LISTERIA MONOCYTOGENES:

A FIELD SURVEY AND

LABORATORY OBSERVATIONS

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Mitchell L. Gray

1949

B.1

This is to certify that the

thesis entitled

Listeria Monocytogenes: A Field Survey and Laboratory Observations

presented by

Mitchell L. Gray

has been accepted towards fulfillment of the requirements for

M.S. degree in Bacteriology and Public Health

H. J. Stafseth
Major professor

Frank Hirsch

Date May 13, 1949

LISTERIA MONOCYTOGENES: A FIELD SURVEY AND LABORATORY OBSERVATIONS

by

Mitchell L. Gray

A Thesis

Submitted to the School of Graduate Studies of
Michigan State College of Agriculture and
Applied Science in partial fulfillment
of the requirements for the degree
of

MASTER OF SCIENCE

Department of Bacteriology and Public Health

1949

Affectionately dedicated

to

Nell

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. H. J. Stafseth for his constructive criticism and benign influence. To Dr. Frank Thorp, Jr. for the keen inspiration and compelling influence here reflected. To Drs. L. B. Sholl, R. A. Runnells and R. F. Langham for their part in the necropsy examinations. Also remembered are the veterinarians in the field for their cooperation in making the material available for study in the laboratory. Thanks also are given to the technicians for their assistance.

The author is grateful to Merck & Co., Rahway, N. J., for the streptomycin; Commercial Solvents Corp., Terre Haute, Indiana, for the bacitracin and Park Davis & Co., Detroit, Mich., for the chloromycetin.

TABLE OF CONTENTS

	Page
Introduction	1
Part I. A Field Survey	
Methods of Isolation for Diagnosis	4
Report of Cases	7
Discussion	17
Summary	25
Illustrations	27
Part II. Laboratory Observations	
Isolation from the Bovine Brain	42
Ocular Instillation	46
Viability	53
Inhibition	57
Sodium Azide	58
Potassium Tellurite	58
Acetic Acid - Deep Brain Medium	60
The Effect of Antibiotics	61
Streptomycin	61
Bacitracin	89
Penicillin	91
Chloromycetin	92
Pathogenicity	93
Immunological Reactions	97
Biochemical Reactions	99
Summary	101
Illustrations	104
Bibliography	115

INTRODUCTION

In the nineteen years that have passed since Gill (1931) showed Listeria monocytogenes to be the etiological agent of circling disease in sheep, listeriosis has become a disease of considerable economic importance. It has been established that its presence is world wide; that its hosts are not only sheep, but also cattle (Graham et al, 1943; Hatch, 1945; Boucher, 1946; Cole, 1946; Pounden et al, 1947A; Gray et al, 1948), swine (De Blieck and Jansen, 1942, 1943; Graham et al, 1943; Kerlin and Graham, 1945; Rhoades and Sutherland, 1948), goats (Graham et al, 1943; Kaplan and Lager, 1945; Gifford and Jungherr, 1947), dogs (Cox, 1945 and Chapman, 1947), chickens (Graham et al, 1943; Jansen and Pepperkamp, 1947), some forms of wild life (Lilleengen, 1942; Graham et al, 1943; Gifford and Jungherr, 1947; Jansen and Pepperkamp, 1947), man (Kaplan, 1945; Handelman et al, 1946; Wheeler, 1948). Subsequently it has been reported in sheep by Graham et al, 1943; Pounden and Edington, 1947; Gray et al, 1947; Ryff and Lee, 1948. There is, as yet, no method of control nor adequate treatment of its victims. The next two decades may find it one of the leading killers of livestock.

The presence in Michigan of this bacterium and

listeriosis, the disease caused by it, was first suspected by Cheng (1945). He reported a two-month old lamb suffering from a disorder of the central nervous system, but did not culture the brain at necropsy. However, histological sections of the medulla revealed the characteristic "perivascular cuffing". Gray et al (1947) confirmed its presence in sheep by isolation and identification of L. monocytogenes. This report included three ewes and one lamb. The ewes exhibited typical encephalitis while the lamb displayed a septicemia. One other ewe showed similar symptoms but Listeria was not isolated.

The following year Listeria was shown by Gray et al (1948) to be present not only in sheep but also in cattle from Michigan. That year gram positive bacteria were isolated from five cows and one ewe. These bacteria were subsequently identified as L. monocytogenes. During the winter and spring of 1948 Listeria was isolated from nine sheep and nine cows. In a one month period from December 15, 1948 to January 15, 1949 Listeria was isolated from seven head of cattle. From that date until April 28, 1949 Listeria was isolated from three more cows and 17 sheep. The records compiled during this study indicate that this disease has killed at least 104 head of livestock. A killer of food producing animals such as this, which is also a menace to

the health of their owners, warrants a complete study to determine, if possible, effective methods of diagnosis, mode of transmission, methods of treatment and a system of control.

For convenience sake this thesis is divided into two parts. The first consists of a report of the problem of listeriosis as it affects livestock in the state of Michigan. The second part presents the experimental procedures undertaken in an effort to contribute to a better understanding of the behavior of the etiological agent.

Part I

A FIELD SURVEY

Part I. A FIELD SURVEY

Methods of Isolation for Diagnosis

The first attempts to isolate L. monocytogenes from suspected material resulted in failure. The technic employed consisted of searing the incised portion of the medulla oblongata at its junction with the spinal cord. A sterile inoculating loop was inserted and the material adhering to the loop after removal was plated on tryptose agar and sheep blood agar plates. Incubation at 37° C for periods up to 96 hours failed to produce any significant bacterial growth.

With the adoption of a modification of a technic described by Biester and Schwarte (1939) isolation of Listeria was accomplished. This procedure consisted of searing the exposed surfaces of the medulla after its removal from the skull. A large portion of this material was placed in a sterile mortar together with 10-15 ml nutrient broth and ground to a fine pulp. This material was transferred to a sterile bottle containing glass beads and put in a shaking machine for from 10 to 20 minutes. Approximately 0.5 ml of the resulting material was plated on tryptose and sheep blood agar plates. Incubation at 37° C for 18 to 24 hours produced a heavy growth of Listeria on both media.

Both the above described technics were carried out on the three cases of listeriosis in ewes reported by Gray et al (1947). In no instance was Listeria isolated when the brain material was not macerated as described above.

When, in the spring of 1947, several bovine brains were submitted to culture for Listeria, the inoculated plates remained sterile. Some of the animals concerned had shown what seemed to be a clinical picture of the disease, yet the causative agent could not be isolated (figure 15). However, it was found that after the prepared brain suspensions had been refrigerated at 4° C for varying lengths of time, Listeria could be demonstrated by colonies in quantity comparable to those found in sheep brain isolations. These original observations were reported by Gray et al (1948A) and are further confirmed in this thesis.

Recently a further modification was made. This consisted in the employment of a Waring blendor with a micro head attachment to macerate the brain material. A portion of the seared medulla was placed in the blendor together with approximately 15 ml of nutrient broth. This was agitated for three or four minutes. A 0.2-ml portion was plated on tryptose agar plates. The incubation time and temperature were the same as above.

Except for the economy of time and convenience the blendor has no particular advantage in sheep brain isolations. However, it would seem that the number of colonies in bovine isolations was increased. To prevent excessive heating the running time of the blendor should not exceed four minutes.

In examining the incubated plates for the presence of Listeria a dissecting microscope was employed as described by Huddleson (1946). White light was directed obliquely (approximately 45°) through the stage of the microscope. The colonies of Listeria were a bright green with a finely textured surface. This was found to be so characteristic that Listeria colonies can be identified even in cultures with extreme contamination (figure 1). This may safely be used as a diagnostic technic and was used in all but the first four isolation attempts reported.

By employing these technics the isolation of Listeria did not fail in any clinically described case. The diagnoses were substantiated by demonstration of the histo-pathology in hematoxylin eosin stained sections of the medulla. In listeriosis a marked so-called "perivascular cuffing" and areas of focal necrosis were noted. These areas were characterized by both mononuclear and polynuclear cells (figure 19).

Report of Cases

Outbreaks, 1946.

Case 1. March 23, 1946. St. Johns.
A yearling ewe was admitted to the pathology laboratory for diagnosis, March 22. The animal was lethargic and showed rather indefinite symptoms of central nervous system origin. Ketosis was suspected but clinical pathological tests of the blood and urine revealed no significant change in glucose value and no ketone bodies in the urine. After three days' observation the animal was sacrificed. At necropsy the brain showed some congestion. There were no other significant findings. Cultures prepared from the medulla were positive for Listeria.

Case 2. April 4, 1946. East Lansing.
An eight-day old lamb was presented for routine necropsy. The lamb had been weak since birth. The omentum and diaphragm adhered to the liver. White necrotic foci were uniformly spread throughout the liver substance. There were numerous whitish grey foci throughout the lungs. Cultures were prepared from the liver, lung, and heart blood. Listeria was isolated from the liver and heart blood.

Case 3. May 7, 1946. East Lansing.
A four-year old ewe was found staggering on the morning of May 6. After 250 ml glucose was administered intervenously, she seemed no better. The next morning 200 ml saline with 5% glucose were given intraperitoneally. She died in the evening of that day. At necropsy there was an acute bronchopneumonia. The kidneys showed parenchymatous degeneration. There were no other lesions. Cultures prepared from the medulla were positive for Listeria.

Case 4. May 14, 1946. East Lansing.
A six-year old ewe was noticed staggering on the morning of May 13. She was given 200 ml saline with 5% glucose intraperitoneally. Sulfathiazole, 210 grains, was administered orally. Sulfathiazole administration was repeated in the evening. The ewe was found dead the following morning. Necropsy findings were confined to a marked hemorrhagic duodenitis. Cultures prepared from the medulla were positive for Listeria.

Outbreaks, 1947.

Case 5. February 27, 1947. Clare.
A nine-month old bull was showing symptoms of listeriosis. It was killed February 26, and the head submitted for culture. Cultures prepared from the medulla were negative after 24 hours' incubation at 37° C, but when the brain suspension, which had been stored in the refrigerator for three months, was again plated, there was a heavy growth of Listeria.

Case 6. March 24, 1947. Kalamazoo.
This animal was the third in a group of steers. The first two died after showing symptoms of cerebral disturbance. The first died about six weeks before; the second about two weeks before. This animal began walking in circles and seemed to be blind in the right eye. It crowded into the corner of the stall and showed symptoms of agony. It would go down and had to be helped up each day. At necropsy the mucous membrane of the fourth stomach showed general congestion. The small intestine showed diffuse congestion and blood stained contents throughout its entire length. A few petechial hemorrhages were found on the heart. Upon removal of the brain an excess of cerebral spinal fluid was noted. Cultures prepared from the medulla showed the presence of Listeria. Examination for rabies was negative.

Case 7. April 5, 1947. Rose Lake.
A six-month old heifer was admitted to veterinary clinic on March 31. She had been circling to the left since March 30. She was killed for necropsy. Except for some congestion of the brain, findings were negative. Cultures prepared from the medulla were badly contaminated and Listeria was not observed. After refrigeration for two months it was possible to observe colonies of Listeria among the contaminants.

Case 8. April 15, 1947. Climax.
A yearling heifer entered veterinary clinic on April 11, 1947. The right ear drooped, the right eye was somewhat inflamed and the animal circled to the right. The tongue was hanging out, and there was excessive salivation. There was some twitching of the peripheral muscles. (The owner had lost one heifer about a week before which had shown similar symptoms.) The animal was killed for necropsy. The abomasum showed considerable congestion. There was marked patchy congestion throughout the small intestine. The lungs and kidneys

were mildly congested. There was an excess of cerebral fluid, and the brain was markedly congested. Cultures prepared from the medulla were negative. Three weeks later it was possible to demonstrate numerous colonies of Listeria from the refrigerated brain suspension.

Case 9. April 24, 1947. Rose Lake.
A yearling ewe had shown a tendency to turn her head to the right, and to circle. These symptoms persisted for three days. She had been kept in the same pen as the heifer in Case 7. She was killed for necropsy. The abomasum showed several small erosions of the mucosa. The lungs and kidneys showed some congestion. There was marked congestion of the brain. No other lesions were noted. Cultures prepared from the medulla were positive for Listeria.

Case 10. May 8, 1947. Springport.
On May 3 a two-year old cow developed anorexia and difficulty in swallowing. Temperature was 104.2. May 5 the temperature was 105. The tongue was hanging out and there was hay in the mouth. The animal showed an inability to swallow water. The right eye was inflamed, the right ear drooped and she circled to the right. The brain was submitted for culture. Listeria was isolated from the medulla.

Outbreaks, 1948.

Case 11. December 31, 1947. Manchester, Indiana.
The head of a five-year old cow was submitted for culture. She had been raised on this farm and no new stock had been brought in. She had shown symptoms of a central nervous disorder and walked in circles. One lip, ear, and eyelid were paralyzed. Temperature was slightly elevated. She lived for six days after these symptoms appeared and became violent before paralysis developed. Cultures prepared from the medulla were positive for Listeria.

Case 12. January 9, 1948. Jackson.
The head of a cow was sent for examination. This was the fourth cow showing symptoms of a central nervous system disorder. The first died about three weeks before. The second apparently recovered. The third was killed and the brain sent to the state health laboratory to be checked for rabies. This was reported as being negative.

were mildly congested. There was an excess of cerebral fluid, and the brain was markedly congested. Cultures prepared from the medulla were negative. Three weeks later it was possible to demonstrate numerous colonies of Listeria from the refrigerated brain suspension.

Case 9. April 24, 1947. Rose Lake.
A yearling ewe had shown a tendency to turn her head to the right, and to circle. These symptoms persisted for three days. She had been kept in the same pen as the heifer in Case 7. She was killed for necropsy. The abomasum showed several small erosions of the mucosa. The lungs and kidneys showed some congestion. There was marked congestion of the brain. No other lesions were noted. Cultures prepared from the medulla were positive for Listeria.

Case 10. May 8, 1947. Springport.
On May 3 a two-year old cow developed anorexia and difficulty in swallowing. Temperature was 104.2. May 5 the temperature was 105. The tongue was hanging out and there was hay in the mouth. The animal showed an inability to swallow water. The right eye was inflamed, the right ear drooped and she circled to the right. The brain was submitted for culture. Listeria was isolated from the medulla.

Outbreaks, 1948.

Case 11. December 31, 1947. Manchester, Indiana.
The head of a five-year old cow was submitted for culture. She had been raised on this farm and no new stock had been brought in. She had shown symptoms of a central nervous disorder and walked in circles. One lip, ear, and eyelid were paralyzed. Temperature was slightly elevated. She lived for six days after these symptoms appeared and became violent before paralysis developed. Cultures prepared from the medulla were positive for Listeria.

Case 12. January 9, 1948. Jackson.
The head of a cow was sent for examination. This was the fourth cow showing symptoms of a central nervous system disorder. The first died about three weeks before. The second apparently recovered. The third was killed and the brain sent to the state health laboratory to be checked for rabies. This was reported as being negative.

All the affected animals showed the same symptoms: elevated temperature, eyes protruded, staggered gait, walked in circles, leaned against any stationary object as if unable to stand and after about four days went down. They all continued eating until they went down.

There were 25 animals of all ages in this lot. All but these four were suffering from winter dysentery. There had been no new animals brought on this farm in the past two years. One cow on a farm across the road had died after showing similar symptoms.

Cultures prepared from the medulla were positive for Listeria.

Case 13. February 2, 1948. Manchester.
A ten-month old ewe was presented for necropsy. It was the sixth animal in this flock to die. Three others were sick. Symptoms developed rapidly. Some died suddenly. Others lived for three or four days. At necropsy the abomasum of this ten-month old ewe showed mild congestion. The brain was markedly congested. No other lesions were noted. Cultures prepared from the medulla were positive for Listeria.

Case 14. February 3, 1948. Manchester.
History the same as that of the ewe in Case 13. In this ten-month old wether no lesions were noted at necropsy other than congestion of the brain and an excess of cerebral fluid. Cultures prepared from the medulla were positive for Listeria.

Case 15. February 11, 1948. Fenton.
An aged ewe was presented for necropsy. Three other animals had died during the past month. They all staggered, showed a discharge from the nose, and an excessive salivation. They died about 24 hours after the onset of symptoms. The abomasum was congested. The small intestine showed congestion throughout its entire length. The lungs, kidneys and brain showed a mild congestion. Cultures prepared from the medulla were positive for Listeria.

Case 16. February 11, 1948. Fenton.
This sheep was from the same flock as reported in Case 15. Only the head was submitted for examination. The brain was badly decomposed, but Listeria was isolated from the medulla.

Case 17. March 11, 1948. Bronson.
The head of a cow which had been shot after showing

symptoms of a central nervous system disorder was presented for culture. The bullet was found lodged under the pituitary fossa. There was marked hemorrhage throughout the brain and the meninges. Listeria was isolated from the medulla.

Case 18. April 9, 1948. Homer.
The head of a cow which showed circling was presented for culture. The brain was markedly congested. Listeria was isolated from the medulla.

Case 19. April 14, 1948. Grand Ledge.
A yearling ewe was presented for necropsy. The day before she was noticed to circle around the fence, and press into corners. She went down and showed kicking and running motions. She died during the night. There were a few hemorrhages in the heart, the lungs were congested and there was a marked congestion of the brain. Cultures prepared from the medulla were positive for Listeria.

Blood picture on April 13 was: WBC - 8,450; RBC - 10,000,000; HB - 8.9 gm %; P - 82; L - 16; M - 2.

Case 20. April 21, 1948. Pewamo.
The head of a ewe was presented for culture. All together the owner had lost seven ewes. They tended to circle to the right. The temperature of this ewe just before death was 107.6. The brain showed considerable congestion. Cultures prepared from the medulla were positive for Listeria.

Case 21. April 22, 1948. Williamston.
The owner had lost one ewe April 20. Another ewe, five years old, began to circle the night of April 21. The head was thrown backward and to one side. She was killed for necropsy. There was some congestion of the abomasum; the brain was slightly congested. No other significant lesions were noted. Listeria was isolated from the medulla.

Blood picture: WBC - 14,100; RBC - 9,520,000; HB - 10.1 gm %; L - 3; P - 79; M - 15; B - 3.

Case 22. May 4, 1948. Clarksville.
The owner had lost two animals previous to this one. The head of this two and a half-month old ewe lamb was presented for culture. They all showed symptoms of circling to the right. There was hemorrhage around the spinal cord at the base of the brain. No other lesion was noted. Listeria was isolated from the medulla.

