The pathogenesis of ovine mastitis due to Pasteurella Mastidis
by Burton D Firehammer

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Bacteriology
Montana State University

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The possible origin of the bacteremia is discussed, P. mastidis was isolated from the lung of one inoculated animal.

Lung lesions were found in the lungs of 5 of 6 inoculated ewes. The lesions consisted of aggregations of lymphocytes and monocytes, A different type of lesion, resembling the type found in the Udder, was observed in the lung of a field case.

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INTRODUCTION

For many years the sheep industry of the western range states of the United States has suffered economic loss as a result of mastitis or blue-bag of ewes. Investigations carried on at the Montana Veterinary Research Laboratory at Bozeman have revealed that most of the cases of this disease in Montana are due to infection with a specific organism of the *Pasteurella* genus. In 1932 Marsh reported these findings and identified the etiological agent with the organism first isolated by Dammann and Freese in 1907 from ewes suffering from contagious mastitis in Germany. Since the time of this first publication in this country the disease has been reported in most of the range states. The disease is also found in many other regions of the world, the literature containing reports of this specific mastitis in France, Germany, England, Greece and Russia.

The published material yields very little information concerning the pathogenesis of the disease. In the past some work of this nature has been carried on at this laboratory but the information that has been obtained is by no means complete. Autopsies of infected ewes have revealed that some of the animals have lung changes in the form of small hyaline foci beneath the surface of the capsule. In a few instances the causative organism has been isolated from such infected lungs. That a bacteremia develops in some cases has been shown by the isolation of *Pasteurella*

mastidis from blood cultures.

This particular problem was undertaken in the hope that more information would be obtained concerning the pathogenesis of the disease by studying, at intervals during the course of the infection, the nature of the tissue reaction in the udder and in other organs, particularly the lung, and to determine the position and relative numbers of organisms in the various regions of the udder tissues. It was also hoped that more information could be obtained on the development and possible duration of the bacteremia which is sometimes found in clinical cases.

REVIEW OF LITERATURE

The earliest reported work on ovine mastitis in which a definite microorganism was shown to be the causative factor was that of Nocard (1887). He studied two outbreaks of the disease which he stated as being prevalent in France at that time and obtained pure cultures of an extremely small coccus, which he called Micrococcus mastitidis gangrenosae ovis. Dammann and Freese (1907) described contagious mastitis in Germany due to infection with a small gram negative organism. They presented a clinical description of the disease as well as autopsy reports on artificially inoculated and natural cases. Histological studies of the mammary tissue revealed a pronounced hyperemia and degeneration of some of the alveoli, leaving a hematoxylin stained mass. They considered bedding soiled by the udder secretion and lambs nursing more than one ewe as possible factors in transmission but were unable to prove it by experimentation.

Later, Haring (1909), also working in Germany described a small gram negative organism as the causative organism of ovine mastitis. Stephan and Geiger (1921) and Raebiger (1925) distinguished between two types of sheep mastitis, one caused by cocci and the other by an organism which they felt was the organism of Dammann and Freese (1907). Leyshon (1929), who made an investigation of 38 sporadic cases of mastitis in England, found that micrococci were the predominating organisms in most cases but in 4 cases from one farm he obtained pure cultures of a small gram negative rod. He stated that the characteristics of this organism were those of a Pasteurella.

Haupt (1932) described as causal agents of two enzootic forms of sheep mastitis, Micrococcus ovis Migula, and the organism of Dammann and Freese. He referred to the latter organism as Bacterium ovinum n. sp. and presented the results of physiological and serological tests to which it was subjected. He also stated that he had recovered from a lung focus of a sheep an organism serologically and culturally identical with B. ovinum.

Marsh (1932) described a specific mastitis of range sheep in the western United States caused by infection with a Pasteurella, which he considered similar to the organism described by Dammann and Freese (1907). In the same year, Meissner and Schoop (1932) reported on an investigation of 27 cases of mastitis on 8 farms in Germany. In 23 cases they isolated a small gram negative organism for which they proposed the name Bacterium mastitidis Dammann and Freese. They recovered the organism from the

heart blood, peritoneum, and lungs of ewes at autopsy. They were able to reproduce the disease by injecting cultures into the udders of lactating ewes, but not by subcutaneous injection. Inoculation of a non-lactating udder was negative. Histological sections of mastitis udders showed hyperemia with infiltration of leucocytes and lymphocytes into the interstitial tissue. Many of the alveoli were enlarged and filled with deep staining masses of cells. In many regions karyorrhexis was evident. In some ewes, dead of mastitis, lung changes were observed. The affected organs were darker and firmer than normal and miliary foci were found in the affected areas. The authors also stated that B. mastitidis caused pneumonia in lambs from 4 herds, in 2 of which ewes were suffering from mastitis.

