



The dissociation of *Pasteurella mastitidis*
by Peter H Matischeck

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

Because of its variability, *Pasteurella mastitidis*, the etiological agent of a form of mastitis in sheep, was examined for dissociation. From broth cultures six variants, falling into three groups, were isolated. They were classified according to their colony characteristics and designated: Group 1, the iridescent and blue iridescent variants; Group 2, the white opaque and wrinkled variants; Group 3, the blue-grey and blue variants. Their growth characteristics were determined on various media.

The iridescent and white opaque groups produced a small amount of hemolysis on blood agar, but no toxin was found.

No significant differences in carbohydrate utilization between variants were found. The reactions were weak, and somewhat variable. There was no gas production.

The capsule of the encapsulated variants (the iridescent and white opaque groups) was destroyed rapidly by heat, phenol and acid in the medium. The iridescent and white opaque groups were highly pathogenic for sheep and mice, and the blue-grey was low.

Cross agglutination reactions showed considerable group specificity. Possible antigen complex was postulated to account for this. The blue-grey variant was highly antigenic, and showed little group specificity.

This variant shows promise in the development of a good vaccine, and suitable diagnostic test.

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TABLE OF CONTENTS

	Page
ABSTRACT.....	3
INTRODUCTION.....	4
REVIEW OF LITERATURE.....	5
MATERIALS AND METHODS.....	7
EXPERIMENTAL:	
Isolation & Description of Variants.....	10
Influence of Environment on Dissociation.....	13
Growth in Various Media.....	14
Effect of Different Media on Colony Characteristics.....	18
Toxin Production.....	22
Pathogenicity.....	23
Agglutination Reactions.....	25
The Capsule.....	34
Fermentation Reactions.....	35
DISCUSSION.....	38
CONCLUSIONS.....	42
LITERATURE CITED & CONSULTED.....	44

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9 Graduate Committee Thesis

ABSTRACT

Because of its variability, Pasteurella mastitidis, the etiological agent of a form of mastitis in sheep, was examined for dissociation. From broth cultures six variants, falling into three groups, were isolated. They were classified according to their colony characteristics and designated: Group 1, the iridescent and blue iridescent variants; Group 2, the white opaque and wrinkled variants; Group 3, the blue-grey and blue variants. Their growth characteristics were determined on various media.

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The capsule of the encapsulated variants (the iridescent and white opaque groups) was destroyed rapidly by heat, phenol and acid in the medium. The iridescent and white opaque groups were highly pathogenic for sheep and mice, and the blue-grey was low.

Cross agglutination reactions showed considerable group specificity. Possible antigen complex was postulated to account for this. The blue-grey variant was highly antigenic, and showed little group specificity. This variant shows promise in the development of a good vaccine, and suitable diagnostic test.

THE DISSOCIATION OF PASTEURELLA MASTITIDIS

(MEISSNER & SCHOOP) HAUDUROY ET AL.

INTRODUCTION

The organism, Pasteurella mastitidis, was first described by Dammann & Freese in 1907, and was known as the Bacillus of Dammann & Freese. In 1932 it was identified with outbreaks of mastitic infections of sheep in Germany, by Meissner & Schoop, who called it Bacterium mastitidis. In the same year Haupt described it as Bacterium ovinum. Hauduroy's Dictionnaire des Bacteries Pathogenes pour l'Homme, les Animaux et les Plantes (1937) lists it as Pasteurella mastitidis Dammann & Freese; and Bergey's Manual (1939) lists it as Pasteurella mastitidis (Meissner & Schoop) Hauduroy et al.

Marsh, in 1932, isolated this organism from mastitic infections enzootic in sheep in Montana. He was the first to report this organism in the United States, identifying it with the Bacillus of Dammann & Freese, and describing it as a Pasteurella. A number of cultures have been studied at this laboratory, and a high degree of variability noted, indicating a need for dissociation studies. This work was begun in the hope that a highly antigenic and serologically non-specific variant, suitable for immunization and serological diagnosis, might be found.

REVIEW OF LITERATURE

The earliest dissociation work in this genus was reported by Manninger (1919) who described an unencapsulated, avirulent, and highly immunogenic variant of the fowl cholera organism. De Kruif (1922), working with a rabbit septicemia Pasteurella, described a virulent "D", and an avirulent, rough "G" type. Webster and Burn (1926), working with Bacterium lepi-septicum, found the same types, and an intermediate "I", and a relatively stable mucoid "M" type. Anderson, Coombes & Mallick (1929-30) found the same variant types in Bacterium avisepticus, but used the modern "S" and "R" terminology to replace De Kruif's "D" and "G" types.

Hughes (1930), working with P. avicida in relation to the epidemiology of fowl cholera, described fluorescent, blue, and intermediate variants. The fluorescent type was highly virulent, and definitely associated with epidemic fowl cholera. It was stable on blood agar, but on infusion agar it dissociated to the blue type. The blue variant showed no fluorescence, and had no virulence. It was associated with endemic cholera. This variant was stable on solid media, and Hughes believed it to be similar to the "G" form of P. lepi-septica. The intermediate type varied in its characteristics from the fluorescent to the blue types, and its virulence varied accordingly. This came from only one outbreak during epidemic and post-epidemic periods. It was very stable. With agglutinnin absorption tests all three types proved to

be closely related. Although no capsule could be demonstrated on the fluorescent variant, its inagglutinability with homologous antiserum was believed due to the protection of the capsule.

Priestly (1936) showed that the virulent variants of this organism were encapsulated, and those cultures having greater capsule width were more virulent. The capsule was destroyed almost at once at boiling temperature and disappeared when incubation continued beyond 24 hours at 37 C.

Mørch and Krugh-Lund (1931), and Ochi (1931 and 1933), found variations in hemorrhagic septicemia pasteurellae similar to those described by Hughes. Cornelius (1931), working with P. suis septica, found a similar change, but used the terminology "I" for the fluorescent, and "A" for the blue variant. The direction of dissociation was like that found by Hughes.

Brigham and Rettger (1935), in a study of the genus Pasteurella, found "S", "R", and "I" variants. Rosenbusch & Merchant (1939) studied the hemorrhagic septicemia pasteurellae. They found "S", "M", and "R" forms, and mention intermediate forms. However, their work was primarily from the standpoint of classification. The variants were not well described, although they were correlated to some extent with biochemical properties. The permanent variants differed in biochemical properties, and immunological behavior. A cyclic behavior in biochemical properties was noted, which the

authors did not believe originated with permanent phases.