Case 23. May 9, 1948. Laingsburg.
The head of a cow was presented for culture. She seemed to be blind in one eye. She showed anorexia and drooling. The face was paralyzed prior to death. The brain was congested and there was an excess of cerebral fluid. Listeria was isolated from the medulla. A rabies examination was negative.

Case 24. May 24, 1948. Albion.
The head of a yearling bull was presented for culture. The animal had shown twitching of the nose, slobbering and an inability to swallow. The right eye appeared to be turned inward. These symptoms persisted for six days before death. Listeria was isolated from the medulla.

Case 25. June 21, 1948. Dansville.
The head of a yearling bull was presented for culture. The animal was off feed and showed a pharyngeal paralysis. The head was held down, there was marked salivation and the animal was weak and staggering. The temperature was 107.0 at the onset of symptoms. It had been treated with sulfonamide and penicillin. Cultures prepared from the medulla were negative for Listeria. When the refrigerated brain suspension was cultured, August 5, there was a heavy growth of Listeria.

Case 26. July 16, 1948. Brown City.
The head of a cow was presented for culture. A clinical diagnosis of rabies had been made. The medulla was cultured for Listeria and the remainder of the brain was sent to the State Health Department for rabies examination. Cultures were positive for Listeria; rabies examination, negative.

Outbreaks, 1949.

Case 27. December 17, 1948. Rochester.
The head of a steer showing symptoms of an encephalitis was presented for culture. Several other animals in this same herd were showing similar symptoms. The affected animals circled, had a paralysis of the eyelid and a profuse salivation. These symptoms persisted three or four days. Listeria was isolated from the medulla of the head presented.

Case 28. December 21, 1948. Mason.
The head of a bull was presented for culture. The

animal had difficulty in swallowing, excess salivation, rigid jaw, temperature of 106, and black diarrhea. The owner reported that the animal had been injured by a rusty nail. Tetanus was suspected. It had been treated with procaine penicillin and tetanus antitoxin. The brain appeared normal. Cultures from the medulla were positive for Listeria.

Case 29. December 21, 1948. Grass Lake.
A cow head was submitted for culture. There was no history given. Listeria was isolated from the medulla.

Case 30. December 23, 1948. Stockbridge.
A five year old cow was presented for necropsy. For two days she had shown an unsteady gait, and occasionally she had gone down. The small intestine was slightly congested throughout. There was one small area of mottling on the liver. The meninges were congested, and there appeared to be a fluid with gas bubbles in the fissures of the brain. Listeria was isolated from the medulla.

Blood count: WBC - 10,900; HB - 14.3 gm %; L - 39; P - 41; M - 4; B - 1; E - 15.

Case 31. January 12, 1949. Pewamo.
A yearling bull was presented for necropsy. It was the second one with like symptoms to die. Two days previous the bull was seen to fall against a fence. The animal seemed to be in pain. There was slight pulmonary edema, and the right cerebral hemisphere was congested and edematous. Listeria was isolated from the medulla.

Case 32. January 14, 1949. Jackson.
A cow head was presented for culture. There was no history given. Listeria was isolated from the medulla.

Case 33. January 14, 1949. Grass Lake.
The head of a three-year old heifer was presented for culture. There was no history given. Listeria was isolated from the medulla.

Case 34. February 5, 1949. Charlotte.
A ewe from this flock had died after showing symptoms similar to this ewe which was admitted to veterinary clinic. She had been circling to the right for three days and seemed to be blind. There was twitching of the peripheral muscles. At necropsy there seemed to be a general congestion of the visceral organs. Lis-

teria was isolated from the medulla.

Blood picture:
Feb. 4: WBC - 6,400; RBC - 12,790,000; L - 20; P - 69; M - 8; B - 3.
Feb. 5: WBC - 2,200; RBC - 14,830,000; L - 25; P - 68; M - 7.

Case 35. February 11, 1949. Charlotte.
A two-year old ewe showed symptoms similar to those in Case 34. At necropsy the small intestine, stomach and meninges showed congestion. The lungs were edematous and congested. Cultures from the medulla were positive for Listeria.

Case 36. February 12, 1949. Charlotte.
This ewe was noticed to be circling in the morning, died in the afternoon of same day. Other than areas of congestion in the gastric mucosa, necropsy findings were negative. Listeria was isolated from the medulla.

Case 37. February 15, 1949. Eaton Rapids.
A two-year old ewe which had been circling was presented for necropsy. The small intestine was congested throughout its entire length. The lungs showed an extensive pulmonary edema and congestion. The heart showed hemorrhages in the epicardium. The brain and all visceral organs were mildly congested. Listeria was isolated from the medulla.

Case 38. February 22, 1949. Ionia.
The head of a ewe which had shown symptoms of a central nervous disorder was presented for culture. One other ewe had died after showing similar symptoms. Listeria was isolated from the medulla of the head presented.

Case 39. February 22, 1949. Lansing.
A yearling heifer developed symptoms of a central nervous disturbance, February 17. There was extensive salivation. She walked in a straight line with the head pulled to the left side and extended. Temperature ranged from 104 to 101. She was treated with penicillin and sulfapyridine. At necropsy there was slight congestion of the ileum, a few hemorrhages in the gastric mucosa, lungs were edematous, kidneys were pulpy and hemorrhagic and there were numerous hemorrhages on the heart. Listeria was isolated from the medulla.

Blood count, Feb. 18: WBC - 9,600; L - 37; P - 56; M - 1; B - 1; E - 5.

Case 40. February 22, 1949. Romeo.
A cow head was presented for culture. The animal had shown circling, salivation and difficulty in walking. Cultures prepared from the medulla were positive for Listeria.

Case 41. February 24, 1949. East Lansing.
This ram was found prostrate on February 22. There was a serous discharge from the nose, pupils were dilated, the head was held to the right and extended with ears drooped. There were muscular tremors throughout the body, hypersensitivity, and anorexia. Temperature was 100.3. It was treated with 175 grains sulfapyridine and 200,000 units of penicillin. At necropsy the small intestine was congested, the lungs showed edema, and there was some gastritis. Cultures prepared from the medulla were positive for Listeria.

Blood picture: WBC - 13,900; HB - 10.8 gm %; L - 33; P - 64; M - 3.

Case 42. March 1, 1949. Ionia.
The head of a ewe was submitted for culture. This ewe was from the same flock as reported in Case 38. Listeria was isolated from the medulla.

Case 43. March 4, 1949. East Lansing.
A yearling ewe from the same flock as Case 41 had shown similar symptoms for two days. At necropsy pulmonary edema was found in the lungs, numerous adhesions were present between the liver and diaphragm and intestines, the stomach showed some gastritis, the small intestines were very reddened with some mucoid degeneration, the large intestine had some nodular worm lesions, the kidneys and mesenteric lymph nodes were congested. Cultures prepared from the medulla were positive for Listeria.

Blood picture:
March 2: WBC - 14,050; HB - 13.1 gm %; L - 55; P - 45.
March 3: WBC - 14,000; HB - 14.2 gm %; L - 24; P - 75; M - 1.

Case 44. March 4, 1949. East Lansing.
A three-year old ewe was found lying on its back. When placed on its feet it circled to the left. The animal breathed with a marked rattling sound. The upper air passages contained a very heavy mucopurulent exudate. At necropsy there were no significant lesions. Cultures prepared from the medulla were positive for Listeria.

Blood picture: WBC - 18,300; HB - 9.8; L - 21; P - 79.

Case 45. March 9, 1949. Okemos.
The head of a cow was presented for culture. The animal had shown an inability to swallow. After five days it showed loss of the use of the hind quarters and was destroyed. Cultures prepared from the medulla were positive for Listeria.

Case 46. March 14, 1949. Ypsilanti.
A yearling ewe which had shown symptoms of a central nervous system disorder was presented for necropsy. Three other lambs had died recently. They all died within one day after symptoms were noted. They circled and pressed their heads against partitions. No gross lesions were found in the yearling ewe presented for necropsy. Cultures prepared from the medulla were positive for Listeria.

Case 47. March 16, 1949. Williamston.
A six-year old ewe which had shown signs of a central nervous system disorder was presented for necropsy. Two other ewes had died showing similar symptoms. This six-year old ewe showed no gross lesions at necropsy other than a dull nodular appearance of the meninges. These tiny nodules were a yellowish gray in color and were uniformly distributed. They had the appearance of a suppurative meningitis. Cultures prepared ffom the medulla were positive for Listeria.

Case 48. March 24, 1949. Charlotte.
A yearling ram was presented for necropsy. It had been circling to the right, the right ear drooped, and there was very marked salivation. It had been treated with sulfathiazole and 400,000 units of penicillin. The small intestine showed numerous scattered areas of congestion and petechial hemorrhages to about the middle of the ileum; here the mucosa became thicker for about three feet and did not show any congestion nor hemorrhages. The remainder of the ileum was hemorrhagic and congested. The lungs were edematous. There were hemorrhages scattered throughout the heart. Lymph nodes were congested. The large intestine was congested and showed a few petechial hemorrhages. Cultures prepared from the medulla were positive for Listeria.

Blood picture: March 28: WBC - 17,150; HB - 10.8 gm %; L - 34; P - 64; M - 2.

Case 49. March 31, 1949. Big Rapids.
One of two ewes which had died after showing symptoms of circling was presented for necropsy. Advanced post mortem changes prevented detection of lesions. Cultures prepared from the medulla were positive for Listeria.

Case 50. April 7, 1949. Albion.
A ewe was admitted to the veterinary clinic showing symptoms of central nervous system disorder. Fourteen other ewes died showing similar symptoms. They lived for only one or two days after the onset. Pregnancy disease was suspected. This ewe which had been admitted to the veterinary clinic was killed and necropsied. There were no gross lesions except that the meninges appeared to be thickened and stipled with minute yellowish foci resembling a suppurative meningitis. Cultures prepared from the medulla were positive for Listeria.

Case 51. April 18, 1949. Romeo.
The head of one of two rams that had died after showing symptoms of circling for 24 hours was submitted for culture. This flock of 160 sheep was crowded into an area sufficiently large for only about 60 sheep. No further outbreaks were reported. Listeria was isolated from the medulla of the brain submitted.

Case 52. April 20, 1949. Manchester.
Two one-month old lambs were admitted to the veterinary clinic. They both showed symptoms of circling. They would lean against any stationary object. There was marked salivation, drooping of the ears, strabimus, and terminally, prostration. Four ewes in this flock had died since March 15, and two other ewes were showing similar symptoms. One of the lambs died April 20. The other was comatose on April 21 and killed. Necropsy findings were negative in each. Listeria was isolated from the medulla of both lambs.

The case reports revealed that there were no pathognomonic symptoms nor lesions in listeriosis such as those encountered in hog cholera, for example. Symptoms ranged from the so-called "textbook description" to a mere pharyngial paralysis. The duration of the disease was short in some cases; in others it extended as long as ten days. This was true in both species studied but generally the course was much shorter in

sheep than in cattle.

Listeriosis in sheep could easily be confused with ketosis or with overeating disease. In flocks where these diseases were concurrent they could be distinguished only at necropsy. The fatty liver found in ketosis and the hemorrhages characteristic of overeating disease were not found in cases of listeriosis.

Case 52 was of significance in that one month old lambs that were down with head pulled back were often considered to be suffering from entrotoxemia. It was quite likely that if more of these lambs had been available for culture, Listeria could have been isolated from them. In entrotoxemia death is so sudden that symptoms are seldom noticed.

In cattle listeriosis was most easily confused with rabies or with poisoning. Some cows became quite violent in the terminal stages. All rabies suspects sent to the laboratory proved to be listeriosis. In the milder cases symptoms closely resembled poisoning but where gastric analyses were performed no poisons could be demonstrated.

The similarity of the majority of the cases, however, did render certain symptoms characteristic of listeriosis. At the onset of the disease the infected

animal would usually separate itself from the rest of the herd. It would crowd into a corner or lean against a fence or manger as if it were unable to stand unsupported. If the animal did walk it would usually go in a circle. The animal became very depressed and refused feed. There usually was some elevation of temperature. A conjunctivitis in one or both eyes developed in some cases. The head was held back to one side with the ear on that side drooped. There was usually stringy salivation and a marked discharge from the nose.

As far as could be determined the mortality was near 100%. All affected animals admitted to the veterinary clinic terminated fatally. However, a few veterinarians reported that some animals suffering with that which they had diagnosed as listeriosis, did recover. No ante mortem isolations were made so these diagnoses could not be confirmed.

Lesions at necropsy ranged from none to rather extensive damage. As far as could be determined all but one of the cases (case 3) were uncomplicated. A marked enteritis was shown in many cases (figure 2). It was felt that this lesion may have some significance but it was not clearly understood. This was also shown in some rabbits artificially exposed. A number of cases were characterized by a marked pulmonary edema,

but this too was not consistent. (figure 20)

A number of animals, particularly sheep, were presented for necropsy showing symptoms suggestive of listeriosis. Necropsy findings failed to establish a diagnosis. Repeated cultures of the medulla, however, failed to reveal the presence of Listeria. Histo-pathological examination of the medulla failed to show perivascular cuffing or areas of necrosis. The only exception to this was a horse submitted for necropsy. Listeria was not isolated but tissue sections of the medulla showed lesions resembling those of listeriosis. This case has not been positively diagnosed.

From the foregoing it was evident that listeriosis could be positively diagnosed only by isolation and identification of L. monocytogenes.

In seven of the cases (35, 36, 37, 39, 41, 44, 51) the lungs, spleen, liver and kidney were cultured. An area from each organ was seared and macerated in the Waring blendor. About 15 ml nutrient broth was added to make a suspension. A 0.2-ml portion was plated on tryptose agar and incubated at 37° C for 24 hours. In no instance was Listeria isolated. Tissue sections prepared from these organs failed to show lesions. Fetuses removed at necropsy from cases 36 and 37 were negative

for Listeria following the usual culture procedure.

In four cases (43, 44, 46, 50) cultures were prepared from the cerebrum. Listeria was not isolated. The cerebellum was cultured in two cases (46, 50). Listeria was isolated in small numbers from both cases.

Four cases (17, 23, 25, 26) all bovine, showed Listeria in larger numbers in the cervical cord immediately posterior to the medulla than in the medulla itself.

Frequently conjunctivitis and keratitis accompanied the natural occurrence of the disease. Cultures prepared from conjunctival swabs failed to reveal the presence of Listeria. Saliva and nasal mucus were negative for Listeria. With one exception, Case 2, Listeria was not isolated from any source other than the brain.

It may be of some significance that in young animals listeriosis invariably occurs as a septicemia. Burn (1936) reported its presence in the liver of infants. De Blieck and Jansen (1942, 1943), Kirlin and Graham (1945) and Rhoades and Sutherland (1948), all reported isolation of Listeria from the liver of young pigs. Grini (1943) recovered Listeria from the liver of a six-day old foal. None of these reports indicate whether Listeria was also present in the brain of the affected animals. This evidence suggested that the disease may

have been septicemic at some time prior to the appearance of nervous symptoms or that there may have been an altered host-parasite relationship in the young. If the former was true, a monocytosis could be expected in the peripheral blood at the time of exposure. Intravenous inoculation of experimental animals invariably produced a monocytosis. However, during the course of this study several flocks of sheep having active cases of listeriosis, were observed. The exposed animals were bled but no significant deviation from normal hemocytological values was evident. Subsequently, in one flock, deaths due to listeriosis occurred at about three and six-week intervals. The postulation of an altered host-parasite relationship could not be made until the specific nutrient requirements of the bacterium, or the physiological differences in the young and adult were more clearly understood. Hunter,(1942) reported some growth requirements of Listeria. Graham et al (1943) reported that the sera of a relatively high percentage of cattle with no history of exposure to Listeria contained agglutinins for the organism. This could indicate that either there were no specific agglutinins for Listeria, that many animals were exposed to the disease but never showed symptoms, or that antibodies were present which cross agglutinated with Listeria antigen. When a number of sheep brains from a flock which had had an outbreak of

listeriosis were cultured, Listeria was not isolated from any of them. The sheep had died of supposedly natural causes. Tissue sections of the medulla revealed no evidence of pathology. There was no evidence that these animals were carriers or that they had been exposed to the disease.

The evidence presented by two outbreaks in the spring of 1947 suggested that listeriosis may have a long period of incubation. Two cases, 6 and 8, occurred 22 days apart. There was no known direct contact between the two affected herds. However, feeder cattle that had been imported from the same source in the state of Texas were involved in each outbreak. Numerous other owners had reported that the affected animals, especially in cases involving cattle, had been imported from the Southwestern or Western feeding ranges. Coupled with this was the fact that during the four years of this study the disease had recurred in only one flock of sheep. In only one instance were both sheep and cattle affected on the same premises (cases 7 and 9). In this outbreak a sheep died from listeriosis 19 days after a cow had died. Another outbreak (cases 34, 35, 36, 48) occurred where cows were kept in the same barn with the infected sheep. In fact, the infected sheep had access to a box stall shared by cattle. The exposed cattle

failed to show symptoms of listeriosis.

Case 50 also was interesting for its epizootical implications. Animals in direct contact became infected while animals sharing a common feed rack, but not in direct contact, did not show evidence of the disease. This would substantiate the conclusion that listeriosis was not transmitted in this instance by contaminated feed or water. One experiment (Thesis, Part II) supported this. Similar findings are reported by Gill (1937), Paterson (1940A), Schwarte and Biester (1942), Graham et al (1943). However, Julianelle (1941) was able to infect white mice fatally when broth cultures were given as the sole source of fluid intake.

It had not been possible to reproduce listeriosis experimentally except by extremely drastic means such as intracerebral inoculation. Numerous investigations had established this; i.e., Biester and Schwarte (1939), Schwarte and Biester (1942), Olofson (1940), Muth and Morrill (1942), Graham et al (1943) and Part II of this thesis.

Numerous investigators had attempted transmission by intranasal sprays. These reports were in such complete disagreement that they will not be discussed here.

It was shown by Graham et al (1943) and Gray et

al (1948A) (Thesis, Part II, Rabbit No. 9) that the nervous form of listeriosis could follow conjunctival instillation. Graham et al (1943) reported that one of two month old pigs died 11 days after instillation of culture. There was no conjunctivitis or keratitis. Listeria was recovered from the medulla. Gray et al (1948A) reported the death of a rabbit, 23 days following ocular instillation. The symptoms were suggestive of listeriosis. There were no significant lesions at necropsy. Listeria was isolated only from the medulla. Tissue sections of the medulla showed perivascular cuffing.

