidemics of perinatal death (still-birth and early death) of infants due to L. monocytogenes in both sectors of Germany, where listeriosis has now been designated a reportable disease.

In addition to infection in the reproductive system of the human female, abortions and perinatal death of young caused by L. monocytogenes have been reported in cattle, sheep, goats, swine, rabbits and horses.

This investigation was designed to study the role of L. monocytogenes in abortions and perinatal death of young in animals and with the hope that it might shed some light on the pathogenesis of the condition in man. The experiments were conducted primarily on rabbits, sheep and goats and two routes of exposures (conjunctival and oral) were used. Conjunctival exposure was chosen from academic interest to investigate the role of L. monocytogenes in the so-called local eye reaction and the possibility of its dissemination



to other parts of the body following conjunctival instillation. Oral exposure was conducted because it is one of the most common avenues of bacterial infection and thus might be the route used by L. monocytogenes in causing the clinical syndromes of abortion and perinatal death of young in both animals and man.

While attempts were made to study infection by L. monocytogenes through these portals of entry, it was also felt that an attempt should be made by histopathologic studies to elucidate the pathogenesis of this condition.

Therefore bacteriology and pathology were employed as companion studies in the experiments reported here.

2

11

## II. HISTORICAL BACKGROUND

In May of 1924, Murray, Webb and Swan noticed six cases of sudden death in young rabbits in the animal breeding establishment of the Department of Pathology at Cambridge University. Many more deaths followed in the subsequent 15 months. They isolated a small Gram-positive motile rod which reproduced the disease characteristically in rabbits, guinea pigs, and mice. The most striking character of this disease was found to be the production of a marked monocytosis and, as no reports of a similar microorganism could be found, these authors called the microbe Bacterium monocytogenes in 1926.

Working independently, Pirie discovered the same bacterium in four wild gerbilles (Tatera lobengulae) in South Africa. Because of the marked necrosis of the liver produced in these animals, he called the bacterium Listerella hepatolytica in 1927 in honor of Lord Lister, the well-known pioneer of modern aseptic surgery. As the generic name Listerella had already been used for a mycetozoon (a group of slime molds), and as the generic name Bacterium, used by Murray et al. (1926) was not suitable because this microorganism did not possess the characteristics of the genus Bacterium, the International Rules of Nomenclature invalidated

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the generic names given to this microbe. In 1940, Pirie proposed the generic name Listeria which was adopted in the sixth edition of Bergey's Manual of Determinative Bacteriology (1948). This Manual describes L. monocytogenes as: small rods, 0.4 to 0.5 by 0.5 to 2.0 microns with rounded ends, slightly curved in some culture media; occur singly or in V-shaped or parallel pairs and in short chains; motile, peritrichous, with four flagella at ordinary temperature, with tendency toward non-motility or single flagellum at 37° C; not acid-fast; Gram-positive.

The bacterium also is urea-, indol-, V.P.- (Voges-Proskauer test), M.R.- (Methyl red test), nitrite-, citrate-, H<sub>2</sub>S-, and gelatin-negative. It is catalase-positive; non-sporing; non-capsulated; facultative anaerobic. It grows well on tryptose agar with dew-drop-like colonies which appear small, circular, slightly raised, finely textured, translucent, and blue-green in color when examined by means of obliquely transmitted light and a binocular scanning microscope. (Fig. 1)

Murray (1953) reported that almost exactly twenty years after the publication of his original description of this microorganism in 1926, he found a paper published in Sweden by Hulphér in 1911. In this paper, Hulphér described a microorganism (which was undoubtedly L. monocytogenes) and, struck

2

11



by the liver necrosis associated with it, he named it Bacillus hepatis.

The first isolation of L. monocytogenes from a human case of infectious mononucleosis was reported by Nyfeldt (1929). Girard and Murray (1951) reviewed the literature and discussed the relationship of L. monocytogenes to infectious mononucleosis in humans. Burn (1936) was the first to report the isolation of this bacterium from three cases of meningitis in infants under one year of age. Since then many cases of meningo-encephalitis associated with L. monocytogenes in humans have been reported in the literature. Those reported until 1952 were reviewed by Finegold et al. (1954).

The first report of the isolation of L. monocytogenes from sheep was published by Gill (1931) from an outbreak of so-called "circling disease" in New Zealand. He isolated the bacterium from the brain of the infected sheep and established its relationship to the disease. Gray (1954) cited that Pletnov and Stiksove (1950) claim the isolation of L. monocytogenes from pigs in Russia as early as in 1924.

Graham et al. (1939) were the first to report the isolation of L. monocytogenes from an aborted bovine fetus. Since then, abortions in other species of domestic animals, sheep, goat, swine, horse and perinatal death of calves, lambs, foals and pigs have been reported from all over the world (Tables I, II and III).

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11

Graham, Levine and Morrill (1943) published a bulletin "Listerellosis in Domestic Animals" reviewing previously published cases in animals and presenting the results of their field and laboratory investigations.

Gray (1954) reviewed the literature pertaining to various forms of listeric infections in animals and man. He presented the isolations of L. monocytogenes from various clinical syndromes in several species of animals and man in tabular form as a readily available source of bibliographic material. He also reported at least 20 different rough colonial variants which develop in smooth cultures maintained on artificial media. (Fig. 2)

Reiss, Potel and Krebs (1951) described a disease in human infants which they called "granulomatosis infant-iseptica". From the visceral organs they isolated a small Gram-positive rod which they called Corynebacterium infantiseptica. (Potel, 1952). Seeliger (1952) suggested that this bacterium was actually L. monocytogenes. This subsequently has been confirmed by a number of investigators. It is now generally conceded that this disease is identical to that which was formerly known as "pseudo-tuberculosis" of the newborn. This disease appears to be most prevalent in Central Europe particularly in Eastern Germany and Czechoslovakia. Recently a similar condition has been reported in Canada and in the United States. (Table IV).

Seeliger (1955), surveying the literature, reported the following recorded cases of human listeric infections:

|                                      |     |
|--------------------------------------|-----|
| Probable cases since 1891 . . . . .  | 56  |
| Cases reported between 1918-1939 . . | 29  |
| Cases reported between 1940-1949 . . | 40  |
| Cases reported since 1950..more than | 221 |

He also summarized the cases according to the reported isolation of L. monocytogenes from various clinical syndromes as follows:

|                                         |           |
|-----------------------------------------|-----------|
| Meningo-encephalitis . . . . .          | 85 cases  |
| Infectious mononucleosis . . . . .      | 41 cases  |
| Conjunctivitis . . . . .                | 13 cases  |
| Septic granulomatosis                   |           |
| Adults . . . . .                        | 4 cases   |
| Newborn . . . . .                       | 121 cases |
| Other manifestations . . . . .          | 26 cases  |
| (urethritis, endocarditis, pneumonitis) |           |

The most recent and comprehensive review of literature pertaining to the isolation of L. monocytogenes from various manifestations of disease in man and animals and other related studies of the bacterium has been published in book form under the title "Listeriose" by Seeliger (1955). This book also includes the reviews and discussions on diagnosis, differential diagnosis, pathogenesis, transmission, public

health relationship, prophylaxis, treatment, and other phases of the disease.

Murray (1955) stated that L. monocytogenes has been isolated and identified in a minimum of 27 species of animals (rabbit, hare, guinea pig, gerbille, lemming, mouse, rat, hamster, chinchilla, vole, sheep, goat, cow, pig, horse, fox, dog, ferret, raccoon, chicken, canary, duck, goose, eagle, capercailzie, unspecified birds, and man). To this list should be added mink (Stenberg, 1955), Skunk (Bolin, et al., 1955), and owl (Reed, 1955).

Murray (1955) also stated that the distribution of listeriosis in various forms has been reported from 26 countries in five continents, ranging from the Arctic to the tropics. To this list should be added Azerbaijan (Khalimbekov, 1952), Belgium (Geurden and Devos, 1954), Ceylon (Bandarnayake, 1953), Czechoslovakia (Pouska, 1950), and Puerto Rico (Felsenfeld, 1948). Thus the list comprises at least the following 31 countries:

- |                    |                 |                   |
|--------------------|-----------------|-------------------|
| 1. Argentina       | 12. England     | 22. Norway        |
| 2. Australia       | 13. Finland     | 23. Palestine     |
| 3. Austria         | 14. France      | 24. Poland        |
| 4. Azerbaijan      | 15. Germany     | 25. Puerto Rico   |
| 5. Belgium         | 16. Holland     | 26. Russia        |
| 6. Brazil          | 17. Hungary     | 27. Scotland      |
| 7. Canada          | 18. India       | 28. South Africa  |
| 8. Ceylon          | 19. Italy       | 29. Sweden        |
| 9. Cuba            | 20. Japan       | 30. United States |
| 10. Czechoslovakia | 21. New Zealand | 31. Uruguay       |
| 11. Denmark        |                 |                   |

### III. REVIEW OF LITERATURE

The whole scope of infections due to L. monocytogenes has been reviewed adequately by a number of workers as mentioned in the historical background. The reports reviewed here are mainly concerning problems of this study.

In general, the encephalitic form of listeric infection is more commonly reported in mature large animals, particularly ruminants, and man. The septicemic form is more frequently found in rodents, chickens and in aborted fetuses, stillborn, premature, or newborn young animals and man. However, there is a strong suspicion that atypical or subclinical infections may occur both in animals and man.

Gray (1954) in a review tabulated the known isolations of L. monocytogenes. This included all forms of the disease in all species. He reported that symptoms of the encephalitic form of listeric infection in sheep, goats and cattle are similar but differ in severity. The following symptoms are common: nasal discharge, inappetence, fever, leaning or pushing against an object, "circling", general depression or stupor, incoordination, torticollis, difficulty in swallowing due to paralysis of facial and throat muscles, uncontrolled running motions, complete prostration and death.

Gray (1954) reported that listeric abortions in cattle usually occur between the 4th and 7th months of gestation and in sheep at or near term. Isolations of L. monocytogenes can be made from the liver, blood, fetal membranes and especially from the abomasal contents of the fetus. Positive diagnosis depends upon such isolation. The following tables are based upon the review of Gray (1954) with subsequent additions of new reports as they appeared in the literature.

TABLE I  
REPORTS OF ISOLATIONS OF L. MONOCYTOGENES  
FROM ABORTIONS IN CATTLE

| Author                    | Year | Country     |
|---------------------------|------|-------------|
| Graham <u>et al.</u>      | 1939 | U.S.A.      |
| Evans and Sawyer          | 1942 | U.S.A.      |
| Wramby                    | 1942 | Sweden      |
| Olson                     | 1945 | Sweden      |
| Ferguson <u>et al.</u>    | 1951 | U.S.A.      |
| Van Dorssen and Jansen    | 1951 | Netherlands |
| Levi <u>et al.</u>        | 1952 | Israel      |
| Van Ulsen                 | 1952 | Netherlands |
| Eveleth <u>et al.</u>     | 1953 | U.S.A.      |
| Amanzhulov                | 1953 | Russia      |
| Stockton <u>et al.</u>    | 1954 | Russia      |
| Gray <u>et al.</u>        | 1954 | U.S.A.      |
| De Lay and Shultz         | 1954 | U.S.A.      |
| Rowsell (unpublished)     | 1955 | Canada      |
| v.d. Schaaf (unpublished) | 1955 | Netherlands |
| Smith <u>et al.</u>       | 1955 | U.S.A.      |

TABLE II  
REPORTS OF ISOLATIONS OF L. MONOCYTOGENES  
FROM ABORTIONS IN SHEEP

| Author                                                            | Year | Country   |
|-------------------------------------------------------------------|------|-----------|
| Paterson                                                          | 1940 | England   |
| Poppensiek                                                        | 1944 | U.S.A.    |
| Olson                                                             | 1945 | Sweden    |
| Charles                                                           | 1950 | Australia |
| Eveleth <u>et al.</u>                                             | 1953 | U.S.A.    |
| Amanzhulov                                                        | 1953 | Russia    |
| Paulsen and Moule                                                 | 1953 | Australia |
| Hindmarsh and Blumer (1932)<br>(Cited by Paulsen and Moule, 1953) | 1953 | Australia |
| Stenberg                                                          | 1954 | Finland   |
| <u>Abortions in Goats</u>                                         |      |           |
| Amanzhulov                                                        | 1953 | Russia    |

Gray et al. (1956) reported a condition in cattle which appears to be similar to the epidemic of perinatal death of human infants reported by Potel (1953). They observed cases in which calves were born at or near term but survived only for a few days. These calves were very weak and showed marked bloody diarrhea. L. monocytogenes was isolated from the livers of 6 calves.



Gray (1954) reviewed the incidence of perinatal death of young in animals and man due to L. monocytogenes. The following tables are based on this review with subsequent additions of new reports which appeared in the literature.

TABLE III  
REPORTS OF ISOLATIONS OF L. MONOCYTOGENES  
FROM PERINATAL DEATH OF YOUNG ANIMALS

| Author               | Year | Country     |
|----------------------|------|-------------|
| <u>Calves</u>        |      |             |
| Harbour              | 1941 | England     |
| Wramby               | 1944 | Sweden      |
| Rubarth and Wollarz  | 1951 | Sweden      |
| Gray <u>et al.</u>   | 1954 | U.S.A.      |
| <u>Lambs</u>         |      |             |
| Jepsen               | 1942 | Sweden      |
| Gray <u>et al.</u>   | 1949 | U.S.A.      |
| Stenberg             | 1953 | Finland     |
| <u>Foals</u>         |      |             |
| Krage                | 1944 | E. Prussia  |
| Svenkerud            | 1948 | Norway      |
| De Lay and Shultz    | 1954 | U.S.A.      |
| <u>Pigs</u>          |      |             |
| de Blieck and Jansen | 1942 | Netherlands |
| Kerlin and Graham    | 1945 | U.S.A.      |
| Solomkin             | 1954 | Russia      |
| De Lay and Schultz   | 1954 | U.S.A.      |

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TABLE IV  
 REPORTS OF ISOLATIONS OF L. MONOCYTOGENES  
 FROM HUMAN INFANTS (PERINATAL DEATH)

| Author                                   | Year    | Country        |
|------------------------------------------|---------|----------------|
| Tompkins                                 | 1952    | U.S.A.         |
| Alex and Potel                           | 1953    | E. Germany     |
| Erdmann and Potel                        | 1953    | E. Germany     |
| Hagemann and Simons                      | 1953    | E. Germany     |
| Linzenmeier <u>et al.</u>                | 1953    | W. Germany     |
| Starck                                   | 1953    | E. Germany     |
| Potel                                    | 1953-54 | E. Germany     |
| Patocka <u>et al.</u>                    | 1953    | Czechoslovakia |
| Stoot                                    | 1954    | Canada         |
| De Lay and Shultz                        | 1954    | U.S.A.         |
| Breunning and Fretzsche                  | 1954    | E. Germany     |
| Portero and Despirito                    | 1954    | U.S.A.         |
| Hahnefeld and Nisolk                     | 1954    | E. Germany     |
| Martinek                                 | 1954    | E. Germany     |
| Detmold and Girgensohn                   | 1954    | E. Germany     |
| Norman                                   | 1955    | U.S.A.         |
| Johnston <u>et al.</u>                   | 1955    | Canada         |
| Reed <u>et al.</u>                       | 1955    | Canada         |
| Greay (Cited by Johnston <u>et al.</u> ) | 1955    | Canada         |



Newborn human infants are either stillborn, or born prematurely or die a short time after birth. Usually they are weak and develop cyanotic attacks before death. It is interesting to note that although perinatal infection is almost 100 percent fatal, both in animals and humans, the mothers show only transient or inapparent symptoms. Some women complain of such nonspecific symptoms as headaches, stomach-ache and fever 1-3 weeks before parturition and in some, the movements of the fetus may decrease.

There are no reliable clinical tests for the detection of listeriosis in its early stages or identification of possible carrier states. Hematological pictures, especially in ruminants, do not give any indication of the specificity of the disease. In some cases acetone is reported to be present in the urine, which further confuses the disease with other symptomatically similar diseases, particularly acetonemia, in cattle and sheep.

The significance of the triple-phosphate-producing properties of this bacterium in edematous fluids as pointed out by Murray (1953) is still unknown. Further biochemical studies on the peritoneal, pleural and pericardial effusions which clot on exposure to air are needed.

Murray et al. (1926) described greyish white foci of necrosis in the liver of rabbits and guinea pigs. Jansen (1945) reported necrotic endometritis in a pregnant rabbit which

died after aborting a fetus. There are often no detectable gross lesions found in animals which die of listeric encephalitis. Congestion of the brain may be found in some cases but is variable. Gray (1954) pointed out that slight clouding of the meninges or pin-point greyish white foci in the brain may be seen rarely. He also reported that lesions in other viscera are rare but occasionally fatty liver, duodenitis or pulmonary edema are seen.

Pallaske (1940) reported marked focal necrosis of the liver in an adult sheep. No such lesions have been reported in adult cattle.

In listeric abortions in animals, pus in the uterine cavity and vaginal discharge may be noticed in some cases. There are usually no detectable gross lesions in aborted fetuses. However, Smith et al. (1955) reported a small amount of yellow flocculent material in the pleural and peritoneal fluid of aborted fetuses.

Harbour (1941) isolated L. monocytogenes from a calf which showed focal hepatic necrosis and ulcerative enteritis. This calf had diarrhea and died 9 days after birth.

Gray et al. (1949) isolated L. monocytogenes from the liver of an 8-day-old lamb that showed focal necrosis. Gray et al. (1956) also reported a similar condition in 8 newborn calves which lived only a few days. They were weak and showed marked gastritis and enteritis on necropsy.

Some calves showed a few pin-point grayish white foci in the liver. These authors also noted the presence of thrombi on the heart valves of a lamb and serosanguinous fluid in the pericardial sac. Similar cases have been reported in other animals (Table III).

Isolations of L. monocytogenes from human infants in perinatal death is reported by several workers. (Table IV).

Reiss, Potel and Krebs (1951) called this condition "granulomatosis infantiseptica" on the basis of lesions observed in the organs of the infants. The chief gross lesions they described as miliary granulomata with necrosis, found particularly in the liver, spleen, adrenals, lungs, lymph nodes and brain.

Hagemann and Simon (1953) found gross lesions in the liver, lungs, spleen and adrenals as well as in the leptomeninges in perinatal death of infants associated with L. monocytogenes. They described the lesions in these organs as numerous submiliary to miliary greyish-white nodule-like foci. In some cases they found similar foci in the mucosa of the mouth, tonsils, esophagus, stomach and small and large intestine, including the rectum.

Detmold and Girgensohn (1954) found amnionic fluid turbid in some cases in addition to the findings of other workers just described.

Johnston et al. (1955) reported gross lesions mainly in both lobes of the liver of a newborn infant which died 72

hours after birth. The liver was uniformly enlarged and firm, and its surface and substance were studded with sub-miliary greyish-white nodules which were neither raised nor palpable. In another case the amniotic fluid was reported as being "murky" but not foul-smelling.

The study of microscopic lesions of listeric encephalitis in the brain of cattle, sheep and goats is reported in considerable detail by many workers; among them are King (1940), Olafson (1940), Biester and Schwarte (1941), Graham et al. (1943), Ryff and Lee (1948), Jensen and Mackay (1949), Gray and Moore (1953), and Tajima and Nishihara (1953). The lesions are found primarily in the medulla and pons and are characterized by marked perivascular infiltration or "cuffing" and areas of focal necrosis which are variable in degree and extent. The perivascular "cuff" is formed mainly by mononuclear leukocytes (lymphocytes and monocytes) in all species of ruminants.

Gray and Moore (1953) reported that polymorphonuclear leukocytes (polymorphs) predominate more in the foci of necrosis in sheep and goats than in cattle and may in some cases even cause liquefaction and suppuration. These authors also observed in contrast that focal lesions in cattle usually are limited to edema and small focal collections of microglial cells and lymphocytes and are usually not as extensive as in sheep.



Olafson (1940) and Pallaske (1940) pointed out the tendency toward suppuration of the necrotic lesions in the brain of sheep.

Histopathology of the aborted fetuses in listeric abortions is rarely reported. Smith et al. (1955) reported that kidney sections from aborted bovine fetuses showed advanced decomposition and masses of bacteria resembling L. monocytogenes. They found acute endometritis in the sections of uterus of the dam but with no marked involvement or necrosis. The medulla and other tissues from the dam showed no significant lesions.

The microscopic lesions in cases of perinatal death of young in animals and man are variable in intensity and extent.

Gray et al. (1956) reported that the microscopic hepatic lesions in calves which died after birth were of three types, including degenerative changes (fatty metamorphosis) in hepatic cells, discrete focal necrosis without involvement of adjacent hepatic cells and diffuse necrosis with almost complete disappearance of hepatic cells. These authors also reported extensive degeneration of endothelial lining and thrombosis of hepatic arteries in varying degrees. They also noted the presence of Gram-positive bacteria resembling L. monocytogenes in the necrotic areas. The same authors observed hemorrhagic gastritis and enteritis with

necrosis of intestinal epithelium and congestion and thrombosis of some blood vessels.

The histopathology in cases of listeric perinatal death of human infants has been reported by several German workers - Potel (1953-54), Hagemann and Simon (1953), Hagemann et al. (1953), Hahnefeld and Nisolk (1954), Detmold and Girgensohn (1954) and others: Patocka et al. (1953, Czechoslovakia), and Johnston et al. (1955, Canada). In general the microscopic lesions described by these workers are in agreement. The principal lesions in various organs (particularly in the liver, spleen, adrenals, lungs, lymph nodes, and brain) are characterized by areas of focal necrosis, with varying degree of leukocytic infiltration and fibroblastic proliferation. Hagemann et al. (1953) described the lesions caused by L. monocytogenes according to the tissue reactions involved in these conditions. The most common lesion was focal necrosis with the presence of varying numbers of bacterium in almost all the foci. The organisms were usually found at the periphery of the lesion rather than at the center. In other cases they found similar lesions in the mucosa of the mouth, tonsils, esophagus, stomach, small and large intestine (including rectum) and placenta. Another type of reaction observed by these workers was characterized as a proliferating granuloma through an increase in mesenchymal cells, a proliferation in which both vascular and

connective tissues take part. The organisms are rarely demonstrated in or isolated from this type of lesion. This so-called "granuloma" was not seen in all cases of infection.