There have been no reports in the literature relating to the variants of Pasteurella mastitidis. Jasmin, in unpublished work done at this laboratory, found three types of colonies. One was a smooth, translucent colony showing chains of organisms, which he designated as "R" or "IR". This variant had few or no capsules, and was avirulent. Another type, designated "S", showed a bluish-green iridescence, with a high degree of encapsulation and virulence. A third type was a dense colony, very granular with irregular edges and very friable. Encapsulation and pathogenicity were variable.

MATERIALS AND METHODS

The standard culture medium used throughout this investigation is designated as VRL medium. It is a modification of the "hormone" medium described by Huntoon (1918). He avoided filtration in order to retain the growth factors. The VRL medium used in this study requires filtration and therefore does not meet Huntoon's criterion of a "hormone" medium. Pasteurella mastitidis grows well on this medium, and it has the added advantages of being clear and lightly colored. The different types of colonies are well differentiated. The medium is made up as follows:

Beef (round steak).....	500 g
Distilled water.....	1000 ml
Bacto-Peptone.....	10 g
Sodium chloride.....	5 g
Eggs.....	2

All fat and connective tissue are cut away, and the meat is ground fine in a meat grinder. The meat, peptone, sodium chloride, and the egg yolks are added to the water and heated with constant stirring to 68 C, no higher. Normal NaOH is added until the medium is slightly alkaline to litmus paper. The pH at this point is about 7.6. Stir in the whites of the eggs beaten up in a little water. Heat in an Erlenmeyer flask in an Arnold sterilizer for an hour. Remove and separate the clot from the sides of the flask with a glass rod. Heat again for an hour. Pour the liquid from the clot and filter. This constitutes the VRL broth used in this investigation. Agar is added to this to give a final concentration of 1.2 per cent agar. The agar is dissolved by autoclaving and the precipitate filtered out. The final reaction should be about 7.2. For use, sterile normal horse serum is added to the cool, liquid agar to give a final concentration of 10 per cent serum. This constitutes the VRLS agar. For VRL agar the serum is omitted.

Beef extract agar is the standard medium used in bacteriological work, with the exception that it contains only 1.2 per cent agar. Beef extract broth is identical with that used in standard bacteriological procedure.

For sugar fermentation reactions, VRL broth was used. To this was added brom cresol purple to give a final concentration of 0.0016 per cent. The broth was adjusted with

a glass electrode potentiometer to give a final pH of 7.0. The sugars in 15 per cent solution in distilled water were autoclave sterilized for 15 minutes at 15 pounds pressure. This sterile solution was added aseptically to the indicator broth to give a final concentration of 1.0 per cent sugar.

For the study of variants streak plates were made so that about half the plate showed well isolated colonies. The agar in these plates was no more than 4 mm thick. The colonies were studied and picking was done using a binocular dissecting microscope. The light source was a small adjustable lamp using a 6 volt bulb, connected to the 110 volt line through a transformer. This lamp had a condensing lens that formed a beam of parallel light rays. The correct angle of the light beam to the surface of the agar was found to be about 45 degrees.

The capsule stain used throughout this study was that of Jasmin (1945). His technique consists of mixing a loop of culture with a mixture composed of 10 per cent serum in distilled water or 0.85 per cent salt solution to which 0.5 to 1.0 per cent phenol was added. This was done on a clean glass slide. After the mixture had dried the slide was dipped into absolute methyl alcohol and flamed. The slide could then be stained by the gram technique or by any of the common bacteriological stains. It was found that the film of coagulated serum could be well stained with dilute

carbol fuchsin.

EXPERIMENTAL

Isolation and Description of Variants

All of the variants used in this study were isolated from a single culture. This culture (2459) was isolated from the udder of an ewe with a fatal case of mastitis. A 24 hour VRLS agar plate showed colonies with iridescence, and some of a white opaque type. A beef extract agar plate showed the same types, with the white opaque type predominant.

The standard medium for colony study used throughout this study was VRLS agar. It showed maximum growth, and the greatest amount of colony differentiation. Dissociation was produced by growing the original culture in VRL broth plus 10 per cent serum at 37 C for a maximum of 2 months. At various intervals VRLS agar plates were made from the dissociated broth cultures, and the variants were isolated from these. They were stabilized by picking selected colonies daily for a minimum of 2 months. The encapsulated variants were picked for greatest encapsulation, using a capsule stain.

The following descriptions refer to colonies on VRLS agar streak plates, pH about 7.2, and incubated for 24 hours at 37 C. These are the stable variants. Several other types were observed, but they were not sufficiently stable for cultural work.

White opaque:

The colonies are generally large and white opaque with a slight yellowish iridescence at the edge. There may be some wrinkling. On prolonged incubation, the center becomes more opaque, and the edge tends to become transparent. All iridescence is lost. The organisms are large and many have squared ends. They occur in short chains, pairs and singly. Their average dimensions are 1.0 by 0.7 micron. From 10 to 30 per cent of the organisms are encapsulated. These capsules are light and irregular.

Wrinkled:

The colonies are large with some yellow iridescence, opaque, and have a wrinkled surface. The wrinkling is not constant, and is lost on continued incubation. Like those of the white opaque variant these organisms are large and sometimes square ended, but they form longer chains. The dimensions are the same as for the white opaque organisms. From 40 to 60 per cent are encapsulated. The capsule width is about 0.3 micron.

Iridescent:

The colonies are smooth and small, with a yellow iridescence. After 24 hours they lose their iridescence at the center. The organisms are medium in size, ovoid, regular, and occur singly and in pairs. They measure 0.9 by 0.7 micron. From 60 to 100 per cent of the organisms show heavy capsules. The capsule width is about 0.27 micron.

Blue iridescent:

The colonies are smooth, small, and highly blue iridescent. After 24 hours they become blue-grey at the center, and more dense, with iridescence only at the edge. The organisms are small and rod shaped, and occur singly and in pairs. They average about 0.9 by 0.5 micron. From 90 to 100 per cent of the organisms have heavy capsules. The capsule width is about 0.28 micron.

Blue-grey:

These colonies are smooth, small, and somewhat granular, with a blue-grey color. On continued incubation the center becomes white and more granular. The organisms are small and rod shaped, and occur singly and in pairs with no capsules. They average about 0.9 by 0.6 micron.

Blue:

These colonies are small, smooth and blue, with slight granulation. They remain blue on continued incubation, but large colonies become white at the center. The organisms are small and rod shaped, occurring singly and in pairs. From 0 to 10 per cent of the organisms have light capsules. Their average dimensions are about 0.9 by 0.6 micron.