However, transmission under field conditions could not be established. Cultures of drinking water, straw, feed, litter, etc. from infected premises failed to establish the presence of Listeria in these materials. The nostrils and blood of exposed animals were cultured with negative results. Until the natural reservoir of this bacterium could be determined it was unlikely that its epizoology could be established.

Summary

Fifty two cases of listeriosis, occurring in a four-year period, were reported. In 29 of these sheep were involved, while cattle were involved in 23. The known losses totaled more than 104 animals of both species.

Certain aspects of the epizoology of the disease are discussed.

Several technics were presented for the successful isolation of the bacterium from both species of animals. A method of readily identifying colonies of *Listeria* was described.

Fig. 1. Map indicating areas where listeriosis has occurred. Red denotes outbreaks in sheep, blue denotes outbreaks in cattle.


EMMET
CHEBOYGAN
PRESQUE ISLE
CHARLE
OTSEGO
MONTMOR.
ALPENA
ANTRIM
LEELANAU
KALKASKA
CRAWF'D
OSCODA
ALCONA
BENZIE
GRD TRAV
MANIS.
WEXFORD
MISSAUKEE
ROSCOM.
OGEMAW
IOSCO
MASON
LAKE
OSCEOLA
CLARE
GLADWIN
ARENAC
BAY
HURON
OCEANA
NEWAYGO
MECOSTA
ISABELLA
MIDLAND
TUSCOLA
SANILAC
MUSKE.
MONTCALM
GRATIOT
SAGINAW
KENT
LAPEER
GENESEE
ST.CLAIR
OTTAWA
IONIA
CLINTON
SHIAWA.
OAKLAND
MACOMB
ALLEGAN
BARRY
EATON
INGHAM
LIVINGST'N
VAN BUREN
KALAMA.
CALHOUN
JACKSON
WASHTENAW
WAYNE
BERRIEN
CASS
ST.JOSEPH
BRANCH
HILLSDALE
LENAWEE
MONROE

Fig. 2. Case 7. Green appearnace of *Listeria* colonies when viewed through a dissecting microscope using oblique light. This is at the time that *Listeria* was first visible among the contaminating bacteria. Kodachrome print.

Fig. 3. Case 43. Intestine showing the marked congestion of the small intestine seen in many of the necropsied animals. Kodachrome print.

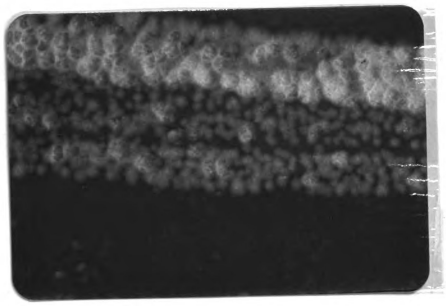


Fig. 2

Fig. 3

Fig. 4. Case 29. Primary isolation from bovine medulla using the grinding and shaking technic for the preparation of the brain suspension. Only two of the colonies visible are *Listeria*.

Fig. 5. Case 29. Primary isolation from bovine medulla using the Waring blendor for the preparation of the brain suspension. Twenty two of the colonies visible are *Listeria*.

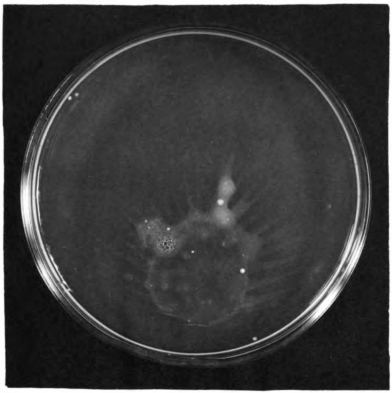


Fig. 4

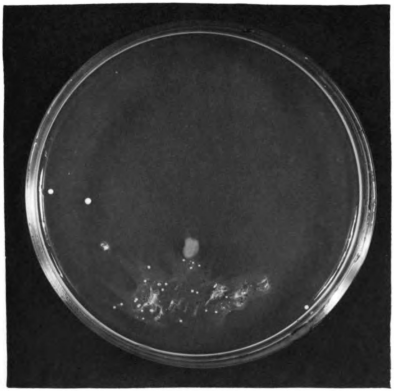


Fig. 5

Fig. 6. Case 33. Primary isolation from bovine medulla using the grinding and shaking technic for the preparation of the brain suspension. Only two of the colonies visible are *Listeria*.

Fig. 7. Case 33. Primary isolation from bovine medulla using the Waring blender for the preparation of the brain suspension. Fifty six of the colonies visible are *Listeria*.

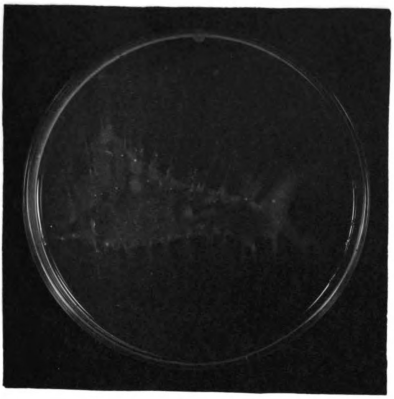


Fig. 6

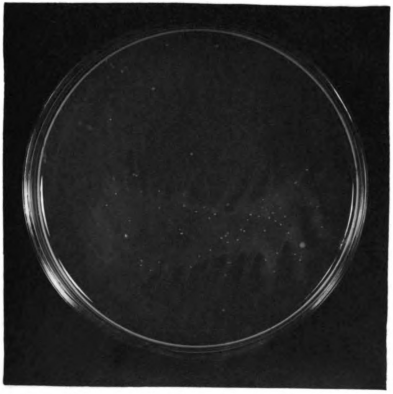


Fig. 7

Fig. 8. Primary isolation from the bovine medulla.
Case 17.

Fig. 9. Primary isolation from the spinal cord
Case 17. immediately posterior to the medulla.

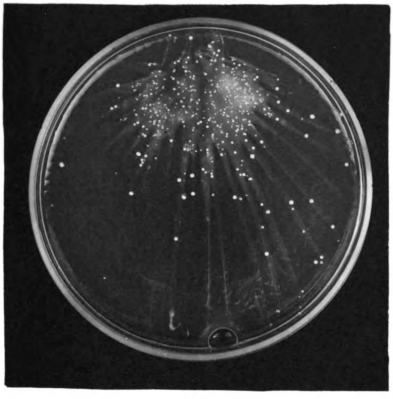


Fig. 8

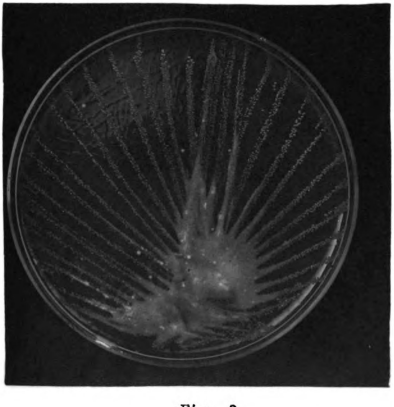


Fig. 9

Fig. 10. Primary isolation from the bovine medulla.
Case 25.

Fig. 11. Primary isolation from the spinal cord
Case 25. immediately posterior to the medulla.

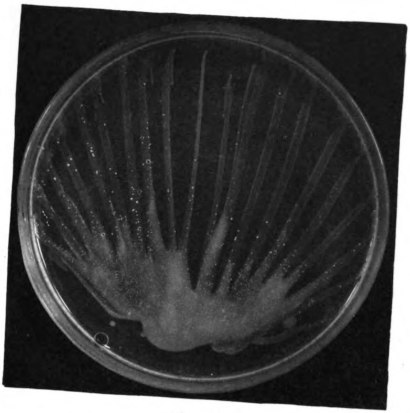


Fig. 10

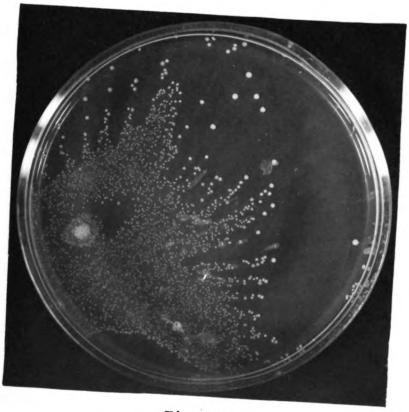


Fig. 11

Fig. 12. Liver showing the large white necrotic
Case 2. foci.

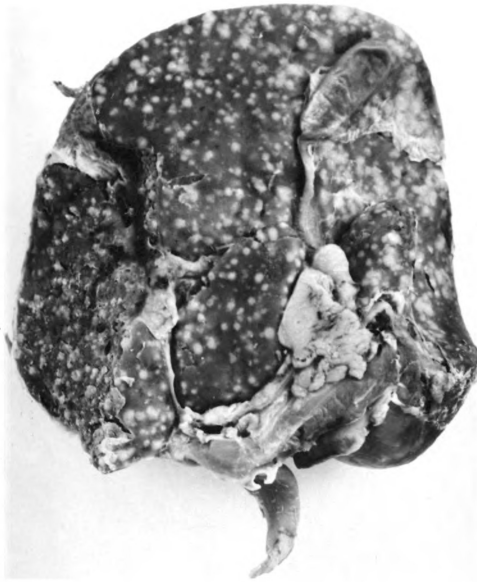


Fig. 12

Fig. 13. Photomicrograph of liver showing
Case 2. focal areas of necrosis (H&E).

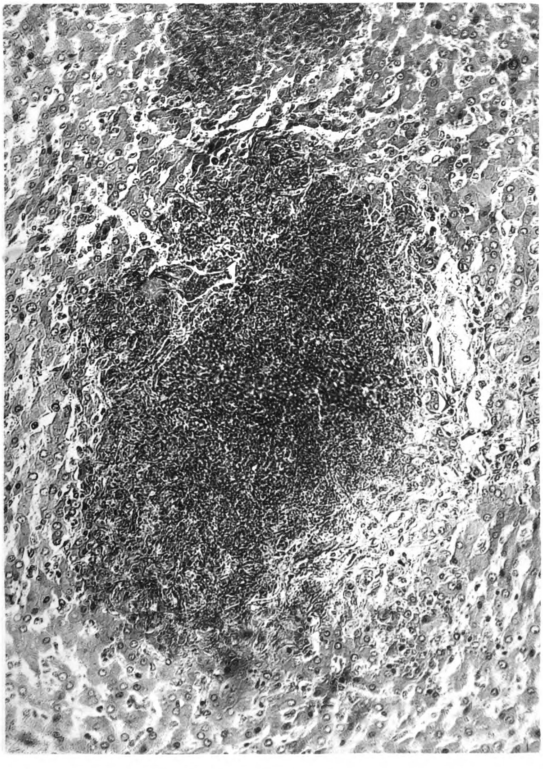


Fig. 13

Fig. 14. Case 2. High power photomicrograph of a minute focal area showing bacteria as dark stained bodies (Gram-Weigert).

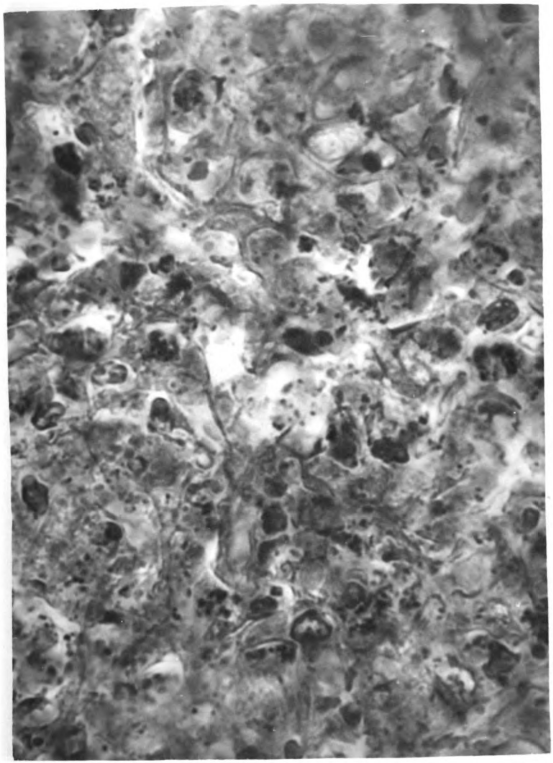


Fig. 14

Fig. 15. Case 8. Yearling heifer. Note the position of the head, drooping right ear, protruding tongue, and excessive salivation. There is also marked conjunctivitis of the right eye. Primary culture of the medulla was negative for Listeria. Print from a Kodachrome transparency from the collection of Dr. C. S. Bryan.

Fig. 15

Fig. 16. Ewe. Note position of head, drooped
Case 34. right ear and protruding tongue.

Fig. 16

Fig. 17. Case 34. Ewe showing tendency to lean against stationary objects. Note mucopurulent exudate from nostrils, protruding tongue, and snow on the wool indicating that the ewe could not maintain her equilibrium.

Fig. 17

Fig. 18. Case 52. Lamb showing the head drawn back and tongue protruding. The neck and forelegs are tense and rigid while the hind quarters are relaxed.

Fig. 18

Fig. 19. Photomicrograph of bovine medulla showing perivascular cuffing and areas of necrosis. Note also necrosis of neurons (H&E).

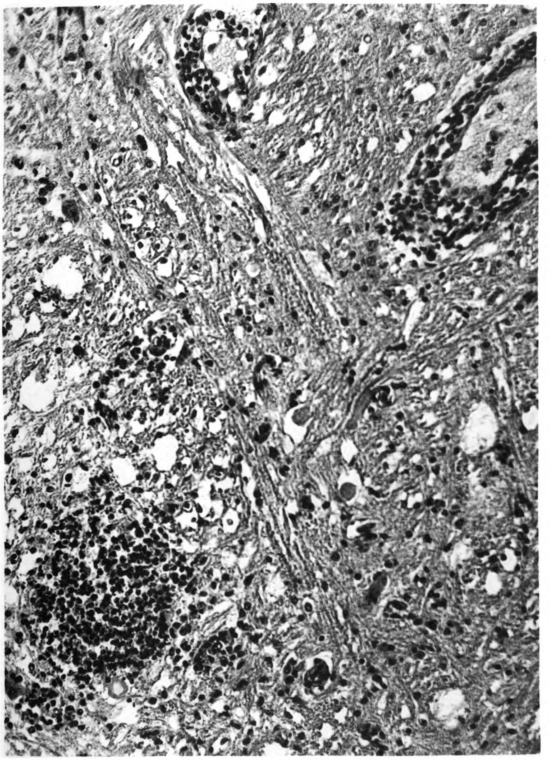


Fig. 19

Fig. 20. Edematous lungs seen in many of the Case 41. sheep presented for necropsy.

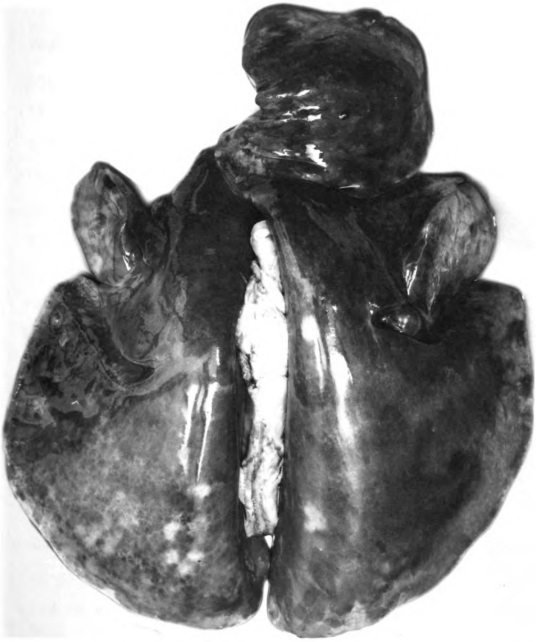


Fig. 20

Part II

LABORATORY OBSERVATIONS

Part II. LABORATORY OBSERVATIONS

Isolation from the Bovine Brain

Using the technics of isolation described in Part I, it was relatively easy to isolate L. monocytogenes from the brain in cases of ovine listeriosis. However, the direct isolation of the microrganism from the bovine brain met with only partial success. Several bovine brains (case 5, 7, 8, 25, 39) failed to yield Listeria upon primary culture. After refrigeration of the prepared brain suspensions it was possible to demonstrate Listeria in all cases where listeriosis was suspected and where tissue sections revealed perivascular cuffing and necrosis in the medulla. In all cases the number of colonies obtained in primary isolations from bovines was small compared to that obtained by primary isolations from ovines. This is shown in table I and figures 24 to 39.

The evidence presented here strongly suggested that the bovine brain possessed a factor bacteriostatic for Listeria. The presence of such substances in animal tissues might account for the specific immunity to infection inherent in certain tissues. Listeriosis in the ovine was generally extremely acute with a short fatal termination. In the bovine the disease was less

acute and recoveries were reported (Biester, 1941; Graham, 1943; and others). Pounden (1947A) reported one outbreak of an extremely acute nature, but that could be considered as atypical. Primary isolation of *Listeria* from the ovine seldom resulted in failure, but in the bovine primary isolations were made in less than a third of the suspects cultured even though listeriosis was confirmed by histopathologic examinations. These observations were similar to those reported by Gifford and Jungherr (1947).

The mechanism of inhibition exhibited in cultures from the bovine brain was not understood. There seemed to be a correlation between the number of colonies obtained at primary isolation and time that had elapsed between death and preparation of culture. Animals that had died of the disease in the veterinary hospital and were necropsied immediately presented the greatest difficulties with respect to primary isolation. Bovine heads received through the mail seldom gave negative results on primary isolation. No direct relationship between time of death and preparation of culture could be established because the time of death was not given in all cases. This evidence strongly suggested the presence of an unstable substance bacteriostatic for *Listeria* in the bovine brain. However, to date this

was not established because experimental results were not conclusive.

Whole bovine brains that had yielded a small number of colonies at primary isolation were kept at 4° C for varying lengths of time. Weighed portions of the medulla suspended in a constant volume of broth were cultured at intervals of a few days. It was found that within five days it was no longer possible to isolate Listeria from these brains. Similarly, sheep brains became sterile after about seven days. This indicated that Listeria could not survive for many days in the brains of its victims, although the microorganism remained viable in prepared brain suspensions.