Johnston et al. (1955) reported the microscopic lesions of two newborn infants. One died 42 hours and the other 3 hours after birth. The first showed many small areas of hepatic necrosis with little inflammatory reaction. These lesions had no consistent relationship to any part of the liver lobule. Many Gram-positive, short, slightly beaded rods were observed in the lesions. They also found similar foci in the spleen, adrenal medulla, thymus, bone marrow, and the submucosa of the bladder. Sections of the brain revealed an acute meningitis with polymorph infiltration. No focal accumulations of mononuclear cells were found. The myocardium was normal.

Sections of lung from the other infant who died 3 hours after birth showed bronchitis and bronchiolitis with a radiating bronchopneumonia. The pulmonary alveoli were filled with amnionic debris and there was early hyaline membrane formation. Many small abscesses with a peripheral cell response were seen in the adrenal gland. There was pus on the surface of the gastric mucosa with a slight inflammatory reaction in the mucous membrane. They also noticed Gram-positive bacilli "in the adrenal abscesses, in the pus

covering the gastric mucosa, and diffusely throughout the lungs. The liver, brain and remaining organs were normal."

#### IV. EXPERIMENTS

##### A. Rabbit Experiments

##### 1. Conjunctival Exposure

###### a. Materials and Methods

Rabbits 2-3 weeks pregnant. Nine pregnant rabbits (2-3 weeks gestation) ranging in age from 8 to 18 months were exposed to L. monocytogenes by instillation of 2 drops of culture into the left conjunctival sac. The culture (12255) used in the experiment was isolated from the medulla oblongata of a calf which died of listeric encephalitis complicated by vitamin E deficiency. Unless otherwise indicated, this culture was used in all experiments. The culture was grown at 37° C on tryptose agar (Difco) slants for 18-24 hours. The suspension was made with sterile distilled water to a density approximating the number 8 tube of the McFarland nephelometer when compared in a Cenco-Sheard-Sanford photelometer. Two drops of the culture were instilled into the conjunctival sac by means of a capillary pipette. Swab cultures from the left eye and vagina were taken each 3rd day after exposure for a period of 2 weeks. All aborted fetuses were examined post mortem and liver and stomach contents of each and cotyledon

and amnionic fluid and exudates when available were cultured. Necropsies were done on all does which aborted and died or were sacrificed. Liver, spleen, heartblood, kidney, uterus, cotyledon, lung, brain, vagina, urine and eye were cultured. Cultures were made on tryptose agar (Difco) plates, incubated at 37° C for 18-24 hours after which they were examined by means of a binocular scanning microscope and oblique lighting (Fig. 1 and 2) as described by Gray et al. (1948).

Tissues were fixed in 10 percent formalin, dehydrated in graded alcohols, cleared in xylene and embedded in paraffin of melting point 56°-58° C. Sections were cut 6-7 microns thick and stained with hematoxylin and eosin by the Armed Forces Institute of Pathology method (1953) with the modification of (1) using standard hemotoxylin solution diluted 50 percent with distilled water, and (2) introducing carbol xylene (melted phenol and xylene, equal volumes) for 5 minutes for differentiation before the final two changes in neutral xylene and mounting in Permount. Whenever necessary, Mac Callum's Modification of Goodpasture's Method for bacterial stain in tissue section was used. (Mallory, 1938).

Rabbits near term. Seven rabbits 2-4 days prepartum were exposed in the same manner.

Non-pregnant rabbits. Six non-pregnant rabbits were exposed similarly.

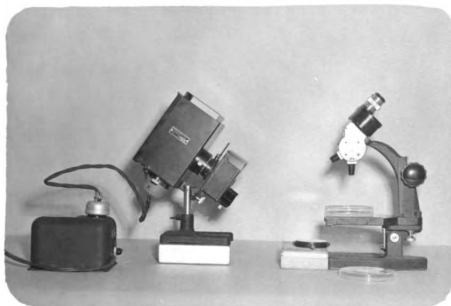


Fig. 1. Arrangement of binocular scanning microscope and light source for examination of bacterial colonies.

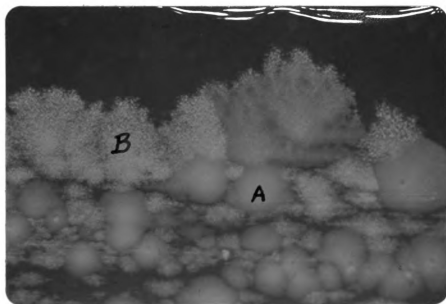


Fig. 2. Smooth (A) and rough (B) colonies of *L. monocytogenes* as viewed through binocular scanning microscope. (Fig. 1)

b. Results: Bacteriology

Rabbits 2-3 weeks pregnant. Of the 9 does, 3 aborted after the 3rd day after exposure and died the following day or in two days. One aborted 5th post-exposure day and was sacrificed; two died before abortion; and two aborted 3 and 6 days after exposure, respectively, and survived. The results of isolation of L. monocytogenes from aborted fetuses and does are shown in Tables V and VI, respectively.

Rabbits near term. Seven does gave birth to 58 young of which 16 were either stillborn or died shortly after birth; the remaining young died within 8 days except for one which survived. The does did not show significant manifestation of illness and all except one survived. L. monocytogenes was isolated from the young as shown in Table VII. Tissues were not refrigerated.

Non-pregnant rabbits. Six non-pregnant rabbits showed no apparent signs of illness and were sacrificed. L. monocytogenes was isolated as shown in Table VIII.

c. Results: Pathology

Lesions are described under the heading of the organ or tissue involved. Organs showing no significant lesions are not mentioned.



TABLE V

ISOLATIONS OF L. MONOCYTOGENES FROM ABORTED FETUSES FOLLOWING CONJUNCTIVAL  
EXPOSURE OF RABBITS (CULTURE 12255)

| Rabbit<br>Number | Date<br>of<br>Breeding | Date<br>of<br>Exposure | Date<br>of<br>Abortion                      | Necropsy<br>Number | Number<br>of<br>Aborted<br>Fetuses       | Liver | Stomach<br>Content  | Cotyledon | Amniotic<br>Fluid | Exudates | Remarks                                   |
|------------------|------------------------|------------------------|---------------------------------------------|--------------------|------------------------------------------|-------|---------------------|-----------|-------------------|----------|-------------------------------------------|
| 221              | 6/14/54                | 6/29/54                | 7/2/54                                      | 1916X              | 10                                       | 10+   | 10+                 | 1+        | 2+                |          | Died 7/3/54<br>Necropsy 1916X             |
| 214              | 6/14/54                | 6/29/54                | 7/3/54                                      | 1917X              | 1                                        | 1+    | 1+                  | 1+        |                   |          | Died 7/4/54<br>Necropsy 1917X             |
| 216              | 6/28/54                | 7/20/54                | 7/23/54<br>7/24/54                          | 1928X              | 3                                        | 3+    | 3+                  | 3+        |                   |          | Died 7/26/54<br>Necropsy 1928X            |
| 222              | 6/28/54                | 7/20/54                | 7/25/54                                     | 1929X              | 2                                        | 2+    | 2+                  | 2+        |                   |          | Sacrificed<br>Necropsy 1929X              |
| 212              | 7/8/54                 | 7/20/54                | Died before<br>abortion<br>7/27/54          | 1935X              | Macerated<br>fetuses<br>in the<br>uterus |       |                     |           |                   |          | Died 7/27/54<br>Necropsy 1935X            |
| 203              | 7/21/54                | 8/11/54                | Died before<br>abortion<br>8/16/54          | 1947X              | 9 fetuses<br>contained<br>in uterus      | 9+    | 8+<br>1 not<br>done | 9+        |                   |          | Died before<br>abortion<br>Necropsy 1947X |
| 220              | 7/20/54                | 8/11/54                | 8/14/54                                     | -                  | 6                                        | 6+    | 6+                  | 4+        |                   |          | Survived                                  |
| 207              | 7/21/54                | 8/11/54                | 8/17/54                                     | -                  | 10                                       | 10+   | 9+<br>1 not<br>done | 2+        | 2+                | +        | Survived                                  |
| 209              | 7/21/54                | 8/11/54                | Sacrificed<br>before<br>abortion<br>8/20/54 | 1952X              | 7 fetuses<br>contained<br>in uterus      | 7+    | 7+                  | 7+        | 7+                | +        | Sacrificed<br>Necropsy 1952X              |

TABLE VI

ISOLATIONS OF *L. MONOCYTOGENES* FROM RABBITS (2 TO 3 WEEKS PREGNANT)  
FOLLOWING CONJUNCTIVAL EXPOSURE (CULTURE 12255)

| Rabbit Number | Date of Breeding | Date of Exposure | Date of abortion, death, etc.                        | Necropsy Number | Liver | Spleen | Blood | Kidney | Uterus | Cotyledon | Lung | Brain | Vagina | Urine | Milk | Eye |
|---------------|------------------|------------------|------------------------------------------------------|-----------------|-------|--------|-------|--------|--------|-----------|------|-------|--------|-------|------|-----|
| 200           | 5/14/54          | 6/2/54           | Aborted on 6/7/54<br>Sacrificed same day             | 1902X           | +     | +      | -     |        |        | +         |      |       | +      | +     |      | +   |
| 221           | 6/14/54          | 6/29/54          | Aborted on 7/2/54<br>Died on 7/3/54                  | 1916X           | +     | +      | +     | +      | +      | +         |      |       | +      | +     |      | +   |
| 214           | 6/14/54          | 6/29/54          | Aborted on 7/3/54<br>Died on 7/4/54                  | 1917X           | +     | +      | +     | +      | +      | +         |      |       | +      | +     |      | +   |
| 216           | 6/28/54          | 7/20/54          | Aborted on 7/23/54<br>and 7/24/54<br>Died on 7/26/54 | 1928X           | +     | +      | -     | -      | -      | +         |      |       | -      | -     |      | +   |
| 222           | 6/28/54          | 7/20/54          | Aborted on 7/25/54<br>Sacrificed on 7/27/54          | 1929X           | -     | -      | -     | -      | +      | +         |      |       | +      | -     |      | +   |
| 212           | 7/8/54           | 7/20/54          | Died before abortion on 7/27/54                      | 1935X           | +     | +      | +     | +      | +      | +         |      | +     | +      | +     |      | +   |
| 203           | 7/21/54          | 8/11/54          | Died before abortion on 8/16/54                      | 1947X           | -     | +      | -     | -      | +      | +         |      |       | -      | -     |      | +   |
| 220           | 7/20/54          | 8/11/54          | Aborted on 8/14/54<br>but did not die                |                 |       |        |       |        |        |           |      |       |        |       |      |     |
| 207           | 7/21/54          | 8/11/54          | Aborted on 8/17/54<br>but did not die                |                 |       |        |       |        |        |           |      |       |        |       |      |     |
| 209           | 7/21/54          | 8/11/54          | Sacrificed before abortion on 8/20/54                | 1952X           | +     | +      | -     | -      | +      | +         |      |       | +      | +     |      | +   |

TABLE VII

ISOLATIONS OF *L. MONOCYTOGENES* FROM PERINATAL DEATH OF YOUNG FOLLOWING  
CONJUNCTIVAL EXPOSURE OF RABBITS (CULTURE 12255)

| Rabbit Number | Date of Breeding | Date of Exposure | Date of Birth | Necropsy Number | Time in which young died                   | Number of young   | Liver      | Stomach contents | Cotyledon | Exudate | Brain | Blood | Spleen | Intestine | Lung | Kidney | Urine | Amniotic Fluid | Spinal Cord | Remarks                      |
|---------------|------------------|------------------|---------------|-----------------|--------------------------------------------|-------------------|------------|------------------|-----------|---------|-------|-------|--------|-----------|------|--------|-------|----------------|-------------|------------------------------|
| 223           | 7/28/54          | 8/24/54          | 8/27/54       | 1959X           | 6 days                                     | 9                 | 7+         | 3+               |           |         |       |       |        |           |      |        |       |                |             | Survived                     |
|               |                  |                  |               |                 |                                            |                   | 2 cont     | 4-               |           |         |       |       |        |           |      |        |       |                |             |                              |
| 227           | 7/28/54          | 8/24/54          | 8/28/54       | 1960X           | 6 died same day } mutilated and sacrificed | 9                 | 5+         | 4+               |           |         |       |       |        |           |      |        |       |                |             | Sacrificed Necropsy 1960X    |
|               |                  |                  |               |                 |                                            |                   | 1-         | 1-               |           |         |       |       |        |           |      |        |       |                |             |                              |
|               |                  |                  |               |                 |                                            |                   | 1 cont     | 1 cont           |           |         |       |       |        |           |      |        |       |                |             |                              |
|               |                  |                  |               |                 |                                            |                   | 2 not cul- |                  |           |         |       |       |        |           |      |        |       |                |             |                              |
|               |                  |                  |               |                 |                                            |                   | tured      |                  |           |         |       |       |        |           |      |        |       |                |             |                              |
| 225           | 8/19/54          | 9/14/54          | 9/17/54       | 1963X           | 4 days                                     | 8                 | 6+         | 4+               | 4+        |         | 1+    |       |        |           |      |        |       |                |             | Survived                     |
|               |                  |                  |               |                 |                                            |                   | 2 cont     | 2 cont           |           |         |       |       |        |           |      |        |       |                |             |                              |
| 232           | 8/19/54          | 9/14/54          | 9/18/54       | 1964X           | 8 days                                     | 6 died 1 survived | 5+         | 4+               | 1+        |         |       |       | 2+     | 1+        | 1+   | -      | 2+    |                |             | Survived                     |
|               |                  |                  |               |                 |                                            |                   | 1-         | 1-               |           |         |       |       |        |           | 1-   |        |       |                |             |                              |
| 234           | 8/19/54          | 9/14/54          | 9/18/54       | 1965X           | 3 days                                     | 10                | 10+        | 9+               | 4+        |         | 1+    | 1+    |        |           |      |        |       |                |             | Died 10/11/54 Necropsy 1985X |
|               |                  |                  |               |                 |                                            |                   |            | 1 cont           |           |         |       |       |        |           |      |        |       |                |             |                              |
| 249           | 9/9/54           | 10/7/54          | 10/9/54       | 1983X           | 3 days                                     | 8                 | 5+         | 1+               | 5+        |         |       |       |        |           |      |        | 2+    |                |             | Survived                     |
|               |                  |                  |               |                 |                                            |                   | 3-         | 5-               |           |         |       |       |        |           |      |        |       |                |             |                              |
|               |                  |                  |               |                 |                                            |                   |            | 2 cont           |           |         |       |       |        |           |      |        |       |                |             |                              |
| 250           | 9/9/54           | 10/7/54          | 10/10/54      | 1948X           | 3 days                                     | 7                 | 4+         | 5+               | 2+        |         |       |       |        |           |      |        | 1+    |                |             | Survived                     |
|               |                  |                  |               |                 |                                            |                   | 3-         | 1-               |           |         |       |       |        |           |      |        |       |                |             |                              |
|               |                  |                  |               |                 |                                            |                   |            | 1 cont           |           |         |       |       |        |           |      |        |       |                |             |                              |

TABLE VIII

ISOLATIONS OF L. MONOCYTOGENES FROM NON-PREGNANT RABBITS  
FOLLOWING CONJUNCTIVAL EXPOSURE (CULTURE 12255)

| Rabbit<br>Number | Date<br>of<br>Exposure | Date<br>of<br>Sacrifice | Necropsy<br>Number | Liver | Spleen | Blood | Kidney | Uterus | Lung | Brain | Vagina | Urine | Eye |
|------------------|------------------------|-------------------------|--------------------|-------|--------|-------|--------|--------|------|-------|--------|-------|-----|
| 217              | 7/20/54                | 7/26/54                 | 1931X              | -     | -      | -     | -      | +      |      |       | +      |       | +   |
| 226              | 7/20/54                | 7/27/54                 | 1937X              | -     | -      | -     | -      | -      |      |       | -      | -     | +   |
| 228              | 8/11/54                | 8/16/54                 | 1948X              | +     | -      | -     | -      | +      |      |       |        | +     | +   |
| 229              | 8/11/54                | 8/20/54                 | 1953X              | -     | -      | -     | -      | -      |      |       | -      | -     | +   |
| 230              | 8/11/54                | 8/23/54                 | 1955X              | -     | -      | -     | -      | -      |      |       | -      | -     | +   |
| 231              | 8/24/54                | 9/3/54                  | 1961X              | -     | -      | -     | -      | -      |      |       | -      | -     | -   |

Rabbits 2-3 weeks pregnant

Necropsy 1916X - Rabbit 221 aborted 3 days after conjunctival instillation of L. monocytogenes and died a day after abortion.

Gross pathology:

Eye - marked conjunctivitis and profuse purulent exudate; eyelids were adhered together and purulent exudate oozed out on pressure. (Fig. 3)

Vagina - moderate sanguinous discharge.

Diffuse congestion of liver, lungs, spleen, intestine, uterus.

Uterus - very severe inflammation characterized by necrotic lesions with caseation and marked purulent exudate.

Cotyledons - extensively necrotic and disintegrating.

Histopathology:

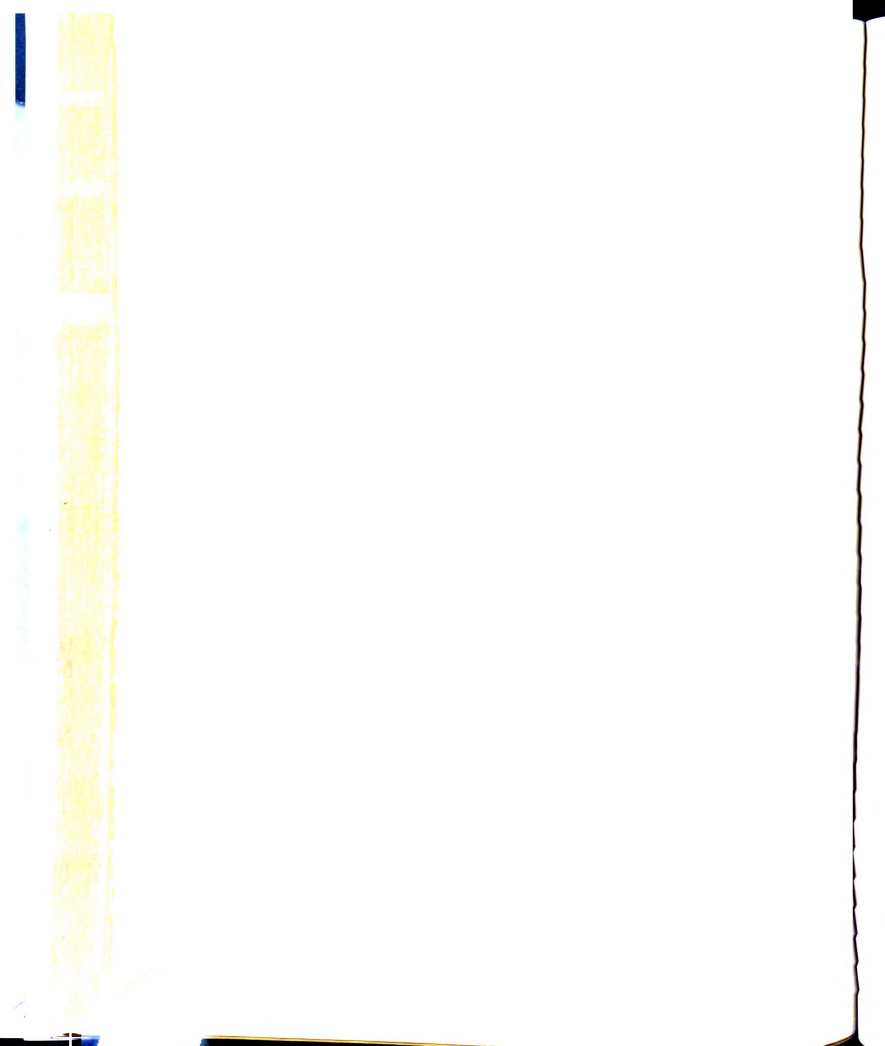
Uterus - acute necrotic endometritis with very extensive necrosis with caseation involving a large portion of endometrium including uterine glands; some of the remaining glands filled with purulent exudate; cellular infiltration consisting predominantly of polymorphs, but with some lymphocytes, plasma cells and macrophages; edema of endometrium. Myometrium shows thrombosis of blood vessels, infiltration of leukocytes (mainly polymorphs), and some necrosis.



Fig. 3. Left eye of a rabbit showing marked conjunctivitis and purulent exudate with adhesions of upper and lower eyelids 3 days after conjunctival instillation of L. monocytogenes.



Fig. 4. Left eye of a rabbit showing kerato-conjunctivitis 9 days after conjunctival instillation of L. monocytogenes.



Liver - marked diffuse fatty metamorphosis, and congestion.

Spleen and lungs - some congestion and hemorrhage.

Kidney - slight congestion.

Necropsy 1917X - Rabbit 214 aborted 4 days after conjunctival instillation of L. monocytogenes and died the day after abortion.

Gross pathology:

Eye - marked conjunctivitis with purulent exudate.

Vagina - slight sanguinous discharge.

Liver - congestion and a mild, diffuse, yellowish discoloration with a few pale, greyish white foci up to 1 mm. in diameter.

Uterus - very severe inflammation with extensive necrosis with caseation of endometrium and some purulent exudate.

Histopathology:

Uterus - acute necrotic endometritis with almost complete necrosis of the uterine glandular epithelium and the lumen of remaining glands filled with purulent exudate. Cotyledonary portion of endometrium and myometrium show marked congestion and necrotic foci with leukocytic infiltration (mainly polymorphs); clumps of bacteria in cotyledon, placenta, and uterine glands. (Fig. 5 and 6)

Urinary bladder - slight congestion.



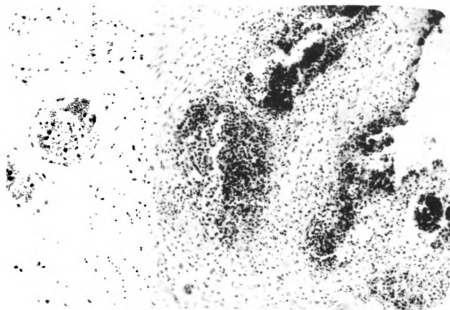


Fig. 5. (Necropsy 1917X) Endometrium from rabbit 214 showing necrosis and purulent exudate. Hematoxylin-eosin. 155x.