Brittle colonies occur in all these variants. They are opaque with irregular surfaces and edges. On sub-culturing they revert to the parent type in which they were found. When grown on media with a pH below 7.1, they tended

to increase in number. Growth on 2 per cent agar media also increased the proportion of brittle colonies. They could not be stabilized.

The variants described above were also isolated from a number of typical cultures of Pasteurella mastitidis, indicating that they are common to this species. Strains recently isolated from acute cases of mastitis contained the encapsulated forms predominantly. In strains from chronic cases the types found were generally non-encapsulated.

Recently isolated cultures held in storage are not stable. After several months the predominant types are no longer identical with those of the original culture. Strain 1500 carried on agar for several years consisted entirely of blue colonies. It was very stable. A culture in VRL broth plus 10 per cent serum stored at 37 C for two months, showed no other variants.

Influence of Environment on Dissociation

The dissociative changes in various media at different temperatures of growth and storage were determined. Two tubes of VRL broth, plus 10 per cent serum, were inoculated with encapsulated organisms from the original culture. These were stored at 37 C. Three VRLS agar slants, three VRL agar slants, three slants of beef extract agar plus 10 per cent serum, and three beef extract agar slants were inoculated as above. One culture of each of these was stored at 37 C,

one culture of each at room temperature, and the third culture of each was incubated for 24 hours at 37 C, and stored at 4 C. Streak plates were made from these cultures on VRLS agar, and beef extract agar at various intervals up to 58 days, or until the culture died. The cultures held at 37 C, and those in the media more favorable for growth, i.e. the VRL media, dissociated more rapidly, and showed predominantly the non-iridescent forms. With less favorable media, i.e. the beef extract media, and at the lower temperatures, dissociation was less rapid, and the cultures remained predominantly in the iridescent and encapsulated forms. During this time no one type became stabilized. In comparisons between VRLS agar and beef extract agar as plating media, the beef extract agar showed more iridescent and encapsulated forms than the VRLS agar.

Growth in Various Media

The growth characteristics of the six variants in broth were determined. Tubes of VRL broth, plus 10 per cent serum, were inoculated in duplicate and incubated at 37 C for 6 days. The pH of the broth was 7.3. The results are tabulated in Table I. This experiment was duplicated using beef extract broth plus 10 per cent serum. The pH of the medium was 7.4. The same conditions were maintained. The results are shown in Table II. From growth characteristics in these two media, three general groups are evident: first

Table I

Growth characteristics in VRL broth plus 10 per cent serum.

	'7 hrs.'	'24 hrs.'	'48 hrs.'	'72 hrs.'	'96 hrs.'
White opaque	'cloudy'	'sediment'	'cloudy'	'cloudy'	'no change'
Wrinkled	'cloudy'	'sediment'	'cloudy'	'cloudy'	'no change'
Iridescent	'cloudy'	'cloudy'	'cloudy'	'cloudy'	'no change'
Blue irid.	'cloudy'	'cloudy'	'cloudy'	'cloudy'	'no change'
Blue-grey	'clear'	'pellicle'	'ring'	'sediment'	'no change'
Blue	'clear'	'pellicle'	'pellicle'	'pellicle'	'no change'

At 144 hours all tubes were clear.

Table II

Growth characteristics in beef extract broth plus
10 per cent serum.

	'7 hrs.'	24 hrs.	'48 hrs.	'72 hrs.	'96 hrs.
White opaque	'cloudy'	'cloudy'	'pellicle'	'pellicle'	'no change'
Wrinkled	'cloudy'	'cloudy'	'pellicle'	'sediment'	'no change'
Iridescent	'cloudy'	'pellicle'	'pellicle'	'ring'	'no change'
Blue irid.	'cloudy'	'cloudy'	'pellicle'	'cloudy'	'no change'
Blue-grey	'floc.'	'pellicle'	'pellicle'	'sediment'	'no change'
Blue	'floc.'	'pellicle'	'pellicle'	'sediment'	'no change'

At 144 hours all tubes were clear.

the white opaque and wrinkled; second, the iridescent and blue iridescent; and third, the blue-grey and blue. These three groups have previously been indicated by colony characteristics, and cell morphology.

After the above 24 cultures had been incubated for 144 hours, dissociation was studied by plating each culture on VR13 agar. The variants found and their approximate percentages are as follows:

VRL Broth

White opaque

Wrinkled

Blue iridescent (98%) &
iridescent (2%)

Iridescent (98%) &
blue-grey (2%)

Blue-grey
no change

Blue

Beef extract Broth

White opaque

Wrinkled
no change

Blue iridescent (60%) &
blue-grey (40%)

Iridescent (70%) &
blue-grey (30%)

Blue-grey

Blue

It can be seen that all these variants, with the exception of the blue iridescent gave rise to brittle colonies. The blue iridescent variants dissociated to the iridescent variant, and both dissociated to the blue-grey variant. These dissociative changes have been found to be typical of the blue iridescent and iridescent variants. The reverse does not occur under these conditions.

The Effect of Different Media on Colony Characteristics

Colony characteristics of a representative member of each of the three groups were studied on VRLS agar, and beef extract agar. The white opaque, iridescent, and blue-grey variants were grown for 24 hours on VRLS agar, and from that streaked on plates of beef extract agar, and grown for 24 hours. They were finally transferred back to VRLS agar. A second set consisted of two serial transfers on VRLS agar, and served as standards for comparison. All plates were incubated at 37 C, and each colony type was checked for cell morphology with a capsule stain.

The iridescent, and blue-grey variants did not change on one transfer to beef extract agar. On beef extract agar the white opaque variant produced colonies resembling in every characteristic the iridescent. However, this change was not permanent, for on transferring back to VRLS agar, the colonies reverted to the white opaque type.

The white opaque and iridescent variants were more extensively studied on the following 6 media: 1, VRLS agar; 2, beef extract agar with 10 per cent serum; 3, beef extract agar with 20 per cent serum; 4, VRL agar with 5 per cent defibrinated rabbit's blood; 5, medium A; and 6, medium B.

Medium A was composed of:

casamino acids	0.4 g
yeast extract	0.4 g
peptone	0.3 g
proteose-peptone	0.3 g
tryptose	0.3 g
neopeptone	0.3 g
tryptone	0.3 g
sodium chloride	3.0 g
meat extract	0.3 g
distilled water	100 ml
phosphate buffer pH 7.6	2.0 ml
1.2 per cent agar &	
10 per cent serum	

Medium B was composed of :

casamino acids	0.5 g
yeast extract	0.5 g
phosphate buffer pH 7.6	1.0 ml
distilled water	100 ml
1.2 per cent agar plus	
10 per cent serum	

Typical white opaque and iridescent colonies on VRLS agar were plated on each medium and serially transferred on that medium for three transfers. The fourth transfer was made to VRLS agar plates. Plates were incubated for 24 hours at 37 C between transfers. Readings were made after 24 hours incubation and the colonies were checked for cell morphology with a capsule stain. The order of transfer and colony types found are shown in Tables III and IV.