Apparently normal bovine brains removed immediately after slaughter were prepared, employing the usual technic. A 0.2 ml portion was cultured to check for sterility. The suspension was immediately inoculated with a known number of Listeria. A 0.2-ml portion was plated and incubated at 37° C for 24 hours. There was no evidence that the growth of Listeria was inhibited by the brain material.

Attempts were made to establish the number of microorganisms in the ovine and bovine brains by means of histological sections of the medulla. The results of this work were inconclusive due to the difficulty in

properly staining the bacteria. Added to this was the difficulty of distinguishing between the bacteria and the numerous fibers of the supporting cells.

Table I

Increase in number of colonies after refrigeration of brain suspensions

	Days						
Case	0	2	4	6	8	10	15
10	90					250	1,500 - 18th day
11	33	27		21		22	340 I - 20th day
12	56	161		1360	3300	I	
18	15	35	55	200	312	890	1,000 - 12th day
23	55	61	82		189		3,200 - 18th day
24		2,300 - 3 days					
25	0	0	0				I - 45th day
27	C	C				I	(still many contaminants
29	1	3		3	9	3	82 I - 28th day
29b	24	70		68	62	87	85 717 - 20th day
30	C		4	4	4		I - 25th day
30b	4			9	14		300 I - 20th day
31	125	240	800	1500 - 5th day			
32	9	15	26		500	900	I
33	2	14		4	22	47	1,000 - 14th day
33b	56	108		220		I	
39	0	1	2		3		
40	S	F					
45	39				1500		

I - too numerous to count
C - contamination
S - spreader
F - few colonies, obscure

A 0.2 ml portion of each suspension was used for each count. All counts were made in duplicate. The figure shown was the average of the two. A streak plate was used for primary isolations. For the counts a sterile bent glass rod was employed to distribute the suspension more evenly. Difco tryptose agar was used throughout.

Ocular instillation

Morris and Julianelle (1935) and Julianelle and Pons (1939) found Listeria to produce a characteristic inflammatory reaction when instilled into the eye of rabbits. Suitable bacterial suspensions were prepared by adding approximately eight ml tryptose broth to an 18-24 hour tryptose agar slant culture. The growth was loosened with a sterile loop. The tube was then agitated to break the clumps of organisms, after which it was allowed to stand for 20-30 minutes. Two to four drops from a capillary pipette were dropped directly into the conjunctival sac. All cultures isolated in the first year of this study were tentatively identified by their ability to produce a conjunctivitis in susceptible animals. However, the following year it was found that recently isolated bovine strains did not consistently produce this reaction. In some instances there was merely a slight congestion of the lid. In cases where there was a more marked reaction it was delayed for as long as six days. Whereas with strains isolated from sheep the conjunctivitis appeared within 24-36 hours. It was postulated that this phenomenon was responsible for the failure, in many cases, to isolate Listeria from cows that had succumbed to this disease.

Rabbit No. 1

Two to four drops of culture no. 11 (bovine - 4 days after isolation) instilled into the left eye of rabbit.

Days	Condition	WBC	L	P	M	B	E
0	Normal.	16,600	24	60	4	7	5
1	Normal.	22,900	45	40	3	7	5
2	Normal.						
3	Normal. Eye swabbed.	13,450	41	54	1	1	3
4	Slight congestion of lids(?) Culture shows Listeria.	18,300	30	63	0	4	0
5	Slight injection of upper lid.	24,800	37	62	1	0	0
6	Normal.	22,350	45	52	0	3	0
12	Normal.	24,000	56	42	0	2	0

Rabbit No. 2

Two to four drops of culture no. 11 (bovine - 76 days after isolation) instilled into the left eye of rabbit.

Days	Condition	Temp.	WBC	L	P	M	B	E
0	Normal.	102.6	8,200	46	54	0	0	0
1	Normal.	102.2						
2	Moderate congestion in both lids. Eye swabbed.	102.7	9,650	69	29	1	1	0
3	Increased vascularization. Purulent exudate. Culture negative.	102.4						
4	No change. Swabbed	103.2	11,900	51	47	0	1	1
6	Normal(?) Watery exudate. Culture negative.	101.8	12,450	64	30	2	3	1
7	Normal.							
8	Normal.							

From these data it was observed that this culture four days after isolation failed to produce a conjunctivitis in a susceptible animal, whereas 76 days after isolation a marked conjunctivitis was produced in 48 hours.

Rabbit No. 3

Two to four drops of culture no. 12 (bovine - 24 hours after isolation) instilled into left eye of rabbit.

Days	Condition	WBC	L	P	M	B	E
0	Normal	9,150	59	39	0	2	0
1	Normal	10,600	61	36	0	3	0
2	Normal	17,350	67	31	0	0	1
3	Lacrimation(?)	15,400	42	57	0	0	1
4	Normal	11,750	44	54	0	1	0
5	Normal	11,350	70	28	0	2	0
6	Normal						
9	Slight reddening(?)						
10	Reddening more marked						
11	No change(?)						
12	Slight injection of upper lid(?)	11,800	55	39	3	3	0
13	Normal						

Rabbit No. 4

Two to four drops of culture no. 12 (bovine - 69 days after isolation) instilled into left eye of rabbit.

Days	Condition	Temp.	WBC	L	P	M	B	E
0	Normal	103.0	8,400	81	18	1	0	0
1	Nictating membrane and conjunctiva slightly injected.	102.0						
2	Increased vascularization but no congestion nor exudate. Swabbed.	102.7	7,550	60	40	0	0	0
3	Increased vascularization of cornea. Culture negative.	103.2						
4	No change. Swabbed.	103.0	12,750	84	16	0	0	0
6	Slight improvement. Culture negative. Swabbed.	102.6	9,300	64	32	2	1	1
7	Approaching normal. Culture negative. Swabbed.							
8	Normal. Culture negative.							

From these data it was observed that this culture 24 hours after isolation failed to produce a conjunctivitis in a susceptible animal, whereas 69 days after isolation a marked conjunctivitis was produced in 24 hours.

Rabbit No. 5

Two to four drops of culture no. 14 (ovine - ten days after isolation) instilled into left eye of rabbit.

Days	Condition
0	Normal.
1	Normal.
2	Considerable white purulent exudate. Eye closed. Marked congestion.
3	Eye partially open. Exudate somewhat dry.
4	No change.
5	Less exudate. Cornea slightly injected. Swabbed.
6	No change. A few colonies of Listeria.
8	No change.
10	Eye open. Pinkish in color. Swabbed.
11	No change. Numerous colonies of Listeria.
12	Improved.
14	Normal.
16	Normal. Swabbed.
17	Normal. Culture negative.

Rabbit No. 6

Two to four drops of culture no. 15 (ovine - two days after isolation) instilled into left eye of rabbit.

Days	Condition
0	Normal.
1	Normal.
2	Eye completely closed. Considerable white purulent exudate. Marked congestion.
3	Eye slightly open. Less exudate. No other change.
4	No change.
5	Increased amount of exudate. Marked vascularization of cornea. Swabbed.
6	No change. Heavy growth of pure Listeria.
7	No change.
8	Eye open. No other change.
10	Small ulcer 1mmm dia. on cornea. Eye nearly opaque and has a pale pink appearance. Swabbed.
11	No change. Few colonies Listeria.
12	Opacity less marked. Right eye has considerable watery exudate.
13	Improved.
14	No change.
16	Normal(?) Swabbed.
17	Slight amount of purulent exudate. Culture negative.
18	Normal.

Rabbit No. 7

Two to four drops of culture no. 16 (ovine - two days after isolation) instilled into left eye of rabbit.

Days	Condition
0	Normal.
1	Normal.
2	Eye nearly closed. Considerable white purulent exudate. Marked congestion.
3	No change.
4	No change.
5	Less exudate. Eye open, still congested. Swabbed.
6	No change. Heavy growth of pure Listeria.
7	No change.
10	More exudate. No other change. Swabbed.
11	No change. Few colonies of Listeria.
12	Small ulcer on cornea. Cornea shows marked injection. Cornea opaque. Exudate gone.
13	No change.
14	Considerable exudate. Cornea is clear.
15	No change.
17	Ulcer gone. Less congestion. Swabbed.
18	No change. Culture negative.
19	Improved. Still considerable exudate.
20	No change.
21	Slight amount of exudate. Eye appears normal.
22	Normal. Swabbed.
23	Normal. Culture negative.

Rabbit No. 8

Two to four drops of culture no. 19 (ovine - three days after isolation) instilled into left eye of rabbit.

Days	Condition
0	Normal.
1	Considerable purulent exudate. Marked congestion and vascularization of lids and cornea.
2	Less exudate. No other change.
3	No change.
4	No change.
5	Exudate is dry. No other change.
6	No change.
8	Cornea red and opaque. Considerable watery exudate.
9	No change. Swabbed.
10	No change. Heavy growth of Listeria.

No further record was kept of this reaction.

Rabbit No. 9

Two to four drops of culture no. 8 (bovine - two days after isolation)* instilled into left eye of rabbit.

Days	Condition	WBC	L	P	M	B	E
0		10,500	57	38	0	2	3
1	Normal						
2	Normal	11,650	74	23	2	1	2
3	Normal						
4	Right eye negative. Nictating membrane of left eye congested. Swabbed.						
5	No change. Culture shows few colonies of Listeria.						
6	No change.	8,250	69	24	2	4	1
7	No change.						
8	Eye less congested.						
9	No change. Swabbed.	9,600	68	29	1	2	0
11	No change. Culture negative.						
12	Slightly more congested. White purulent exudate in anterior corner. Eyes swabbed.	14,900	58	38	2	2	0
13	No change. Not eating. Cultures negative.						
14	Marked conjunctivitis. Exudate increased. Depressed. Has not eaten. Eye swabbed.						
15	Numerous colonies Listeria Eye partially closed. Not eating. Drinking water gone.						
16	No change.						
17	Eye improved.	15,800	40	56	3	0	1
18	Culture negative. Eye shows marked improvement with only slight congestion throughout. Increased appetite.						
19	Eye only very slight congestion. Swabbed. General condition appears normal.						
20	Culture negative. Head down on floor. Does not seem to be able to raise it. Seems to circle to right. Puts head in corner and pushes. Rubs nose with forepaws. Breathing difficult with slight wheezing sound. Ears hot,						

Days	Condition	WBC	L	P	M	B	E
	temperature not taken. not eating, but seems to want water. Cannot lift head to drink except with great difficulty. Hind legs twitched when held. Feces were mucoid and adhered to anus.						
21	No change.						
22	No change.						
23	Comatose. Exudate from right eye. Heavy mucus from nose. Killed by ether inhalation. No significant lesions at necropsy. Cultured both eyes, nose, liver, blood and brain.	3,900	31	66	1	1	1
24	Cultures negative for Listeria. Medulla ground and cultured.						
25	Culture shows moderate growth of Listeria. Approximately 200 colonies.						

*Culture originally considered negative.

Rabbit No. 9 represented a true case of encephalitis due to Listeria following ocular instillation.

From Rabbit No. 5, 6, 7 and 8 it was observed that recently isolated ovine cultures produced a marked conjunctivitis within 48 hours. Listeria could be isolated from the exposed eye for at least 11 days folowing instillation.

Following ocular instillation, it was found that in spite of the severity of reaction the infection was confined to the eye. The infected eye usually acquired a resistance to reinfection. However, no generalized

immunity was established. This was evidenced by the fact that the unexposed eye could be infected or that intravenous injections of cultures readily caused death. Conversely, rabbits having high agglutination titers in the circulating blood showed no immunity to ocular instillation. These data were not at variance with those reported by Julianelle (1941).

Viability

In the study of the etiology of this disease it was necessary to have an understanding of the micro-organism's resistance to its environment. Straw, hay, wood shavings, calf pellets (feed) and rabbit pellets (feed) were placed in individual culture tubes. These were autoclaved at 15 pounds pressure for one hour on three consecutive days. Sterility was checked by adding 3-5 ml sterile nutrient broth to several tubes and incubating them at 37° C for 48 hours. After incubation a standard loopful of the material was plated on tryptose agar and incubated an additional 48 hours. A broth culture, case no. 6, which had been incubated at 37° C for 48 hours was added to each tube in amounts of 2-3 ml. The tubes were allowed to remain at room temperature for 24 hours. The broth was removed aseptically by aspiration. The tubes were placed in a pasteboard

box covered with Kraft paper and stored in an appropriate place in the animal pathology barn on June 19, 1947.

The viability of the organisms on each substance was checked at weekly intervals. A tube of each of the materials was removed and 2-3 ml nutrient broth was added. The tubes were then incubated at 37° C for 24 hours. A loopful of this material was then plated on tryptose agar plates and incubated at 37° C for 24 hours. Growth was checked for purity by the use of the oblique lighting previously described. Under the above experimental conditions the viability of L. monocytogenes was found to be as follows:

Straw	- 6 weeks
Hay	-16 weeks
Wood shavings	- 6 weeks
Calf pellets (feed)	-16 weeks
Rabbit pellets (feed)	-26 weeks

An observation of incidental interest was that in the late stages (above 20 weeks) many of the tubes were found to be contaminated with fungi and large gram positive bacilli. Apparently the cotton plugs in the tubes covered with Kraft paper did not afford sufficient protection in a dust laden atmosphere.

Cultures recovered from rabbits which had been inoculated intracerebrally were transferred to sheep

blood agar slants on October 16, 1946. These were incubated at 37° C for 24 hours and placed in the refrigerator at 4° C. At approximately one-month intervals transfers to tryptose agar slants were made to check for viability. Incubation of the subculture at 37° C for 18-20 hours produced a heavy growth of Listeria. This continued until November, 1948, a period of 25 months. The transfers of December, 1948, showed good growth after 48 hours' incubation at 37° C. The last transfers made April 20, 1949 still showed a heavy growth of Listeria after 48 hours' incubation at 37° C. The agar in the original slants was completely dehydrated and very hard and brittle. None of this material was transferred. The transfer loop was merely scraped along the hard surface of the agar.

A similar experiment was begun January 3, 1947. The slants in this instance were maintained at room temperature. Good growth was evident after 18-20 hours' incubation at 37° C through April 22, 1947. The transfer of May 16 was negative. At that time the slants were completely dehydrated. A few ml of nutrient broth was added to the original slants June 5, 1947. Incubation at 37° C for 18-20 hours showed Listeria still to be viable.

From the foregoing it is evident that Listeria

might remain viable for long periods of time under varying environmental conditions. Witts and Webb (1927) and Schwarte (1942) reported that cultures maintained on artificial media for a considerable period of time lost their pathogenicity. One of the cultures that had been kept at 4° C without transfer for 17 months was inoculated intravenously into a rabbit. The case history follows.

Rabbit 10. July 29, 1948.

0.5 ml culture no. 3c standardized to McFarland Nepholometer Tube no. 1 inoculated I.V.

Days	Condition	WBC	L	P	M	B	E
0	Normal	14,350	70	27	1	1	1
1	Profuse exudate from both eyes. Listless. Cultured	12,350	23	71	1	3	2
2	Cultures negative Trembling	10,450	26	66	6	3	0
3	Trembling. Loss of coordination. Feces wet and s sticky	17,200	11	70	15	3	1
4	No change						
5	Dead						

Necropsy findings were limited due to advanced post mortem changes. Listeria was isolated from the heart blood, liver and medulla.

On this same date this culture when instilled into the eye of a susceptible rabbit produced a mild conjunctivitis in 48 hours.

Thirty months after the beginning of the experiment this same culture (3c - 0.5 ml standardized to Mc

Farland Tube no. 1 I.V.) when inoculated intravenously into a susceptible rabbit caused death in less than 72 hours. Listeria was isolated from the heart blood, liver and kidneys. This same culture also produced a mild conjunctivitis in a susceptible rabbit in 72 hours.

These data indicate that Listeria may remain viable for long periods with no loss of pathogenicity.

Inhibition

Early in the course of this study it became obvious that if Listeria was to be successfully isolated from material containing other aerobic microorganisms a satisfactory differential or enrichment medium would have to be devised.

Snyder and Lechstein (1940) demonstrated the value of sodium azide as an inhibitory substance for gram negative bacteria. Packer (1943) described a method for the isolation of streptococci and Erysipelothrix rhusiopathiae in which sodium azide and crystal violet were employed to inhibit the growth of gram negative bacteria. It was shown by Pike (1945) and by Mueller and Miller (1946) that potassium tellurite was of value in isolatien of gram positive microorganisms from throat swabs. Owen (1946) reported good results in inhibiting gram negative bacteria when acetic acid was added to meat infusion tubes. Potassium tellurite, sodium azide,

and acetic acid in deep brain medium were chosen for study.

Sodium azide.

A 1.0% stock solution was prepared and autoclaved at 15 pounds pressure for 20 minutes. Appropriate portions of this stock were added to sterile tryptose agar, pH 7.0, to make the following concentrations: 0.2%, 0.1%, 0.05% and 0.03%. Plates were then poured in the usual manner.

Potassium tellurite.

Two different methods were used in the preparation of the potassium tellurite plates. The first method consisted in adding the appropriate concentration of potassium tellurite to the tryptose agar before autoclaving. This procedure rendered the agar black in color and made it difficult to distinguish differences in colonial morphology.

The second method was the same as that used for the sodium azide plates, i.e., addition of a sterile stock solution to sterile tryptose agar. The concentrations employed were 0.1%, 0.05%, 0.03% and 0.01%.

Pure cultures of L. monocytogenes either alone or in mixture with both gram positive and gram negative bacteria were streaked on the various concentrations of media using tryptose agar as a control. It was ob-

served that sodium azide would have little or no application in the isolation of Listeria, as it was completely suppressed in concentrations as low as 0.03%. Many of the other test organisms showed growth in concentrations of both 0.03% and 0.05%.

Potassium tellurite, on the other hand, showed considerable selectivity. Growth of Listeria was not greatly suppressed in 0.1% concentrations and growth was uninhibited at concentrations of 0.05% and less. These concentrations were sufficiently high to suppress most gram negative bacteria. However, micrococci and streptococci also grew freely at the higher concentrations. The use of the dissecting microscope, as previously described, was employed at this point. The Listeria colonies appeared black as do all colonies on this medium, but the characteristic green color was evident at the periphery of the colony. The micrococci were pinkish yellow at the periphery, intensely black at the center, and extremely glossy in appearance. The streptococci were smaller, pinkish grey in color with a dull surface.