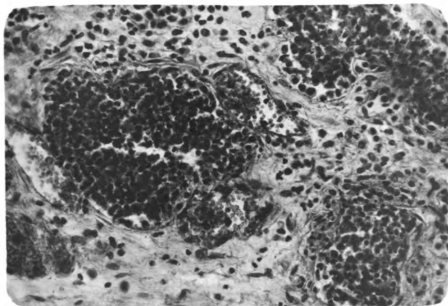


Fig. 6. (Necropsy 1917X) Uterine glands from rabbit 214 showing distention with purulent exudate. Hematoxylin-eosin. 325x.

Kidney - congestion.

Spleen - mild hematogenous pigmentation (probably hemosiderosis).

Liver - mild, diffuse fatty metamorphosis and moderate focal coagulation necrosis.

Necropsy 1928X - Rabbit 216 aborted 4 days after conjunctival instillation of L. monocytogenes and died 2 days after abortion.

Gross pathology:

Eye - marked conjunctivitis with purulent exudate.

Uterus - congestion and extensive caseation necrosis of endometrium, and filled with purulent exudate and macerated remains of conceptus.

Histopathology:

Uterus - acute necrotic endometritis and myometritis, with marked necrosis involving almost all of the endometrium including the uterine glands; some of the remaining glands distended with caseo-purulent exudate; marked congestion and edema, especially in the endometrium; cotyledons and placenta showed extensive marked necrosis and polymorph infiltration; several thrombi in vessels of the myometrium.

Liver - a few coccidial lesions unrelated to this experiment.

Spleen - mild hematogenous pigmentation (probably hemosiderosis).

Necropsy 1929X - Rabbit 222 aborted 5 days after conjunctival instillation of L. monocytogenes and was sacrificed 2 days later.

Gross pathology:

Eye - marked conjunctivitis and purulent exudate.

Vagina - slight sanguinous discharge.

Uterus - marked inflammation with caseation necrosis and thick purulent exudate.

Histopathology:

Uterus - acute necrotic endometritis and myometritis with marked but patchy necrosis of the endometrial epithelium; a zone of necrosis and polymorph infiltration noticed in the cotyledon above the decidua basalis; clumps of bacteria more prevalent on the maternal side of the zone of necrosis and polymorph infiltration. (Fig. 7, 8, 9 and 10)

Liver - slight centrolobular fatty change; mild focal infiltration of lymphocytes; mild infiltration of eosinophiles in the sinusoids.

Necropsy 1935X - Rabbit 212 died 7 days after conjunctival instillation of L. monocytogenes. Beginning 2 to 3 days before death the animal developed anorexia, torticollis, incoordination, and depression.

Gross pathology:

Eye - severe conjunctivitis with marked purulent exudate.

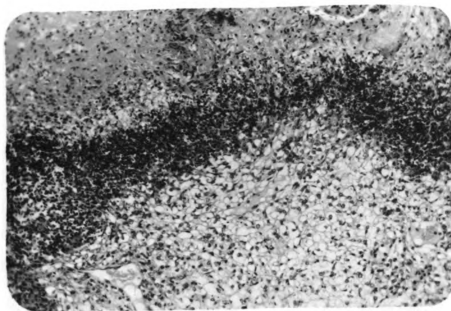


Fig. 7. (Necropsy 1929X) Endometrium from rabbit 222 showing zone of endometrial necrosis and polymorph infiltration. Hematoxylin-eosin. 155x.

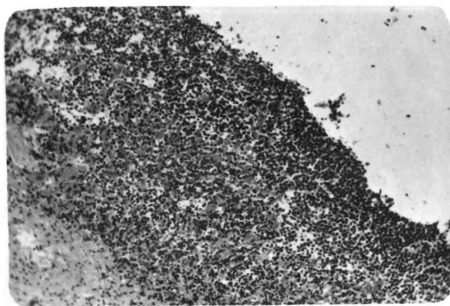


Fig. 8. (Necropsy 1929X) Endometrium from rabbit 222 showing extensive necrosis with caseation and leukocytic infiltration, predominantly polymorphs. Hematoxylin-eosin. 155x.

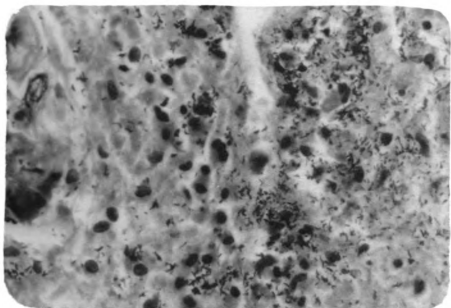


Fig. 9. (Necropsy 1929X) Endometrium from rabbit 222 showing Gram-positive bacteria resembling L. monocytogenes. McCallum's Modification of Goodpasture's Stain. 820x.

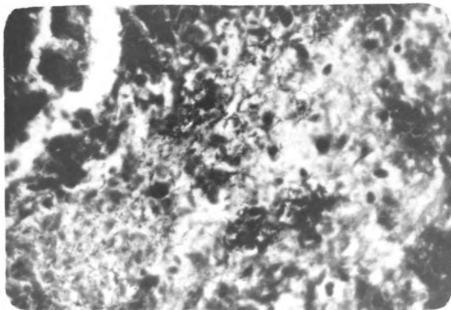


Fig. 10. (Necropsy 1929X) Endometrium of rabbit 222 showing Gram-positive bacteria resembling L. monocytogenes in the cotyledonary portion. McCallum's Modification of Goodpasture's Stain. 820x.



Vagina - slight sanguinous vaginal discharge.

Uterus - highly congested and inflamed; uterine cavity filled with purulent exudate, macerated fetuses and necrotic cotyledons.

Liver - slight yellowish discoloration.

All other visceral organs including brain - highly congested.

Histopathology:

Uterus - acute purulent metritis involving both endometrium and myometrium; necrosis of almost all the endometrium including uterine glands; a zone of necrosis with heavy infiltration of polymorphs at the junction of the endometrium and retained cotyledon; focal necrosis with polymorph infiltration and thrombosis of several vessels in the myometrium.  
(Fig. 11 and 12)

Cotyledon - limited necrosis with variable polymorph infiltration.

Liver - moderate centrilobular fatty metamorphosis.

Spleen - mild hematogenous pigmentation; small amount of hyaline material, probably amyloid, in the splenic corpuscles.

Kidney - moderate subacute interstitial nephritis characterized by focal lymphocytic infiltration near the blood vessels.

Medulla and cerebellum - mild congestion and hemorrhage.

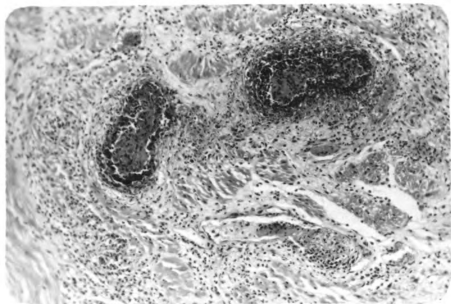


Fig. 11. (Necropsy 1935X) Myometrium from rabbit 212 showing thrombosis and polymorph infiltration. Hematoxylin-eosin. 155x.

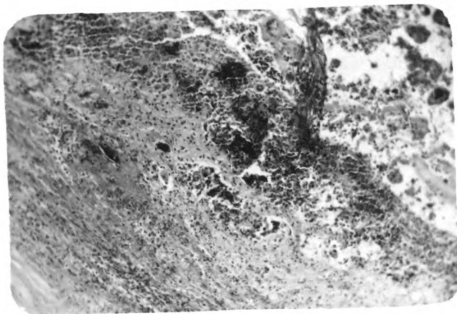


Fig. 12. (Necropsy 1935X) Endometrium from rabbit 212 showing caseous necrosis and polymorph infiltration. Hematoxylin-eosin. 155x.



Necropsy 1947X - Rabbit 203 died 5 days after conjunctival instillation of L. monocytogenes.

Gross pathology:

Eye - conjunctivitis and purulent exudate but not so marked as in other cases.

Uterus - highly inflamed with necrotic to purulent lesions and containing 9 fetuses.

Histopathology:

Uterus - acute necrotic endometritis with necrosis and polymorph infiltration in the upper portion of endometrium including uterine glands; congestion and edema.

Liver - marked diffuse fatty metamorphosis; coagulation necrosis, and congestion.

Kidney - some subacute interstitial nephritis characterized by mild focal lymphocytic infiltration.

Necropsy 1952X - Rabbit 209 was sacrificed 9 days after conjunctival instillation of L. monocytogenes.

Gross pathology:

Eye - severe kerato-conjunctivitis with some purulent exudate. (Fig. 4)

Vagina - slight sanguino-purulent discharge.

Uterus - marked inflammation with necrosis and purulent exudate, the left horn being relatively more severely affected than the right one.

Histopathology:

Uterus - subacute necrotic metritis with very extensive necrosis, and polymorph infiltration of endometrium involving most of the uterine glands; remaining uterine glands highly distended and cystic; mild lymphocytic infiltration of myometrium with some macrophages. (Fig. 13 and 14)

Cotyledon - some necrosis and marked polymorph infiltration.

Placenta - purulent exudate, with some eosinophiles also.

Liver - mild subacute interstitial hepatitis; congestion; some karyolysis of hepatic cells.

Kidney - subacute interstitial nephritis with lymphocyte, macrophage and numerous eosinophilic infiltration.

Fetal livers - marked congestion and diffuse fatty metamorphosis.

Rabbits near term

Necropsy 1959X - Young of Rabbit 223. This doe gave birth to 9 young 3 days after conjunctival exposure. All the 9 young died within 6 days after birth.

Gross pathology:

Liver - mild pale yellowish discoloration.

Histopathology:

Liver - congestion, patchy centrilobular fatty changes and focal necrosis in various combinations and quantitative variations. Necrosis usually appeared in

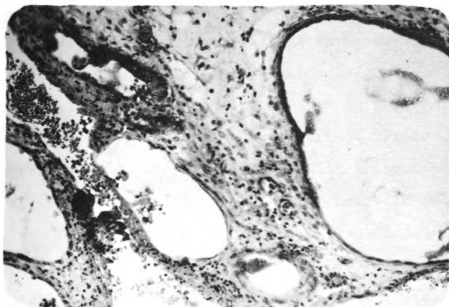


Fig. 13. (Necropsy 1952X) Endometrium from rabbit 209 showing cystic dilatation of uterine glands and some polymorph infiltration. Hematoxylin-eosin. 155x.

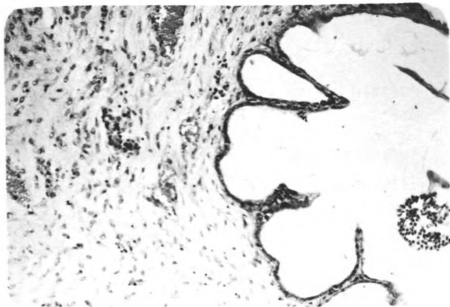


Fig. 14. (Necropsy 1952X) Endometrium from rabbit 209 showing confluence of several cystic uterine glands. Hematoxylin-eosin. 155x.

small discrete foci in the vicinity of blood vessels. In some cases extensive necrosis occurred along the outer zone of a large blood vessel; several thrombi were seen in the smaller blood vessels in the necrotic areas. Necrotic foci were usually infiltrated with polymorphs and clumps of bacteria. In one case necrotic cell debris was noticed in the bile ducts. (Fig. 16)

Gall bladder - limited focal necrosis and polymorph infiltration involving mucosa and submucosa. (Fig. 15)

Necropsy 1960X - Rabbit 227 gave birth to 9 young 4 days after conjunctival exposure. Six died and 3 mutilated ones were sacrificed the same day. The doe was sacrificed 6 days post partum.

Gross pathology:

Uterus - some greyish white material in the uterine cavity.

Histopathology:

Uterus - subacute endometritis with infiltration of lymphocytes and plasma cells, early proliferation of fibroblasts, slight purulent exudate in lumen of the uterus.

Liver - mild subacute interstitial hepatitis with a few foci of lymphocytic infiltration.

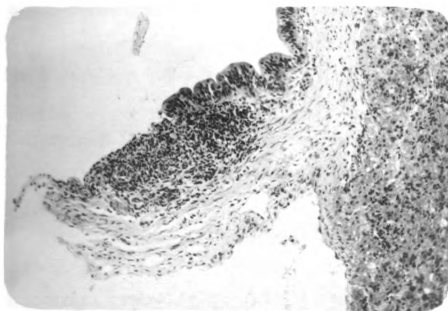


Fig. 15. (Necropsy 1959X) Gall bladder from one of the young of rabbit 223 showing a focal cell reaction in which polymorphs predominate. Hematoxylin-eosin. 155x.

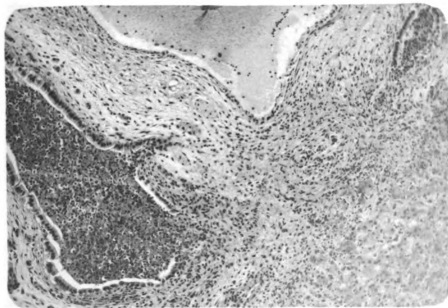


Fig. 16. (Necropsy 1959X) Liver from one of the young of rabbit 223 showing necrotic cell debris in bile ducts. Hematoxylin-eosin. 155x.

Necropsy 1964X - Young of Rabbit 232. These 7 young were born 4 days after conjunctival exposure of the doe. Six died within 8 days and one survived.

Gross pathology:

Liver - diffuse yellowish discoloration; greyish white foci observed in some cases with quantitative variation.

Histopathology:

Cotyledon - focal areas of necrosis with polymorph infiltration.

Liver - moderate centrilobular fatty changes and focal coagulation necrosis with variable polymorph infiltration. In young which survived a week or more, lymphocytic infiltrations and early proliferation of fibroblasts and angioblasts were seen. (Fig. 17 and 18)

Heart - a single focus of necrosis with moderate polymorph infiltration. (Fig. 19)

Stomach - focal superficial necrosis of mucosa; mild purulent exudate on the surface. (Fig. 20)

Lungs - acute broncho-pneumonia with marked congestion (Fig. 21)

Kidney - one focus of necrosis with moderate polymorph infiltration. (Fig. 22)

Necropsy 1965X - Young of Rabbit 234. Ten young were born 4 days after conjunctival instillation of the doe with L. monocytogenes, and died 3 days later. The doe herself died 3 weeks post partum. (Necropsy 1985X)

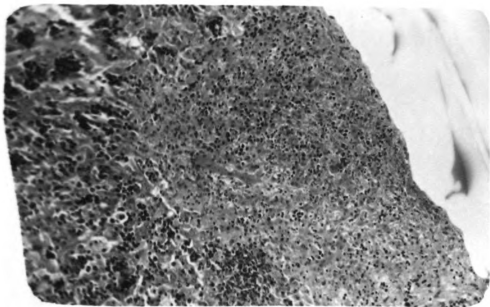


Fig. 17. (Necropsy 1964X) Liver from one of the young of rabbit 232 showing focal necrosis with mild polymorph infiltration. Hematoxylin-eosin. 155x.

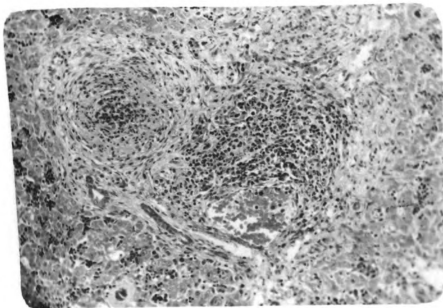


Fig. 18. (Necropsy 1964X) Liver from one of the young of rabbit 232 showing proliferative changes and lymphocytic infiltration. Hematoxylin-eosin. 325x.

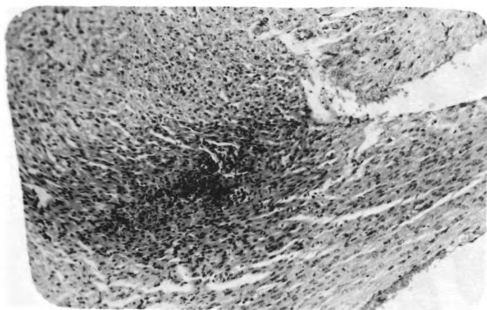


Fig. 19. (Necropsy 1964X) Heart from one of the young of rabbit 232 showing focal necrosis and polymorph infiltration. Hematoxylin-eosin. 155x.

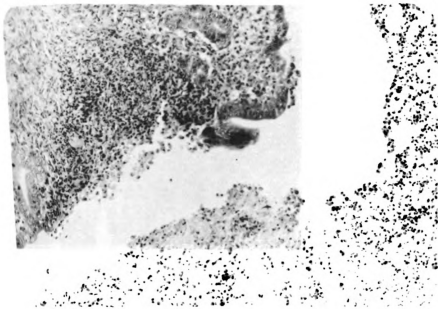


Fig. 20. (Necropsy 1964X) Stomach from one of the young of rabbit 232 showing focal necrosis with polymorph infiltration and mildly purulent exudate on the surface of the mucosa. Hematoxylin-eosin. 155x.



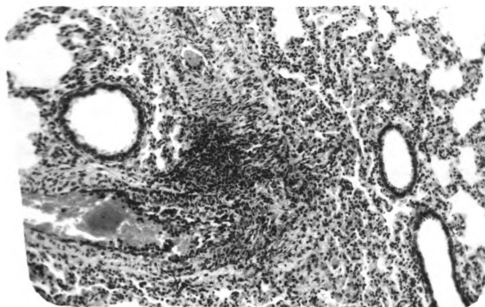


Fig. 21. (Necropsy 1964X) Lung from one of the young of rabbit 232 showing focal infiltration of polymorphs. Hematoxylin-eosin. 155x.

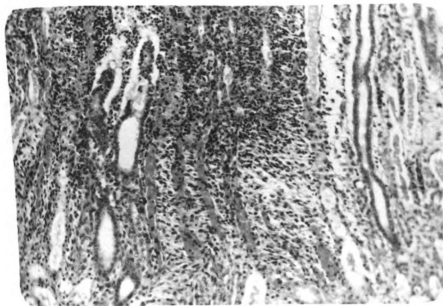


Fig. 22. (Necropsy 1964X) Kidney from one of the young of rabbit 232 showing focal necrosis and polymorph infiltration. Hematoxylin-eosin. 155x.

Gross pathology:

Liver of young - mild patchy yellowish discoloration and several discrete necrotic foci.

Histopathology:

Liver - moderate centrilobular fatty changes to extensive diffuse fatty metamorphosis, several small discrete foci of coagulation necrosis with no polymorphs or very little infiltration to a much larger focus of necrosis with numerous polymorphs; fatty changes and necrosis appear together in some foci.

Lungs - mild congestion, very early pneumonia.

Cotyledon - several areas of focal necrosis with polymorph infiltration.

Necropsy 1985X - Rabbit 234 gave birth to 10 young 4 days after conjunctival exposure. All the young died within 3 days (Necropsy 1965X); the rabbit died 3 weeks post partum.

Gross pathology:

Liver - mild, diffuse yellow discoloration.

Uterus - numerous fibrotic adhesions with intestine; very pale in appearance; many firm abscesses in the right horn ranging from pinpoint to 5 mm. in diameter containing caseated pus. No such lesions were seen in the left horn. (Fig. 23)

Histopathology:

Uterus - chronic suppurative metritis involving myometrium and endometrium with almost complete loss of uterine glands. (Fig. 24)

Necropsy 1983X - Young of Rabbit 249 were born 2 days after conjunctival instillation of the doe, and all died within 3 days after birth.

Gross pathology:

Liver - a few submiliary greyish white foci.

Histopathology:

Cotyledon - limited focal necrosis with a few polymorphs.

Placenta - marked purulent exudate.

Liver - lesions varied from mild centrilobular fatty changes to several small focal areas of necrosis with polymorph infiltration.

Non-pregnant rabbits

Necropsies 1931X - Rabbit 217

1937X - Rabbit 226

1948X - Rabbit 228

1953X - Rabbit 229

1955X - Rabbit 230

1961X - Rabbit 231

Gross pathology:

Necropsy revealed nothing of any significance in these non-pregnant rabbits except in the case of Rabbit 231, whose uterus appeared somewhat enlarged and congested.

1985X

METRIC 1 2 3 4 5 6 7 8

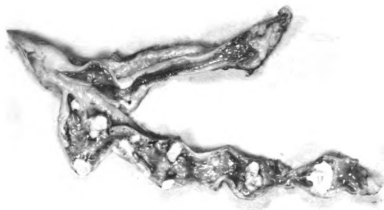


Fig. 23. (Necropsy 1985X) Uterus from rabbit 234 opened to show collections of caseo-purulent exudate.

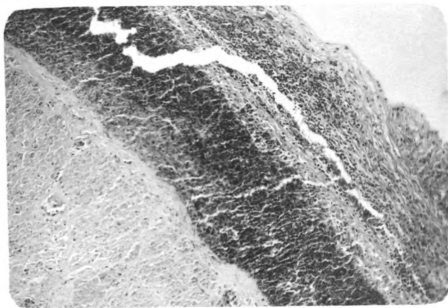


Fig. 24. (Necropsy 1985X) Uterus from rabbit 234. Light-stained area (lower left) is caseous cell debris at the endometrial surface. All layers of uterine wall show inflammatory infiltrations accompanied in some areas by necrosis and even suppuration. Hematoxylin-eosin. 155x.

### Histopathology:

No microscopic lesions of any significance could be noticed in the tissues (liver, spleen, kidney, urinary bladder and uterus) of these non-pregnant rabbits except the following:

#### Necropsy 1931X - Rabbit 217

Liver - a few small foci of lymphocytic infiltration.

Uterus - marked infiltration of eosinophiles with some polymorphs, lymphocytes and plasma cells. (Fig. 25)

#### Necropsy 1948X - Rabbit 228

Liver - a few small foci of lymphocytic infiltration.  
(Fig. 26)

Uterus - a slight focal infiltration of lymphocytes in the endometrium.

#### Necropsy 1961X - Rabbit 231

Liver - moderate centrilobular fatty changes, a few foci of lymphocytic infiltration and some scattered eosinophiles.