In Russia, Milovzorov and Tchasovnikov (1932) reported prevention of mastitis by immunization with a formalin killed suspension of "B. mastitis ovis". Lesbouyries, Berthelon, and Macrides (1935), working in France, described ovine mastitis due to Bacterium mastitidis and discussed the close resemblance of the organism to the Pasteurella genus.

Macrides (1936) described two types of mastitis of sheep in Greece, namely, gangrenous mastitis due to infection with Micrococcus mastitidis gangrenosae ovis Nocard, and contagious mastitis due to Bacterium mastitidis Dammann and Freese. Smith and Harnden (1943) described mastitis due to Pasteurella in sheep and goats in Oklahoma, and reported use of autogenous vaccines in immunization experiments.

The causative organism of pasteurella mastitis of sheep, which has been known by various names since its first isolation, is listed in the

sixth edition of Bergey's Manual of Determinative Bacteriology (1948) as Pasteurella mastidis (Meissner and Schoop) Hauduroy et al.

MATERIALS AND METHODS

Clinical data, blood cultures, and body tissues used in this investigation were obtained from ewes inoculated by the author. Additional tissue from 6 field cases and 3 artificially inoculated cases was obtained from the tissue file at the Veterinary Research Laboratory. As most of the tissue from the file had originally been stained only with hematoxylin and eosin additional sections were prepared and stained with bacterial stains.

A total of 7 mature ewes was selected from the laboratory band for inoculation. Of this number 6 were lactating and 1 non-lactating. The milk from the right and left udders was cultured as a sterility check before inoculation.

Pasteurella mastidis culture 3892 was isolated in April, 1949, from laboratory ewe N1422 which had acute mastitis at the time. The culture was lyophilized shortly after isolation and was stored in that state until the middle of August, 1949, when it was brought out to be used in this study. This culture was used to inoculate ewes HIC234, C4021, 3S65, and T34. Two of the ewes, 2N217 and S1424 were inoculated with a P. mastidis culture recovered from the milk cistern of ewe HIC234 at autopsy.

P. mastidis culture 3967 was recovered from the same ewe as culture 3892. It was discovered at the end of the summer that she had carried the organism in the udder since her apparent recovery following treatment with

sulfamethazine in April. This culture was isolated and used to inoculate ewe HC622 merely to see if there had been any change in virulence in the organism.

Cultures were maintained during the study on serum agar, a medium modified from the "hormone" medium of Huntoon (1918). It differs from the "hormone" medium only in that it is filtered so that a clearer product is obtained. After sterilization, 10 per cent of sterile horse serum was added to each tube of medium and the tubes allowed to solidify in a slanted position.

Serum agar slants with a 10 to 12 hour growth of P. mastidis were used as a source of inoculum to inoculate the animals. The growth from one slant was suspended in 3 ml of physiological saline. After the orifice of the right teat had been cleansed with 70 per cent alcohol and dried with sterile cotton, a fine cotton swab saturated with the saline suspension was inserted into the teat canal approximately 1/4 of an inch. It was felt that a heavier inoculation would not as nearly approximate the probable natural infection. Sucking lambs were cut away from the ewes for 6 hours immediately following inoculation and then returned to be left with the ewe.

At 12 hour intervals following inoculation 10 ml samples of jugular blood were drawn. The blood was cultured in 250 ml flasks containing 40 ml of standard broth with 1 per cent sodium citrate. After 24 to 48 hours incubation a 1 ml portion was withdrawn and subcultured on a serum agar slant. If no growth was apparent on the slant after 24 hours incubation a second subculture was made before the flask was discarded.

The four ewes that developed typical cases of mastitis were killed. As it was desired to culture and section tissues from the udder, lungs, and some of the other organs, it was necessary to select a method of euthanasia which would not greatly alter the pathological picture. Ewes HIC234, C4021, and 2N217 were destroyed by intravenous injections of nembutal. If the dose is sufficiently large, narcosis is rapidly followed by death. However, it was found that there is considerable variation in individual tolerance. Ewe 3S65, the last ewe to be destroyed was killed by the injection of 5 ml of ether into the base of the brain. This produced almost instant death and is probably the better of the two methods.

Immediately after death the udder was carefully dissected out and removed. After removal of the udder, a complete autopsy was performed on the animal. Cultures of the lungs, liver, and spleen were made by searing the surface of the organ with a hot spatula and scraping the cut surface below this region with a sterile scalpel. The material thus obtained was placed in serum broth and on serum agar slants. Tissue blocks were taken from the organs for sectioning.