A less artificial method of determining the effectiveness of this medium for the isolation of Listeria consisted of swabbing the nasal passages of supposedly normal sheep. The swabs were placed in nutrient broth, three drops of a 24 hour broth culture of Listeria was added. The tubes were incubated from 4 to 8 hours at

37° C and plated on potassium tellurite plates and tryptose agar. Identification of colonies of Listeria was invariably impossible on the tryptose agar but they were found without difficulty on the potassium tellurite plates.

Excellent results were obtained when 0.05% potassium tellurite was added to plain nutrient broth and used as an enrichment medium. The material to be cultured, nasal swabs, straw, feces, etc. were added directly to the broth and incubated 6 to 24 hours at 37° C. A loopful of this material was then plated on plain tryptose agar and incubated for 20-24 hours at 37° C. Examination of the plates with the aid of the dissecting microscope revealed the absence or presence of Listeria. With this method nearly pure cultures of Listeria can be obtained providing the number of micrococci in the material is not too high.

Deep brain medium.

Deep brain medium was prepared and acetic acid added to make concentrations of .01%, .15%, 0.1%, 0.5%, 1.0%. This medium was inoculated as described above and incubated at 37° C for 20-24 hours. A loopful of the material was plated on tryptose agar plates. These were incubated for 20-24 hours at 37° C. Listeria grew freely in all concentrations. The gram negative bacteria were markedly suppressed especially in the higher concentrations. The results were comparable to those of

potassium tellurite. Potassium tellurite was adopted, however, because of the convenience of its preparation.

The Effect of Antibiotics

The effects of various therapeutic agents in treating infections with members of the genus Listeria had been reported. Porter and Hale (1939) showed that sulfanilamide and sulfapyridine were of value in treating Swiss mice experimentally exposed to Listeria. Graham et al (1943) reported the use of sulfanilamide in field cases of listeriosis. Their work indicated that this drug was of little or no value in naturally occurring cases of the disease. Foley, Epstein and Lee (1944) reported that Listeria cultures will grow freely in 40 times the concentration of penicillin necessary to inhibit other gram positive organisms. Handelman et al (1946) reported apparent recovery from Listeria meningitis in a six week old infant treated with sodium sulfadiazine after penicillin therapy had failed. Gibbons (1948) claimed success in treating some cases of bovine listeriosis with penicillin and sodium sulfamethazine. Discouraging results with treatment were cited Thesis, Part I. This review would justify further search for a satisfactory treatment of this disease.

Streptomycin

Tryptose agar, pH 7.0, plates containing 0.5,

1.0, 3.0, 6.0 and 10.0 units of streptomycin (hydrochloride) were prepared. Seven cultures of ovine origin and eight cultures of bovine origin were used. These stock cultures were maintained on tryptose agar slants. Their age ranged from two years to those recently isolated. They were transferred through nutrient broth daily for three days prior to exposure to the antibiotic. A 1.0 mm platinum loop was used to inoculate the plates. They were incubated for 24 hours at 37° C. It was observed that most of the cultures grew well on plates containing 0.5 unit of streptomycin but were almost completely inhibited by one unit. Incubation for an additional 24 hours showed a marked growth of Listeria at one unit and slight growth at 3.0 units. The cultures containing higher concentrations were negative (table I). These results were similar to those reported by Coles (1948). Because of the similarity of results, four cultures of ovine and four cultures of bovine origin were chosen for further study. Colonies were picked from the plates containing one unit and transferred to plates containing three and six units. Colonies were then picked from the three and six unit plates and plated on 10 unit plates. By this adaptive procedure it was possible to produce good growth in 24 hours on the 10 unit plates. Tables II, III, and IV show the results up to 6 units after 24 hour incubation periods at 37° C. An additional

24 hours' incubation invariably produced sufficient growth for transfer to a higher concentration of streptomycin.

During the course of this study it was observed that there was an apparent individual resistance to streptomycin. Where discrete colonies were observed there was a marked difference in colony size. This had never been observed in growth on plain tryptose agar (figure 40).

One of the large and one of the small colonies were picked from a plate containing one unit and from a plate containing 10 units and transferred to broth and plain agar slants, incubated for 24 hours and plated on streptomycin plates. The results are shown in Table V.

These exposed cultures were then transferred daily for seven days through broth and on tryptose slants and re-exposed to the same concentrations of streptomycin. The results were essentially the same. These cultures were then left without transfer at 4° C and at 21° C for a period of six weeks. Re-exposure to streptomycin yielded approximately the same results.

In vivo tests were carried out using rabbits. The streptomycin used was the calcium chloride complex. In each experiment one animal was treated at exposure, one 12 hours following exposure and one left as an untreated control. Temperatures were recorded at eight

hour intervals the first day and every 24 hours thereafter. Leukocyte and differential counts were taken at 24 hour intervals. Dosage ranged from 2,000 units per day per rabbit to 150,000 units per day per rabbit. Because the number of rabbits used, 21 in all, was small no definite conclusions could be made but in each case the animal treated initially showed the least temperature rise, but two of these animals were the first to die. The monocytosis following infection was inconclusive but tended to be somewhat lower in the treated animals. The histories of the individual rabbits are given on pages 68 to 88. Cultures isolated from the treated animals were used to inoculate tryptose agar plates containing streptomycin. There was some evidence that these organisms had acquired a resistance to the antibiotic but the results were not consistent and are not given here.

TABLE I

Cultures initially exposed to streptomycin.

	Units streptomycin / ml agar						
	24 hr. incubation				48 hr. incubation		
Culture	0	0.5	1.0	3.0	0.5	1.0	3.0
1	++++	+++	±	-	++++	+	±
2	++++	+++	-	-	++++	±	1 colony
3	++++	+++	±	-	++++	±	1 colony
4	++++	+++	±	-	++++	+	1 colony
5	++++	+++	e	-	++++		
6	++++	+++	±	-	++++	+	±
7	++++	+++	±	-	++++	+	±
8	++++	+++	±	-	++++	+	±
9	++++	+++	±	-	++++	+	±
10	++++	+++	±	-	++++	±	3 colonies
11	++++	+++	-	-	++++	±	2 colonies
12	++++	+++	++	-	++++	+--	+
A	++++	+++	±	-	++++	±	±
B	++++	+++	±	-	++++	±	±
C	++++	+++	+	-	++++	+	±

± = slight growth ± = marked growth ± = trace

e = contaminated

A = strain obtained from Dept. Bact. and Pub. Health, M.S.C.

B = strain obtained from Mich. Dept. Pub. Health Lab.

C = strain obtained from Va. Poly. Inst., Blacksburg, Va.

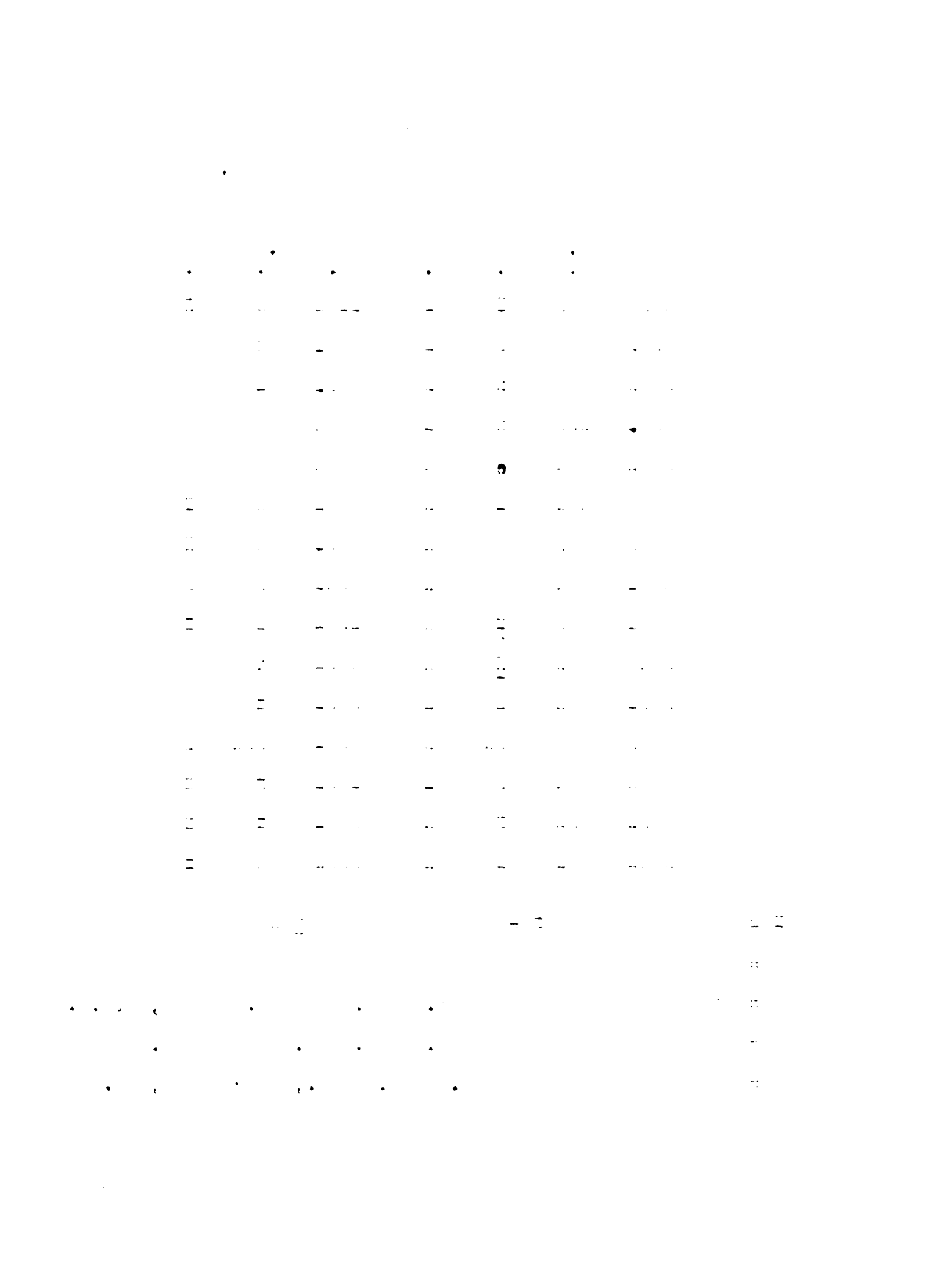


TABLE II

Initial exposure

Units streptomycin / ml agar

Culture	0	0.5	1.0	3.0
2	++++	+++	-	-
4	++++	+++	T	-
B	++++	+++	T	-
9	++++	+++	+	-
7	++++	+++	-	-
10	++++	+++	+	-
12	++++	+++	T	-
C	++++	+++	-	-

T = Trace
Cultures 2, 4, B and 9 are of ovine origin; 7, 10, 12 and B of bovine origin.

TABLE III

Colonies picked from 0.5 u plates

Units streptomycin / ml agar

Culture				
2	++++	++++	+++	-
4	++++	++++	++	-
B	++++	++++	++	-
9	++++	++++	++	-
7	++++	++++	+	-
10	++++	++++	+	-
12	++++	++++	++	-
C	++++	++++	+	-

TABLE IV

Colonies picked from 1.0 u plates

Units streptomycin / ml agar

Culture	0	1.0	3.0	6.0	10.0
2	++++	++++	++	T	-
4	++++	++++	++	+	-
B	++++	++++	+	T	-
9	++++	++++	+++	+++	-
7	++++	-	T	-	-
10	++++	++++	-	-	-
12	++++	++++	+	-	-
C	++++	++++	+	+	-

TABLE V

Units streptomycin / ml agar

12 LV	++++	++++	+++	+	+
12 SV	++++	++++	T	-	T
10 LII	++++	++++	-	-	-
10 SII	++++	++++	-	-	-
10 control	++++	T	-	-	-

LV = large colony from 10 u LII = large colony from 1 u

SV = small colony from 10 u SII = small colony from 1 u

Rabbit 5a

0.5 ml of a saline suspension of culture no. 9 standardized to a MacFarland tube No. 1 inoculated I.V. This animal was an untreated control.

Days	Temp.	Hb	WBC	L	P	M	B	E
0	102.8	11.4	9,800	35	61	0	0	4
1	106.0		8,200	26	70	3	1	0
	(not eating) (blood culture - 3 colonies)							
2	105.0	12.0	5,000	26	59	14	0	0
	(snuffles - exudate in left eye - breathing hard running nose)							
3	100.0		8,700	18	51	31	0	0
	(semi comatose)							

Necropsy

Considerable exudate from both eyes and thick mucus from the nose.

Liver: Marked fatty changes and a few white foci.
Heart: Flabby, yellow, slightly congested.
Spleen: Swollen with numerous white foci 1 mm. in diameter.
Stomach and intestine were nearly empty.
All other organs appeared normal.

Cultures: both eyes, nose, saliva, lung, liver, spleen, kidney and heart blood.

Positive cultures: blood, 1 colony; spleen and lungs, a few colonies; and liver, heavy growth.

Rabbit 6a

0.5 ml of a saline suspension of culture no. 9 standardized to a MacFarland tube No. 1 inoculated I.V. 1,000 u streptomycin given at time of inoculation and every 12 hours thereafter until death.

Days	Temp.	Hb	WBC	L	P	M	B	E
0	102.5	14.1	6,950	41	54	1	3	1
1	103.6		7,550	18	88	0	0	0
2	105.0	12.9	5,400	41	56	3	0	0

Culture of 1 shows 7 colonies Listeria. Trembling, drinking lots of water, not eating.

Necropsy

Liver: severe fatty changes and numerous white foci throughout all lobes.
Spleen: black and swollen.
Lungs: congested.
Heart: flabby and yellow and somewhat congested.
All other tissues appeared normal.

Cultures: lungs, liver, kidneys, spleen and heart blood.

Positive cultures: liver, heavy growth; one kidney and spleen, a few colonies.

The other cultures were covered by a spreading contaminant and no Listeria could be observed.

Rabbit 7a

0.5 ml of a saline suspension of culture no. 9 standardized to a MacFarland tube No. 1 inoculated I.V. 1,000 u streptomycin was given every 12 hours starting 12 hours after inoculation.

Days	Temp.	Hb	WBC	L	P	M	B	E
0	102.3		10,900	55	44	0	1	0
1	104.6		9,100	18	75	2	3	2
2	106.2	11.1	10,950	45	43	12	0	0
	(Diarrhea - snuffles - blood culture negative)							
3	103.0		9,950	50	22	28	0	0
	(Breathing hard)							
4	99.8							

This animal was down and seemed to be near death. Suddenly it lunged out of its cage striking the cement floor with its head. He was dead when picked up.

Necropsy

There was considerable mucus from the nose.

Lungs: Old abscesses throughout all lobes. Some areas of both cold and active pneumonia.
Heart: Flabby and slightly ingested and congested.
Liver: Fatty degeneration with a few white necrotic foci about 1 mm. in diameter.
Kidneys: Appeared normal but each contained blood tinged urine.
Spleen: Swollen and numerous areas of focal necrosis approximately 1 mm. in diameter.
Uterus: Red and swollen.
All other viscera appeared normal.

Cultures: liver, spleen, heart blood and lung.

Positive cultures: liver, heavy growth; spleen, a few colonies.

Rabbit 8a

0.5 ml of a saline suspension of culture no. 9 standardized to a MacFarland tube No. 1 inoculated I.V. 1,000 u streptomycin given at time of inoculation and every 12 hours thereafter until death.

Days	Temp.	Hb	WBC	L	P	M	B	E
0	102.0	11.4	14,900	23	73	3	1	0
1	106.0		15,850	14	78	8	0	0
2	105.5	8.9	17,000	10	77	12	0	1
3	104.4		14,350	21	59	19	1	0
4	103.6	9.2	16,250	11	75	12	2	0
5	103.8		40,000	6	64	28	2	0
6	104.6		17,250	17	63	20	0	0
20				38	48	12	0	2
	(monocytes old and degenerate - estimated count - 10-15,000)							
32			4,800	51	43	3	0	3

This animal never completely recovered from the effects of the disease. It was thin and emaciated, took very little food or water.

Necropsy

Liver: Swollen and leukemic in appearance.
Lungs: Some cold pneumonia.
Heart: Pale and with the veins injected.
Spleen: Black and swollen to twice normal size with numerous white foci throughout.
Kidney: Swollen - right has a large abscess at anterior end (section).

Cultures: both eyes, kidney, lung, liver and blood.

Positive cultures: kidney, 2 colonies; lung, liver and blood, each 5 colonies.

Rabbit 9a

0.5 ml of a saline suspension of culture no. 5 standardized to a MacFarland tube No. 1 inoculated I.V. 1,000 u streptomycin given at time of inoculation and every 12 hours thereafter until death.

Days	Temp.	Hb	WBC	L	P	M	B	E
0	103.4	12.5	7,000	23	62	0	7	0
1	104.8		10,800	8	89	2	1	0
2	105.5	11.4	8,750	21	68	8	3	0
	(blood culture - 2 colonies)							
3	105.0		10,700	9	63	26	2	0
4	104.7	10.8	16,550	6	68	25	1	0
	(crippled)							
5	103.4		26,300	3	74	22	1	0
	(crying as if in pain)							
6	96.2		21,000	8	73	18	1	0
	(still crying - emaciated)							

Killed with ether.

Necropsy

There was excessive purulent exudate from left eye.

Liver: A few necrotic areas 1 mm in diameter. Old coccidia lesions along margin and hepatic artery.
Spleen: Swollen, a few small foci.
Kidney: A few old abscesses.
Brain: There seemed to be an excess of fluid and the medulla appeared congested.

Cultures: Kidney, lung, blood, liver, spleen, cerebral fluid, brain, aqueous humor, both eyes, mouth and nose.

Positive cultures: kidney and liver, heavy growth; lung, spleen, brain, cerebral fluid, and blood, a few colonies.

Rabbit 10a

0.5 ml of a saline suspension of culture no. 5 standardized to a MacFarland tube No. 1 inoculated I.V. 1,000 u streptomycin given every 12 hours, starting 12 hours after exposure.

Days	Temp.	Hb	WBC	L	P	M	B	E
0	103.5	10.1	12,000	47	52	1	0	0
1	106.5		7,900	5	82	13	0	0
2	105.3	12.9	15,450	17	50	32	0	1
3	104.8		26,200	10	58	32	0	0
4	103.2	10.1	22,650	16	56	28	0	0
5	102.4		28,000	10	44	46	0	0
6	103.4		19,200	13	54	32	1	0
20				17	67	16	0	0

This animal never completely recovered. It was thin, refusing food and water most of the time. Has not eaten for the past 3 days.