## 2. Oral Exposure

### a. Materials and Methods

Rabbits 2-3 weeks pregnant. Six (2-3 weeks pregnant) rabbits ranging in age from 8 to 18 months were exposed to L. monocytogenes by adding dense suspensions of the bacterium to the



Fig. 25. (Necropsy 1931X) Endometrium from rabbit 217 showing infiltration of lymphocyte and eosinophile and some cellular exudate on the surface. Hematoxylin-eosin. 155x.

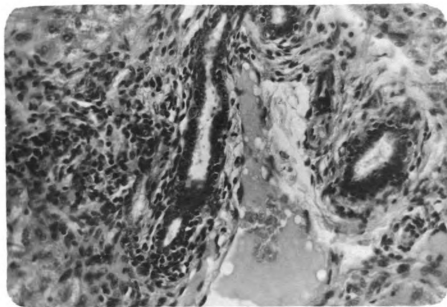


Fig. 26. (Necropsy 1949X) Liver from rabbit 228 showing focal lymphocytic infiltration in the interstitial tissue. Hematoxylin-eosin. 325x.

drinking water. Two of the pregnant does were exposed to culture 1766X isolated by Potel (1953) from the liver of an aborted infant; the remaining four animals were exposed to culture 12255. Thirty-two ounce "Sani-glas" prescription bottles containing approximately 100 ml. of tryptose agar (Difco) were inoculated with a distilled water suspension of freshly grown culture and incubated at 37° C for 18-24 hours. They were then filled with tap water, agitated to wash the culture from the agar surface, and the resulting suspension poured into a ceramic watering dish. Thus each rabbit daily had access to 32 ounces of a dense tap-water suspension of L. monocytogenes. This constituted the only source of fluid intake for a period of 2 weeks. The average density of these suspensions corresponded approximately to the No. 2 tube of the McFarland nephelometer when compared in a Cenco-Sheard-Sanford photelometer. No attempt was made to prevent contamination of the water by other organisms; however, the water was cultured at frequent intervals and, when badly contaminated, a clean dish was provided. All aborted fetuses and animals which died or were sacrificed were cultured as described by Gray et al. (1948, 1951). Tissues to be cultured were ground in a mortar together with sterile water to form an 80 percent suspension. A large loopful (4.0 mm. dia.) of this suspension was plated on tryptose agar (Difco) plates. The remaining suspension was stored in the refrigerator at 4° C. The plates were

incubated at 37° C for 18-24 hours after which they were examined by means of a binocular scanning microscope and obliquely transmitted light. Tissue suspensions which failed to reveal the presence of L. monocytogenes were replated at intervals of several weeks. If L. monocytogenes was not isolated after 3 months of refrigeration, the suspension was considered negative.

Rabbits near term. Two near-term pregnant rabbits were exposed to culture 1766X and the remaining six were exposed to culture 12255.

Non-pregnant rabbits. Three non-pregnant females and one male rabbit were exposed to culture 12255 and another male rabbit was exposed to culture 1766X.

#### b. Results: Bacteriology

Rabbits 2-3 weeks pregnant. Of these 6 rabbits, 3 aborted on the 4th post-exposure day and died 2-4 days after abortion and 2 aborted 5-6 days after exposure and survived. Some fetuses were macerated and others were mummified. There appeared to be no difference in the reactions of the does exposed to the 2 different cultures. Before death these does showed anorexia and depression. Isolation of L. monocytogenes from the fetuses and from the does is shown in Tables IX and X.



Rabbits near term. There appeared to be no difference in the reactions of the does exposed to the 2 different cultures. Of the 55 young born to 8 does, 24 were stillborn or died shortly after birth, none surviving more than 4 days. None of the does died. They displayed no noticeable signs of illness except that most of them did not take care of the young. Isolation of L. monocytogenes from the young is shown in Table XI.

Non-pregnant rabbits. The non-pregnant and male controls showed only a mild anorexia on the second and third days of exposure, with one exception. On the 12th post-exposure day one male showed some difficulty in breathing which persisted for 3 days, at which time he was sacrificed. Necropsy revealed a mild pneumonia and L. monocytogenes was isolated from the lungs, nares, cecum, and small intestine. In spite of the isolation from the lung, the pneumonia appeared to be of different origin as only one colony of L. monocytogenes was observed among a profuse and otherwise pure growth of an unidentified Gram-negative rod. L. monocytogenes was not isolated on initial culture from the three sacrificed female controls; however, it was isolated from the liver of one after maceration and refrigeration for 2 months.

c. Results: Pathology

Rabbits 2-3 weeks pregnant

TABLE IX

ISOLATIONS OF L. MONOCYTOGENES FROM ABORTED  
FETUSES FOLLOWING ORAL EXPOSURE OF RABBITS

| Rabbit<br>Number | Date<br>of<br>Breeding | Date<br>of<br>Exposure       | Date<br>of<br>Abortion             | Necropsy<br>Number | Number<br>of<br>Fetuses                              | Liver                   | Stomach<br>Content | Cotyle-<br>don | Amniotic<br>Fluid | Vaginal<br>Exudate | Remarks                        |
|------------------|------------------------|------------------------------|------------------------------------|--------------------|------------------------------------------------------|-------------------------|--------------------|----------------|-------------------|--------------------|--------------------------------|
| 238              | 8/30/54                | Culture<br>12255<br>9/21/54  | 9/25/54                            | 1968X              | 2 aborted<br>10 retained                             | 12+                     | 12+                | 12+            | +                 |                    | Died 9/23/54<br>Necropsy 1968X |
| 240              | 9/1/54                 | Culture<br>1766X<br>9/21/54  | Died before<br>abortion<br>9/27/54 | 1976X              | 9 mumi-<br>fied (in<br>situ)                         | 6+<br>3 not<br>done     |                    |                | +                 |                    | Died 9/27/54<br>Necropsy 1976X |
| 241              | 9/1/54                 | Culture<br>1766X<br>9/21/54  | 9/25/54                            | 1970X              | 7 aborted                                            | 2+<br>5 not<br>cultured |                    |                | +                 | +                  | Died 9/27/54<br>Necropsy 1970X |
| 251              | 10/11/54               | Culture<br>12255<br>10/27/54 | 11/1/54<br>11/5/54                 | 1997X              | 2 aborted<br>1 macer-<br>ated                        | 2+<br>1+                | 2+                 |                |                   | +                  | Survived                       |
| 252              | 10/11/54               | 10/27/54                     | 10/31/54<br>11/1/54                | 200X               | 2 aborted,<br>mummified<br>1 macer-<br>ated          | 2+                      | 2+                 |                |                   | +                  | Died 11/5/54<br>Necropsy 2003X |
| 253              | 10/11/54               | 10/27/54                     | 11/2/54<br>11/3/54<br>11/5/54      | 1999X              | 4 aborted<br>macerated<br>1 macerated<br>3 macerated | 4+<br>1+<br>3+          |                    | 2+             |                   | +                  | Survived                       |

TABLE X

ISOLATIONS OF L. MONOCYTOGENES FROM RABBITS  
(2 TO 3 WEEKS PREGNANT) FOLLOWING ORAL EXPOSURE

| Rabbit Number | Date of Breeding | Date of Exposure               | Date of Abortion, Death, etc.                                           | Necropsy Number | Liver | Spleen | Blood | Kidney | Uterus | Cotyledon | Lung | Brain | Vagina | Urine | Milk |
|---------------|------------------|--------------------------------|-------------------------------------------------------------------------|-----------------|-------|--------|-------|--------|--------|-----------|------|-------|--------|-------|------|
| 238           | 8/30/54          | Culture 12255<br>9/21/54       | Aborted and died on 9/25/54                                             | 1968X           | +     | +      | +     | +      | +      | +         | -    | +     | +      | +     | +    |
| 241           | 9/1/54           | Culture 1766X<br>9/21/54       | Aborted on 9/25/54<br>Died on 9/27/54                                   | 1970X           | +     | +      | -     | -      | +      | +         | -    | +     | +      |       | -    |
| 240           | 9/1/54           | Culture 1766X<br>9/21/54       | Died before abortion on 9/25/54                                         | 1976X           | +     | +      | -     | -      | +      | +         |      | +     | +      |       |      |
| 251           | 10/11/54         | Culture 12255<br>10/27/54<br>" | Aborted macerated fetuses on 11/1/54 and on 11/5/54 but survived        |                 |       |        |       |        |        |           |      |       |        |       |      |
| 252           | 10/11/54         | 10/27/54                       | Aborted on 10/31/54 and 11/1/54, died on 11/5/54                        | 2003X           | +     |        | -     |        | +      | +         |      |       |        | +     |      |
| 253           | 10/11/54         | 10/27/54                       | Aborted macerated fetuses on 11/2/54 11/3/54 and 11/5/54, but survived. |                 |       |        |       |        |        |           |      |       |        |       |      |

TABLE XI

ISOLATIONS OF L. MONOCYTOGENES FROM PERINATAL DEATH OF YOUNG  
FOLLOWING ORAL EXPOSURE OF RABBITS

| Rabbit Number | Date of Breeding | Date of Exposure | Date of Birth                         | Necropsy Number | Time in which young died             | Number of young | Liver            | Stomach Content | Cotyledon | Exudate | Brain | Blood | Spleen | Intestine | Lung | Kidney | Urine | Amniotic Fluid | Spinal Cord | Remarks  |
|---------------|------------------|------------------|---------------------------------------|-----------------|--------------------------------------|-----------------|------------------|-----------------|-----------|---------|-------|-------|--------|-----------|------|--------|-------|----------------|-------------|----------|
| 255           | 9/27/54          | Culture          | 1766X 10/28/54<br>10/27/54            | 1993X           | 4 days                               | 9               | -                | -               | 1+        |         |       |       |        |           |      |        |       |                |             | Survived |
| 254           | 9/27/54          | Culture          | 1766X 10/28/54<br>10/27/54            | 2009X           | 2 days                               | 6               | 2+<br>4-         | -<br>-          |           |         |       |       |        |           |      |        |       |                |             | Survived |
| 264           | 11/12/54         | Culture          | 12/12/54<br>12255 12/13/54<br>12/9/54 | 2046X           | 4 still-birth<br>1 died 6 hrs. later | 5               | 4-<br>1 not done | 1+<br>3 cont    | 2+        |         |       |       |        |           |      |        |       |                |             | Survived |
| 266           | 11/12/54         | "                | 12/9/54<br>12/12/54                   | 2047X           | 3 still-birth<br>2 died 12/13/54     | 5               | 1+<br>4-         | 4+<br>1-        | 2+        |         |       |       |        |           |      |        |       |                |             | Survived |
| 265           | 11/12/54         | "                | 12/9/54<br>12/13/54                   | 2048X           | all still-births                     | 8               | 6+<br>2-         | 6+<br>2-        | 4+        |         |       |       |        |           |      |        |       | 2+             |             | Survived |
| 267           | 11/12/54         | "                | 12/11/54<br>12/9/54<br>12/15/54       | 2049X           | 7 still-births<br>2 "                | 9               | 8+<br>1 not done | 4+<br>4-        | 5+        |         |       |       |        |           |      |        |       |                |             | Survived |
| 256           | 11/12/54         | "                | 12/11/54<br>12/11/54                  | 2056X           | 4 days                               | 10              | 6+<br>4-         | 2+<br>-         | 4+        |         |       | 1+    |        |           | 1+   | 2+     | 3+    |                | 1+          | Survived |
| 268           | 11/12/54         | "                | 12/11/54<br>12/11/54                  | 2058X           | 4 days                               | 3               | 3+<br>-          | 3+<br>2-        | 1+<br>2-  |         |       | 2+    | 3+     |           | 1+   | 2+     | 1+    |                |             | Survived |

Necropsy 1968X - Rabbit 238 aborted 2 fetuses the 5th day after exposure and died the same day.

Gross pathology:

Uterus - contained 10 additional fetuses and endometrium appeared necrotic. In some cases amnionic fluid was dark purple.

Liver - mild pale yellow discoloration and several greyish white areas about 2 to 3 mm in diameter.

Lungs - marked congestion.

Fetal liver - mild yellow discoloration and several sub-miliary to miliary greyish white foci.

Histopathology:

Uterus - acute necrotic endometritis with necrosis involving at places varying depths of endometrium.

Cotyledon - several areas of focal necrosis with polymorph infiltration, clumps of bacteria, and several thrombi with polymorph infiltration.

Liver - marked diffuse fatty changes and several foci of coagulation necrosis with mild polymorph infiltration, some eosinophiles and a few lymphocytes, some congestion.

Fetal tissues:

Liver - moderate congestion, diffuse fatty change, several foci of necrosis with polymorphs and bacteria, at some places dense clumps of bacteria in the parenchyma and some in the vicinity of bile ducts. (Fig. 27 and 28)

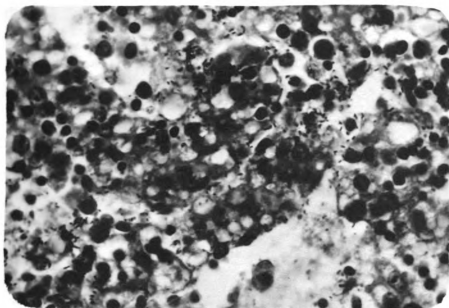


Fig. 27. (Necropsy 1968X) Liver from one of the young of rabbit 238 showing Gram-positive bacteria resembling L. monocytogenes. McCallum's Modification of Goodpasture's Stain. 620x.

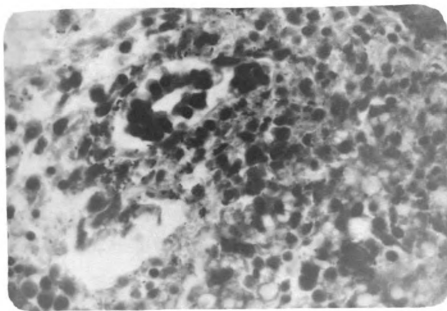


Fig. 28. (Necropsy 1968X) Liver from one of the young of rabbit 238 showing Gram-positive bacteria resembling L. monocytogenes in the vicinity of the bile duct. McCallum's Modification of Goodpasture's Stain. 620x.

Kidney - foci of necrosis with polymorph infiltration and bacterial clumps.

Heart - mild coagulation necrosis with clumps of bacteria.

Lungs - marked congestion.

Necropsy 1970X - Rabbit 241 aborted 4 days after exposure and died 2 days after abortion.

Gross pathology:

Uterus - congestion with pale streaks showing through serosa; uterine cavity contained a large amount of caseated necrotic thick gray material resembling pus, endometrium underneath the gray material appeared to be gray and necrotic. There was one cotyledon in the uterus which appeared to be entirely necrotic, and almost black in color.

Kidneys - petechial hemorrhages and a few greyish white areas.

Lungs - mild congestion with some edema.

Vagina - slight sanguinous discharge.

Fetal liver - slight yellow discoloration.

Histopathology:

Uterus - acute necrotic endometritis with an extensive necrosis of endometrium and marked purulent exudate involving most of the uterine glands; edema of the endometrium; areas of necrosis with polymorph infiltration and several thrombi in the myometrium; some thrombi were relatively large and infiltrated with polymorphs.

Cotyledon - several thrombi with polymorph infiltration;  
numerous clumps of bacteria.

Fetal membrane - mild purulent exudate.

Kidney - subacute interstitial nephritis and small focal  
areas of coagulation necrosis.

Liver - some centrolobular fatty change.

Lungs - mild congestion and edema.

Fetal tissues:

Liver - mild diffuse fatty change.

Necropsy 1976X - Rabbit 240 died 6th day after exposure  
without aborting.

Gross pathology:

Uterus - greatly distended and showed very marked congestion  
and numerous gray streaks and plaques of what appeared  
to be necrotic tissue. In the uterine cavity nine partially  
mummified fetuses were found tightly adhered to the endometrium.  
There was no evidence of any amniotic fluid. In addition it  
contained large amounts of caseous material adherent to the  
endometrium; underneath it, the endometrial surface appeared  
corrugated and necrotic. The cotyledons were gray in color and  
completely necrotic. (Fig. 29 and 30) The livers of the fetuses  
were all pale yellow in color and no lesions could be detected  
in the fetuses because of mummification.





Fig. 29. (Necropsy 1976X) Uterus from rabbit 240 in situ showing greyish streaks in the otherwise congested organ.



Fig. 30. (Necropsy 1976X) Uterus from rabbit 240 partly opened to show the caseous necrotic material on the fetal membranes and endometrium.

Liver - slightly pale in color and some submiliary greyish white foci.

Histopathology:

Uterus - acute necrotic endometritis and myometritis with extensive necrosis of almost all the endometrium including the uterine glands with marked purulent exudate. The few remaining uterine glands were filled with exudate. The myometrium showed edema and contained numerous extensive thrombi with polymorph infiltration. The serosal layer was extensively necrotic and infiltrated with polymorphs. (Fig. 31, 32, 33 and 34)

Cotyledon - several extensive thrombi with polymorph infiltration, clumps of bacteria, extensive areas of necrosis and purulent exudate, especially on the outer surface. (Fig. 35)

Fetal membranes - enclosed an abundance of necrotic debris. (Fig. 36)

Liver - several foci of coagulation necrosis with moderate polymorph infiltration. (Fig. 37)

Kidney - mild subacute focal interstitial nephritis with focal lymphocytic infiltration. (Fig. 38)

Fetal tissues:

Liver - moderate diffuse fatty changes.

Lungs - mild congestion.

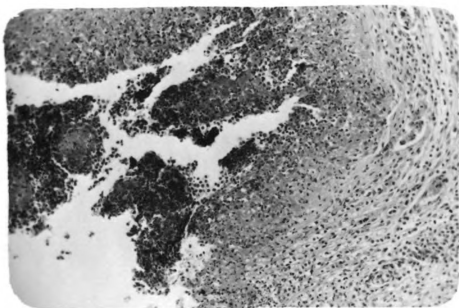


Fig. 31. (Necropsy 1976X) Endometrium from rabbit 240 showing necrosis and caseo-purulent exudate on the surface. Hematoxylin-eosin. 155x.

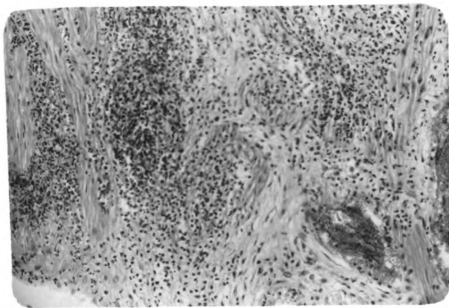


Fig. 32. (Necropsy 1976X) Myometrium from rabbit 240 showing necrosis and a predominantly polymorphic infiltration. Hematoxylin-eosin. 155x.

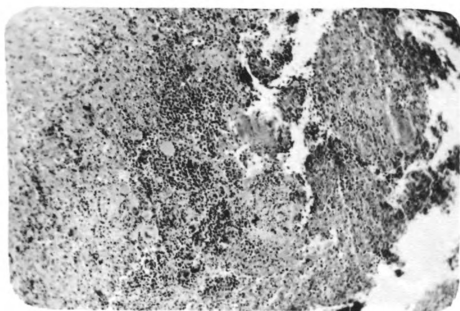


Fig. 33. (Necropsy 1976X) Endometrium from rabbit 240 showing necrosis and polymorph infiltration. Hematoxylin-eosin. 155x.

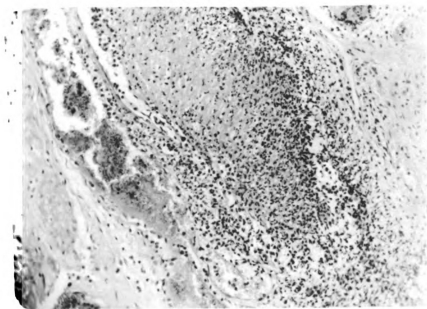


Fig. 34. (Necropsy 1976X) Myometrium of rabbit 240 showing inflammatory infiltration of a venous thrombus with polymorphs and localized disappearance of endometrium. Hematoxylin-eosin. 155x.

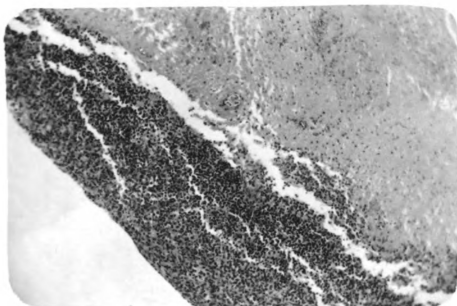


Fig. 35. (Necropsy 1976X) Cotyledon from rabbit 240 showing necrosis with purulent exudate on the surface. Hematoxylin-eosin. 155x.



Fig. 36. (Necropsy 1976X) Fetal membranes from rabbit 240 showing purulent exudate. Hematoxylin-eosin. 155x.

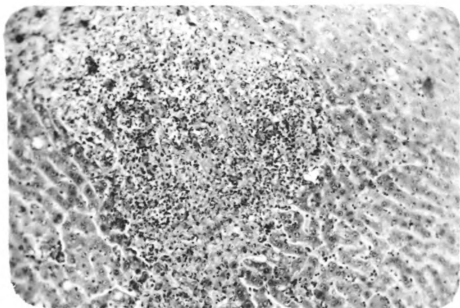


Fig. 37. (Necropsy 1976X) Liver from rabbit 240 showing necrosis and moderate polymorph infiltration. Hematoxylin-eosin. 155x.

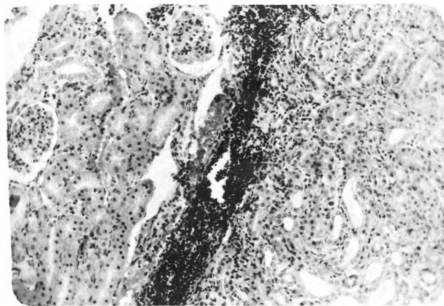


Fig. 38. (Necropsy 1976X) kidney of rabbit 240 showing predominantly lymphocytic infiltration. Hematoxylin-eosin. 155x.