Again the white opaque resembled the iridescent variant on the beef extract media, and on these media the iridescent lost some iridescence and some encapsulation. On media A and B the white opaque variant did not show increased iridescence. In three transfers the variants did not basically

Table III

Colony characteristics of the white opaque variant on various media.

Typical white opaque colonies on VRLS agar were transferred to each of the following:

	1	2	3	4	5	6
1st	typical ' ' ' '	smaller & iridescent ' ' ' '	smaller & iridescent ' ' ' '	typical ' ' ' '	more trans- parent & grey ' '	very small wrinkled & more opaque '
2nd	typical ' '	same as above '	same as above '	same as above '	same as above '	same as above '
3rd	typical ' ' '	same as above ' '	same as above ' '	small zone of hemoly- sis '	same as above ' '	larger ' ' '
4th	1	1	1	1	1	1

20

All colonies were typical white opaque

Table IV

Colony characteristics of the iridescent variant
on various media.

Typical iridescent colonies on VRIS agar were
transferred to each of the following:

	1	2	3	4	5	6
1st	typical ! ! !	more iridescent ! ! !	more iridescent ! ! !	typical ! ! !	wrinkled less irid. ! ! !	small less irid. ! ! !
2nd	same as above ! !	same as above ! !	same as above ! !	same as above ! !	same as above ! !	same as above ! !
3rd	same as above ! !	same as above ! !	same as above ! !	slight hemolysis ! !	same as above ! !	same as above ! !
4th	1	1	1	1	1	1

All colonies were typical iridescent

change as is shown by the typical colonies on VRLS agar at the end of the series. Both of these variants, when grown for several transfers on blood agar, acquire the ability to produce hemolysis. Hemolysis is more pronounced with the white opaque variant than with the iridescent. The blue and blue-grey variants were grown on blood agar plates, and after four transfers did not show hemolysis. This indicates a basic difference between the encapsulated and non-encapsulated variants.

Toxin Production

Some evidence had been obtained from guinea pigs and mice which indicated the presence of an exotoxin. Several attempts were made to demonstrate a soluble toxin. Each variant was grown on VRLS agar for 24 hours, then washed off and suspended in distilled water, and killed at 55 C for a half hour. A second set was prepared in the same manner, except that the organisms were suspended in physiological saline. These suspensions were incubated for periods up to 35 days. At various intervals the suspensions were centrifuged, and the bacteria-free supernate injected intraperitoneally into mice in doses as high as 0.5 ml. In none of these suspensions was there any evidence of a soluble toxin. Suspensions of living organisms treated in the same manner also gave negative results. A 24 hour VRL broth plus 1 per cent glucose culture, when freed of

organisms by centrifuging, and injected into mice, also gave no indication of toxin.

The conditions under which these cultures have been grown differ markedly from those encountered in natural infection, especially regarding CO_2 and O_2 tension. Also the medium may lack certain nutrient factors required for toxin production.

Pathogenicity

A week after isolation the original strain was tested for pathogenicity. A 6-hour culture killed a mouse injected intraperitoneally with a 0.10 ml dose, and 0.50 ml killed a guinea pig. Six-hour cultures of the blue iridescent, and of the white opaque variants were suspended in physiological saline and inoculated into 2 ewes. This was done by swabbing the opening of the teat canal with the culture suspension. Both variants produced severe mastitis. At this time the blue-grey variant also produced severe mastitis. One year later, after stabilization of the variants, both the iridescent and white opaque still produced severe mastitis in ewes. The blue variant produced moderate mastitis, and the blue-grey, mild to none.

After stabilization of the variants more pathogenicity tests were done on mice and guinea pigs. Six to 7-hour VRIS agar cultures were used, and the animals injected intraperitoneally. The cultures were made on VRIS agar

slants in standard 15 mm tubes, and the growth in each was suspended in 1.0 ml sterile saline. Death, in most cases, occurred in less than 24 hours. The results are summarized in Table V.

Table V.
Pathogenicity of Variants

Culture	Animal	mld
parent culture	mouse	0.05-0.10 ml
	G.P.	0.25-0.50 ml
white opaque	mouse	0.05 ml
	G.P. less than	0.25 ml
wrinkled	mouse	0.05 ml
	G.P. less than	0.25 ml
blue iridescent	mouse	0.05 ml
	G.P. less than	0.25 ml
iridescent	mouse	0.10 ml
	G.P.	0.25-0.50 ml
blue-grey	mouse	0.15-0.25 ml
	G.P.	0.50 ml

The white opaque, wrinkled, and blue iridescent variants were about equally pathogenic, although the blue iridescent variant did not produce death as quickly as the first two. The pathogenicity of the iridescent variant was somewhat lower. The least pathogenic of these variants was the blue-grey. This general order agreed with the results obtained with ewes. The value of pathogenicity tests using mice or guinea pigs is doubtful since in sheep the route and type of

infection is entirely different. These tests, however, do give information on the relative order of pathogenicity of the different variants.

Agglutination Reactions

In cross agglutination tests between different strains of Pasteurella mastitidis at this laboratory there was found to be a great lack of homogeneity. It was thought that this lack of homogeneity was due to the predominance of different variants in different strains. In order to determine the agglutinnin specificity of the variants, cross agglutination tests were done.

Preparation of Antisera.

Rabbits were injected in duplicate, intravenously, at 5-day intervals, starting with 0.05 ml killed culture, and doubled until 2.0 ml was reached. Live cultures were then injected i.v., starting with a 0.10 ml dose and doubled until 2.0 ml was reached. This dose was repeated, and two more doses of 4.0 ml and 8.0 ml were given. For the killed cultures the 16-18 hour growth from VRIS agar slant in a standard 15 mm tube was suspended in 1 ml sterile saline after discarding the supernatant fluid. This suspension was killed at 55 C for 30 minutes. The live cultures were obtained in the same way; except that they were not heated. About 10 days after the last injection the rabbits were bled, and the serum obtained. Some of the sera were preserved with

1 per cent chloroform and some with 1/10,000 merthiolate.