Necropsy

Liver: Numerous white necrotic areas about 1 mm in diameter throughout.
Kidneys: Left swollen to twice normal size.
Right slightly swollen, soft and degenerated.
Stomach and intestine: Empty.

Cultures: liver, blood and kidney.

All cultures were covered with spreaders and no Listeria could be observed.

Rabbit 1b

0.4 ml culture no. 5. This animal was an untreated control.

Days	Temp.	WBC	L	P	M	B	E
0	102.5	10,400	80	19	0	1	0
1	107.0A.M. 106.9P.M.	12,350	40	53	2	1	4
2	107.4 (drinking - anorexia)	14,500	26	62	9	0	3
3	106.4 (trembling, nervous, no activity)	33,100	9	65	22	0	4
4	106.0 (bad fits)	29,600	20	67	11	2	0
5	105.0 (snuffles - difficulty standing)	26,400	43	40	11	6	0
6	Dead.						

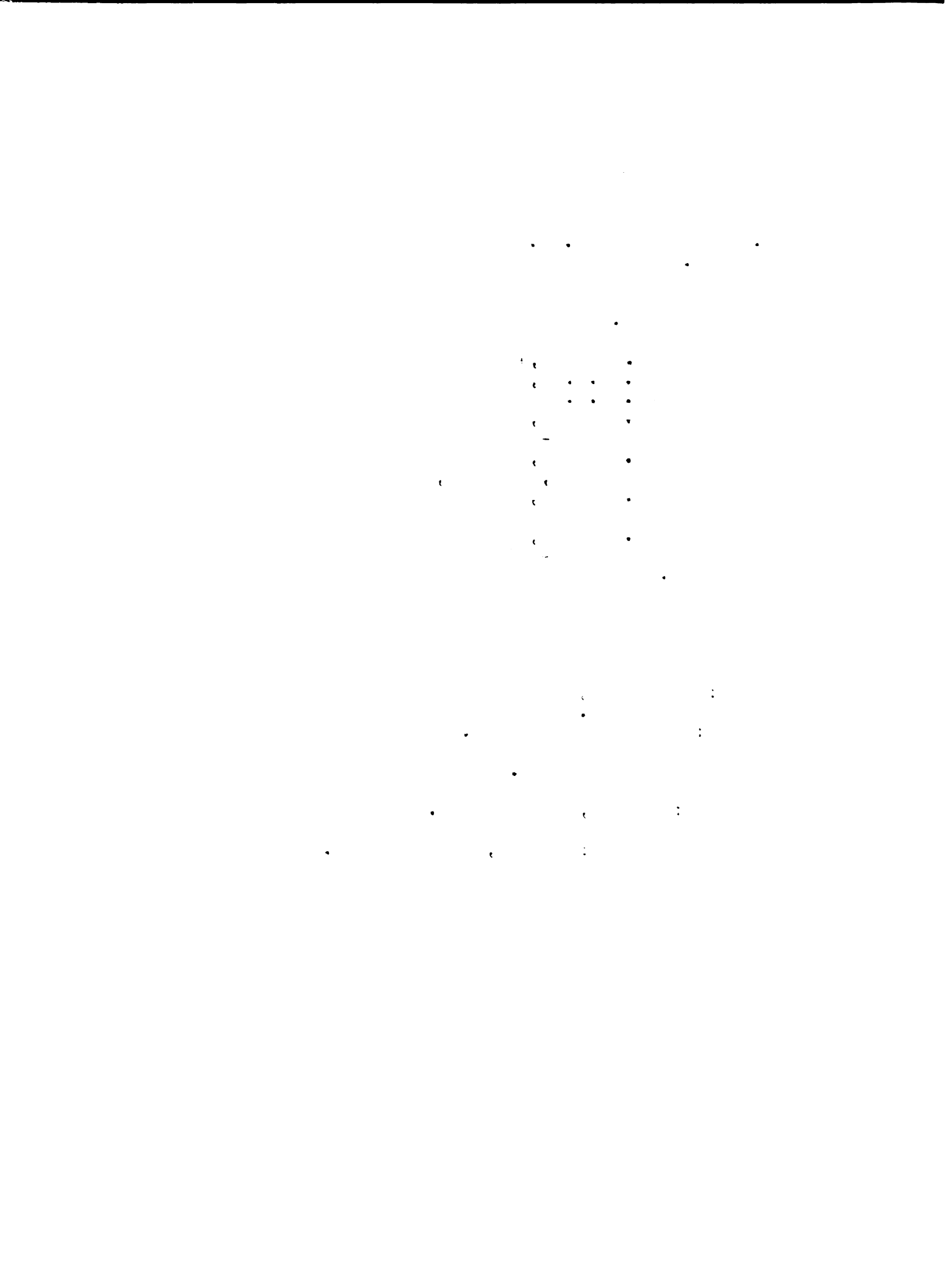
Necropsy

Liver: Swollen, a few small white foci and old coccidia lesions.
Spleen: Black and swollen.

No other gross lesions.

Cultures: liver, heart blood.

Positive culture: liver, few colonies.



Rabbit 2b

0.8 ml culture no. 5 I.V. 10,000 u streptomycin given at time of inoculation and 5,000 u given every 8 hours thereafter. 0.8 ml was given in error.

Days	Temp.	WBC	L	P	M	B	E
0	102.3	11,600	62	34	2	2	0
	(sore on left eye)						
1	106.6	15,100	62	23	9	6	0
	(left eye closed - some exudate - many platelets and baskets)						
2	106.0	41,800	53	31	14	2	0
	(many platelets and baskets - snuffles - eating)						
3	105.3	33,100	73	10	16	1	0
	(anorexia - snuffles - nervous - crippled)						
4	94.8	50,150	51	4	42	3	0
	(comatose)						

Killed with ether.

Necropsy

Liver: Large areas that appeared necrotic - old coccidia lesions along the margin.

Spleen: Pitted - a few necrotic foci approximately 1 mm. diameter.

Intestine: Empty - injected throughout the entire length.

Spinal cord: Pinched in thoracic region and congested throughout.

No other gross lesions were observed.

Cultures: blood, liver, urine, medulla.

Positive cultures: liver, few colonies.

Rabbit 3b

3 drops of culture no. 9 instilled into the left eye. 10,000 u streptomycin at time of exposure and 5,000 u given every 8 hours thereafter.

Days	Temp.	WBC	L	P	M	B	E
0	102.2	9,350	64	30	2	2	2
1	103.0	10,950	66	23	9	2	0
2	105.2	8,650	69	27	1	3	0
	(eye closed tight - streaming exudate)						
3	103.0	8,450	64	23	4	7	2
	(no change)						
4	103.3	12,400	69	20	7	4	0
	(eye closed - exudate dry)						
5	103.7	11,300	52	38	10	0	0
	(no change)						
8	102.9	10,400	54	38	6	2	0
	(open, but opaque - no exudate)						
11	103.6	10,200	48	36	8	4	4
	(opaque (Kodachrome) culture)						
14	103.1	9,700	55	35	4	6	0
	(clearing - ulcer in center-culture)						
17		8,600	41	44	7	6	2

Rabbit 4b

0.4 ml culture no. 5 I.V. 10,000 u streptomycin given 12 hours after exposure and 5,000 u given every 8 hours thereafter.

Days	Temp.	WBC	L	P	M	B	E
0	103.3	6,350	58	41	1	0	0
1	103.3A.M. 106.3P.M.	11,550	34	59	4	0	0
2	105.4	8,750	19	54	22	0	5
3	106.3	17,700	15	48	30	0	7
	(trembling - snuffles - heavy breathing)						
4	106.0	16,250	14	69	13	4	0
	(worse - relaxed - exudate in left eye)						
5	103.3	11,850	34	42	17	7	0
	(no change)						
8	103.6	29,100	31	62	5	2	0
	(thin, soft feces - improved)						
11	103.4	18,100	25	38	33	4	0
	(normal)						
14	103.3	13,900	53	37	1	9	0
	(normal)						
17		8,600	48	28	11	3	0
	(normal)						

Rabbit 5b

0.4 ml culture no. 8L I.V. This animal was an untreated control.

Days	Temp.	WBC	L	P	M	B	E
0	103.4	5,750	91	8	1	0	0
1 A. M.	104.0	8,250	87	13	0	0	0
P. M.	104.7						
2	106.6	8,850	56	34	8	1	1
3	105.2 (difficulty walking)	10,150	75	15	6	3	1
4	104.9 (slight trembling)	12,400	47	20	28	3	2
5	104.1	14,350	59	35	3	3	0
8	103.4	11,100	56	34	7	1	2
11	102.9	8,550	50	27	21	2	0
14	102.9 (normal)	12,850	46	33	12	8	1
17		8,800	24	51	15	9	1
68 Dead							

Necropsy

Both eyes: considerable exudate
left eye somewhat injected especially along the lower margin of the cornea.
Kidneys: slightly pale and swollen.

There were no other gross lesions.

Cultures: heart blood, liver, spleen, kidney, brain, lung, and both eyes.

Positive cultures: None.

Rabbit 6b

0.4 ml culture 8L I.V. 10,000 u streptomycin given 12 hours after exposure and 5,000 u given every 8 hours thereafter.

Days	Temp.	WBC	L	P	M	B	E
0	103.6	7,800	78	22	0	0	0
1 A.M.	104.8	9,150	29	64	1	0	1
P.M.	106.0						
2	106.5	6,050	37	35	21	3	4
3	105.2 (breathing hard)	8,650	40	24	31	5	0
4	104.0 (slight trembling)	16,550	74	15	7	4	0
5	104.6	9,850	71	14	13	2	0
8	103.6 (thin)	10,800	64	20	12	4	0
11	103.0	8,950	42	30	16	9	3
14	103.2 (normal)	7,950	52	35	12	1	0
17		7,550	49	40	7	4	0

Rabbit 7b

Three drops of culture no. 9 instilled into the left eye. This animal was an untreated control.

Days	Temp.	WBC	L	P	M	B	E
0	104.2	6,900	76	23	1	0	0
1	103.5	10,500	61	36	3	0	0
2	103.5	9,500	60	32	5	3	0
	(slight exudate)						
3	103.5	10,100	68	24	2	1	0
	(very slight injection and exudate)						
4	103.5	12,550	69	23	6	2	0
	(no change)						
5	103.4	12,850	81	12	7	0	0
	(normal)						
8	103.5	10,150	67	24	2	2	0
	(normal - eye culture negative)						
11	105.2	10,500	59	28	9	4	0
	(normal - 4 colonies Listeria)						
14	103.0	9,000	77	17	3	3	0
	(normal - eye culture negative)						
17		7,250	46	40	7	6	1

Rabbit 8b

0.4 ml culture no. 9 I.V. This animal was an untreated control.

Days	Temp.	WBC	L	P	M	B	E
0	103.0	10,100	72	26	0	1	1
1	A.M.103.4	7,650	45	53	0	2	0
	P.M.106.1						
2	107.0	7,850	33	64	2	1	0
	(left eye closed)						
3	106.6	9,800	53	43	2	2	0
	(head swaying)						
4	105.8	15,500	33	53	12	2	0
	(trembling - twitching)						
5	104.5	14,650	36	48	7	9	0
	(no change)						
8	104.6	17,450	61	27	6	6	0
	(left eye closed - otherwise normal)						
11	104.1	17,100	33	54	8	5	0
	(no change)						
14	103.5	9,700	54	29	6	1	0
	(eye open)						
17		6,250	44	37	10	9	0
	(normal)						

Rabbit 9b

0.4 ml culture no. 9 I.V. 10,000 units streptomycin given 12 hours later and 5,000 units given every eight hours thereafter.

Days	Temp.	WBC	L	P	M	B	E
0	102.8	7,600					
1	104.6A.M. 106.6P.M.	9,950	25	75	0	0	0
2	106.4	9,050	48	51	8	3	0
3	106.5	15,650	53	39	7	1	0
4	106.1	15,450	33	60	7	0	0(anorexia)
5	104.3	13,900	51	34	12	2	1
8	104.6 (not drinking)	26,550	47	45	5	3	0
11	104.0	no blood	(right eye bad)				
14	104.9 (right-bad - very thin)	22,700	41	55	2	1	1
17		11,450	64	29	4	3	0

19 P.M. Dead.

Necropsy

Right eye: marked conjunctivitis.
Heart: firm, yellow mass at apex measuring about 1.5 cm. in dia. This same material extended into the walls and septa of both ventricles. The entire organ appeared pale and flabby.
Intestine: empty and somewhat congested.
Liver: a few old coccidia lesions.

No other gross lesions.

Cultures: eyes, heart, heart blood, liver brain and urine.

Positive cultures: none.

Rabbit 10b

0.4 ml culture no. 9 I.V. 10,000 u streptomycin given at exposure and 5,000 u every 8 hours thereafter.

Days	Temp.	WBC	L	P	M	B	E
0	102.2	9,000	84	16	0	0	0
1 A.M.	102.7	10,250	41	53	0	2	4
P.M.	105.1						
2	106.3	8,500	62	34	0	4	0
3	105.0	19,050	60	31	4	5	0
4	104.9 (off feed)	18,150	68	25	3	4	0
5	104.0 (eating again)	15,100	56	40	0	4	0
8	103.3	15,400	64	33	0	3	0
11		17,600	55	32	5	6	2
14	102.7	15,400	58	37	2	3	0
17		11,550	31	56	7	5	1

Rabbit 11b

0.4 ml culture 8L I.V. 10,000 u streptomycin given at time of exposure and 5,000 u given every 8 hours thereafter.

Days		Temp.	WBC	L	P	M	B	E	
0		103.0	5,650	80	19	0	0	1	
1	A.M.	103.5	8,400	40	58	0	1	1	
	P.M.	106.3							
2		106.2	9,550	48	44	2	4	2	
3		105.3	15,100	20	63	6	8	3	
4		104.3	11,000	57	32	9	2	0	(not eating)
5		104.0	15,400	58	18	12	2	0	(eating)
8		103.0	12,250	68	26	0	5	1	(normal)
11		102.6	9,850	63	29	4	3	1	(normal)
14		103.3	7,050	67	28	2	3	0	(normal)
17			12,250	64	31	2	2	1	(normal)

Rabbit 1e

0.75 ml of culture no. 9 I.V. This animal was an untreated control.

Days	Temp.	WBC	L	P	M	B	E
0 7:00	103.0A.M.	8,650	67	25	2	6	0
3:20	105.8P.M.	(breathing hard and twitching)					
9:45	106.8P.M.						
1	106.0A.M.	5,200	53	45	0	2	0
	105.8P.M.	(many nucleated and other immature reds - unsteady)					
2	102.7	10,200	60	36	4	0	0
	(limp, unable to stand)						

2 P.M. - Dead.

Necropsy

Liver: A number os small pinpoint areas of necrosis, severe fatty degeneration, and a few old coccidia lesions along the margin. Slightly swollen.
Spleen: Black but otherwise normal.

No other gross lesions.

Cultures: liver, heart blood, kidney.

Rabbit 2c

0.75 ml culture no. 9 I.V. 50,000 u streptomycin I.M. every 3 hours.

Days		Temp.	WBC	L	P	M	B	E
0	7:00	103.3A.M.	10,700	74	24	1	0	1
	3:20	105.3P.M.	(breathing hard)					
	9:45	105.5P.M.						
1		106.0A.M.	4,800	31	66	1	2	0
		105.6P.M.						
2		105.3	10,550	41	42	14	2	1
		(some immature red cells)						
3		103.1	13,900	53	29	16	2	0
		(not many immature red - eating?)						
5			9,400	66	18	11	5	1
7			10,600	74	24	1	1	0
10			12,200	63	28	6	1	2
14			9,900	64	24	6	5	1

Rabbit 3e

0.75 ml culture no. 9 I.V. 50,000 u streptomycin at exposure and 25,000 u every 3 hours thereafter.

Days		Temp.	WBC	L	P	M	B	E
0	7:00 3:20 9:45	102.7A.M. 105.6P.M. 106.9P.M.	10,850 (breathing hard)	66	28	2	4	0
1		106.0A.M. 105.7P.M.	6,850	45	49	4	2	0
2		105.9	5,900	39	41	18	2	0
3		101.2	15,350	45	38	17	0	0
5			20,400	65	19	15	1	1
7			18,350	87	8	3	2	0
10			17,400	69	27	4	0	0
14			14,450	79	14	6	1	0

Rabbit 4c

0.75 ml culture no. 9 I.V. 50,000 u streptomycin every 3 hours.

Days	Temp.	WBC	L	P	M	B	E
0 7:00 3:20 9:45	102.5A.M. 106.4P.M. 106.5P.M.	7,900	60	35	2	1	2
1	106.0A.M. 106.2P.M.	6,450	10	88	0	2	0
2	105.0	9,650	22	49	28	1	0
3	103.3	13,600	38	41	21	0	0
5		9,600	60	26	12	1	1
7		11,850	56	29	11	4	0
10		10,050	55	28	12	5	0
14		6,600	63	13	21	3	0

Bacitracin

Considerable interest had been shown in bacitracin since its isolation by Johnson *et al* (1940). *In vitro* tests were carried out using four ovine (1, 9, 13, 48) and four bovine (8, 11, 27, 45) strains of *Listeria*. Difco tryptose agar, pH 7.0, was used as a base medium and bacitracin added to make the following concentrations: 0.125, 0.25, 0.5, 1.0 and 2.0 units. These plates were inoculated with a 1 mm loopful of 24 hour broth culture of each strain. Plates were read after incubation at 37° C for 24 and 48 hours. The results are shown in tables VI, VII and VIII.

TABLE VI

Initial exposure

	Units bacitracin / ml agar								
	24 hr. incubation						48 hr. incubation		
Culture	0	0.125	0.25	0.5	1.0	2.0	.5	1.0	2.0
1	++++	++++	++++	±	-	-	++	-	-
9	++++	++++	++++	+	-	-	+++	-	-
13	++++	++++	++++	±	-	-	+++	-	-
48	++++	++++	++++	-	-	-	+	-	-
8	++++	++++	++++	+++	-	-	+++	±	-
11	++++	++++	++++	+++	-	-	+++	±	-
27	++++	++++	++++	-	-	-	±	-	-
45	++++	++++	++++	-	-	-	-	-	-

TABLE VII

Colonies picked directly from 0.5 u plates

Culture	Units bacitracin / ml agar 24 hr. incubation 1.0	2.0
1	-	-
9	±	-
13	±	-
8	±	-
11	±	-

TABLE VIII

Cultures picked from 0.5 u plates and maintained in nutrient broth for 16 days.