Necropsy 2003X - Rabbit 252 aborted macerated fetuses 4 days after exposure and died 5 days after abortion. (Fig. 39)

Gross pathology:

Uterus - greatly distended, dark purple in color and containing large masses of necrotic debris and purulent exudate. (Fig. 40)

Liver - slightly pale

Histopathology:

Uterus - acute necrotic endometritis with very extensive necrosis of most of the endometrium with infiltration of cells, chiefly polymorphs. A zone of necrosis with heavy infiltration of polymorphs was noted in the cotyledonary attachment with the endometrium; the myometrium showed extensive areas of necrosis, polymorph infiltration, edema and numerous thrombi with mild polymorph infiltration. (Fig. 41 and 42)

Cotyledon - areas of extensive necrosis on the outer surface with marked polymorph infiltration. Some bacteria noted.

Fetal membranes - necrosis and caseo-purulent exudate.

Necropsy 2056X - Young of Rabbit 256 - Of 10 young born after 3 days of oral exposure of the doe, 3 were found dead and the rest died within 4 days of post-natal life.

Gross pathology:

Liver - necrotic foci in the liver (studded with submiliary



Fig. 39. (Necropsy 2000X) Aborted fetuses from rabbit 252 showing varying degrees of disintegration.



Fig. 40. (Necropsy 2003X) Uterus from rabbit 252 showing abundant caseo-purulent exudate covering a diffusely reddened endometrium, and masses of necrotic cotyledons.



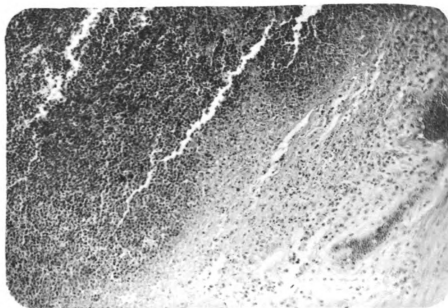


Fig. 41. (Necropsy 2003X) Endometrium from rabbit 252 showing very extensive necrosis and purulent exudate. Hematoxylin-eosin. 155x.

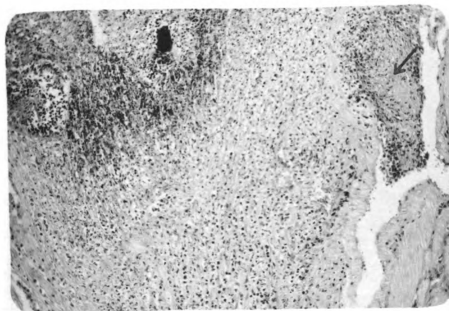


Fig. 42. (Necropsy 2003X) Myometrium of rabbit 252 showing thrombosis (arrow) and necrosis with moderate polymorph infiltration. Hematoxylin-eosin. 155x.

greyish white foci with an area of fatty change.

(Fig. 43)

Histopathology:

Liver - the lesions ranged from a few discrete foci of necrosis with mild polymorph infiltration in some livers to numerous foci of necrosis with moderate to marked polymorph infiltration in others. In one case several foci of necrosis with clumps of bacteria were noted near a large blood vessel. The smaller blood vessels showed localized disappearance of endothelial lining and tendency to thrombus formation. Some congestion and fibrinous exudate was noted in sinusoids. Clumps of bacteria were scattered through the parenchyma. (Fig. 44)

Kidney - a discrete foci with mild polymorph infiltration in one case.

Medulla and cerebellum - some congestion, quite marked in meningeal blood vessels.

Necropsy 2058X - Young of Rabbit 268 - Three young born after 3 days oral exposure of the doe, died within 4 days of post-natal life.

Gross pathology:

Liver - numerous submiliary to miliary greyish white foci, covered with a fibrinous exudate forming adhesions. (Fig. 45)

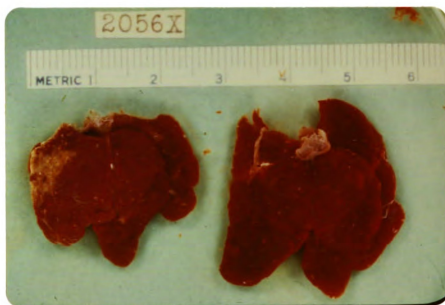


Fig. 43. (Necropsy 2056X) Liver from one of the young of rabbit 256 showing numerous submiliary to miliary greyish-white foci and an irregularly shaped area (left) showing fatty changes in addition.

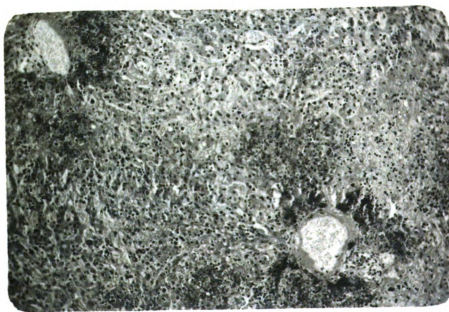
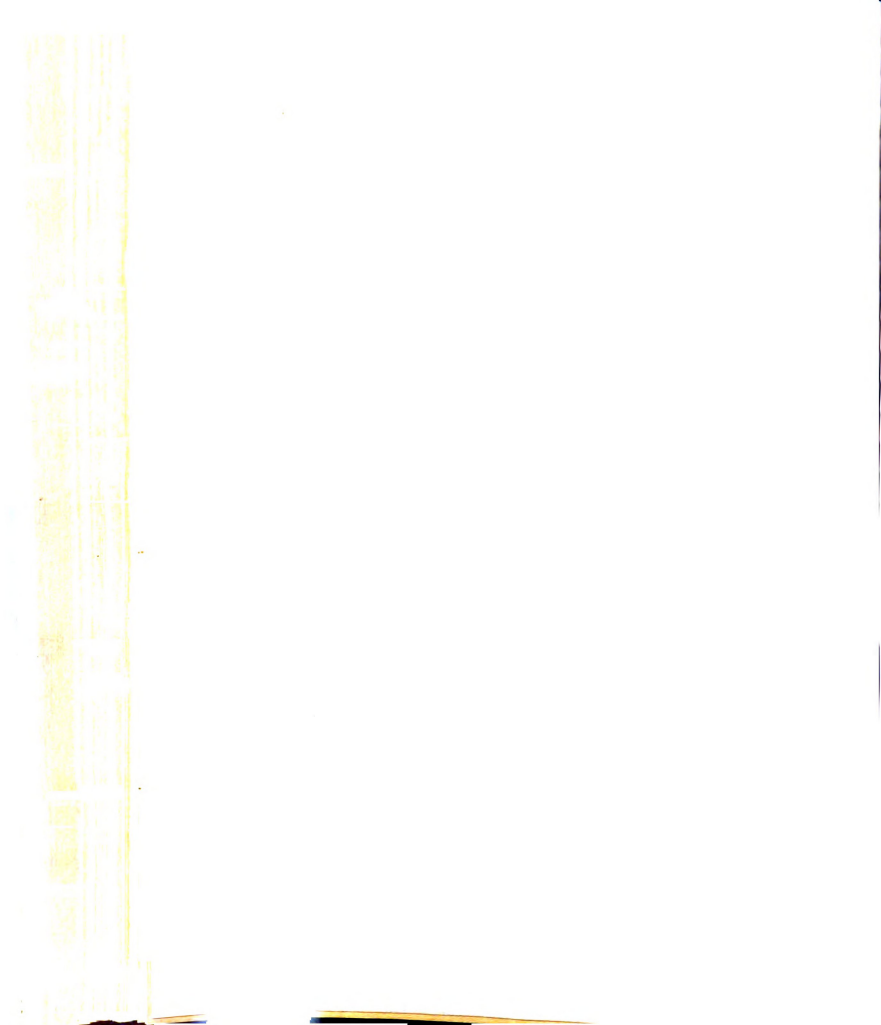


Fig. 44. (Necropsy 2056X) Liver from one of the young of rabbit 256 showing foci of necrosis and fatty changes. Hematoxylin-eosin. 155x.



Histopathology:

Liver - the lesions ranged from discrete foci with a mild polymorph infiltration to numerous extensive foci of necrosis and marked polymorph infiltration.

Most of the necrotic foci were in the vicinity of blood vessels. In some instances the inflammation involved extensive areas of the parenchyma and extended through the vessel walls apparently causing thrombus formation and polymorph infiltration in the thrombus itself. Bacteria could be seen in several foci of necrosis, in some cases in clumps; fibrinous exudate on the surface with polymorph infiltration underneath. (Fig. 46 and 47)

Small intestine - a small focus of polymorph infiltration in serosa with a fibrinous exudate overlying it. (Fig. 48)

Non-pregnant rabbits.

Necropsy 1977X - Rabbit 242 was sacrificed after 8 days of oral exposure to L. monocytogenes.

Gross pathology: No significant lesions.

Histopathology:

Liver - several small foci of lymphocytic infiltration in the parenchyma.

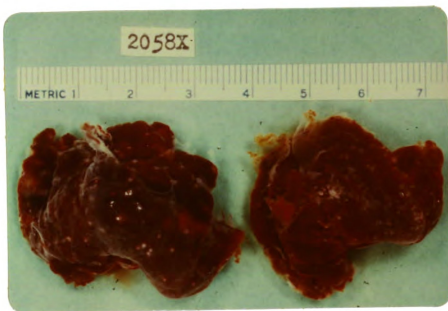


Fig. 45. (Necropsy 2058X) Liver from one of the young of rabbit 268 showing numerous submiliary to miliary greyish-white foci and covered with fibrinous exudate.

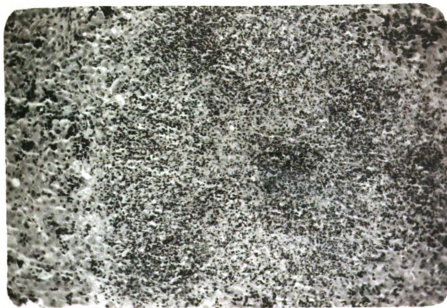


Fig. 46. (Necropsy 2058X) Liver from one of the young of rabbit 268 showing an extensive area of necrosis and polymorph infiltration. Hematoxylin-eosin. 155x.

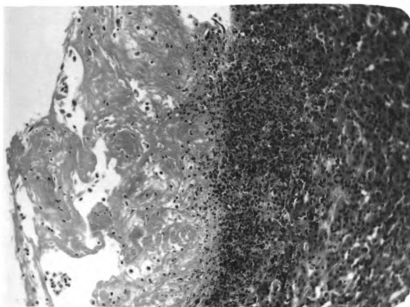


Fig. 47. (Necropsy 2058X) Liver from one of the young of rabbit 268 showing fibrinous exudate on the surface and an underlying zone of polymorph infiltration. Hematoxylin-eosin. 155x.



Fig. 48. (Necropsy 2058X) Small intestine from one of the young of rabbit 268 showing fibrinous exudate on the serosa accompanied by polymorph infiltration. Hematoxylin-eosin. 155x.

### 3. Conjunctival Exposure of Pregnant Rabbits Surviving Previous Oral Exposure

#### a. Materials and Methods

Six pregnant rabbits near term were exposed conjunctivally to L. monocytogenes as described under initial conjunctival exposure. Of these, two (rabbits 258 and 259) were previously exposed orally as non-pregnant animals and the remaining 4 were exposed near term and gave birth to infected young from which L. monocytogenes was isolated.

All had been exposed orally 2-4 months previously. In the intervening period, all but one (rabbit 268) were bred as soon as possible after the first exposure. However, at least 2 weeks elapsed between exposure and breeding, since the does refused the buck. All delivered normal young. Circulating antibody titers in the blood were not determined at the time of second exposure.

#### b. Results: Bacteriology

The results of this exposure followed the pattern of the results obtained in the case of rabbits which were initially exposed near term by conjunctival instillation. Of 37 young, 19 were stillborn or found dead, 14 died within a period of 4 days of postnatal life and 4 survived. The does did not show any apparent symptoms and all of them survived.



TABLE XII

ISOLATIONS OF L. MONOCYTOGENES FROM YOUNG RABBITS (PERINATAL DEATH) FOLLOWING  
CONJUNCTIVAL EXPOSURE OF THE DAM SUBSEQUENT TO PREVIOUS ORAL EXPOSURE

| Rabbit<br>Number | Date<br>of<br>Breeding | Date<br>of<br>Exposure | Date<br>of<br>Kindling           | Necropsy<br>of<br>Number | Number<br>of<br>Young                         | Liver | Stomach<br>content | Colon | Exudate | Brain | Spleen | Intestine | Lung | Kidney | Urine | Amniotic<br>fluid | Remarks  |
|------------------|------------------------|------------------------|----------------------------------|--------------------------|-----------------------------------------------|-------|--------------------|-------|---------|-------|--------|-----------|------|--------|-------|-------------------|----------|
| 254              | 1/22/55                | 2/18/55                | 2/23/55                          | -                        | 2 died in<br>9 days<br>2 survived             | 1+    | 2-                 |       |         |       |        |           |      |        | 2-    |                   | Survived |
| 259              | 1/22/55                | 2/18/55                | 2/22/55                          | 2122X                    | 8 found<br>dead<br>3 died in<br>2 days        | 11+   | 1+                 | 5+    | 2-      |       |        |           |      |        | 1+    |                   | Survived |
| 258              | 2/5/55                 | 3/2/55                 | 5 on<br>3/6/55<br>4 on<br>3/7/55 | 2148X                    | 9 found<br>dead                               | 7+    | 8+                 | 4+    | 1-      | 2+    |        |           |      |        | 1+    |                   | Survived |
| 266              | 2/27/55                | 2/25/55                | 3/30/55                          | 2270X                    | 1 found<br>dead<br>6 died<br>within<br>a week | 6+    | 6+                 | 5+    |         |       |        |           |      |        | 1+    |                   | Survived |
| 268              | 2/27/55                | 3/25/55                | 3/30/55                          | 2271X                    | 1 found<br>dead<br>3 died in<br>4 days        | 2+    | 1+                 | 5+    |         |       |        |           |      |        | 1-    |                   | Survived |

Isolation of L. monocytogenes from the young is shown in Table XII. L. monocytogenes could not be isolated from the does when they were sacrificed over a period ranging from 22 days to one year.

#### c. Results: Pathology

Grossly the liver of most of these young presented the same lesions, as the young of previous exposures (pale discoloration and submiliary to miliary greyish white foci of necrosis. No other lesions were detected. No tissue sections were taken from young.

Histopathology of liver, spleen, urinary bladder, small intestines, large intestines, kidney, uterus of does, revealed nothing significant.

### 4. Oral Exposure of Pregnant Rabbits Surviving Previous Conjunctival Exposure

#### a. Materials and Methods

Two pregnant rabbits, one near term and the other 2-3 weeks pregnant were exposed orally to L. monocytogenes (culture 12255) in the same manner as described under initial oral exposure.

Rabbit 232 was exposed orally near term. She had been exposed conjunctivally near term about 5 months before, and

had given birth to infected young. All but one of the young had died within 7 days. L. monocytogenes had been isolated from the young which died.

Rabbit 220 was exposed orally in the third week of gestation. She had been exposed conjunctivally in the 3rd week of gestation about 6 1/2 months before. She had aborted and L. monocytogenes had been isolated from the aborted fetuses. Both rabbits had been bred during the intervening period and had given birth to apparently normal young. Circulating antibody titers in the blood were not determined at the time of the second exposure.

b. Results: Bacteriology

Rabbit 232 gave birth to 5 young, 4 of which died within the first 6 days of life. One survived but died due to an infected leg injury at 3 weeks of age. L. monocytogenes was isolated from the liver of 2 of 3 young which died on the 3rd day after birth and from the liver, blood, kidney and brain of the 4th young which died on the 6th day after birth, showing central nervous system damage.

Rabbit 220 aborted 5 days after exposure and died 2 days later. L. monocytogenes was isolated from the liver and brain of two aborted fetuses and from several macerated fetuses retained in the uterus. The bacterium was also isolated from the uterus, liver, spleen and urine of the doe.

c. Results: Pathology

Necropsy 2126X - Young of rabbit 232.

Gross pathology:

Liver - lesions varied from pale yellow discoloration to extensive focal necrosis. Necropsy of one young (Fig. 49) showing symptoms suggestive of central nervous system damage revealed moderate hydroperitoneum and a few petechial hemorrhages in the kidney and heart, in addition to numerous submiliary to miliary greyish white foci in the liver.

Histopathology:

Liver - lesions consisted of diffuse fatty changes and multiple focal necrosis, with infiltration of the latter with polymorphs in varying number. In one case (Fig. 50) surface of the liver was covered with fibrinous exudate accompanied by polymorph infiltration.

Necropsy 2350X - Rabbit 232 sacrificed 3 months after giving birth to infected young showed no gross or microscopic lesions.

Necropsy 2173X - The uterus of rabbit 220 was filled with macerated fetuses and a large amount of caseous necrotic debris. No tissue sections were taken.

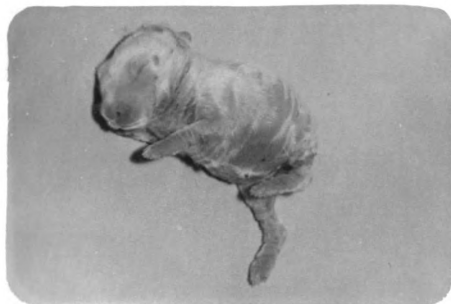


Fig. 49. Six day-old kindling of rabbit 232 showing depression and incoordination suggestive of central nervous system damage.

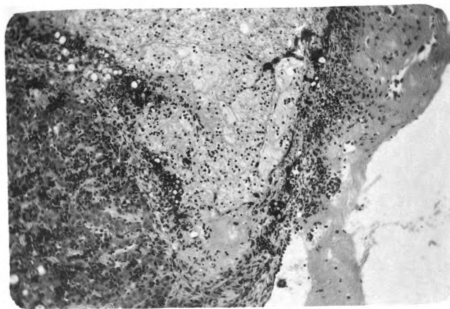


Fig. 50. (Necropsy 2126X) Liver from kindling (Fig. 49) showing surface fibrinous exudate accompanied by polymorph infiltration and fatty changes in the parenchyma. Hematoxylin-eosin. 155x.

## 5. Rebreeding of Infected Rabbits Without Further Exposure

### a. Materials and Methods

Two rabbits (249 and 250) were rebred 8 days after giving birth to infected young following conjunctival exposure.

### b. Results: Bacteriology

Three weeks after rebreeding, rabbit 249 aborted 3 fetuses (Fig. 51) and rabbit 250 aborted one mummified fetus. Rabbit 249 passed one cotyledon on the 6th day, 2 macerated fetuses on the 7th day (Fig. 52), and one mummified fetus on the 10th day following abortion. L. monocytogenes was isolated from the liver and stomach contents of all aborted fetuses, from macerated fetuses, cotyledons, and vaginal exudate.

Six days after expulsion of the last fetus, rabbit 249 was again bred. Twenty-nine days later, eight masses of thick yellow material which appeared to be purulent exudate were found to be on the droppings pan of the cage (Fig. 53). There were also several fragments of tissue which might have been cotyledons. Later the same day this rabbit delivered one very macerated fetus. L. monocytogenes was isolated from the purulent exudate and macerated fetus. The bacterium could not be isolated from blood, urine, liver, spleen,

kidney, either horn of uterus, nor vagina of rabbit 249 when she was sacrificed 9 months after the third gestation.

c. Results: Pathology

Necropsy 1983X - Young of rabbit 249, from first gestation (see under initial conjunctival exposure experiment near term).

Necropsy 2014X - Aborted fetuses following second gestation (without further artificially induced exposure), showed no significant gross or microscopic lesions.

Necropsy 2064X - Aborted fetuses following third gestation (without further artificially induced exposure) showed marked maceration and disintegration and were covered with purulent exudate. Histopathology revealed necrosis of cotyledons and caseo-purulent exudate on the skin of macerated fetus. (Fig. 54)

Necropsy 2420X - Rabbit 249 could not be bred any longer in spite of numerous attempts, and was sacrificed 9 months after the third gestation.

Gross pathology:

Uterus - left horn appeared quite large; right horn was normal in size but very dark purple in color. Both horns had a few white areas that appeared to be old scars. There were several fibrotic

adhesions from the uterus to the parietal layer of peritoneum and intestine. (Fig. 55)

Ovaries - left ovary contained many abscesses about 1 mm. in diameter, and a few small cysts. The right ovary showed 4 small abscesses and several cysts.

Kidneys - several small focal pitted areas in the cortex and many pale streaks in the medulla.

Histopathology:

Liver - mild subacute interstitial hepatitis.

Uterus - extensive proliferative processes in the endometrium (Fig. 56).

Kidney - mild subacute interstitial nephritis.

Necropsy 2451X - Rabbit 250 was sacrificed due to complications unrelated to the after-effects of this experimentation.







Fig. 51. (Necropsy 2014X) Aborted fetuses from rabbit 249. This doe gave birth to infected young from induced exposure, was rebred, and aborted the next time without further known exposure.



Fig. 52. (Necropsy 2014X) Macerated fetuses of rabbit 249 expelled on the 7th day following abortion of fetuses shown above (Fig. 51).



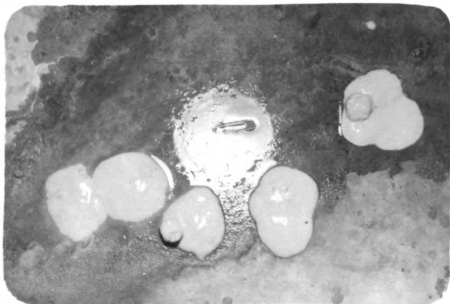


Fig. 53. Grayish white vaginal discharge from rabbit 249 after abortion (the third gestation to be affected by a single exposure).

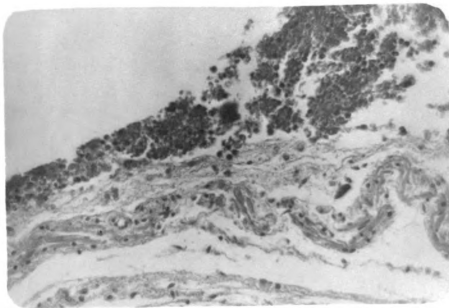


Fig. 54. (Necropsy 2064X) Caseo-purulent exudate on the surface of the racinated skin of fetus from rabbit 249. Hematoxylin-eosin. 155x.





Fig. 55. (Necropsy 2420X) Fibrotic adhesions on the uterus from rabbit 249, extending to parietal peritoneum and intestinal serosa.

