The dissociation of Pasteurella mastitidis
by Peter H Matisheck

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

Abstract:
Because of its variability, Pasteurella nastitidis. 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 nice, 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*

by

PETER H. MATISHECK

A THESIS

Submitted to the Graduate Committee

in

partial fulfillment of the requirements

for the degree of

Master of Science in Bacteriology

at

Montana State College

Approved:

Hadleigh Marsh
In Charge of Major Work

W G Walter
Chairman, Examining Committee

J. A. Nelson
Chairman, Graduate Committee

Bozeman, Montana
June, 1947

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

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.

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 lepisepticum, 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. lepisep-tica. 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