Cultures were made at three levels, 1/2, 1, and 1 3/4 inches below the dorsal surface of the udder in a region near the posterior aspect of the udder and slightly to the right of the median line separating the right and left mammary glands. This is the thickest portion of the udder. These cultures were referred to as median dorsal, or MD 1, 2, and 3, respectively.

A culture was made from the lateral ventral region, on the side of the udder, above the milk cistern. Cultures from this region were identi-

fied by the letters LV. The last culture made from each udder was from the milk cistern. These cultures were lettered MC.

Blocks of tissue were taken at autopsy from the same regions of the udder as those cultured and were identified by the same letters used for the cultures. The tissues were fixed in Zenker's solution for 24 hours, washed, and stored in 80 per cent alcohol. The tissues were embedded by the dioxan-paraffin method of Mallory (1938) and sectioned on the rotary microtome.

It was originally planned to use the phloxine-methylene blue stain of Mallory (1938) to stain the tissue sections but in practice the stain proved too harsh for good results with the particular combination of tissue and organism involved in this study. Accordingly, a number of other technics were tried, including the methyl green-pyronine stain as modified by Saathof (1905), the gram stain of Glynn (1935), and Goodpasture's stain as modified by MacCallum (1919). All three of these methods showed inadequate staining of the bacteria and poor background contrast.

Wolbach's (1919) modification of the Giemsa stain gave quite good results and all tissues taken from animals inoculated by the author were stained by this method. The azure eosinate stain of Lillie, as presented in Staining Procedures (1947), was in some respects superior to the Giemsa stain and was also used to stain sections from the inoculated ewes. Either of these staining procedures gives a more delicate contrast than is obtained by the phloxine-methylene blue stain. The azure eosinate stain has the advantage that through the use of the buffer solutions employed,

the pH can be controlled and thus the contrast between reds and blues in the tissue can be held at any desired point. This fact also permits the stain to be used on tissue fixed with any of the fixatives commonly in use. A pH value of 5.2, slightly higher than the recommended range of 4.0 to 5.0, was found best for the Zenker's fixed tissue used in this work.

The Ollett (1947) neutral red-fast green stain was employed in staining most of the tissues obtained from the tissue file. This stain gave excellent results, the organisms staining a bright red against a green background. It was found that the intensity of the stain could be increased somewhat by maintaining the neutral red-fast green solution at 56C in the paraffin oven during use. Unfortunately this stain can be used only on sections fixed in neutral formalin.

EXPERIMENTAL

Mastitis Cases Produced by Inoculation

Data from artificially inoculated cases were obtained from the ewes inoculated by the author, and from tissues of 3 previously inoculated cases in the tissue file of the Veterinary Research Laboratory. Of the animals inoculated by the author, ewes HIC234, C4021, 2N217, and 3S65 developed clinical cases of mastitis and were destroyed. Ewes C622, T34, and S1424 did not develop mastitis. The tissues obtained from the tissue file were from ewes M-75, N-115, and HK-85.

Clinical history and gross pathology.

Ewe HIC234.

On August 24, 1949 the ewe was brought to the laboratory where the udder and milk were examined and found normal. At 9 a.m. the right mammary gland was inoculated with P. mastidis culture 3892 by passing a swab saturated with a saline suspension of the organism through the orifice of the teat.

The inception of active mastitis was rapid. Within 12 hours after inoculation the ewe's temperature rose to 107.0 F and the right mamma was inflamed in appearance and hot to the touch. The temperature had fallen to 102.8 by the following morning, but rose to 105.3 during the afternoon. At this time the ewe was extremely ill and was not allowing the lambs to nurse. The respiration was accelerated and the right mamma was slightly enlarged with some induration. The milk was slightly thinned and beginning to curdle.

The animal's temperature was 104.2 on the second morning, 49 hours after inoculation. The ewe was quite gaunt and dejected but respiration was normal. The right gland was enlarged, tense, and tender on palpation. The left gland was normal and full of milk.

It appeared at this time from the rapid course of the disease and the condition of the ewe that there was a possibility that death might ensue. As it was considered desirable to obtain tissues from an early severe mastitis case the animal was destroyed. An intravenous injection of nembutal was rapidly followed by narcosis and death.

At autopsy the spleen, liver, kidneys, and heart were normal in appearance. Two small, gray, hyaline foci were observed beneath the capsule of one lung. They were similar in appearance to the miliary foci occasionally observed at autopsy in the lungs of fatal field cases of mastitis.

A longitudinal section through the affected side of the udder revealed a region of subcutaneous edema approximately 2 cm thick. The posterior third of the section was red and hemorrhagic. The remaining portion of the udder was a dull gray color with scattered hemorrhagic areas throughout. The milk cistern contained a mass of soft curds.