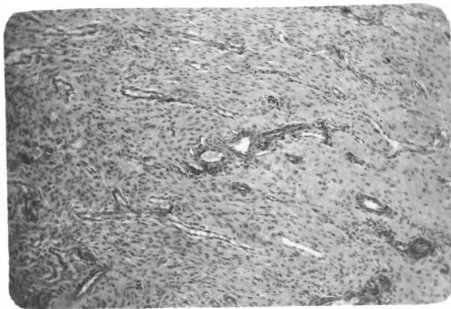


Fig. 56. (Necropsy 2420X) Endometrium of rabbit 249 showing the proliferative process.

## B. Sheep Experiments

### 1. Oral Exposure

#### a. Materials and Methods

Two pregnant ewes near term were exposed to L. monocytogenes by adding suspensions of the bacterium to drinking water. Thirty-two ounce "Sani-glas" prescription bottles containing approximately 100 ml. of tryptose agar (Difco) were inoculated with a distilled water suspension of freshly grown culture (12255) and incubated at 37° C for 18-24 hours. They were then filled with tap water and agitated to free the culture from the agar surface. Each day 64 ounces of this suspension were added to approximately 10 quarts of tap water in a galvanized pail. This constituted the only source of fluid intake for the ewes for a period of seven days. All aborted fetuses, young which died or were sacrificed, and the dams which died or were sacrificed, were examined post mortem and the liver, spleen, kidney, lungs, heartblood, stomach contents, urine, brain, spinal fluid, uterus, cotyledons and exudates were cultured as described for the rabbit experiments (oral exposure).

#### b. Results: Bacteriology

Sheep 1 gave birth to slightly premature stillborn twins 6 days after oral exposure. L. monocytogenes was

isolated from the uterus, liver, brain and udder of the sheep and from liver, stomach content, blood, brain and spinal fluid of the stillborn lambs. Sheep 2 gave birth to a lamb 7 days after oral exposure which died 10 days later.

L. monocytogenes was isolated from cotyledons and from the liver, kidney, and brain of the lamb. This sheep was sacrificed 37 days after the exposure. L. monocytogenes could not be isolated from any visceral organ including brain.

#### c. Results: Pathology

Necropsy 2142X - Sheep 1 - During the next nine days after parturition this ewe showed a thick blood-tinged, mucus-like putrid discharge from the vagina. She was inappetent and progressively became very weak, emaciated and dyspneic and expired on the ninth post-partum day. There were no symptoms suggestive of a central nervous disorder. (Fig. 57)

#### Gross pathology:

Uterus - very extensive necrosis of the endometrium and myometrium; uterine cavity filled with a thick, putrid, sanguino-purulent exudate containing necrotic cotyledons. (Fig. 58)

Liver - moderately pale yellow and friable.

Kidney - mild pale yellow discoloration.

Intestine - moderate number of nodular worm lesions.

#### Histopathology:

Uterus - tissue section not taken.





Fig. 57. Sheep 1 showing prostration and vaginal exudate on the 8th post-partum day, one day before death.



Fig. 58. (Necropsy 2142X) Uterus from sheep 1 showing necrotic to purulent endometritis with sanguino-purulent exudate containing shreds of necrotic tissue.

Liver - moderate centrilobular fatty metamorphosis.  
 Kidney - moderate fatty change.  
 Intestine - subacute necrotic enteritis with congestion.  
 Lungs - marked congestion and edema.  
 Spleen - marked congestion

Necropsy 2121X - Stillborn twin lambs of Sheep 1

Gross pathology:

Kidney - hemorrhagic in both the lambs.  
 Mesenteric lymph nodes - hemorrhagic in both the lambs.  
 Liver - pale yellow in the female lamb and congested in the male.  
 Brain - slight congestion in the male.  
 Lungs - petechial hemorrhages in female.  
 Heart - petechial hemorrhages in epicardium of each.

Histopathology:

Lymph node - extensive hemorrhage and congestion.  
 Lungs - acute broncho-pneumonia with congestion, serous and purulent exudate, and some necrosis.  
 Liver - moderate diffuse fatty changes.  
 Kidney - moderate subcapsular petechial hemorrhage, congestion.

Necropsy 2216X - Sheep 2 - Seven days after oral exposure to L. monocytogenes, this ewe gave birth to what appeared to be a full-term lamb. No clinical manifestation of any sickness was noticed except slight inappetence. The ewe was sacrificed one month postpartum.

Gross pathology:

Uterus - incomplete involution; some caseo-purulent material in the uterine cavity.

Histopathology:

Uterus - subacute necrotic endometritis with marked congestion; edema, cystic degeneration of uterine glands; some proliferation of fibroblasts; infiltration with eosinophiles and some polymorphs.

Lungs - mild patchy subacute broncho-pneumonia with congestion, hemorrhage, infiltration with lymphocytes and numerous eosinophiles.

Small intestine - mild subacute enteritis, with desquamation and coagulation necrosis of mucosa and infiltration with lymphocytes, plasma cells and eosinophiles.

Necropsy 2141X - Lamb of Sheep 2 was born after the ewe was exposed orally to L. monocytogenes for seven days. It was very weak and stood only with difficulty and for short periods of time. Most of the time it lay down and was never seen suckling the ewe. It became progressively weaker and unable to stand. (Fig. 59) It showed symptoms suggestive of a central nervous system disorder, including twitching of the muscles of the face and forelegs, incoordination, and torticollis. It expired on the eighth post-natal day.

Gross pathology:

Lungs - slight pneumonia of the apical lobes.

Histopathology:

Lungs - acute broncho-pneumonia with congestion, purulent exudate, and some necrosis; atelectasis and compensatory emphysema. (Fig. 60)

Liver - marked congestion.



Fig. 59. Moribund lamb from sheep 2 showing extreme depression and weakness 7 days after birth.

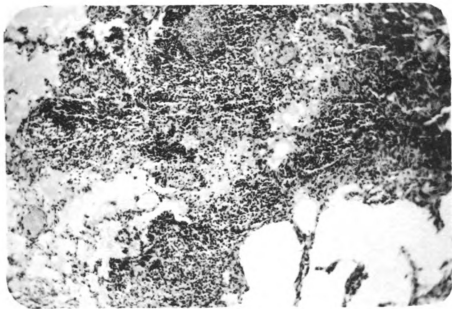


Fig. 60. (Necropsy 2141X) Lung from the same lamb (Fig. 61) showing purulent exudate, some necrosis, atelectasis and compensatory emphysema.

### C. Goat Experiments

#### 1. Conjunctival Exposure

##### a. Materials and Methods

Three goats, one near termination of pregnancy, one non-pregnant female, and one castrated male were exposed by conjunctival instillation of L. monocytogenes. The culture (12255) was grown on tryptose agar (Difco) slants at 37° C for 18-24 hours. It was then suspended in sterile distilled water to a density approximating the number 10 tube of the McFarland nephelometer when compared in a Cenco-Sheard-Sanford photelometer. Four drops of this suspension were instilled into the conjunctival sac of the left eye by means of a capillary pipette. All aborted fetuses, young which died or were sacrificed and all dams which died or were sacrificed, were examined post mortem and the liver, spleen, kidney, lungs, heartblood, stomach contents, urine, brain and spinal fluid, uterus, cotyledons, and exudates were cultured as described under the rabbit experiments (oral exposure).

##### b. Results: Bacteriology

Two days following the instillation of L. monocytogenes into the conjunctival sac of the pregnant goat, a slight serous discharge from the eye was noticed. This persisted

for two days after which the eye appeared normal. The goat did not show any apparent signs of sickness. On the 26th day after exposure the goat appeared to be in labor. The following day she was prostrate and unable to get up. Caesarian section was performed under pentothal anesthesia. Three apparently healthy full term kids were delivered. The dam was given 12 million units of penicillin and appeared to be resting quietly. Three cultures from the cotyledons and amnionic fluid remained sterile. The kids suckled colostrum in the evening and again late at night. The following day the kids appeared normal and suckled a little milk from the dam. However, the dam appeared quite ill and by mid-morning showed symptoms of a central nervous system disorder; incoordination, convulsions, twitching of the facial muscles and running motions suggestive of listeric encephalitis. (Fig. 61) Rectal temperature was 106.5. In the evening she was given 1000 ml of cow's milk, but no medication. The next day she was given 500 ml. of cow's milk. The rectal temperature came down to 104.0 and the animal was quiet and calm. However, she made no attempt to get up and expired in the evening, approximately 66 hours after Caesarian section. L. monocytogenes was isolated on initial culture from the brain and liver. One of the three kids was sacrificed on the seventh postpartum day. Necropsy and cultures revealed nothing significant. Another kid was sacrificed at

the age of five months due to a scrotal injury. No other lesions were detected and all cultures remained sterile. The remaining kid is presently a year old and apparently normal.

The non-pregnant female and castrated male goats exposed in the same manner showed slightly increased lacrimation in the exposed eye for 3 to 4 days following conjunctival instillation of L. monocytogenes. After this the eye appeared normal and there was no further indication of illness in either animal. The castrated male goat was re-exposed in both eyes 15 days after the first exposure and was sacrificed 10 days later. L. monocytogenes could not be isolated from any tissue.

#### c. Results: Pathology

Necropsy 2102X - Goat 2, castrated male.

Gross pathology: revealed nothing significant.

#### Histopathology:

Medulla - moderate lymphocytic perivascular "cuffing" and perivascular hemorrhages.

Cerebellar stalk - moderate lymphocytic perivascular cuffing and some neuroglial proliferation.

Liver - moderate centrilobular fatty metamorphosis.

Necropsy 2286X - Goat 7 died about a month following conjunctival exposure, after showing involvement of central nervous



system. (Fig. 61)

Gross pathology:

The goat had shown a serous discharge from the eye for 2 days but no appreciable inflammatory reaction could be detected.

Uterus - a little caseo-purulent exudate and necrotic cotyledons.

Histopathology:

Medulla - very marked perivascular infiltration with mononuclear leucocytes (mainly lymphocytes) and occasional polymorphs; areas of necrosis with very little infiltration to areas of necrosis with infiltration of large number of polymorphs and tendency toward liquefaction; some sections showed very extensive early suppurative lesions; limited neuroglial proliferation. (Fig. 62, 63 and 64)

Cerebellar stalk - moderate lymphocytic perivascular infiltration.

Cerebrum - some edema and slight meningitis with leucocytic infiltration, chiefly lymphocytes.

Liver - moderate centrilobular fatty metamorphosis.

No other tissue sections were taken.



Fig. 61. Goat 7 with listeric encephalitis 28 days following conjunctival exposure to L. monocytogenes.

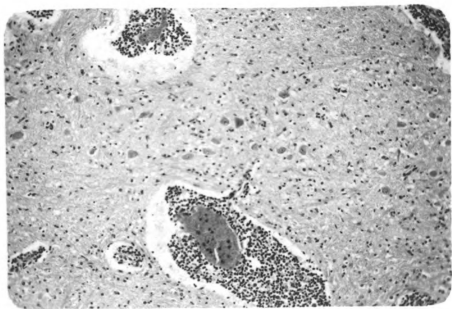


Fig. 62. (Necropsy 2286X) Medulla oblongata of goat 7 showing perivascular "cuffs" formed by infiltration of leukocytes, predominantly lymphocytes. Hematoxylin-eosin. 155x.

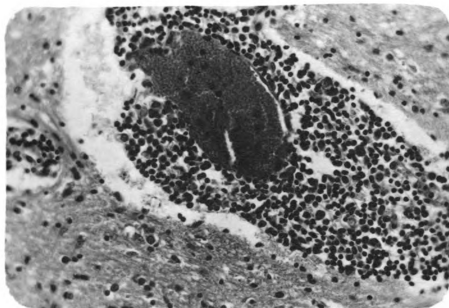


Fig. 63. (Necropsy 2286X) Same tissue as Fig. 62.  
325X.

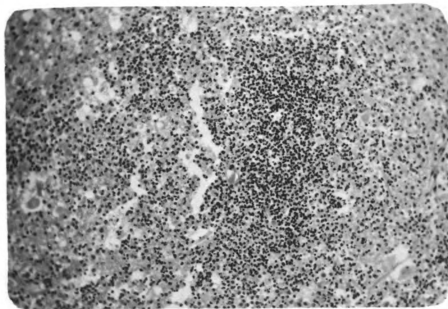


Fig. 64. (Necropsy 2286X) Medulla oblongata of goat  
7 showing necrosis and polymorph infiltration with  
tendency toward liquefaction and early suppuration.  
Hematoxylin-eosin. 155x.

## 2. Oral Exposure

### a. Materials and Methods

Three pregnant goats and a castrated male goat were exposed to L. monocytogenes by adding suspension of the culture (12255) to the drinking water as described under the sheep experiment (oral exposure).

### b. Results: Bacteriology

Of the three pregnant goats exposed orally to L. monocytogenes, goat 4 on the eighth post-exposure day was listless and inappetent and reluctant to move. Although she still could get up the ninth day, she died the tenth day after exposure. L. monocytogenes was isolated on primary culture only from the liver and some cotyledons. Some cotyledons contained the bacterium in large numbers while cultures prepared from others remained sterile. Cultures from the kidney and brain became positive for L. monocytogenes after 36 days of refrigeration. Three kids were in utero. L. monocytogenes was isolated from the liver, stomach contents and brain of both of the two female kids, from the lungs and kidney of one of them, but there were no isolations from the one male kid.

The other two pregnant goats showed no indications of illness following oral exposure of L. monocytogenes. However,

goat 5 aborted twins on the 12th post-exposure day and goat 6 aborted triplets on the 13th post-exposure day. All five aborted fetuses appeared to be in about the fourth month of gestation. Only one appeared to have been dead for some time before expulsion. A few small yellow necrotic foci were observed in the livers of four. There were no other detectable lesions. L. monocytogenes was isolated from the uterus of goat 6 (Necropsy 2217X) which was sacrificed 7 days after abortion. The liver, stomach contents, kidney, brain, lungs and heartblood of all 5 fetuses were positive for L. monocytogenes. The castrated male showed no apparent signs of illness. L. monocytogenes was isolated from the kidney, urine and nasal passages when it was sacrificed 10 days after oral exposure.

#### c. Results: Pathology

Necropsy 2103X - Goat 3, castrated male.

##### Gross pathology:

Intestine - mucosa highly congested throughout the length.

Kidney - a few pale greyish-white focal areas in the cortex.

##### Histopathology:

Small intestine - marked congestion, desquamation of epithelium at the tip of villi.

Large intestine - acute catarrhal enteritis with marked congestion and a few areas of necrosis infiltrated with some polymorphs; hyperplasia of lymphoid follicles.

Liver - moderate congestion, mild centrolobular fatty change.  
 Gall bladder - very marked congestion and desquamation of  
 epithelium.

Necropsy 2172X - Goat 4 died 10 days after oral exposure  
 without having aborted.

Gross pathology:

Liver - moderate fatty metamorphosis.  
 Lungs - moderate edema.  
 Vagina - contained a small amount of mucopurulent exudate.  
 Uterus - contained 3 kids at about 4 months of gestation.  
 The livers of the kids showed some yellowish discoloration.

Histopathology:

Liver - moderate centrolobular fatty metamorphosis.  
 Lungs - moderate edema and congestion.  
 Kidney - some hemorrhage in the cortex, marked degenerative changes (resembling fatty change and necrosis), in the proximal convoluted tubules. It could not be ascertained with certainty whether the karyolysis was antemortem.  
 Spleen - diffuse marked congestion and hemorrhage.  
 Mesenteric lymph node - marked congestion and hemorrhage.  
 Heart - marked congestion of coronary vessels.  
 Fetal liver - marked congestion and hemorrhage.

Necropsy 2190X - Aborted fetuses of goat 5

Gross pathology:

Goat 5 aborted twin kids 12 days after oral exposure. The male fetus showed numerous focal greyish white areas of necrosis whereas the female showed only a few foci. The female appeared to have been dead for a longer period of time than the male.

Histopathology:

Liver - marked congestion, focal necrosis, containing large clumps of bacteria near the margin of the zone of coagulation necrosis. (Fig. 65 and 66)

Necropsy 2217X - Goat 6 aborted triplets (Fig. 67) on the 13th day of oral exposure and was sacrificed 7 days later.

Gross pathology:

Uterus - incompletely involuted, edematous, filled with purulent exudate and caseo-necrotic debris. (Fig. 68)

Histopathology:

Uterus - subacute endometritis with marked edema and congestion of endometrium; moderate infiltration of lymphocytes; purulent exudate in the uterine cavity. (Fig. 69 and 70)

Liver - moderate centrilobular fatty changes.

Spleen - some congestion and hemorrhage.

Small intestine - marked congestion in the mucosa.

Kidney - marked congestion.

Necropsy 2191X - Aborted fetuses of goat 6

Gross pathology:

Liver - several miliary greyish white foci in the livers of  
all three fetuses. (Fig. 71)

Histopathology:

Liver - marked congestion, atrophy of hepatic cords,  
moderate focal necrosis containing large clumps  
of the bacteria. (Fig. 72)



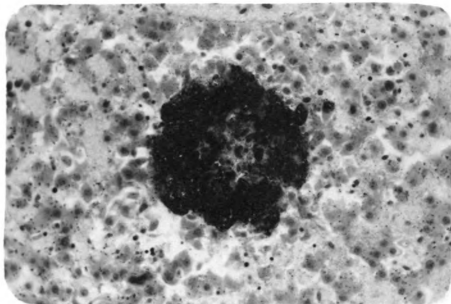


Fig. 65. (Necropsy 2190X) Liver of one of the aborted fetuses from goat 5 showing a large clump of bacteria. Hematoxylin-eosin. 820x.

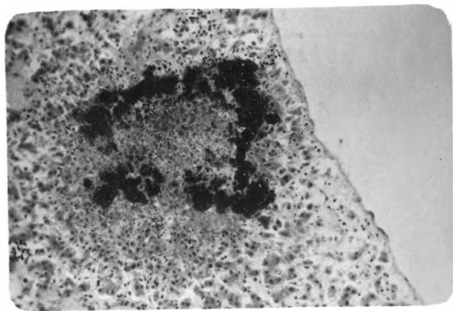


Fig. 66. (Necropsy 2190X) Liver of one of the aborted fetuses from goat 5 showing clumps of bacteria near the periphery of a necrotic focus. Hematoxylin-eosin. 820x.



Fig. 67. One of the aborted fetuses from goat 6 (about 4 months gestation).



Fig. 68. (Necropsy 22174) Uterus from goat 6 showing edema, incomplete involution and caseo-purulent exudate in the uterine cavity.

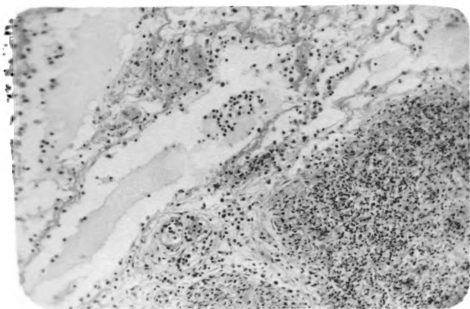


Fig. 69. (Necropsy 2217X) Endometrium from goat 6 showing edema, necrosis and polymorph infiltration. Hematoxylin-eosin. 155x.

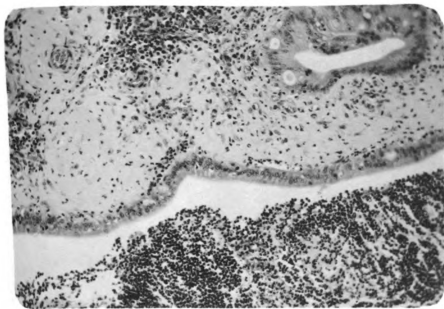


Fig. 70. (Necropsy 2217X) Endometrium from goat 6 showing purulent exudate on the surface. Hematoxylin-eosin. 155x.



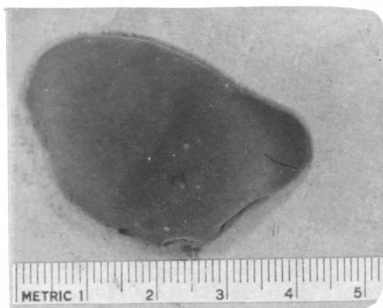


Fig. 71. (Necropsy 2191X) Portion of liver from one of the aborted fetuses of goat 6 showing submiliary greyish white foci.

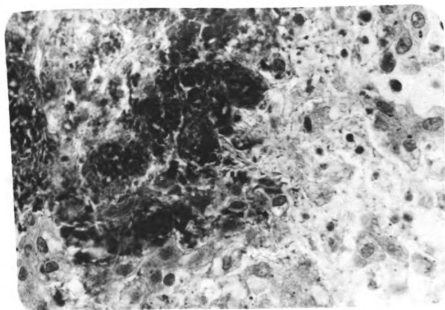


Fig. 72. (Necropsy 2191X) Liver from one of the aborted fetuses of goat 6 showing necrosis and large clumps of bacteria. Hematoxylin-eosin. 820x.

## V. DISCUSSION

The results of the rabbit experiments revealed that abortion or perinatal death of young can be consistently induced by either conjunctival or oral exposure to L. monocytogenes. They also demonstrated that if the infected conceptus were quickly and completely expelled, the doe usually survived whereas if the infected conceptus and macerated fetuses were retained in the uterus the doe died. This may explain the death of several does in the course of the experiment.

Anton (1934) and Morris and Julianelle (1935) observed that the instillation of the cultures of L. monocytogenes into the conjunctival sac of rabbit or guinea pig produced a marked "local" purulent conjunctivitis and keratitis. On the basis of this specific reaction Julianelle and Pons (1939) suggested it as a quick method of identification of L. monocytogenes and since then this reaction has been accepted as one of the standard technics along with other bacteriological and biological tests for identifying this bacterium. Julianelle and Moore (1942) further studied the ophthalmic response in several species of animals (especially in rabbits and guinea pigs) and reported that the ocular reaction after instillation of L. monocytogenes is characterized by

an inflammatory response in the conjunctiva and cornea without appreciable involvement in the deeper structures of the eye. They report that, "in general, the cellular infiltration in the first few days consists principally of lymphocytes but by the 5th day, the proportion of polymorphonuclear leukocytes increases until these cells predominate by the 10th day. Edema of the involved structures is a prominent feature at the height of the infection. Vascularization of cornea develops concomitantly with other evidences of inflammatory response. The pathological changes rapidly recede and the eye generally heals without scarring 2 to 3 weeks after instillation of the organism." This reaction led Julianelle and Moore (1942) and others to believe that it is a "local" eye reaction.