Preparation of Antigens

Just before the antigens were prepared, the variants used were picked several times to insure purity of the cultures. The parent culture was taken directly from a lyophilized tube. Single colonies of each culture were picked to VRLS agar slants. After 7 hours at 37 C the condensation fluid was discarded, and the growth suspended in sterile saline. The growth from one slant was inoculated into a 16 oz. prescription bottle containing 1.5 per cent VRLS agar. After 18-20 hours at 37 C the growth was washed off with 40 ml sterile saline plus 0.5 per cent phenol. The organisms were killed at 55 C for 1 hour, then washed three times in the centrifuge. The growth was suspended in phenolated saline, and the suspension was adjusted to 6 cm on the Gates nephelometer. These antigens were used for the agglutination tests.

The lowest dilution of antiserum used in these tests was 1-25. The dilution was doubled in each successive tube. The reactions were read after 48 hours incubation at 37 C. The endpoint was taken as the highest dilution of anti-serum at which complete agglutination took place. The titres obtained are only approximate. The cross agglutination reactions between the variants are summarized in Table VI.

Table VI

Cross agglutination reactions between the six variants and the parent culture.

Sera	Antigens							
	'White 'opaque	'Wrink.'	'Blue	'Blue- 'grey	'Blue 'irid.'	'Irid.'	'Parent 'Culture	
Parent Culture	25	50	50	1600	3200	6400	12,000	
White opaque	200	200	100	200	100	200	400	
Wrinkled	400	800	200	200	200	800	400	
Blue	800	1600	800	800	25	800	1600	
Blue-grey	200	200	200	3200	3200	1600	3200	
Blue irid.	50	50	50	400	800	3200	1600	
Irid.	25	50	25	100	100	400	400	

The above titres are the greatest dilution at which complete agglutination occurs after 48 hours incubation at 37 C.

Table VII

Adjusted cross agglutination reactions

	Antigens							
Sera	'White 'opaque'	'Wrink.'	Blue	Blue- grey	Blue irid.	Irid.	Parent Culture	
	'	'	'	'	'	'	'	
Parent Culture	'-----'	'-----'	'-----'	100	200	400	800	
White opaque	800	800	400	800	400	800	1600	
Wrinkled	400	800	200	200	200	800	400	
Blue	800	1600	800	800	25	800	1600	
Blue-grey	50	50	50	800	800	400	800	
Blue irid.	50	50	50	400	800	3200	1600	
Irid.	50	100	50	200	200	800	800	

----- Below 1-25

Table VII shows these reactions adjusted to equal homologous titres. They are adjusted so that complete agglutination is represented as occurring at a dilution of 1-800. The other titres are adjusted in proportionate amounts accordingly. This was done by noting the number of dilutions the homologous titre of a particular serum was raised or lowered to bring it to 1-800. If it had to be raised a certain number of dilutions all the heterologous titres of that serum were raised the same number of dilutions and vice versa. This probably gives a clearer picture of group differences, although the validity of bringing up the titre of low titre sera is doubtful, especially where several antigenic components are involved.

These reactions indicate the probability of two antigens, or antigen complexes, the non-specific protein or somatic antigens, and the specific polysaccharide or capsular antigens. The white opaque, wrinkled, and blue variants have antigens common to their group. The same is true of the blue iridescent and iridescent antigens. Because of the method of preparation of the blue iridescent and iridescent agglutination antigens, the capsules were destroyed, although the antisera probably consist mainly of capsular antibodies. Thus, they react with the somatic antibodies of the white opaque, wrinkled and blue sera. Their antisera contain low somatic titres as shown by their

reactions with the white opaque wrinkled and blue antigens. The blue-grey variant does not conform to this scheme, indicating the presence of other antigens. Irregularities in the reactions of the blue iridescent variant tend to confirm this. Data presented later indicate that the problem has considerable complexity. The three general groups into which the variants fall, as shown by their cultural characteristics, are not evident here. Considering the conditions under which these tests were made the results are not conclusive. However, considerable specificity is exhibited in these reactions. This indicates that the lack of homogeneity between different strains may be partially due to the predominance of different variants in the cultures.

The least specificity is exhibited by the blue-grey antigen. Although the titres are intermediate, its reactions with the various antisera are more uniform than those exhibited by any other antigens (see Table VI). It shows the greatest promise in the search for a sufficiently non-specific antigen for diagnostic purposes. X

It is probable that the antigen complex is composed of several antigens of different characteristics. These antigens do not necessarily differ in conformity with the difference in gross characteristics of the variants. This has been amply demonstrated by numerous investigators.

The influence of killing agents on the characteristics of the agglutination antigens is shown by the following experiment. The antigens used in this experiment were prepared as those above, except for the killing, and preserving agents. They are divided into two groups, the formalin antigens, and the phenol antigens. Of the formalin antigens, one was killed with 1 per cent formalin, and the other was killed at 93 C for 5 minutes, and preserved with 1 per cent formalin. Of the phenol antigens, one was killed with 0.5 per cent phenol, and the other was killed at 90 C for 20 minutes, and preserved with 0.5 per cent phenol. The antisera are the same as those used in the cross agglutination reactions above. The iridescent and wrinkled antigen reactions from the cross agglutination tests above are included. The tests were done in the same manner as those above. The results are summarized in Table VIII.

In the interpretation of these reactions it is assumed that formalin does not alter the specific character of the antigens. Phenol, as is shown later, does destroy, or extract, the polysaccharide of the capsule. This probably leaves an antigenically different cell than when the capsule is destroyed by heat. The difference can be seen in the difference in titres and specificity between the iridescent formalin and phenol antigens with each of the 6 antisera.

Table VIII

The influence of killing and preserving agents on the antigens of the encapsulated variants. The antisera are the same as those used for the reactions recorded in Table VI.

Sera

			White opaque	Wrink.	Blue	Blue grey	Blue irid.	Irid.
<u>Irid.</u> <u>HCHO</u> antigens	Killed at 93 C Unheated		----	----	200 ---	6400 200	6400 400	---- 100
<u>Irid.</u> <u>Phenol</u> antigens	Killed at 90 C Unheated		50 200	200 200	--- 100	1600 1600	800 800	200 200
<u>Wrinkled</u> <u>Phenol</u>	Killed at 90 C Unheated		50 50	100 200	--- ---	--- ---	--- ---	--- ---
<u>Irid.</u> <u>(phenol)</u>	Killed at 55 C		200	800	800	1600	3200	400
<u>Wrinkled</u> <u>(phenol)</u>	Killed at 55 C		200	800	1600	200	50	50
--- Less than 1-25								

The influence of temperature of killing on the antigens is more strikingly evident in the titres of the wrinkled antigens. The low titre of the unheated antigen may be due to incomplete destruction of the capsule. If this is the case, a thermolabile antigen may be responsible for the high titres and non-specificity of the antigen killed at 55 C.