Ewe C4021.

The ewe was brought to the laboratory on August 31, 1949. At 9:00 a.m. the right mamma was inoculated via the teat canal with P. mastidis culture 3892.

The animal's temperature was 106.3 F and the right side of the udder hot and swollen 12 hours after inoculation. On the following morning the temperature was 104.0. The udder was hot and swollen but apparently palpation did not cause severe discomfort. The milk was somewhat thinner than normal. The ewe took nourishment during the day and apparently was not in very great pain, although the temperature rose to 105.6 by evening.

On the second morning, 48 hours after inoculation, the temperature was 105.8. The ewe was still in fairly good condition but was showing more discomfort than she had previously shown. The disease was apparently

following a considerably milder course than that evidenced in ewe HIC234. The ewe was destroyed by an intravenous injection of nembutal.

At autopsy, examination of the abdominal organs did not reveal any abnormalities. Two small, gray, hyaline foci were observed beneath the capsule of the lungs, one on each lobe. The larger one was approximately 1 mm in diameter while the other was pin-point.

The cut surface of the right mammary gland was gray in color and did not contain the numerous hemorrhagic areas seen in the udder of HIC234. There was little subcutaneous edema.

Ewe 2N217.

The right mammary gland of this animal was inoculated on September 17, 1949, with a culture of P. mastidis isolated from the milk cistern of ewe HIC234 at autopsy. The ewe did not show any appreciable rise in temperature until 36 hours after inoculation at which time the temperature was 105.2 F. At 48 hours the temperature was 105.0 and the ewe was dejected and ill. The right mamma was greatly enlarged, hot, and inflamed. The secretion from the infected side consisted of a straw colored whey containing a few curds.

The general condition of the ewe was only slightly improved 4 days after inoculation although there was a slight decrease in temperature. The disease apparently was running a course similar to that observed in ewe C4021, and considerably milder than in ewe HIC234. The prognosis for recovery was considered good at this time but it was decided to sacrifice the ewe so that tissue specimens could be obtained. The

animal was destroyed by an intravenous injection of nembutal.

At autopsy the kidneys, spleen, heart and lungs were found free of any abnormality. The border of one lobe of the liver presented a wrinkled appearance apparently due to shrinking of scar tissue from an old injury.

On section the udder presented a gray color with a few small hemorrhagic areas. The milk cistern contained some clotted milk and whey.

Ewe 3865.

On September 21, 1949, the right mammary gland of this ewe was inoculated with P. mastidis culture 3892. Within 24 hours the inoculated side of the organ was greatly enlarged and indurated. The milk was thin and watery. The ewe showed some depression at this time but the temperature was only 103.6 F.

During the first 8 days following inoculation, the general condition of the ewe showed little change. The animal was sick and gaunt but took some nourishment. The temperature averaged slightly over 104.0 during this period and showed very little day to day variation. The ewe developed a slight cough during this period.

On the 9th day following inoculation the temperature ranged from 102.3 to 103.6 which is an essentially normal diurnal variation. The temperature did not rise above the normal range again and the ewe appeared to be well on the road to recovery.

The animal was sacrificed on the 12th day after inoculation as it was considered desirable to study tissues from an animal in the recovery phase of the disease.

At autopsy the spleen, kidneys, and heart were normal in appearance. The lungs did not collapse on removal to the extent characteristic of the normal lung, possibly indicating a general interstitial thickening. The color of the tissue was not the typical bright pink, but a more drab hue. A considerable number of minute white foci 1 to 2 mm in diameter, just below the surface of the capsule, were observed scattered over the lungs.

The right mammary gland was greatly enlarged and indurated. Thick, white pus was found 4 mm below the surface of the udder in the median dorsal region. The same type of pus was found 4 mm below the surface in the lateral ventral region and in the milk cistern. The pus in the milk cistern contained a few curds. The cut surface of the udder was a drab gray color. No hemmorrhagic areas were seen. The left mammary gland appeared normal.

Ewe C622.

The right udder of this ewe was inoculated on August 25, 1949 with P. mastidis culture 3967. The ewe's temperature was 105.2 when it was taken 24 hours after inoculation. There was no apparent change in either the right mamma or the milk. The animal was eating and apparently in no pain.

The temperature was normal the second day after inoculation and remained so during the next 4 days. At the end of this period the udder and milk were normal and the ewe showed no discomfort. P. mastidis was isolated from the milk of the right gland at this time. The ewe was returned to the pasture 6 days after inoculation. Two months later the

udder was examined and found normal, dry and shrunken. The ewe was sold at this time.