Culture	Units bacitracin / ml agar 24 hr. incubation 0.5	1.0
1	-	-
9	-	-
13	-	-
8	±	-
11	±	-
27	-	-

These data suggest that certain strains may be slightly more resistant to bacitracin than are others. However, all strains failed to grow in the presence of 1.0 unit of the antibiotic. The resistant bovine strains

are more resistant than are the resistant ovine strains. These cultures were not able to adapt themselves to bacitracin as they did to streptomycin.

Penicillin

Similar tests were carried out using penicillin. The results obtained were not at variance with those reported by Foley et al (1944) and Handelman et al (1946). The results are shown in Table IX.

TABLE IX

Initial exposure

	Units penicillin / ml agar						
	24 hr. incubation					48 hr. incubation	
Culture	0	0.125	0.25	0.5	1.0	0.5	1.0
1	++++	++++	++++	+++	+++	+++	+++
9	++++	++++	++++	+++	+	++	++
13	++++	++++	++++	+++	+	+++	++
48	++++	++++	++++	+++	++	+++	++
8	++++	++++	++++	++	-	-	+
11	++++	++++	++++	+++	±	+++	+
27	++++	++++	++++	+++	-	+++	±
45	++++	++++	++++	++	±	++	±

These data corroborate other investigations showing that penicillin alone had little or no value in treating Listeria infections. This was further substantiated by data on clinical cases cited in Part I.

Chloromycetin

Recently considerable attention had been focused on chloromycetin, an antibiotic isolated by Ehrlich *et al* (1947). Although this substance had shown its greatest effectiveness against certain viruses, rickettsiae and gram negative bacteria, Smadel and Jackson (1947), its effect on *Listeria* was determined. From the results shown in table X it appeared unlikely that it will have any value in treating infections involving *Listeria*. Susceptible microorganisms were inhibited in concentrations as low as 0.1-0.05 micrograms per ml of medium.

TABLE X

Initial exposure

	Micrograms / ml agar 24 hr. incubation				
Culture	0	0.5	1.0	1.5	2.0
1	++++	++++	++++	+	-
9	++++	++++	++++	+++	±
13	++++	++++	++++	++	-
48	++++	++++	++++	++	+
8	++++	++++	++++	±	-
11	++++	++++	++++	++++	+
27	++++	++++	++++	+	±
45	++++	++++	++++	+++	±

Pathogenicity

The case histories of the rabbits in the streptomycin experiments were included to show the typical reactions following intravenous inoculation of *Listeria*. The most striking characteristic was the high percentage of monocytes in the peripheral blood. This reaction was reported by Murry *et al* (1926) in the first description of *L. monocytogenes*. It was this characteristic which led them to give "monocytogenes" as a species name to the organism. This reaction had been used by Witts and Webb (1927), Bloom (1928), Conway (1938, 1939) and numerous other hematologists in investigations on the origin and nature of the monocyte.

Graham *et al* (1943) reported that there was no evidence showing that a circulating monocytosis was present in affected ruminants. This was borne out in part by cases presented in Part I. However, case 21 showed a monocytosis of 15% and case 34 a monocytosis of 7 and 8 % on consecutive days. These values were sufficiently high to suggest that an increased number of monocytes may be present in ruminants suffering from listeriosis. Chapman (1947) reported increased numbers of monocytes in the dogs from which he isolated *Listeria*.

After ocular instillation an increase in circulating monocytes was not consistently shown. This was in accord with the findings of Julianelle (1941) and Graham

et al (1943). Julianelle and Pons (1939) state that large numbers of monocytes were found in the exudate of eyes following ocular instillation. A few smears made from this exudate and stained with Wright's stain showed many polemorphonuclear leucocytes but few monocytes. However, so few smears were made that this was not intended to be contrary to the findings reported above.

It will be of interest to note that rabbits exposed to Listeria by intravenous inoculation showed "sludge blood" as reported by Knisely et al (1947). Animals that survive for longer than three days became so badly affected that it was difficult to obtain suitable samples for hemocytological examination. This condition persisted for several days after which time, if the outcome of the disease was favorable, the blood regained its normal fluidity.

The presence of basophilic staining granules in the polymorphonuclear leucocytes was observed in almost all animals exposed to Listeria. These granules were present in naturally occurring cases as well as in those artificially exposed. This was true by any route of infection; ocular instillation, intravenous inoculation, or ingestion. It was felt that they were similar to the so-called "toxic granules" found in infections (Downey 1938).

To determine the effect of oral administration of Listeria a rabbit was given 250-300 ml of 24 hour broth culture as the sole source of fluid intake for a period of 20 days. Two days following initiation of this regime the rabbit was irritable and displayed slight difficulty in walking. The leukocyte count was increased by 6,000 cells per mm^3, and the differential blood count showed 6% monocytes. The lymphocyte-polymorphonuclear leukocyte ratio was not altered. This condition persisted for two days after which time the animal appeared normal. By the sixth day the blood values had returned to normal limits. There was no further reaction noted. The temperature did not vary throughout the 20 day period.

The significant finding of this experiment was that Listeria could pass through the entire length of the digestive tube. Listeria could be isolated from the feces in large numbers throughout the 20 day period and for at least five days after the termination of the experiment. Nine days later Listeria could not be isolated from the feces. In no instance was Listeria isolated from the urine.

In attempting to isolate Listeria from the feces, a few fecal pellets were macerated in the Waring blendor together with approximately 50 ml nutrient broth. One ml portion of this suspension was transferred to broth

tubes containing 0.05% potassium tellurite. These were incubated at 37° C for 24 hours and plated on tryptose agar. The plates were incubated for 24 hours at 37° C and examined with a dissecting microscope using oblique light. Listeria was often isolated in nearly pure culture.

Other modifications of this procedure were tried, but the one described proved to be most satisfactory.

Also of significance was the fact that an ocular reaction could not be produced in this rabbit with a culture that readily produced a conjunctivitis in susceptible animals.

Six rabbits were inoculated intracerebrally under ether anesthesia. An inoculum of 0.2 ml of a saline suspensions of organisms produced death in from 20 hours to 4 days in all but one. Listeria was isolated from the liver and brain of these animals. One animal survived for a week but it was nervous and irritable and killed by ether inhalation. Listeria could not be isolated.

Six rabbits inoculated subcutaneously with 0.5 ml of a saline suspension of organisms showed no ill effects after one week period of observation. These same animals were inoculated intravenously with 0.5 ml

of a saline suspension of Listeria. Death followed in periods from 24 hours to three days. At necropsy all but one animal showed a large abscess with a necrotic center at the point of the subcutaneous inoculation. Listeria could be isolated from the liver and heart blood but not from the abscess.

Immunological Reactions

The serological reactions of Listeria had received considerable attention from Seastone (1935), Schultz et al (1938), Paterson (1939, 1940), Julianelle (1941), Graham et al (1943), and Drew (1946).

Schultz et al (1938) and Julianelle (1941) suggested the presence of two serological groups. Julianelle (1941) designated these as Type I, or rodent strain; and Type II, or ruminant strain. He postulated that the ultimate origin of the strains isolated from infective cases could be determined by this method. This was in disagreement, however, with Paterson (1940) who found, within the genus Listeria, four serological groups based on somatic and flagellar antigens. He concluded that there was no host specificity for the four suggested. Drew (1946) found two groups based on precipitation reactions which corresponded to the two groups of Schultz et al (1938), Julianelle (1941) and to groups

I and IV of Paterson (1940).

It was not the purpose of this study to venture into the serological characteristics of this group. However, antigens prepared from cultures one through 22 and cultures A, B, C, and D* were tested for agglutinability with antisera prepared from cultures 9 (ovine) and 5 (bovine). Antisera were prepared by repeated intravenous injections of living organisms into a group of rabbits that previously had been exposed to the specific culture and had survived. It was of interest that these animals displayed no alterations in hemocytological values during the 24 day immunization period. A second group of rabbits was immunized using a heat killed culture. These, too, showed no variation in hemocytological values. At the termination of immunization there was no significant difference in antibody titer between the groups receiving living and those receiving killed antigen. This was also observed by Graham et al (1943).

The agglutination titer of the ovine strain antisera ranged from 1:2560 to 1:5120 while that of the bovine strain antisera ranged from 1:640 to 1:1280 with their homologus antigens.

Antigens were prepared from the various cultures

---

*Human strain, Larkin 6288. Received from Dr. W. Wheeler, Childrens' Hospital, Columbus, Ohio.

by suspending in physiological saline the washed off growth from a 24 hour agar slant culture. Cultures were killed by heating at 90° C for 1 hour and standardized to the density of a McFarland Nepholometer tube no. 2. Tests were performed as described by Julianelle (1941).

It was found that of the 26 strains tested, all but three (8, 12, 20) showed agglutinability with the two types of antisera. Cultures 3 and 26 showed only slight agglutinability. The remaining cultures showed high agglutinability in dilutions of 1:2560 and 1:5120 with the antisera prepared from the ovine culture, and in dilutions of 1:640 to 1:1280 with the antisera prepared from the bovine culture.

These tests revealed that of the strains isolated during this study at least two distinct immunological groups were represented.

Biochemical Reactions

The biochemical and fermentation reactions of *Listeria* had been reported by numerous investigators; Seastone (1935), Julianelle (1941), Harvey and Faber (1941), Graham *et al* (1943), and many others. As pointed out by Julianelle (1941) the saccharolytic reactions

of the various strains were so similar that they do not form a sufficient basis for classification of the various strains. Harvey and Faber (1941), however, suggested that the fermentation of melizitose might form a basis of division. The division of groups by the fermentation of melizitose closely resembles the serological division of Paterson (1940). Fermentation reactions were carried out on the following fermentable substances: glucose, galactose, levulose, arabinose, xylose, lactose, maltose, sucrose, trehalose, raffinose, dextrin, inulin, inositol, dulcitol, mannitol, sorbitol, glycerol, salicin. These substances were added to a basal medium containing bacto-tryptose, beef extract, sodium chloride and distilled water. Brom cresol purple was used as an indicator. All media were sterilized by autoclaving at 15 pounds pressure for 15 minutes with the exception of dextrin and maltose which were filtered through a fritted glass filter. All reactions were observed for 14 days. Doubtful reactions were checked with a Beckman pH meter.

All strains produced acid but no gas after 24 hours in glucose, levulose, trehalose and salacin. Acid production was slow and variable in arabinose, galactose, lactose, maltose, sucrose, dextrin, sorbitol and glycerol. There was no fermentation of xylose, raffinose, inositol, inulin, dulcitol, nor mannitol.

Biochemical reactions were carried out using media and methods indicated in "The Manual of Methods for the Pure Culture Study of Bacteria". Nitrates were not reduced. Indole was not produced. Gelatin was not liquefied. All strains showed a slight beta hemolysin on thin sheep blood agar plates. Starch was not hydrolysed.

All strains produced H_2S in 24 hours using a medium consisting of liver infusion, Witte's peptone, sodium chloride and distilled water. Lead acetate strips were used as an indicator. As there were no reports of the production of H_2S by Listeria, several other accepted media for the production of H_2S were inoculated with cultures taken at random. H_2S was not produced in any of these media. Thus it is evident that H_2S can be produced with the proper medium.

The above fermentation and biochemical reactions were carried out on cultures 1 through 47 and A, B, C and D.

Summary

The difficulty of successful isolation of Listeria from the bovine brain was discussed. It was demonstrated that refrigeration of brain suspensions yielded an increased number of colonies when a 0.2 ml portion was plated. Evidence was presented that recently isolated

bovine strains might not produce a conjunctivitis in a susceptible rabbit. It was postulated that these phenomenon are related to an unstable substance in the bovine brain inhibitory to Listeria.

It was shown that Listeria could survive for six weeks on straw and wood shavings; 16 weeks on hay and calf feed; and for 26 weeks on rabbit feed. It remained viable without transfer at 4° C for at least 30 months with no loss in pathogenicity.

The effects of various selective media incorporating sodium azide, potassium tellurite, and acetic acid were reported. Potassium tellurite, 0.05%, in nutrient broth was found to be most effective.

The effect of streptomycin, bacitracin, penicillin, and chloromycetin on Listeria cultures was presented. There was little evidence that these antibiotics could be of therapeutic value.

Listeria was shown to be pathogenic for laboratory rabbits following intravenous and intracerebral inoculation. Oral administration produced only a slight reaction. Subcutaneous inoculation was followed by abscess formation at the site.

Immunological, fermentation, and biochemical reactions were presented. The cultures studied could be divided into at least two serological groups. Fermenta-

tion and biochemical reactions were similar for all strains.

Fig. 21. Rabbit 6a. Liver at necropsy. The white necrotic foci are a characteristic lesion at necropsy of rabbits that have died following intravenous inoculation. This lesion is the same as seen in the lamb reported in case 2. See also figure 12. Kodachrome print.

Fig. 22. Typical conjunctivitis following ocular instillation of Listeria culture. Kodachrome print.

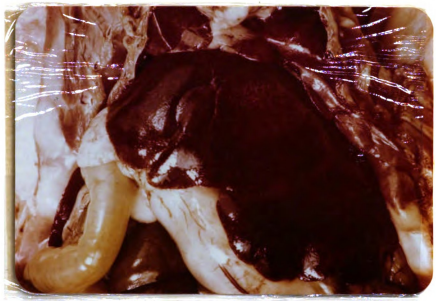


Fig. 21

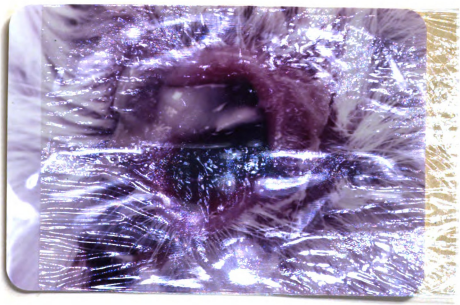


Fig. 22

Fig. 23. Heavy confluent growth of *Listeria*
Case 5. after the brain suspension had been refrigerated for three months. This had originally been considered negative.

Fig. 24. Primary isolation from bovine medulla.
Case 10. Ninety colonies *Listeria*.

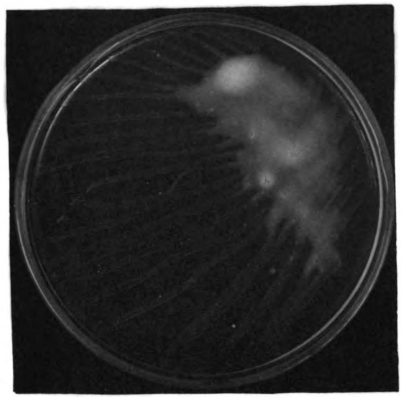


Fig. 23

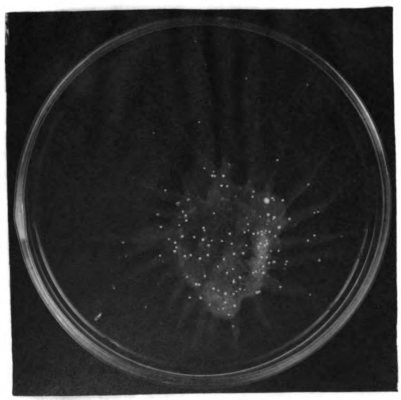


Fig. 24

Fig. 25. Primary isolation from bovine medulla.
Case 12. Forty four colonies *Listeria*.

Fig. 26. Plating of brain suspension after refrigeration at 4° C for eight days.
Case 12. A 0.2 ml portion was plated in each instance.

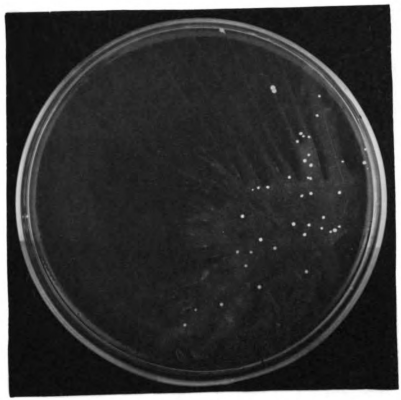


Fig. 25

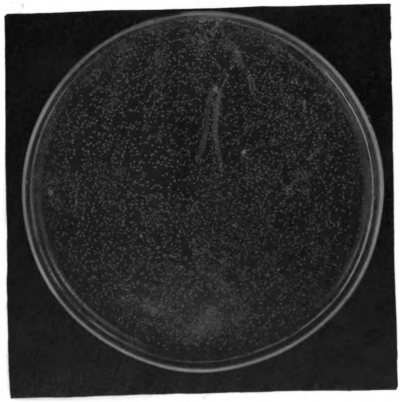


Fig. 26

Fig. 27. Case 12. Daily increase in colony numbers during an eight day period of refrigeration of the brain suspension. The first plate is also shown in figure 25; the last is shown in figure 26.

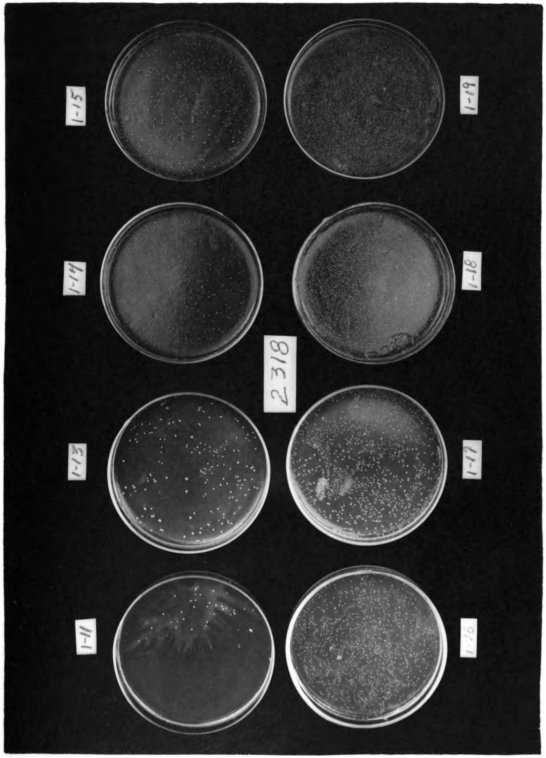


Fig. 27

Fig. 28. Primary isolation from bovine medulla.
Case 18. Eighteen colonies *Listeria*.