The formalin killed iridescent antigen titres with the blue iridescent and iridescent antisera show the low antigenicity of the capsular antigen (Table VIII). The blue-grey variant apparently contains an antigen common to the iridescent variant. This and the heat killed antigens show no relationship to the white opaque and wrinkled antisera. The heat killed antigen showed a thermostable somatic antigen in its reaction with the blue and blue-grey antisera. Its reaction with the blue iridescent antiserum shows the presence of a somatic antibody, probably formed in the process of immunization.

The phenol and heat killed iridescent antigens in their reactions with the blue iridescent and iridescent antisera indicate that the destruction of the capsule was equally complete in both. The somatic antigen is apparently influenced by the phenol as shown by the reactions with the blue and blue-grey antisera. The reactions with the white opaque and wrinkled antisera show either a destruction of the specificity of the antigens or another antigen. The iridescent phenol antigen (killed at 55 C) shows a lack of

specificity which, like the equivalent wrinkled antigen, may indicate a thermolabile antigen or destruction of specificity by phenol at that temperature.

These results show that somatic and capsular antigens are involved in the immunological reactions of these variants. They also indicate that both the somatic and capsular antigens of the variants probably differ. The influence of phenol is not clearly shown by these reactions. Although the results are not conclusive, it is beyond the scope of this investigation to go further into this question.

The Capsule

The stability of the capsule of the encapsulated variants was studied in the following experiments.

The iridescent, and blue iridescent variants, growing in VRL broth plus 10 per cent serum, lost their capsules rapidly after 24 hours at 37 C. These same variants in beef extract broth plus 10 per cent serum had fewer capsules after 24 hours at 37 C, but lost them more slowly, probably due to a slower rate of growth. On subculturing, the organisms returned to the original state of encapsulation.

Iridescent and blue iridescent variants in VRL broth plus 10 per cent serum containing 1.0 per cent glucose almost completely lost their capsules in 20 hours at 37 C. The pH of the medium after 20 hours was 6.0. Again, on subculturing, the capsules returned.

Physiological saline suspensions of 16-hour cultures of the iridescent, blue iridescent, and wrinkled variants were heated to 55 C, 60 C, and boiled. The capsules were completely lost in 5 minutes even at 55 C. A Pasteurella septica culture lost only a slight amount of encapsulation after 20 minutes at 55 C.

When encapsulated variants were suspended in phenol-saline the suspension was ropy. The white opaque variant, although only lightly encapsulated showed more ropiness than the others. Unheated phenol-saline suspensions of all encapsulated variants lost their capsules in 24 hours. Similar suspensions in formal-saline showed good encapsulation after 3 weeks. Phenol evidently destroys the capsule. This may be accomplished by hydrolysis due to increased acidity, or by solution. These results show that the capsule is highly labile. It is readily destroyed by heat, acidity, and phenol.

Fermentation Reactions

The fermentation reactions for all 6 variants and the parent culture were determined for 20 carbohydrates. All tests were done in duplicate. The controls consisted of uninoculated tubes of each carbohydrate, tubes of the basal medium inoculated with each culture tested, and tubes of the basal medium alone. Because these organisms do not produce gas the customary inner tubes were not used. Brom cresol

purple was used as the indicator. The tubes were incubated at 37 C for at least 6 days. The longest incubation time used was 11 days. This was done with slow and weak fermentations. Readings were made daily for the first 5 days, and at the end of the run. At the end of this time the final pH was determined with a glass electrode pH meter. Fermentation was recorded as positive or negative on evaluation of the final pH. This was necessary because acid formation was never very strong, and decolorization of the indicator tended to mask weak reactions. The results are summarized in Table IX.

The carbohydrates are listed according to the degree of acid production, the strongest being listed at the top. A few minor differences in reactions between variants are evident. The iridescent variant exhibited very slow fermentation. Positive reactions did not appear until the fifth day. The blue iridescent variant also gave weaker reactions than the other variants.

In many cases the distinction between weak fermentation and none is finely drawn. No significance is attached to variations in these weak reactions. Where fermentation was strong, reactions were positive within 48 hours.

Because of weak acid production, decolorization of the indicator, and some differences between variants it becomes apparent that different strains may show variations in fermentation reactions. This behavior has previously been observed at this laboratory.

Table IX

FERMENTATION REACTIONS OF VARIANTS

	'Irid.'	'Blue 'Irid.'	'White 'opaque'	'Wrink.'	'Blue'	'Blue 'grey'	'Parent Culture'
Glucose	A	A	A	A	A	A	A
Sucrose	A	A	A	A	A	A	A
Xylose	A	A	A	A	A	A	A
Mannitol	A	A	A	A	A	A	A
Maltose	A	A	A	A	A	A	A
Levulose	A	A	A	A	A	A	A
Lactose	A	A	A	A-	A	A	A
Raffinose	A	A	A	A	A	A	A
Galactose	O	A	A	A	A	A	A
Dextrin	A	A	A	A	A	A	A
Inositol	A	A	A	A-	A	A	A
Sorbitol	A	A	A	A-	A-	A-	A
Arabinose	A-	A	A-	A-	A-	A-	A-
Melezitol	A-	A	A-	O	A-	A-	A-
Glycerol	O	A-	O	O	A-	A-	A-
Dulcitol	O	O	O	O	O	O	O
Inulin	O	O	O	O	O	O	O
Salacin	O	O	O	O	O	O	O
Mannose	O	O	O	O	O	O	O
Rhamnose	O	O	O	O	O	O	O

A = acid production; A- = weak or doubtful; O = no reaction

DISCUSSION

Evidence has been presented which indicates that many of the difficulties encountered in working with Pasteurella mastitidis are partly due to its great variability. Because of this variability on isolation, particularly from acute cases of the disease, it is believed that the organism exists in some unstable phase in the host animal. Several observations, not recorded in the experimental section, have shown that passage through mice will decrease the stability of previously stabilized variants.

Some of the factors influencing dissociation rate on artificial media have been determined. Lower temperature of growth or storage results in a lower dissociation rate, and conversely, a higher temperature increases the rate of dissociation. Similar observations have been made by Braun (1945) on Brucella abortus.