Although culture 3967 was capable of establishing itself in the udder, it failed to produce mastitis in this case, which may have been due to its existence in the udder of ewe N1424 during the summer.

Ewe T34.

On August 30, 1949 the ewe was brought to the laboratory and examined. The udder was normal in appearance, shrunken and non-lactating. A small amount of clear fluid was expressed from each teat for culturing but later was found to be sterile. The right mammary gland was inoculated with P. mastidis culture 3892.

The animal was observed for a 5 day period following inoculation. There was no rise in temperature during this interval although the right mammary gland showed a very slight enlargement and fever on the second and third days. Cultures taken from the udder on the fourth day after inoculation were negative for P. mastidis.

The organism was apparently unable to establish itself in the inactive glandular tissue. This is in agreement with the work of Meissner and Schoop (1932) who were unable to establish infection in non-lactating animals.

Ewe S1424.

This ewe was inoculated with a suspension of P. mastidis culture 3892 on September 17, 1949. There was no change in temperature or general

condition of the udder and milk following inoculation. Milk cultures made on the third day after inoculation were sterile. Apparently the organism did not reach the secreting tissue and so was unable to establish itself in the gland. In view of the method of inoculation used, invagination of the external orifice of the teat with an infected swab, it did not seem unlikely that some of the attempted inoculations would fail.

Bacteriology.

This section includes the results of the jugular blood cultures which were made at 12 hour intervals after inoculation, the results of the cultures made at autopsy of the animal, and the results of fermentation studies made on selected cultures from each animal.

Cultures which showed a light, colorless growth with a bluish-green iridescence on slants, appeared in stains as small gram negative rods or cocco-bacilli, and which either did not grow on Endo's medium or appeared only as pin-point colonies, were considered as P. mastidis. Cultures were selected from each animal to be used for fermentation studies.

Ewe HIC234.

The culture of the jugular blood taken 49 hours after inoculation, immediately before the animal was destroyed, yielded a pure culture of P. mastidis. Blood cultures taken previous to this time were sterile.

Cultures taken at autopsy from the spleen were sterile but the liver cultures were heavily contaminated with Escherichia coli. Lung cultures made from the tissue in the immediate vicinity of the small hyaline foci contained pure cultures of P. mastidis.

Cultures from the supramammary lymph node were sterile. Cultures from the three levels of the median dorsal region, from the lateral ventral region, and from the milk cistern of the right mamma all yielded pure cultures of P. mastidis. It appeared that invasion of the tissue of the right gland was complete. It should be remembered that the MD-1 culture was made only 1/2 inch below the dorsal border of the udder in the gland tissue farthest from the orifice of the teat. Cultures from the milk cistern of the left mammary gland were sterile.

Ewe C4021.

All blood cultures made from this animal were sterile as were cultures, at autopsy, from the kidneys, spleen, and lungs. The culture from the liver yielded a pure culture of a gram positive rod.

The supramammary lymph node culture was sterile. Pure cultures of P. mastidis were obtained from the three levels of the median dorsal region of the right mamma as well as from the lateral ventral region and from the milk cistern.

Ewe 2N217.

A total of 8 blood cultures, all of which proved to be sterile, were made during the 4 day interval between inoculation and the time the animal was destroyed.

Cultures made at autopsy from the right supramammary lymph node and from the three levels of the median dorsal region of the right mamma were sterile. Pure cultures of P. mastidis were obtained from the lateral ventral region and from the milk cistern of the gland.

The fact that the cultures from the median dorsal region of the gland were sterile was somewhat surprising inasmuch as similar cultures from ewe C4021 were positive when the animal was destroyed 2 days after inoculation. However the first clinical symptoms of mastitis did not appear in ewe 2N217 until 36 hours after inoculation so it would seem that the disease was pursuing a milder course in this animal than in C4021.

Ewe 3S65.

A total of 21 blood cultures was made from this animal after she was inoculated. The first positive blood culture was obtained 48 hours after inoculation. The bacteremia persisted through the evening of the 5th day after inoculation, a total of 84 hours.

When the ewe was destroyed 12 days after inoculation, cultures made from all three levels of the median dorsal region of the right mamma as well as those from the lateral ventral region and the milk cistern yielded P. mastidis in pure culture. Cultures from the supramammary lymph node were sterile, as were cultures from the kidney, spleen, liver, and lungs.

The fact that the organism was isolated from all levels of the udder cultured, 12 days after inoculation, was not surprising in view of the fact that ewes have been known to carry the organism in their udders for months or even several seasons after recovery.

Fermentation reactions.

In order to complete identification, cultures were selected from each animal and their fermentation reactions determined for 14 carbohydrates. In addition to cultures isolated from the milk cistern of each

animal, cultures from the lung and blood of ewe HIC234, from the blood of ewe 3S65, and culture 3892 were used.