However, Graham, et al. (1943) and Gray et al. (1948) reported death due to listeric encephalitis of a pig and a rabbit, respectively, following conjunctival instillation of L. monocytogenes. Anton (1934) observed death due to listeric septicemia in a one-week-pregnant rabbit and abortion in another rabbit late in gestation following conjunctival instillation of L. monocytogenes. Belin (1947) observed abortion in a guinea pig following conjunctival instillation of the bacterium.

The results of this study confirm experimentally the observation of these latter authors and clearly demonstrate

that the bacterium is not confined in the so-called "local" eye reaction (kerato-conjunctivitis) as previously supposed by Julianelle and Moore (1942) and others. The results also emphasize the fact that L. monocytogenes is not only capable of setting up an inflammatory process in a mucous membrane but also may gain entrance to the body through the apparently intact mucous membrane, and may cause abortion and perinatal death of young in pregnant rabbits. This was further suggested by the finding that conjunctival instillation of L. monocytogenes produced a listeric encephalitis in a pregnant goat 30 days after exposure. In a castrated male goat, histopathological studies of the brain revealed a mild encephalitis, in spite of the fact that the animal did not show noticeable symptoms and the bacterium could not be isolated following conjunctival exposure to L. monocytogenes.

Asahi and Hosoda (1952 — 1953) claimed the production of encephalitis in goats following conjunctival exposure of L. monocytogenes. These authors reported the bacterium reaches the medulla by way of the trigeminal nerve and then spreads to other brain tissues. While this claim needs further confirmation, it does appear that the pathogenesis of listeric encephalitis in ruminants has some unusual features. This is suggested by the fact that encephalitis was not demonstrated following conjunctival or oral exposure of rabbits or oral exposure of sheep and goats, although L. monocytogenes was isolated from the brain in several cases representing all



three species. It is also interesting to note that L. monocytogenes could not be isolated from the placenta, the young, or the reproductive tract of the goat which died of listeric encephalitis following conjunctival instillation (it was isolated from brain and liver). The observation that no abortion and perinatal death of young was noted in several natural outbreaks of listeric encephalitis in pregnant sheep further suggests variation in pathogenesis. Though this study clearly indicates that conjunctival exposure of L. monocytogenes may incite listeric encephalitis, no isolation of the bacterium has been reported from the eye of ruminants with natural infection. Other portals of entry, especially intranasal or via the respiratory tract, are suggested by Gill (1933), and Olafson (1940) and hematogenous pathogenesis is suggested by Graham *et al.* (1943) and Olson (1951). Osebold and Inouye (1954) are inclined to favor a hematogenous pathogenesis in spite of the fact that they could isolate L. monocytogenes in 5 out of 7 instances from the brain of rabbits following intranasal exposure. Asahi and Hosoda (1953) claim to have induced listeric encephalitis in 4 of 11 goats following inoculation in the submucosa of the lip, and in 2 of 3 goats following exposure to rough feed contaminated with L. monocytogenes. These authors suggest entrance of the bacterium through small wounds caused by the rough feed. Felsenfeld (1951) reported an outbreak of listeric conjunctivitis

in workers in a poultry processing plant. He isolated L. monocytogenes from the spleen of five apparently normal birds which were processed at the plant. This incident suggests the possibility of the conjunctival as one of the portals of entry.

An oculoglandular form of listeric infection in Russia is reported by Pletneva and Stiksove (1950) and Shmeleva (1953). They could not isolate L. monocytogenes from the patients but reported the diagnosis on the basis of agglutination tests. Shmeleva (1953) reported direct contact with animals in 4 cases of conjunctivitis in human patients.

The fact that abortion and perinatal death of young in rabbits, sheep and goats followed oral exposure to L. monocytogenes re-emphasizes the contention that L. monocytogenes can pass through the intact mucous membrane.

Patocka (1953) has suggested the possibility of a filterable form of L. monocytogenes, but this has not been substantiated so far.

Olson (1956) reported the isolation of a listeriosis-enhancing agent (LEA) which in combination with L. monocytogenes via intranasal exposure results in encephalitic form of listeriosis in sheep. Lesions were confined to the central nervous system.

Listeriosis in the young of all species of animals so far observed, including man, is usually septicemic and

terminates fatally. The experimentally induced disease as observed in this study closely resembles that of the natural epidemic of perinatal death of human infants in Germany and elsewhere (Table IV) and that of perinatal death of calves, lambs, foals and pigs reported elsewhere (Table III). The does exposed near term usually survived. This also is true of the naturally infected mothers in all species. Isolation of L. monocytogenes from the viscera of young (especially liver and stomach content) and from the placenta, vaginal exudate and uterus of the dam supports the contention of Erdmann and Potel (1953) who suggest the sequence of events leading to fetal infection as follows: infection of the dam; transfer of the bacterium across the placenta resulting in septicemia with development of lesions in the fetus; shedding of the bacteria through the urine, contaminating the amnionic fluid; aspiration and swallowing of the contaminated amnionic fluid resulting in a secondary infection of the fetus by way of the respiratory and digestive tract. Similar views are held by Linzenmeier et al. (1953), Hahnefeld and Nisolk (1954) and Flamm (1955).

Oral exposure of rabbits surviving previous conjunctival exposure, and vice versa, clearly indicated that the previous exposure (2-6 months later) did not provide protection to further exposure. Circulating antibody titers were not determined immediately before the second exposure; however,

Julianelle (1941) contends that agglutinating antibodies are not a measure of protection against infection with L. monocytogenes.

L. monocytogenes was found to survive at least 4 weeks in the reproductive tract of a rabbit which died after conjunctival exposure. This conforms to the report of Levi et al. (1952) who isolated L. monocytogenes from the ovary of a heifer which was slaughtered two and a half months after listeric abortion.

In the present study, conjunctival exposure to L. monocytogenes caused in one rabbit, in addition to infection of fetuses of the current gestation, two subsequent abortions without any further exposure. These abortions took place about the 3rd week of gestation. It remains to be determined as to how conception could occur and the fetuses develop up to a certain stage of gestation in the presence of L. monocytogenes. The question of some hormonal factor or fetal tissue affinity is a subject for further research.

Other routes of infection, especially the urogenital tract, must be considered in cases of abortion and perinatal death of young. An intrauterine infection induced by vaginal instillation of L. monocytogenes has been observed in an associated study in this laboratory and fetal death and absorption of embryos in rabbits following intravaginal exposure on the 7th day of gestation has been reported by Osebold and Inouye (1954).

The isolation of L. monocytogenes from the urethral exudate of 5 men with gonorrhea by Wenckebach (1953), from vaginal mucus of a cow by Stenberg (1953), from the cervix of a so-called repeat-breeder cow by Gray and McWade (1954) and from vaginal exudate of a human patient by Stoot (1954), suggests a venereal mode of infection.

It is quite clear from this study that uterine contents, aborted fetuses and vaginal exudates contain L. monocytogenes in large numbers. On expulsion they may constitute a source of infection by contaminating food and water. Gray et al. (1956) reported that one goat was infected by direct contact with infected goats (which aborted following oral exposure to L. monocytogenes) and their contaminated environment. This is also supported by the observation of Poppen-siek (1944) in which a cow died of listeric encephalitis after grazing on the same pasture where an ovine abortion thought to be due to L. monocytogenes had occurred. Van der Schaaf (1955) reported 2 cases of listeric infection in a farmer and in a veterinarian after handling a case of bovine abortion. L. monocytogenes was isolated from blood and spinal fluid of the farmer who died and from blood of the veterinarian who survived following therapy.

In the present study, L. monocytogenes was isolated in one instance from the milk of a rabbit and from the udder of a sheep which died following oral exposure. Potel (1953-54)

isolated L. monocytogenes from a case of bovine mastitis. Gray and Thorp (1956) have shown on rabbit experiments that L. monocytogenes passes into the milk following oral exposure of the doe. Thus infection of the newborn could be induced by suckling the orally exposed doe.

This study also demonstrates that L. monocytogenes is excreted through the urine and may be a source of contamination of feed, possibly by rodents. No cultures of fecal material were made in this investigation; however, it may serve as a source of infection. Potel (1953) reported that meconium of the infected human infant is a very good source for the isolation of L. monocytogenes. This should be further investigated by experimentation on animals. Other phases deserving investigation are the possible roles of rodents, insects and parasites and the possibility of the organism's being a saprophytic and facultative pathogen.

The whole field of the transmission and intertransmissibility of listeriosis is incompletely understood. No reliable diagnostic or clinical tests are available for detection of a carrier state or subclinical case. Therefore prophylactic measures are lacking and need further research.

The presence of the bacteria can be demonstrated in the tissue sections of most of the organs of aborted fetuses and young which have died perinatally thus further confirming the septicemic nature of the disease in these animals.

Gray (1956) reported the isolation of L. monocytogenes from thrombi on the heart valves of a lamb. Hoeprich et al. (1956) have reported a case of subacute bacterial endocarditis due to L. monocytogenes in a human patient. These reports indicate that atypical forms of listeric infection should be investigated.

The exact manner in which L. monocytogenes induces the lesions is incompletely understood. Although the lesions appear to be caused by some bacterial toxins, or toxins, the production of endotoxin or exotoxin has not been demonstrated so far.

In this study, the tendency toward thrombus formation in the liver of young and thrombosis with leukocytic infiltration and endothelial degeneration in the blood vessels of the myometrium, indicate that L. monocytogenes may initiate an inflammatory process in the blood vessels and cause thrombosis. This thrombosis would contribute in the causation of necrosis by interfering with the circulation. This, in turn, would interfere with elimination of toxic metabolic products and thus aid in the furtherance of the necrotic processes. This may also explain the edema of the endometrium.

The encephalitic lesions in the medulla in goats, especially in the one which died following conjunctival exposure to L. monocytogenes, closely resemble the microscopic lesions found in natural cases in sheep and goats. The lesions are

primarily found in the medulla. Asahi and Hosoda (1952 - 1953), on the basis of histopathologic examination of brain and trigeminal nerve sections, claim that the bacterium follows the trigeminal nerve and produces lesions along the pathway of the nerve and then in the medulla. They state that infection may then spread outward even to the meninges. The fact that encephalitic lesions were produced following conjunctival instillation in 2 goats in the present study, tends to support this claim. However, further work is necessary to establish definitely that L. monocytogenes may follow branches of the trigeminal nerve. Hydroperitoneum and fibrinous exudate on the visceral organs, especially on the liver and intestine, were noted in two of the young following oral exposures of pregnant rabbits near term. The microscopic lesions in the gall bladder (necrosis and polymorph infiltration), necrotic exudate and presence of the bacterium in the vicinity of the bile duct, found in this study, apparently have not been previously reported in the literature. The significance of these findings remains to be further explored.

The number of positive isolations of L. monocytogenes from tissues cultured in the present study was considerably enhanced by employment of the isolation technic described by Gray et al. (1948). This technic has been further emphasized in the reports of Gray et al. (1955, 1956).



## VI. CONCLUSIONS

1. Conjunctival instillation of L. monocytogenes in pregnant rabbits results not only in conjunctivitis but also in abortion or perinatal death of young.
2. Oral exposure of pregnant rabbits, sheep and goats to L. monocytogenes causes abortion and perinatal death of young.
3. In case of retention of infected conceptus, the dam may die from septicemia.
4. In perinatal infection of the young in rabbits, sheep and goats, the disease is usually septicemic and almost always fatal. The dam usually survives with little or no apparent symptoms of illness.
5. The bacterium may remain latent in the rabbit for at least 3 weeks after kindling and may cause subsequent abortion, if the animal is rebred within a week after abortion.
6. Evidence of immunity in rabbits (at least within 2-6 months) following survival of any form of exposure under conditions of these experiments is lacking.
7. Conjunctival and oral exposure of non-pregnant rabbits and oral exposure of castrated male goats usually fails to cause systemic disease. However, the organism may be isolated from visceral organs in some cases as long as 10 days after exposure.

8. L. monocytogenes may be excreted in the urine of rabbits and goats.

9. Conjunctival instillation of L. monocytogenes in goats may cause listeric encephalitis.

10. Listeric encephalitis may not accompany uterine infection in goats and sheep following oral exposure to L. monocytogenes.

11. The clinical manifestations of listeriosis apparently vary with species, route of exposure and stage of pregnancy.

12. Gross lesions in aborted fetuses and cases of perinatal death of young in rabbits, sheep and goats include, with variations in degree and extent, the following: yellow discoloration and submiliary to miliary greyish-white foci in the liver and, less regularly, fibrinous adhesions on the surface of the liver and hydroperitoneum.

13. Gross lesions of aborting and parturient does and ewes include, with variations in degree and extent, the following: necrosis of endometrium, with caseous necrotic material, retained conceptus, and purulent uterine exudate. Less consistently observed are: incomplete involution of the uterus in sheep and goats; fibrotic adhesions on the uterus of rabbits which abort several times; and yellow discoloration and small greyish-white foci in the liver of dams dying with septicemia.

14. Histopathologic examination of aborted fetuses and cases of perinatal death of young in rabbits, sheep and goats reveals, with variations in degree and extent, the following: fatty changes and focal necrosis of the liver, with polymorph infiltration and tendency of thrombus formation in the smaller blood vessels. Less frequent findings include: foci of necrosis and polymorph infiltration in heart, lung, kidney, stomach and gall bladder, necrotic exudate in the bile ducts of some young rabbits, and proliferation of fibroblasts and angioblasts in the liver. In addition to hepatic necrotic foci, acute bronchopneumonia may be found prominently in the young of sheep and goats. Bacteria (sometimes in very large clumps) may be demonstrated in several organs especially in liver, heart and kidney.

15. Histopathologic examination of uteri of aborting and parturient does and ewes reveals, with variations in degree and extent, the following: endometritis with necrosis of endometrium and purulent exudate on the surface of mucosa; necrosis and distention of uterine glands with or without cellular exudate; necrosis and thrombosis with polymorph infiltration and endothelial degeneration in the blood vessels of the myometrium. Bacteria (sometimes in large clumps) may be noticed in the endometrium, uterine glands, cotyledons and myometrium.

16. Histopathologic examination of the liver of aborting and parturient does and ewes shows, with variations in degree

and extent, the following: fatty changes, some coagulation necrosis with or without polymorph infiltration; small foci of lymphocytic infiltration and proliferative processes in chronic cases. Subacute interstitial nephritis may be noted in some cases. Edema of the lung is usually observed in sheep and goats.

17. The most prominent microscopic lesions in the conjunctivally exposed goats are seen in the medulla oblongata. They consist of perivascular infiltration, chiefly with lymphocytes, and areas of necrosis with polymorph infiltration and, sometimes, early suppuration.

18. This study supports the contention that intra-uterine infection of the young in animals and man may take place through the placenta.

19. The use of obliquely transmitted light and a binocular scanning microscope is helpful in identifying the colonies of L. monocytogenes.

20. The maceration of tissues and storage under refrigeration for at least 3 months is helpful in the isolation of L. monocytogenes when primary cultures are negative.

## VII. SUMMARY

1. Conjunctival exposure of 9 rabbits (2-3 weeks pregnant) to Listeria monocytogenes caused abortion in 3-6 days and death of 5 does due to septicemia. Oral exposure of 6 rabbits in the same stage of pregnancy caused abortion in 4-6 days and death of 4 does due to septicemia. L. monocytogenes was isolated from liver, spleen, blood, kidney, uterus, cotyledon, brain and vaginal exudates of does, and from the liver, stomach content, and amnionic fluid of aborted fetuses.

2. Conjunctival exposure of 7 rabbits near term caused perinatal death of 57 of 58 young due to septicemia within 7 days. Only one survived. All does except one survived and showed no apparent signs of illness. Oral exposure of 8 rabbits in the same period of gestation caused the perinatal death of all 55 young within 4 days due to septicemia. All does survived and showed no apparent signs of illness. L. monocytogenes was isolated consistently from the liver, stomach content, and cotyledons and in most instances in which brain, blood, spleen, intestine, lung, kidney and urine of the young were cultured.

3. Conjunctival exposure of 6 and oral exposure of 3 non-pregnant rabbits failed to produce apparent infection.

L. monocytogenes was isolated from the uterus of 2 rabbits and liver of one rabbit conjunctivally exposed (tissues not refrigerated) and from the liver of one rabbit orally exposed (after refrigeration).

4. Retention of the infected conceptus usually was followed by death from septicemia whereas the does usually survived if the conceptus was expelled quickly and completely.

5. L. monocytogenes was isolated from a uterine abscess of one rabbit which died 3 weeks post partum following conjunctival exposure.

6. Conjunctival exposure of 6 rabbits (near term) surviving previous oral exposure caused perinatal death of 33 of 37 young; 4 survived. The results followed the pattern of initial conjunctival exposure both in production of lesions and isolation of L. monocytogenes from the young.

7. Oral exposure of 2 rabbits (one 2-3 weeks pregnant and the other near term) surviving previous conjunctival exposure was followed by abortion and perinatal death as in the case of initial oral exposure.

8. One rabbit which was bred within a week after giving birth to infected young following conjunctival exposure, aborted about the third week of gestation. Rebreeding within 6 days after the abortion resulted in subsequent abortion in the 3rd week of the 3rd pregnancy.

9. Oral exposure of 2 pregnant ewes near term resulted in death of the young. One ewe died 9 days post partum due

to septicemia. L. monocytogenes was isolated from the uterus, liver, brain, and udder of the ewe and from the liver, stomach content, blood, brain, spinal fluid, and kidney of the young and from the cotyledons.

10. Conjunctival exposure of 2 goats resulted in encephalitis.

11. Oral exposure of 3 pregnant goats was followed by abortion of all 8 fetuses and death of one of the dams. L. monocytogenes was isolated from the liver, stomach content, kidney, brain, lungs and blood of most of the aborted fetuses.

12. Aborted fetuses and young which were stillborn or died soon after birth revealed liver lesions consisting of varying degree and extent of yellow discoloration and submiliary to miliary greyish white foci. Hydroperitoneum and fibrinous exudate on the surface of the liver were noticed in some young rabbits. Slight pneumonia was noted in one lamb.

13. Uteri of rabbits which aborted showed marked congestion and caseous necrotic masses on the endometrial surface with varying degree and extent of purulent exudate in the uterine cavity. After removal of the caseous material from the uterine mucosa, the endometrium appeared reddened and corrugated; in some cases it was necrotic throughout its depth. The cotyledons were usually necrotic. Yellow

discoloration and small greyish-white foci were noticed in the livers of some does. Incomplete involution of the uterus with some purulent exudate was noted in sheep and goats. The uterus of one sheep which died was extensively necrotic and filled with thick, putrid material containing necrotic cotyledons.

14. Histopathologic examination of the livers of aborted fetuses and young which died perinatally (rabbits, sheep and goats) revealed (a) varying degree and extent of fatty change in the hepatic cells, (b) varying sizes and numbers of foci of necrosis, and (c) variable polymorph infiltration of necrotic areas. Similar necrotic lesions were noted in the heart, kidney, lungs, stomach, and gall bladder in some cases. Fibrinous adhesions were seen on the liver and intestine of some young rabbits. Bacteria could be demonstrated in the liver, bile duct, kidney and heart of rabbits and liver of goats. In one young rabbit, the bile ducts were found to be filled with purulent exudate.

15. Histopathologic examination of the uteri of rabbits which died or were sacrificed after abortion revealed acute necrotic endometritis with varying extent of necrosis and leukocytic infiltration (mainly polymorphs) of endometrium. In some cases, edema of endometrium and dilatation of uterine glands with or without cellular exudate were noted. Clumps of bacteria were often seen in the endometrium, uterine glands



and cotyledons. The myometrium often showed necrosis in variable extent and thrombosis of blood vessels with leukocytic infiltrations. In chronic cases following abortion, proliferative processes and scarring of the uterus were observed. The uteri of sheep and goats showed mild endometritis with some necrosis and edema of endometrium and some purulent exudate on the surface of endometrium.

16. The possible significance of the foregoing findings and some of their implications with regard to further research are discussed.

## BIBLIOGRAPHY

- Alex, R. and J. Potel. 1953. Listeriosis und Schwangerschaft Geburtsh. Frauenheilk. 13:651-659.
- Amanzhulov, S. A. 1953. Listerellesnii Abort Koz, Zabolevanie Koslat i Rezultat Letchenia Sulfantrolom. Veterinariya Moscow 30:22-23.
- Anton, W. 1934. Kritisch-experimenteller Beitrag zur Biologie des Bakterium Monocytogenes. Mit besonderer Berucksichtigung seiner Beziehung zur infektiösen Mononukleose des Menschen. Zbl. Bakt. Abt. I, Orig. 131:89-103.
- Asahi, O. and T. Hosoda. 1952. Studies on listeriosis in domestic animals. II. Studies on experimental listeriosis in goats. Med. and Biol., Japan 24:38-41.
- Asahi, O. and T. Hosoda. 1952. Studies on listeriosis in domestic animals. III. Studies on experimental listeriosis in mice. Med. and Biol., Japan 24:100-103.
- Asahi, O. and T. Hosoda. 1953. Studies on listeriosis in domestic animals. IV. Observations on the route of infections via trigeminal nerve fiber of L. monocytogenes in mice. Med. and Biol., Japan 26:72-75.
- Asahi, O. and T. Hosoda. 1953. Studies on listeriosis in domestic animals. V. Inquiries into the mechanism of spontaneous infection of listeriosis in goats. Med. and Biol., Japan 26:196-199.
- Bandarnayake, A. 1953. An outbreak of listeriosis in goslings. Ceylon Vet. J. 1:40-42.
- Belin, M. 1947. Contributions a l'etude Listeria monocytogenes, Ann. Pasteur Inst., 73:99-101.
- Bergy's Manual of Determinative Bacteriology. 1948. The Williams and Wilkins Company, Baltimore. 6th ed.
- Blester, H. E. and L. H. Schwarte. 1941. Bovine listerellosis in Iowa with studies on a recovered case. No. Am. Vet. 22:729-734.