The 6 variants isolated fall into 3 general groups. The descriptions refer to the colony characteristics on a medium used as a standard at this laboratory, and designated VRLS agar. The iridescent group includes the iridescent and blue iridescent variants. The group is characterized by smooth, translucent, highly iridescent colonies. The organisms are relatively small in size and highly encapsulated. The white opaque group includes the white opaque and wrinkled variants. The group is characterized by large,

opaque colonies that exhibit little iridescence. The organisms are large, occur in chains, and encapsulation is variable. The blue-grey group includes the blue and blue-grey variants. This group is characterized by small, smooth, translucent and non-iridescent colonies. The organisms are intermediate in size and non-encapsulated. With one exception, these characteristics did not vary significantly on media other than the VRIS agar used in this study. On beef extract agar the white opaque variant resembled the iridescent. It returned to its typical form when cultured on VRIS agar. A number of brittle variants were found, but they were too unstable for satisfactory study.

Both the iridescent and white opaque groups produce diffuse growth in broth. The blue-grey group produces a flocculent or granular growth. On blood agar both the iridescent and white opaque groups produce slight hemolysis. The blue-grey produces no reaction. The pathogenicity of the white opaque and iridescent groups for sheep is high. The blue-grey group is almost avirulent. The pathogenicity for mice shows less spread between the two extremes.

No toxin could be demonstrated. Carbohydrate utilization was generally weak and no important variations were noted. The iridescent group produced slower and weaker fermentation than the other two groups.

The capsule of the encapsulated variants proved to be highly labile. It was destroyed rapidly in broth cultures after 24 hours, by heat (less than 5 minutes at 55 C), by acidity and by phenol. This capsule proved to be more labile in these respects than the capsule of Pasteurella septica studied by Priestly (1936).

Both heat and phenol were used in the preparation of the agglutination antigens. Heat was used in the preparation of the killed antigens for the preliminary immunization of rabbits for hyperimmune sera. The greater part of the hyperimmunization was done with living cultures possessing the full antigen compliment. Therefore, the agglutination antigens contained only the somatic antigens and the antisera contained both somatic and polysaccharide antibodies. The capsular antibodies build up rather slowly, and the final titre of the antiserum may in some cases be due more to the somatic than the capsular antibodies. In spite of these complications the cross agglutination reactions show definitely group specificity.

Priestly (1936), working with P. septica, showed that the antigenic structure of this organism was composed of both a capsular and a somatic antigen. The results of the present study also indicate the presence of several antigens. One is a non-specific protein antigen which appears when the polysaccharide antigens are destroyed by heat or phenol. This antigen is probably demonstrated best in the reactions

of the blue-grey antigen (see Table VII). The iridescent and white opaque groups each possess a group specific capsular antigen (see Table VIII). The blue-grey antiserum reactions indicate the presence of another antigen, probably a polysaccharide complex. Further work is needed to test the correctness of this interpretation and to elucidate the antigens involved. However, it can be seen that among strains containing different variants heterogeneity in agglutination reactions would be found.

During pathogenicity trials, and in the homologous hyperimmune serum titres it was noted that the blue-grey variant exhibited the greatest antigenicity. These observations and its low pathogenicity led to the use of the blue-grey variant in vaccination trials on sheep. These experiments are being conducted at the present time.

The blue-grey antigen, when prepared with phenol-saline and the organisms killed at 55 C, exhibited the least specificity of any variant (see Table VI). Because of this low specificity the blue-grey antigen shows the greatest promise for use as a good diagnostic antigen.

The primary objectives of this investigation were to find a variant with the proper characteristics for use as a vaccine and a diagnostic antigen. These objectives have been partially realized in the blue-grey variant.

No phase designation has been assigned to the variants found. Hadley (1937), in a review of dissociation, has shown that variants and the characteristics associated with them differ between species, and possibly even within a species. The variants found in the hemorrhagic septicemia *Pasteurella* are designated as rough, smooth and mucoid. When the criteria used for these phases are applied to the variants of *Pasteurella mastitidis* the iridescent group corresponds to the smooth phase, the blue-grey to the rough and the white opaque to the mucoid. The dissociation of the iridescent to the blue-grey or S--- R as found in this study tends to bear out this classification. However, the appearance of the blue-grey colony is not of the rough type. Actually, only the brittle variants appear rough.

Until a more complete investigation of these variants can be made the author does not feel justified in classifying these variants into the classic types.

CONCLUSIONS

1. *Pasteurella mastitidis*, the causative organism of mastitis in sheep was found to be highly variable.
2. Dissociation occurred in broth cultures, and from them 6 variants were isolated. They were classified according to their colony characteristics, and designated: iridescent, blue iridescent, white opaque, wrinkled, blue-grey, and blue. They fall into three groups, the variants in each group having

similar characteristics. The groups are: 1, iridescent and blue iridescent; 2, white opaque and wrinkled; 3, blue-grey and blue. The growth characteristics of these variants were determined on various media.

3. No toxin could be demonstrated, but the iridescent and white opaque groups produced a small amount of hemolysis on blood agar. Carbohydrate utilization was weak and somewhat variable. No significant difference between variants was noted. No gas was produced.

4. Cross agglutination reactions showed considerable group specificity, and a possible antigen complex was postulated to account for this. The blue-grey variant exhibited a high antigenicity.

5. The capsule of the encapsulated variants was found to be highly labile. It was destroyed in less than 5 minutes at 55 C by phenol, and by growth in acid media.

6. The white opaque and iridescent groups were pathogenic for sheep and mice. Both groups were almost equally pathogenic. The blue-grey group had a low pathogenicity for mice, and almost none for sheep.

7. The blue-grey variant, because of its low pathogenicity and high antigenicity, is considered the best possibility for the production of a vaccine. An antigen of the blue-grey variant, prepared by a phenol-saline suspension of the organisms at 55 C for 1 hour showed low specificity in agglutination reactions. It is believed that this antigen may be useful for a diagnostic test antigen.