It was necessary to make the standard meat infusion broth, used in the laboratory, sugar free as P. mastidis will not grow on the dehydrated sugar free media that are available on the market. This was done by inoculating a flask of standard broth with Escherichia coli and incubating for 48 hours. It was then heated in the steamer for an hour and filtered through a pad of macerated filter paper by vacuum. The pH was adjusted to give a final value of 7.3-7.4 and 0.0016 per cent of brom thymol blue added. The broth was tubed 7 ml to the tube and autoclaved. After cooling, sterile 15 per cent solutions of carbohydrate were added to the tubes to give a final concentration of 1 per cent. This broth produced a good growth of P. mastidis in 24 hours, and there was no change in the indicator color in tubes containing no carbohydrate two weeks after inoculation.

Duplicate tubes of each carbohydrate were inoculated for each culture used. The controls consisted on uninoculated tubes of each carbohydrate and tubes of the basal medium without added carbohydrate, inoculated with each culture. All cultures were incubated at 37 C.

The cultures were observed daily for the first 4 days, then at 7 days, 11 days, and at 16 days when the cultures were discarded. In most instances there was no difference in the results recorded at 4 days and those at 16 days.

The combination of the sugar free medium and brom thymol blue indicator proved very satisfactory. Previous attempts to use brom cresol

purple as an indicator were unsatisfactory due to the weak fermentative powers of P. mastidis and its tendency to decolorize the indicator. When brom thymol blue is used it is important that the pH of the medium be 7.3 to 7.4 or even slightly higher. If it is lower the pH may drop sufficiently before use to produce a color change in the indicator.

All cultures showed a slight change in arabinose, indicating weak fermentation. However the arabinose control tubes also showed a change, although of a lesser degree. Therefore, results in this carbohydrate possibly are not too reliable.

Fermentation of lactose was very slight, but could be detected by change in the color of the indicator and by use of the glass electrode potentiometer.

The reactions, as shown in table I, agree in all respects with the findings of Matisheck (1947) who determined the fermentation reactions on a number of variants of P. mastidis.

The fact that the HIC234 lung culture was identical to the other cultures in fermentation reactions as well as in morphology was of considerable interest. Although pasteurellas have previously been isolated from the lungs of mastitic ewes at this laboratory, none were positively identified as P. mastidis.

Histopathology.

The histopathology of the inoculated mastitis cases consists of the results of studies made on tissues from ewes HIC234, C4021, 2N217, and 3S65 as well as on tissues from the 3 ewes previously inoculated at the

TABLE I

FERMENTATION REACTIONS OF *Pasteurella mastidis* CULTURES

	HIC234 MC	HIC234 Lung	HIC234 Blood	C4021 MC	2N217 MC	3S65 MC	3S65 Blood	3892
Glucose	A	A	A	A	A	A	A	A
Galactose	A	A	A	A	A	A	A	A
Levulose	A	A	A	A	A	A	A	A
Lactose	A-	A-	A-	A-	A-	A-	A-	A-
Maltose	A	A	A	A	A	A	A	A
Mannitol	A	A	A	A	A	A	A	A
Sorbitol	A	A	A	A	A	A	A	A
Sucrose	A	A	A	A	A	A	A	A
Raffinose	A	A	A	A	A	A	A	A
Arabinose	A-	A-	A-	A-	A-	A-	A-	A-
Dulcitol	0	0	0	0	0	0	0	0
Inulin	0	0	0	0	0	0	0	0
Mannose	0	0	0	0	0	0	0	0
Salicin	0	0	0	0	0	0	0	0

A=Acid production; A-=Weak or doubtful; 0=No reaction.
Gas was not produced in any carbohydrate.

Veterinary Research Laboratory. Tissues from the latter 3 animals were sectioned and stained for bacteria by the author.

Ewe HIC234.

Udder: The MD-1 section was quite hyperemic with some hemorrhage and a moderate edema of the connective tissue. The interlobular connective tissue was lightly infiltrated with leucocytes, predominately of the neutrophile type. In most lobules the majority of the alveoli showed varying degrees of epithelial exfoliation, although occasional alveoli were observed in which the secreting cells showed no damage.

In nearly every lobule a large proportion of the alveoli were filled with masses of cells and cellular debris. There was evidence of karyorrhexis in these aggregations as small bits of nuclear material were often seen. The nuclei of many of the cells were distorted in polyhedral and long, slender spindle forms. In some instances groups of spindle forms were arranged in such a manner that they gave the impression of "eddies" or "whorls" in the mass of cells.