Fig. 29. Plating of brain suspension after refrigeration at 4° C for 11 days. A 0.2 ml portion was plated in each instance.
Case 18.

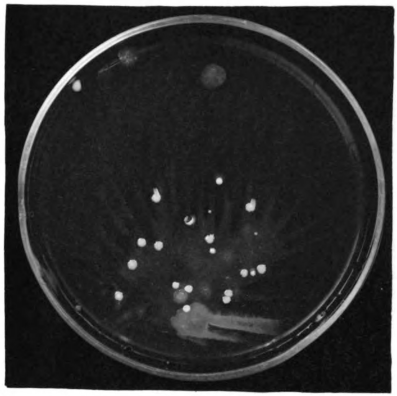


Fig. 28

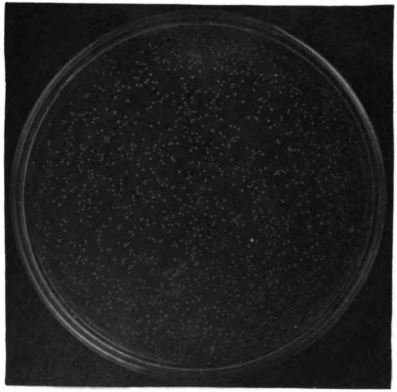


Fig. 29

Fig. 30. Primary isolation from bovine medulla.
Case 23. Fifty four colonies Listeria.

Fig. 31. Plating of brain suspension after refrigeration at 4° C for 17 days. A 0.2 ml portion was plated in each instance.
Case 23.

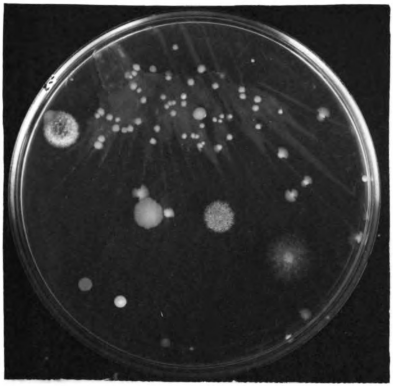


Fig. 30

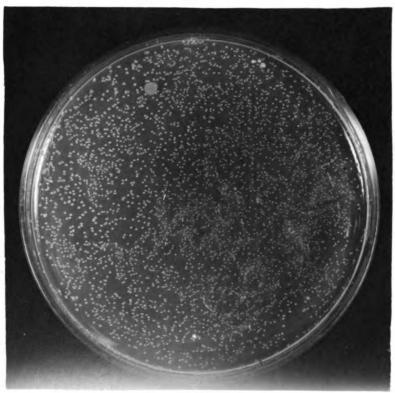


Fig. 31

Fig. 32. Primary isolation from bovine medulla.
Case 32. Nine colonies Listeria.

Fig. 33. Primary isolation from bovine medulla.
Case 31. One hundred twenty five colonies Listeria.

Fig. 32

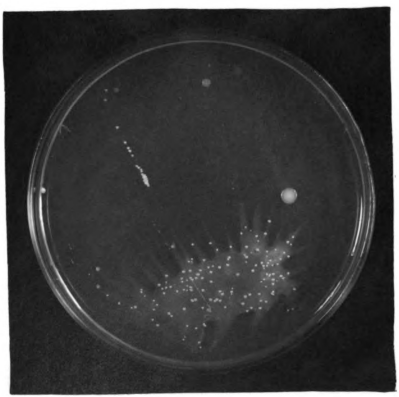


Fig. 33

Fig. 34. Primary isolation from bovine medulla.
Case 28. See also figure 35.

Fig. 35. Primary isolation from bovine medulla.
Case 24. These two cases presented the highest number of colonies at primary isolation from the bovine. At least 36 hours had elapsed between death and culture in each case.

Fig. 34

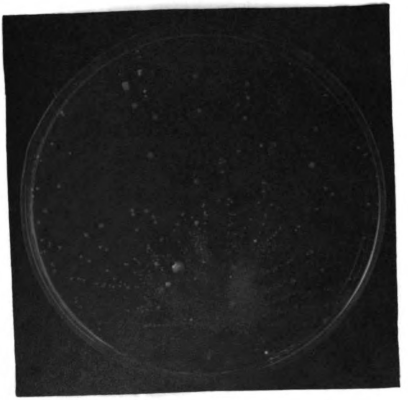


Fig. 35

Fig. 36. Primary isolation from ovine medulla.
Case 9.

Fig. 37. Primary isolation from ovine medulla.
Case 14.

Fig. 36

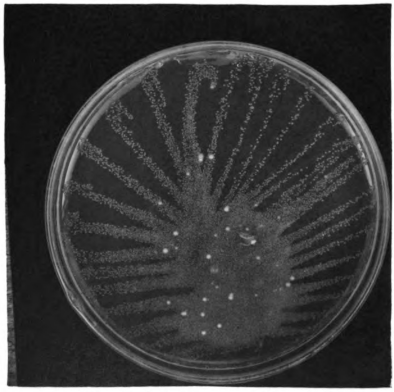


Fig. 37

Fig. 38. Primary isolation from ovine medulla.
Case 15.

Fig. 39. Primary isolation from ovine medulla.
Case 16.

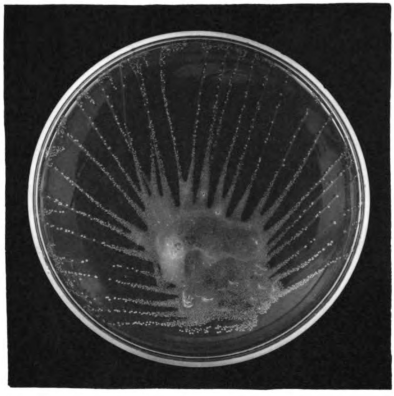


Fig. 38

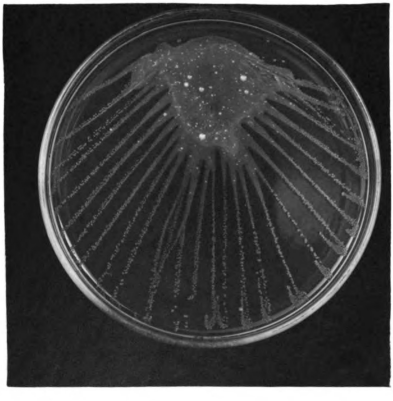


Fig. 39

Fig. 40. Difference in colony size of Listeria on tryptose agar containing streptomycin.

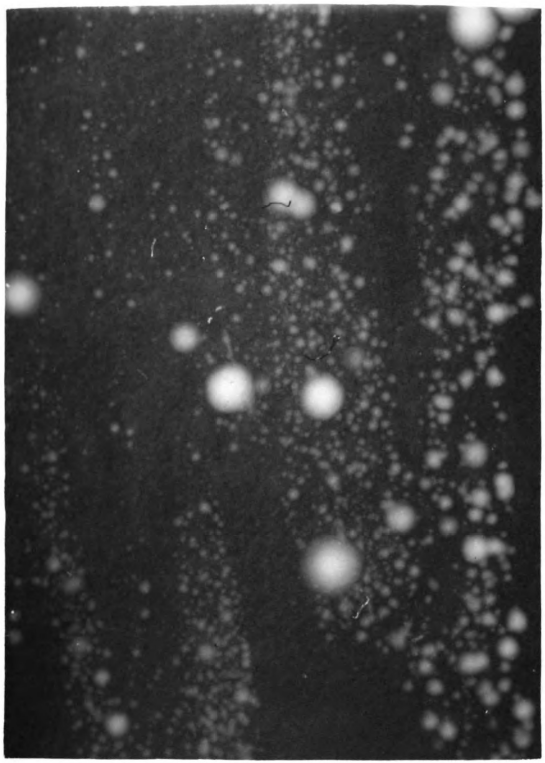


Fig. 40

BIBLIOGRAPHY

Biester, H. E. and Schwarte, L. H. 1939. Studies on Listerella infections in sheep, J. Inf. Dis,, 64: 135-144.

Biester, H. E. and Schwarte, L. H. 1941. Bovine listerellosis in Iowa with studies on a recovered case, N. Am. Vet., 22: 729-734.

Bloom, W. 1928. The origin and nature of the monocyte, Folio Haematol., 37: 1-62 (Orig. not seen).

Boucher, W. B. 1946. A case of bovine listerellosis in Pennsylvania, J. Am. Vet. Med. Assoc., 109: 213.

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.

Chapman, Merwyn P. 1947. Listerellosis in a dog, a field case, N. Am. Vet., 28: 532-538.

Cheng, Ching-tuan. 1945. The diseases of new born lambs with special emphasis on bacterio-pathology. A thesis submitted to the School of Graduate Studies, Michigan State College.

Cole, Clarence R. 1946. Listerellosis in a Hereford cow, J. Am. Vet. Med. Assoc., 109: 216-217.

Coles, E. H. 1948. The sensitivity of bacteria of animal origin to streptomycin, Am. J. Vet. Research, 9: 152-156.

Conway, E. A. 1938. Reaction of lymphatic tissues in early stages of Bacterium monocytogenes infection, Arch. Path., 25: 200-227.

Conway, E. A. 1939. Reaction of lymphatic tissues of rabbits to repeated injections of Bacterium monocytogenes, J. Infect. Dis., 64: 217-240.

Cox, B. F. 1945. Listerellosis of dogs, Auburn Vet., 98-99.

De Blieck, L. and Jansen, Jac. 1942. Listerellose bij biggen, Tijdschr. V. Dierg, 69: 575.

De Blieck, L. and Jansen, Jac. 1943. Listerellose bie tieren, A. Listerellose bie Ferkelen, Jour. Microbiol. and Serol., 9: 93-103.

Downey, Hal. 1938. Handbook of Hematology, Vol. 4. Paul B. Hoeber, Inc., New York.

Drew, Ruth M. 1946. Occurrence of two immunological groups within the genus Listeria. Studies based upon precipitation reactions, Proc. Soc. Ex. Biol. and Med., 61: 30-33.

Ehrlich, John; Bartz, Quentin R.; Smith, Robert M. and Joslyn, Dwight A. 1947. Chloromycetin, a new antibiotic from a soil actinomycete, Sci., 106: 417.

Foley, E. J.; Epstein, J. A. and Lee, S. W. 1944. Effectiveness of penicillin on Listerella, J. Bact., 47: 110.

Gibbons, W. J. 1948. Diseases of the nervous system, Vet. Med., 43: 139.

Gifford, Rebecca and Jungherr, Erwin. 1947. Listeriosis in Connecticut with particular reference to a septicemic case in a wild raccoon, Cornell Vet., 37: 39-48.

Gill, D. A. 1931. "Circling disease" of sheep in New Zealand, Vet. J., 87: 60-74.

Gill, D. A. 1937. Ovine bacterial encephalitis (circling disease) and the bacterial genus Listerella, Austral. Vet. J., 13: 46.

Graham, R.; Levine, N. D. and Morrill, C. C. 1943. Listerellosis in domestic animals, Univ. Ill. Agr. Expt. Sta., Bull. 499.

Gray, M. L.; Thorp, Frank Jr.; Nelson, R. and Sholl, L. B. 1947. Listerellosis in sheep of Michigan, M.S.C. Vet., 7: 161-163.

Gray, M. L.; Thorp, Frank Jr.; Sholl, L. B. and Riley, Jr. 1948. Bovine listerellosis in Michigan, M.S.C. Vet., 8: 83-84.

Gray, M. L.; Stafseth, H. J.; Thorp, Frank Jr.; Sholl, L. B.; and Riley, W. F. 1948A. A new technique for isolating listerellae from the bovine brain, J. Bact., 55: 471-476.

Grini, O. 1943. Listerella monocytogenes, som arsak til septico-pyemi hos foll. (L. monocytogenes as the cause of septicemia in foals), Norsk. Vet. Tidskr., 55: 97. Abst. Vet. Bull., 1945. 15: 289.

Handelman, N. I.; Rolande, C. C.; Scott, E. P. and Knighton, E. T. 1946. Listerella meningitis, J. Pediat., 28: 210-213.

Harvey, P. C. and Faber, J. E. 1941. Studies on the Listerella group. I. Biochemical and hemolytic reactions, J. Bact., 42: 677-687.

Hatch, Ray D. 1945. Report of a case of Listerella infection in a cow in southwest Virginia, J. Bact., 49: 110-111.

Huddleson, I. F. 1946. The mucoid phase of the genus Brucella, Am. J. Vet. Res., 7: 5-10.

Hunter, S. H. 1942. Some growth requirements of Erysipelothrix and Listerella, J. Bact., 43: 629-240.

Jansen, J. and Pepperkamp, C. W. 1947. Fowl listerellosis, Tydscrift voor Diergeneeskunde, 72: 389-390. Abst. J. Am. Vet. Med. Assoc., 1949. 114: 225.

Jansen, Jacob and Pepperkamp, C. W. 1947. Listerellosis in a silver fox, Tydscrift voor Diergeneeskunde, 72: 319-320. Abst. J. Am. Vet. Med. Assoc., 1949. 114: 223.

Johnson, B. A.; Anker, H. and Meleney, F. L. 1945. Bacitracin: a new antibiotic produced by a member of the B. sublilis group, Sci., 102: 376-377.

Julianelle, L. A. and Pons, C. A. 1939. Identification of Listerella monocytogenes, Proc. Soc. Exper. Biol. and Med., 40: 362-363.

Julianelle, L. A. 1941. Biological and immunological studies of Listerella, J. Bact., 42: 367-383.

Kaplan, M. M. and Lager, A. E. 1945. A clinical and laboratory study of spontaneous listerellosis in a goat, Abs. Vet. Med., 40: 199-202.

Kaplan, Martin M. 1945. Listerellosis, New Eng. J. Med., 232: 755-759.

Kerlin, D. L. and Graham, R. 1945. Studies of listerellosis. VI. Isolation of Listerella monocytogenes from liver of pig, Proc. Soc. Exper. Biol. and Med., 58: 351-352.

Knisely, Melvin H.; Black, Edward H.; Eliot, Theodore S. and Warner, Louise. 1947. Sludged blood, Sci., 106: 431-440.

Lilleengen, K. 1942. Listerellos hos Tjader, Abs. Meddelanden fran Veterinarhogskolen i Stockholm, Abst. Expt. Sta. Rec. 93: 39.

Manual of the methods for the pure culture study of bacteria. 1946. Biotech Publications, Geneva, N. Y.

Morris, M. C. and Julianelle, L. A. 1935. A study of an ocular infection induced experimentally with Bacterium monocytogenes, Am. J. Path., 18: 535-541.

Mueller, J. H. and Miller, P. A. 1946. A new tellurite plating medium, J. Bact., 51: 743.

Murray, E. G. D.; Webb, R. A. and Swann, M. B. R. 1926. A disease of rabbits characterized by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus Bacterium monocytogenes (n. sp.), J. Path. and Bact., 29: 407-439.

Muth, O. H. and Morrill, D. R. 1942. An outbreak of listerellosis in Oregon sheep, J. Am. Vet. Med. Assoc., 100: 242-243.

Olofson, Peter. 1940. Listerella encephalitis (circling disease) of sheep, cattle and goats, Cornell Vet., 30: 141-150.

Owen, Cora Rust. 1946. Acetic acid inhibition of gram bacilli in culture media, J. Bact., 52: 353.

Packer, R. Allen. 1943. The use of NaN_3 and crystal violet in a selective medium for streptococcae and E. rhusiopathiae, J. Bact., 46: 343.

Paterson, J. S. 1939. Flagellar antigens of organisms of the genus Listerella, J. Path. and Bact., 48: 25-32.

Paterson, J. S. 1940. Antigenic structure of organisms of genus Listerella, J. Path. and Bact., 51: 427-436.

Paterson, J. S. 1940A. Studies on organisms of the genus Listerella. IV. An outbreak of abortion associated with the recovery of Listerella from the aborted fetuses, Vet. J., 96: 327-332.

Pike, Robert M. 1945. The isolation of hemolytic streptococcae from throat swabs. The value of enrichment broth containing potassium tellurite and crystal violet, J. Bact., 50: 297.

Porter, J. R. and Hale, W. M. 1939. Effect of sulfanilamide and sulfapyridine on experimental infections with Listerella and Erysipelothrix in mice, Proc. Soc. Exper. Biol. and Med., 42: 47-50.

Porter, J. R. and Pelczar, M. J. 1941. Some growth factor requirements of several strains of Listerella monocytogenes, J. Bact., 42: 141.

Pounden, W. D. and Edington, H. B. 1947. Listerellosis in Ohio, J. Am. Vet. Med. Assoc., 110: 107.

Pounden, W. D.; Bell, D. S.; and Mairs, R. E. 1947A. An outbreak of acute bovine listerellosis, J. Am. Vet. Med. Assoc., 111: 128-129.

Rhoades, Harry E. and Sutherland, A. K. 1948. Concurrent Listerella monocytogenes and hog cholera infections, J. Am. Vet. Med. Assoc., 112: 451-452.

Ryff, J. F. and Lee, A. M. 1948. Listerellosis - report of an outbreak in Wyoming sheep and preliminary, experimental therapy in rabbits, Am. J. Vet. Res., 9: 147-151.

Schultz, E. W.; Terry, M. C.; Brice, A. T. Jr. and Gebhardt, L. P. 1938. Listerella monocytogenes a cause of meningo-encephalitis in man, Proc. Soc. Exper. Biol. and Med., 38: 605-608.

Schwarte, L. H. and Biester, H. E. 1942. Listerella infection in cattle, Am. J. Vet. Res., 3: 165-176.

Seastone, C. V. 1935. Pathogenic organisms of the genus Listerella, J. Exptl. Med., lxii: 203-212.

Smadel, J. E. and Jackson, E. B. 1947. Chloromycetin, an antibiotic with chemotherapeutic activity in experimental rikettsial and viral infections, Sci., 106: 418-419.

Snyder, M. L. and Lechstein, H. C. 1940. Sodium azide as an inhibiting substance for gram bacteria, J. Infect. Dis., 67: 113.

Wheeler, Warren. Personal communications.

Witts, L. J. and Webb, R. A. 1927. The monocytes of the rabbit in B. monocytogenes infection. A study of their staining reactions and histogenesis, J. Path. and Bact., 30: 687-712.

ROOM USE ONLY