LITERATURE CITED AND CONSULTED

- Anderson, L.A.P., Coombes, M.G., and Mallick, S.M.K.,
1929-30. ON THE DISSOCIATION OF BACILLUS AVISEPTICUS,
Part I.
Indian Jour. Med. Res., 17, 611-624.
- Anderson, L.A.P., Coombes, M.G., Mallick, S.M.K., and
Martin, C. de C., 1929-30, ON THE DISSOCIATION OF
BACILLUS AVISEPTICUS, Part II.
Indian Jour. Med. Res., 17, 625-639.
- Bergey, D.H., Breed, R.S., Murray, E.G., and Hitchins,
A.P., 1939, MANUAL OF DETERMINATIVE BACTERIOLOGY.
1032 pp. Williams and Wilkins Co., Baltimore, 5th ed.
- Braun, W., 1945, FACTORS CONTROLLING BACTERIAL DISSOCIATION.
Science, 101, 182-183.
- Brigham, G.D., and Rettger, L.F., 1935, A SYSTEMATIC STUDY
OF THE PASTURELLA GENUS AND CERTAIN CLOSELY RELATED
ORGANISMS.
J. Inf. Dis., 56, 225-237.
- Cornelius, J.T., 1930-31, SOME OBSERVATIONS ON BACTERIAL
VARIATION IN A STRAIN OF P. SUISEPTICA.
Indian Jour. Med. Res., 18, 1167-1176.
- Dammann and Freese, 1907, EINE DURCH EIN STÄBCHENBAKTERIUM
HERVORGERUFENE SEUCHENARTIGE EUTERENTZÜNDUNG DER SCHAFE.
Deut. Tierarztl. Wchnschr., 15, 165-170.

- De Kruif, P., 1922, MUTATION OF THE BACILLUS OF RABBIT SEPTICEMIA.
J. Exp. Med., 35, 561-573.
- De Kruif, P., 1922, VIRULENCE AND MUTATION OF THE BACILLUS OF RABBIT SEPTICEMIA.
J. Exp. Med., 35, 621-633.
- Hadley, P., 1937, FURTHER ADVANCES IN THE STUDY OF MICROBIC DISSOCIATION.
J. Inf. Dis., 60, 129-192.
- Haupt, H., 1932, ZUR KENNNTINS DER ERREGER ZWEIER ENZOOTISCH AUFTRETENDER EUTERENTZUNDUNGEN DER SCHAFE DES MICROCOCCUS OVIS MIGULA UND DES BACTERIUM OVINUM N.SP.
Cent. f. Bakt., I abt., orig., 123, 365-376.
- Hauduroy et all., 1937, DICTIONNAIRE DES BACTERIES PATHOGENES POUR L'HOMME, LES ANIMAUX ET LES PLANTES. P. 316.
- Hughes, T.B., 1930, THE EPIDEMIOLOGY OF FOWL CHOLERA.
II. BIOLOGICAL PROPERTIES OF P. AVICIDA.
J. Exp. Med., 51, 225-238.
- Huntoon, F.M., 1918, "HORMONE" MEDIUM. A SIMPLE MEDIUM EMPLOYABLE AS A SUBSTITUTE FOR SERUM MEDIUM.
J. Inf. Dis., 23, 169-172.
- Jasmin, A.M., 1945, AN IMPROVED STAINING METHOD FOR DEMONSTRATING BACTERIAL CAPSULES, WITH PARTICULAR REFERENCE TO PASTEURELLA.
J. Bact., 50, 361-363.
- Manninger, 1919, UEBER EINE MUTATION DES GEFLUGEL-CHOLERA-BAZILLUS.
Cent. f. Bakt., I abt., orig., 83, 520-528.

Marsh, H., 1932, MASTITIS IN EWES, CAUSED BY INFECTION WITH A PASTEURELLA.

J. A. V. M. A., 34, 376-382.

Meissner, H. and Schoop, G., 1932, MASTITIS INFECTIOSA OVIS.

Deut. Tierarztz. Wehnschr., 40, 69-75.

Mörch, J.R. and Krugh-Lund, G., 1931, UNTERSUCHUNGEN UBER BAKTERIEN DER PASTEURELLAGRUPPE.

Z. f. Hyg. u. Infektionskr., 112, 471-491.

Ochi, Y., 1931, STUDIES ON HEMORRHAGIC SEPTICEMIA ORGANISMS, ESPECIALLY ON THEIR VARIABILITY. REPORT I. STUDY ON SO-CALLED FOWL CHOLERA ORGANISMS. (ABSTRACT).

J. Jap. Soc. Vet. Sci., 10, 348-349.

Ochi, Y., 1931, STUDIES ON HEMORRHAGIC SEPTICEMIA ORGANISMS, ESPECIALLY ON THEIR VARIABILITY. REPORT II. STUDIES ON HEMORRHAGIC SEPTICEMIA ORGANISMS ISOLATED FROM SO-CALLED SWINE PLAGUE. (ABSTRACT).

J. Jap. Soc. Vet. Sci., 10, 361-362.

Ochi, Y., 1931, STUDIES ON HEMORRHAGIC SEPTICEMIA ORGANISMS, ESPECIALLY ON THEIR VARIABILITY. REPORT III. STUDIES ON HEMORRHAGIC SEPTICEMIA ORGANISMS ISOLATED FROM CATTLE. (ABSTRACT).

J. Jap. Soc. Vet. Sci., 10, 366.

Ochi, Y., 1933, STUDIES ON HEMORRHAGIC SEPTICEMIA ORGANISMS, ESPECIALLY ON THEIR VARIABILITY. REPORT IV. STUDIES ON HEMORRHAGIC SEPTICEMIA ORGANISMS ISOLATED FROM SHEEP. (ABSTRACT).

J. Jap. Soc. Vet. Sci., 12, 51-52.

Ochi, Y., 1933, STUDIES ON HEMORRHAGIC SEPTICEMIA ORGANISMS, REPORT V. CORRELATIONS BETWEEN HEMORRHAGIC SEPTICEMIA ORGANISMS ISOLATED FROM VARIOUS ANIMALS. (ABSTRACT)

J. Jap. Soc. Vet. Sci., 12, 198-199.

Priestly, F.W., 1936, A NOTE ON THE ASSOCIATION OF THE VIRULENCE OF PASTEURELLA SEPTICA WITH CAPSULATION.

J. Comp. Path. & Therap., 49, 348-349.

Priestly, F.W., 1936, SOME PROPERTIES OF THE CAPSULE OF PASTEURELLA SEPTICA.

Brit. J. Exp. Path., 17, 374-378.

Rosenbusch, C. and Merchant, I.A., 1939, A STUDY OF THE HEMORRHAGIC SEPTICEMIA PASTEURELLAE.

J. Bact., 37, 69-89.

Webster, L.T. & Burn, C.G., 1926, BIOLOGY OF BACTERIUM LEPISEPTICUM. III. PHYSICAL, CULTURAL AND GROWTH CHARACTERISTICS OF DIFFUSE AND MUCOID TYPES AND THEIR VARIANTS.

J. Exp. Med., 44, 343-358.

Webster, L.T. & Burn, C.G., 1926, BIOLOGY OF BACTERIUM LEPISEPTICUM. IV. VIRULENCE OF DIFFUSE AND MUCOID TYPES AND THEIR VARIANTS.

J. Exp. Med., 44, 359-386.

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