It was difficult to distinguish the types of cells present in such alveoli, but exfoliated epithelial cells and undistorted nuclei resembling lymphocytes were identified. In a few instances alveoli were observed with partially intact epithelium and small numbers of undistorted leucocytes in the lumen. Neutrophiles, monocytes, and lymphocytes could be identified. From this it would appear likely that the masses of cells within the alveoli described above were leucocytes. The large numbers of cells present in itself indicated that they were probably of outside origin and not from the alveoli themselves.

The MD-2 section was very similar to MD-1. Damage to the secreting tissue was similar in extent to the first section. Several large aggregations of cells with bizarre nuclei were observed near the edge of the section farthest from the dorsal border of the udder. They were apparently formed by necrosis of the interalveolar tissue, leaving the aggregations of cells in the lumens of the alveoli.

The LV section was quite hyperemic, with hemorrhage into the alveoli in some places. The interlobular connective tissue was edematous and contained fibrin in some regions. There was a light, spotty leucocyte infiltration of the interlobular tissue. In regions where the infiltration was heaviest, neutrophiles and monocytes predominated, while lymphocytes were more prominent in the thinner regions.

Damage to the alveolar epithelium was more pronounced than in the medial dorsal sections, with exfoliation general throughout the section. Many of the alveoli contained masses of cells showing nuclear changes.

The more dense connective tissue in the vicinity of the milk cistern did not show the pronounced edema that was observed in the lateral ventral sections. Leucocyte infiltration, with neutrophiles predominating, was heavier than that observed in the sections from the other regions of the udder.

Destruction of the secreting epithelium was very marked with general exfoliation throughout the section. In some of the lobules all of the alveoli were heavily engorged with cells showing karyorrhexis as well as fusiform and polyhedral shaped nuclei. Cells showing similar changes were often found in the adjacent interalveolar tissue. Eddies and streams

were apparent in the intra-alveolar aggregations. In lobules where only a portion of the alveoli were congested with masses of cells, the alveoli at the periphery of the lobule were most commonly affected.

Some lobules were surrounded or partially surrounded by thin bands of leucocytes showing nuclear changes similar to those observed in the alveoli. These bands were in the connective tissue at the periphery of the lobule and in some instances appeared to be partially within the peripheral alveoli.

Examination of a large milk duct in the section revealed some erosion of the lining cells, leaving a ragged border.

Sections from the various regions of the udder stained to show bacteria revealed enormous numbers of organisms among the masses of cells observed in the lumens of many of the alveoli. Large masses of organisms were frequently observed in the lumens of alveoli with marked epithelial exfoliation but few infiltrating leucocytes. In a few cases alveoli showing marked exfoliation contained either very small numbers of organisms or no organisms. When organisms were present in alveoli where the epithelium was partially intact, they were often observed in close contact with the free surface of the secreting cells, and in quite a few instances, within the cells themselves.

Small clumps of organisms were frequently seen within the interalveolar connective tissue when near by alveoli were heavily invaded. Small aggregations of bacteria were occasionally seen in the interlobular tissue, but such invasion was very limited.

The largest numbers of organisms were in the section of tissue taken from the vicinity of the milk cistern while the smallest numbers were found in the median dorsal sections. The morphology of the organisms was similar to that found in cultures grown on artificial media.

Supramammary lymph node: The pathological picture here was one of lymphadenitis with neutrophiles invading the cortical and medullary sinuses. No organisms were found in sections stained with bacterial stains.

Spleen: Sections of the spleen revealed marked engorgement of the redpulp with erythrocytes. No organisms were found in sections stained with bacteria stains.

Liver: The most striking feature of this section was the general vacuolation of the hepatic cells. The vacuoles did not have the well defined borders found in fatty livers, but a ragged edge. There was no particular distribution of the vacuoles, as they were found in equal numbers in the peripheral as well as the central regions of the lobes.

This section resembles a section obtained by liver biopsy from a normal steer. It may be that the vacuolation is due to glycogen stored in the liver, however it would appear that the liver of the ewe would be depleted of glycogen due to the fasting of the animal before death.

Occasional small clumps of organisms, undoubtedly the coliform organisms isolated in the cultures, were seen in sections stained for bacteria.

Lung: Sections from one block of lung tissue were cut through one of the hyaline foci observed at autopsy. The lesion appeared on microscopic

examination as a circular mass of cells, 1.5 mm in diameter, lying in the parenchyma of the lung just beneath the capsule. The peripheral region of the lesion was composed of monocytes and lymphocytes. At the center of the lesion fibroblasts radiated from a central focus of necrotic cells which had lost their identity but probably were of leucocyte origin. The remaining portion of the section showed no pathological change.