- Bolin, F. M., J. Turn, S. H. Richard and D. F. Eveleth.  
1955. Listeriosis of a skunk. North Dakota Agr. Exp.  
Sta. Bi-monthly Bull. 18:49-50.
- Breuning, M. and Fr. Fr tzsche. 1954. Uber die Haufigkeit  
der Listeriose bei Neugeborenen. Geburtsh. Frauenheilk.  
13:1113-1123.
- Burn, C. G. 1936. Clinical and pathological features of an  
infection caused by a new pathogen of the genus Listerella.  
Am. J. Path. 12:341-348.
- Burton, A. N. 1955. Listeriosis of sheep in Saskatchewan.  
Canad. J. Comp. Med. and Vet. Sc. 19:221-223.
- Charles, G. 1950. (Isolation from aborted lambs). The  
Institute of Med. Vet. Sci. 12th Ann. Report of Council,  
Adelaide. 39-40.
- de Blicck, L. and J. Jensen. 1942. Listerellose bij Biggen.  
Tijdschr. Diergeneesk. 69:575.
- De Lay, P. D. and G. S. Shultz. 1954. Incidence of listeri-  
osis in man and animals in California. Proc. Book, Am.  
Vet. Med. Assn. 413-417.
- Detmold, J. G. and H. Girgensohn. 1954. Listeriose bei  
Neugeborenen als Folge von diaplantenterer Infektion  
mit Listeria infantiseptica. Geburtsh. Frauenheilk.  
12:1125-1131.
- Erdmann, G. and J. Potel. 1953. Listericse der Neugeborenen  
Granulomatosis infantiseptica. Zeit. Kinderheilk.  
73:113.
- Evans, W. M. and J. E. Sawyer. 1942. Bovine abortion  
apparently due to Listeria infection. Cornell Vet.  
32:448.
- Eveleth, D. F., F. M. Bolin and J. Turn. 1953. Listeriosis  
of sheep and swine. North Dakota Ag. Exp. Sta. Bi-  
monthly Bull. 16:47-51.
- Eveleth, D. F., A. I. Goldsby, F. M. Bolin, G. C. Holm and  
J. Turn. 1953. Epizootology of vibrosis and listeriosis  
of sheep and cattle. Vet. Med. 48:321-323.
- Felsenfeld, O. 1948. Listerella monocytogenes strain iso-  
lated from a human source in Puerto Rico. Puerto Rico  
J. Pub. Health and Trop. Med. 24:24-30.

- Felsenfeld, O. 1951. Diseases of poultry transmissible to man. Iowa State Vet. 13:89-92.
- Ferguson, L. C., E. H. Bohl and W. L. Ingolls. 1951. Listeria monocytogenes associated with bovine abortion. J. Am. Vet. Assn. 118:10-11.
- Finegold, S. M., J. G. Bradley, M. K. Campbell and A. J. Greenberg. 1954. Listeria monocytogenes meningitis. Arch. Int. Med., 93:515-527.
- Flamm, H. 1955. Die Patho-histologische Diagnose der Listeriose in Tierversuch. Schweiz Z. Path. Bakt. 18:270-277.
- Graham, R., H. R. Hester and N. D. Levine. 1939. Listerella from premature bovine fetus. Science 90:336.
- Graham, R., N. D. Levine and C. C. Morrill. 1943. Listerellosis in domestic animals. 99 p. Univ. Ill. Agri. Expt. Station Bull 499.
- Geurden, L. and A. Devos. 1954. Listerellose bij rundvee. Vlaams. Diergeneesk. Tijdschr. 23:1-10.
- Gill, D. A. 1931. "Circling disease" of sheep in New Zealand. Vet. J. 87:60-74.
- Gill, D. A. 1933. Circling disease: a meningoencephalitis of sheep in New Zealand. Notes on a new species of pathogenic organism. Vet. J. 89:258-270.
- Girard, K. F. and E. G. D. Murray. 1951. Listeria monocytogenes as the cause of disease in man and animals and its relation to infectious mononucleosis from an etiological and immunological aspect. Am. J. Med. Sci. 221:343-352.
- Gray, M. L., H. J. Stafseth, F. Thorp, Jr., L. B. Sholl and W. F. Riley. 1948. A new technique for isolating Listerellae from the bovine brain. J. Bact. 55:471-476.
- Gray, M. L., R. N. Nelson and F. Thorp Jr. 1949. Listeria isolated from the liver of a lamb. J. Am. Vet. Med. Assn. 115:103-104.
- Gray, M. L. and G. R. Moore. 1953. Aureomycin in the treatment of ovine and bovine listeriosis, with notes on survival. No. Am. Vet. 34:99-105.
- Gray, M. L. and D. H. McWade. 1954. The isolation of Listeria monocytogenes from the bovine cervix. J. Bact. 68:634-635.

- Gray, M. L. 1954. Listeria monocytogenes. A review and colonial dissociation. Doctoral dissertation. Michigan State College. 120 num. leaves.
- Gray, M. L., Chintamani Singh and Frank Thorp, Jr. 1955. Abortion, stillbirth, early death of young in rabbits by Listeria monocytogenes. I. Ocular instillation. Proc. Soc. Exp. Biol. and Med. 89:163-169.
- Gray, M. L., Chintamani Singh and Frank Thorp, Jr. 1955. Abortion, stillbirth, early death of young in rabbits by Listeria monocytogenes. II. Oral exposure. Proc. Soc. Exp. Biol. and Med. 89:169-175.
- Gray, M. L., Chintamani Singh and Frank Thorp, Jr. 1956. Abortion and perinatal death of young due to Listeria monocytogenes. III. Studies in ruminants. Am. J. Vet. Res. (In press).
- Gray, M. L. and Frank Thorp, Jr. Abortion and perinatal death of young due to Listeria monocytogenes. IV. Transmission through milk. In preparation.
- Gray, M. L., C. A. Lassiter, H. D. Webster, C. F. Huffman, and Frank Thorp, Jr. 1956. The isolation of Listeria monocytogenes from the liver of calves. Vet. Med. (In press).
- Hahnefeld, H. 1954. Ergebnisse von Futterrungeversuchen an Tieren mit Listeria monocytogenes (Pirie). Phophylaxe 1:164-169.
- Hahnefeld, H. and E. Nisolk. 1954. Über das Vorkommen der Listeriose in Ostmecklenburg. Beobachtungen bei Nengeborenen. Dtsch. Gesundheits. 9:149-155.
- Hagemann, U. and H. Simon. 1953. Fruchttd oder Fruhgeburt durch diaplacentare Listerien-infektion. Geburtsh. Frauenheilk. 12:1090.
- Hagemann, U., H. Simon and A. Bienengraber. 1954. Die listeriose der Fruhgeburten. Zbl. Allg. Path. 90:17-23.
- Harbour, A. E. 1941. Listerella infection in a calf in North Wales. Vet. J. 97:401-407.
- Hindmarsh, W. L. and C. C. Blumer. 1932. Cited by Paulsen, M. and G. R. Moule. Infectious ovine abortion. The Australian Vet. J. 29:133-139.

- Hoeprich, P. D. and Hyman M. Chernoff. 1956. Subacute bacterial endocarditis due to Listeria monocytogenes. Am. J. Med. 19:488-494.
- Hulphers, . 1911. Cited by Murray, 1953. The story of Listeria. Trans. Royal Society of Canada, 47:sec. 5, 15-21.
- Jansen, J. 1945. Listerellose By het konijn door Listerella-Infectie. Tijdsch. Diergeneesk. 70:209-210.
- Jensen, R. and D. R. Mackey. 1949. Listerellosis in cattle and sheep. J. Am. Vet. Med. Assn. 104:420-424.
- Jepsen, A. 1942. A case of listerella infection in a lamb. Acta Path. Micro. Scand. 19:423-432.
- Johnston, W. H., S. A. Morton, M. H. Wong, and T. E. Roy. 1955. Septicemia of the newborn due to Listeria monocytogenes. The Canadian Med. Assn. J. 73:402-405.
- Julianelle, L. A. and C. A. Pons. 1939. Identification of Listerella monocytogenes. Proc. Soc. Exp. Biol. and Med. 40:362-363.
- Julianelle, L. A. 1941. Biological and immunological studies of listerella. J. Bact. 42:367-383.
- Julianelle, L. A., and E. Moore. 1942. Pathological changes in Listerella infection, particularly of the eye. Am. J. Path. 18:813-825.
- Kerlin, D. L. and R. Graham. 1945. Studies of listerellosis. VI. Isolation of Listerella monocytogenes from liver of pig. Proc. Soc. Exp. Biol. and Med. 58:351-352.
- Khalimbekov, M. M. 1952. (Listeria monocytogenes in sheep). Veterinariya, Moscow. 29(7):37-41.
- King, L. M. 1940. Primary encephalomyelitis in goats associated with Listerella infection. Am. J. Path. 16:467-478.
- Krage, P. 1944. Listerella-infektion bei Fohlen. B. M. Tierar. Ztl. Wochen. 1944:34:30-31.
- Laboratory Manual of Special Staining Technics. 1953. Armed Forces Institute of Pathology, Washington, D. C.

- Levi, M. L., A. Shamir, G. Neeman, and T. Nobel. 1952. Two cases of bovine abortion associated with Erysipelothrix (Listeria) monocytogenes. Refush. Vet. 9:101-104.
- Linzenmeier, G., Kropp and Luchtrath. 1953. Listeriose beim Neugeborenen. Zeit. Kinderheilk. 73:505-512.
- Mallory, F. B. 1938. Pathological Technique. The W. B. Saunders Company, Philadelphia.
- Martinek, R. 1954. Uber das Vorkommen der Listeriose bei Neugeborenen in Bezirk Magdeburg. Dtsch. Gesundheit. 37:1-4.
- Morris, M. C., and L. A. Julianelle. 1935. A study of an ocular infection induced experimentally with Bacterium monocytogenes. Am. J. Opth., 18:535-541.
- Murray, E. G. D., R. A. Webb and M. B. R. Swann. 1926. Disease of rabbits characterized by large mononuclear leucocytosis, caused by hitherto undescribed bacillus Bacterium monocytogenes. J. Path. and Bact. 29:407-439.
- Murray, E. G. D. 1953. The story of Listeria. Trans. Royal Society of Canada. 47(5):15-21.
- Murray, E. G. D. 1955. A characterization of listeriosis in man and animals. Canadian Med. Assn. J. 72:99-103.
- Norman, F. W. 1955. Listeria monocytogenes meningitis in an infant. California Med. 82:124-125.
- Nyfeldt, A. 1940. Listerella monocytogenes. dens betydning i human e veterinaer-medicinen. Skand. Vet. Tidskr. 30: 280-285.
- Nyfeldt, A. 1929. Etiologie de la mononucleose Infectieuse. Compt-rend. Soc. de Biol. 101:590-592.
- Olafson, P. 1940. Listerella encephalitis (circling disease) of sheep, cattle and goats. Cornell Vet. 30:141-150.
- Olson, A. 1945. Bidrag till kannedomen om Listerella-och streptokock-infektionernas forekommet i afterborder och foster. Skand. Vet. Tidskr. 35:272-280.
- Olson, C., Jr., R. H. Cook and V. Bagdonas. 1951. An attempt to immunize sheep during an outbreak of listeriosis. Am. J. Vet. Res. 12:306-313.

- Olson, C. and D. Segre. 1956. An agent enhancing listeriosis of sheep. *Am. J. Vet. Res.* 17:235-242.
- Osebold, J. W. and T. Inouye. 1954. Pathogenesis of Listeria monocytogenes infections in natural hosts. I. Rabbit studies. *J. Inf. Dis.* 95:52-66.
- Osebold, J. W. and T. Inouye. 1954. Pathogenesis of Listeria monocytogenes infections in natural hosts. II. Sheep studies. *J. Inf. Dis.* 95:67-78.
- Paterson, J. S. 1940. Studies on organisms of the genus Listerella. IV. Outbreak of abortion associated with recovery of Listerella from aborted fetuses. *Vet. J.* 96:327-332.
- Patocka, F., R. Benda and J. Starky. 1953. Listeriove infekce novorozencu. *Cesko. Hyg. epid. Mikro* 2:325-340.
- Paulsen, M. and G. R. Moule. 1953. Infectious ovine abortion. *The Australian Vet. J.* 29:133-139.
- Pirie, H. H. J. 1927. A new disease of veld rodents "Tiger River Disease". *Publ. S. African Inst. Med. Res.* 3:163-186.
- Pirie, H. H. J. 1940. The genus Listerella. *Science* 91:383.
- Pletneva, N. A. and V. N. Stiksove. 1950. (Oculoglandular form of listerellosis). *Vetsnik. Oftal.* 29(4):17-21.
- Poppensiek, G. C. 1944. Listerellosis, a case report. *J. Am. Vet. Med. Assn.* 105:147-148.
- Portero, A., and A. Despirito. 1954. *Listeria meningitis*. *Clinical Proc. Children's Hospital, Washington, D. C.*, 10:56-59.
- Potel, J. 1952. Zur Granulomatosis infantiseptica. *Zbl. Bakt. Abt. I Orig.* 158:329-332.
- Potel, J. 1953. Aetiologie der Granulomatosis Infantiseptica. *Wissen. Seit. Martin Luther Univ.* 2:341-364.
- Potel, J. 1954. Zur Epidemiology der Listeriose der Neugeborenen (Granulomatosis infantiseptica). *Dtsch. Gesundheit.* 3:92-95.
- Pouska, F. 1950. Listerellosis. *Cas. Cesko. Vet.* 5:57-58.



- Prevot, A. R. 1944. A Propos de la denomination du Microbe des monocytoses. Ann. Pasteur Inst. 70:118-119.
- Reed, R. W., W. F. Gavin, J. Crosby and Phyllis Dobson. 1955. Listeriosis in man. Canadian Med. Assn. J. 73:400-402.
- Reed, R. W. 1955. Personal communication to Gray.
- Reiss, H. J., J. Potel and A. Krebs. 1951. Granulomatosis infantiseptica eine durch einen spezifischen erreger Hervorgerufene Fetale sepsis. Klin. Wochen. 1:29.
- Rowsell, H. C. 1955. Personal communication to Gray.
- Rubarth, S. and E. Wollarz. 1951. Bidrag till de s.k. celliga levernekrosernas morfologi och etiologi. VI. Nord. Veterinarmotet. 30:36.
- Ryff, J. F. and A. M. Lee. 1948. Listerellosis - Report on an outbreak in Wyoming sheep and preliminary experimental therapy in rabbits. Am. J. Vet. Res. 9:147-151.
- Seeliger, H., F. Jung, G. Linzenmeier and H. Odenthal. 1952. Die Listeriose beim menschen. Dtsch. Med.Wochen. 18: 1-14.
- Seeliger, H. 1955. Listeriose. Johann Ambrosius Barth, Verlag Leipzig.
- Shmeleva, V. V. 1953. Sluchai glazo-shelezistai formy listerellema. Vest. Oft. 32(1):46-47.
- Smith, R. E., I. M. Reynolds and R. A. Bennett. 1955. Listeria monocytogenes and abortion in a cow. J. Am. Vet. Med. Assn. 126:106-110.
- Solomkin, P. S. 1954. K voprosu Listerellesney in Perkiu u Swiney. Veterinariya, Moscow 31(1):24.
- Starck, C. 1953. Zur Klinik der Infection mit Listeria infantiseptica. Zbl. Gyn. 30:1178-1182.
- Stenberg, H. 1953. Kotielaintemme Listeria-Tartunta. Finsk. Vet. Tidskr. 5:1-8.
- Stenberg, H. 1954. Cited by Olson, C. Public health aspects of listeriosis. Proc. Book Am. Vet. Med. Assn. 1954, 450-455.

- Stockton, J. J., Lisa Neu, W. S. Carpenter and M. L. Gray. 1954. The association of Listeria monocytogenes with abortion. Review of the literature and report of a bovine case. J. am. Vet. Med. Assn. 124:102-105.
- Stoot, D. W. 1954. Personal communication to Gray.
- Svenkerud, R. R. 1948. Listerellainfekejoner, spesielt med henblikk pa dares forekomst hos hesten. Norsk. Vet. Tidskr. 60:321-340.
- Tajimas, M. and Y. Nishihara. 1953. Histopathological studies on ovine listerellosis. Jap. J. of Vet. Sci. 15:301-316.
- Tompkins, V. N. 1952. Listeria monocytogenes. New York State Dept. of Health Bull. 5:49.
- Van der Schaaf, A. 1955. Personal communication to Gray.
- Van Dorssen, C. A. and J. Jansen. 1951. Over het Practische Beland van Listeria-infectie. Tijdsch. Diergeneesk. 76:756-761.
- Van Ulsen, F. W. 1952. Infecties met Listeria monocytogenes. Tijdschr. Diergeneesk. 77:899-903.
- Wenkebach, G. 1953. Zuchtung von Listeria monocytogenes aus der Harnroehre des Mannes. Rais. del. Commun. 6th Internat. Cong. Microbiol. 2:Sec. 8-9, 406.
- Wramby, G. O. 1944. Om Listerella monocytogenes bakteriologi och om forekomst av Listerella infection hos djur. Scand. Vet. Tidskr. 34:278-290.

PATHOLOGY AND BACTERIOLOGY OF ABORTION AND PERINATAL  
DEATH OF YOUNG IN RABBITS, SHEEP AND GOATS  
INDUCED BY LISTERIA MONOCYTOGENES

By  
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AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of  
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Studies on the pathology and bacteriology of abortion and perinatal death (stillbirth, and early death) of young in rabbits, sheep and goats induced by Listeria monocytogenes were undertaken to help explain the role of the bacterium in this syndrome.

Pregnant, non-pregnant and male rabbits and goats and pregnant sheep were exposed either by conjunctival instillation of suspensions of the bacterium or by adding them to the drinking water.

Conjunctival exposure of pregnant rabbits resulted not only in a marked conjunctivitis but also in abortion when the doe was exposed early in gestation; when the exposure was late in gestation, the young were either stillborn or died within a few days due to listeric septicemia. Conjunctival exposure of non-pregnant rabbits resulted in marked conjunctivitis and a low-grade and otherwise inapparent infection. Conjunctival exposure of goats resulted in a very mild eye reaction but caused death of a pregnant goat due to listeric encephalitis and induced encephalitic lesions in a castrated male goat.

These findings establish the fact that the infection resulting from conjunctival instillation of L. monocytogenes does not remain localized in the conjunctiva.

Oral exposure of pregnant rabbits, sheep and goats resulted in abortion if the dams were exposed early in gestation;

if the exposures were late in gestation, the young were either stillborn or died within a few days due to listeric septicemia. Non-pregnant animals exposed in the same manner suffered a low-grade inapparent infection.

L. monocytogenes was readily isolated from aborted fetuses, young born at term, placenta and from the dams which died due to septicemia.

The results suggest the uterus as the principal target of infection in pregnant animals. If the entire conceptus was expelled, the defense mechanism of the dam could successfully combat the relatively few remaining bacteria as in the non-pregnant. However, retention of infected conceptus (1) caused localized suppuration, (2) constituted a source of infection which resulted in subsequent abortions if the dam was rebred within 6 days, or (3) caused death from septicemia.

No evidence of immunity could be demonstrated in rabbits (at least within 2-6 months) following survival of any form of exposure under the conditions of these experiments.

The various clinical syndromes of listeric infections apparently vary with species, route of exposure and state and stage of pregnancy.

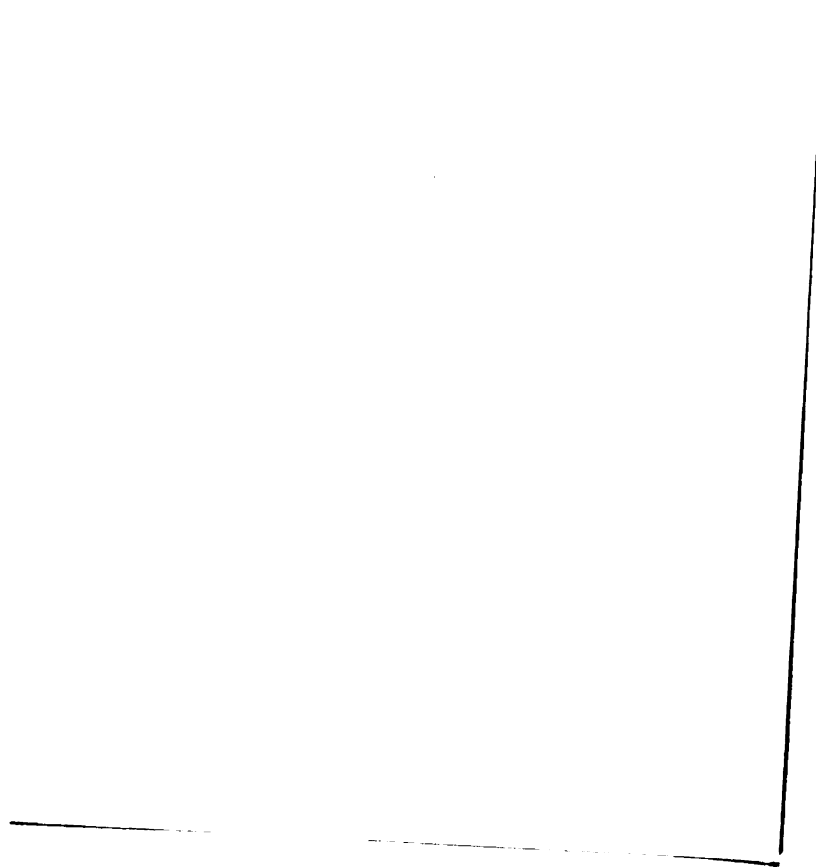
The most prominent pathological findings in aborted fetuses and the young which died perinatally included necrotic foci in the liver and less frequently in the heart, lung,

kidney, stomach and gall bladder, with necrotic debris, in the hepatic bile ducts; fibrinous exudate on the surface of liver and intestine; moderate hydroperitoneum. The bacterium could be demonstrated in tissue sections of most of these organs. Examination of dams which died or were sacrificed revealed necrotic endometritis with variable amount of caseous to purulent exudate. Numerous necrotic foci and thrombi were noticed in the myometrium. The bacterium could be demonstrated in tissue sections of the uterus including the cotyledons.

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