Sections from this block of tissue stained for bacteria revealed an aggregation of 25 or 30 short, almost coccoid bodies lying between the necrotic mass in the center of the lesion and the surrounding fibroblasts. They stained similar to bacteria but could not be positively identified as such. Pasteurellas usually do not occur in tissue in this particular form.

Sections from a second block of lung tissue revealed numerous regions where the alveoli had been obliterated by hypertrophy of the interalveolar connective tissue and cellular infiltration. There was marked peri-bronchial infiltration of lymphoid cells. Sections stained with bacterial stains did not reveal any organisms.

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Udder: The first section extended from the dorsal surface of the udder to a depth of 1 inch and was identified by the letters MD-1-2. The tissue showed slight hyperemia and edema with a very light leucocyte infiltration of the interlobular connective tissue.

Intra-alveolar infiltration of leucocytes was general throughout the section, most alveoli containing small numbers in their lumens while a few were heavily engorged. Neutrophiles predominated, while monocytes

and lymphocytes were present in smaller numbers. There was very little distortion of nuclei in the masses of leucocytes.

There was some damage to secreting epithelium but it was negligible. In many instances the secreting tissue was completely intact in alveoli which were engorged with leucocytes.

Some 20 or 25 lavender staining bodies were observed distributed throughout the section. They were round and of such position and size that it appeared that each was occupying a space originally allotted to an alveolus. Some of the bodies showed a concentric striation while others appeared as hollow cylinders, with one side collapsed, possibly as a result of sectioning.

These bodies are undoubtedly similar to the corpora amylacea or amyloid bodies, so called because of their morphological resemblance to starch grains, often found in the udders of lactating cows. According to Zimmerman (1909), amyloid bodies were first described by Iwanoff in 1880. Since that time many authors, including McFadyean (1930), Morril (1935), and Scholl (1946), have mentioned the bodies. Their exact nature and origin is not known but it appears that they are of calcareous nature.

Examination of the literature has not revealed any mention of corpora amylacea in ovine udder tissue.

The MD-3 section was similar in appearance to section MD-1-2, but showed slightly more damage to the secreting epithelium. A number of amyloid bodies were observed in the section.

The LV section resembled the median dorsal sections very closely. Hyperemia, edema, and leucocyte infiltration of the interlobular connective tissue were so slight as to be practically non-existant. Leucocytes were present in the lumens of most alveoli, usually in small numbers. Most of the leucocytes were free from distortion, but polyhedral and fusiform nuclei were occasionally observed in the lumens of the few alveoli which were engorged with leucocytes. Exfoliation of the secreting cells was present, but was limited in extent, similar to that observed in the median dorsal sections.

Approximately 30 amyloid bodies were observed in the section. In several instances bodies were observed which occupied only small portions of actively functioning alveoli.

The interlobular connective tissue in the MC section was more edematous than in the median dorsal or lateral ventral sections. Leucocyte infiltration of the interlobular connective tissue was also more evident here, but was of the light, diffuse type previously noted. Neutrophiles and monocytes were the most prevalent of the infiltrating cells.

Exfoliation of the secreting epithelium was much more pronounced than it was in the other sections from this animal. However, again a few alveoli were found with completely intact epithelium. The number of alveoli that were completely engorged with leucocytes was much higher than it was in the median dorsal and lateral ventral sections. In a few small lobules all of the alveoli were so congested.

Although the majority of the intra-alveolar leucocytes were comparatively undistorted, a number of alveoli were observed in which polyhedral and fusiform nuclei, often arranged in eddies or whorls, were to be seen. Karyorrhexis was also present in such alveoli. The section contained many amyloid bodies.

Damage to the tissue in this section, while extensive, was not as marked as it was in MC-HIC234.

Sections from the median dorsal and lateral ventral regions stained for bacteria revealed extremely small numbers of organisms. Many alveoli contained no visible organisms while those that did often contained only 3 or 4. All organisms observed in these sections were in the lumens of the alveoli.

Most of the alveoli in the sections from the milk cistern contained small numbers of organisms, although large numbers were found in the alveoli that contained leucocytes showing nuclear changes. The majority of the bacteria were free in the lumens of the alveoli, but in some instances organisms were observed within cells of the secreting epithelium. Small aggregations of organisms were occasionally found in the interalveolar tissue in the immediate vicinity of alveoli which were heavily invaded. No organisms were observed in the interlobular connective tissue or within the wall of the milk cistern.

Supramammary lymph node: There was some leucocyte infiltration of the cortical and medullary sinuses, but it was not as marked as that observed in the node from ewe HIC234. Bacteria were not found in sections stained with Giemsa and with azure eosinate.