**MONTANA STATE UNIVERSITY**

The effects of overmilking on the mammary gland and the incidence of mastitis
by Neil C Quesenberry

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Dairy Production
Montana State University
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Abstract:
Twelve lactating cows, nine Holstein-Friesians, and three Jerseys, were utilized for this project from January 5, through March 1, 1962 (eight weeks). They were assigned to three groups of four animals each, with Group I being the control group, and Groups II and III being overmilked 50 and 100 per cent respectively. There was no hand stripping.

The milk from each quarter was scored daily using the California Mastitis Test. Each udder was palpated four times daily, and the milk from each animal was weighed at every milking.

No clinical mastitis crises developed. California Mastitis Test scores on the milk produced by the 100 per cent overmilked group showed the greatest number of positive reactions-—145, while the milk from the 50 per cent overmilked group showed the least--55. These differences were not significant (P> 0.05).

The average rate decline in milk production for the test period was 10.5 per cent for the 100 per cent overmilked group, followed by 7.0 per cent for the 50 per cent overmilked group. The control group had the smallest average (5.0 per cent) decline in milk production. Neither were these differences significant.

There were no frozen teats during the experiment period in the control group. There were two animals in the 50 per cent overmilked group and one in the 100 per cent overmilked group that had all four teat ends frozen 38 days before the trial ended. Mastitis did not result from any of these frozen teat ends, nor was there a marked change in C.M.T. scores.

Upon close visual examination and palpation, it was noted that the ends of the teats of those animals in the overmilked groups had become quite hard and calloused. However, pinpoint breakage and hemorrhaging were not evident.

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AND THE INCIDENCE OF MASTITIS

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## ABSTRACT

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# INTRODUCTION

Bovine mastitis is a very complex disease. Numerous non-infectious factors often pave the way for bacterial infection of the mammary gland by any one of several organisms. Mastitis is characterized by periodic acute attacks manifested by inflammation of one or more of the glands of the udder and by changes in the physical as well as the chemical properties of the milk.

In terms of economic loss, mastitis is probably the most significant disease with which the dairy industry is faced today. In 1961, the estimated loss caused by this disease in the United States was over 500 million dollars. This loss takes into account the death of an occasional animal, milk loss, the loss of feed fed to non-productive cows, and the cost of the antibiotics and extra labor involved in combating the disease. The estimated loss in 1930 was 72 million dollars.

Between 1930 and 1961, World War II and a post-war recovery period took place making it difficult to obtain farm labor. During this period, the elevated parlor and the pipeline system of milking gradually replaced the old conventional barn with the bucket milkers, because they made milking easier and a more attractive occupation.

The pipeline system was designed to remove, usually elevate, and transport the milk from the udder to a bulk tank often located some distance from the cow. Many workers have demonstrated that these systems when not properly designed and operated, place undue stress on the teats and udders of the dairy cows, and as a result injure the tissues and create conditions favorable to the development of mastitis.

As a means of getting maximum efficiency from labor, milking machine

operators are often required to operate so many machines, that it is physically impossible for them to remove all the machines from the udders at the precise instant milk flow ceases. Many people feel that failure to remove these machines when milk flow ceases allows the vacuum to enter the teats and gland cisterns and results in damage to the tissue. Damaged tissue makes conditions favorable for bacterial infections and mastitis.

The purpose of this study was to determine the effects of leaving a properly designed and operated milking machine attached to the udder after milk flow was complete.

# REVIEW OF LITERATURE

## DEFINITION OF MASTITIS

Mastitis is defined as inflammation of the udder. The term is derived from the Greek word *mastos*, which means mammary gland. Mastitis may occur in any mammalian species, but is of the greatest importance in dairy cows. (3). It is a disease complex in which bacterial infections, and trauma or stress produced by faulty equipment or poor managerial practices, play important roles.

## THE UDDER

Morphologically, the udder is a cutaneous gland, located in the inguinal region, which functions in harmony with the reproductive system. It has four quarters, two on either side of the median plane, which form a half of the udder. Each gland is anatomically separate and drains through a separate duct system into its own gland and teat cisterns (67). The teats are generally quite elastic, and covered with a skin which is quite thin but very tough and durable.

The mammary glands develop from the ectoderm as a single-layered sheet of cuboidal cells overlying the mesenchyme tissue. It can be identified in the embryo at approximately three to four weeks. At birth, the teats are well-developed, and the general contour of the gland is visible even though the majority of the udder consists of fat and connective tissue (26, 67). The udder development keeps pace with the normal growth of the animal, but consists largely of fat until puberty.

From the onset of puberty until pregnancy, the udder continues to grow, but varies in size synchronously with the estrous cycle. During estrus, the udder increases in size, but decreases again about six days

after estrus is complete. These changes are due to the varying levels of the hormones estrogen and progesterone in the blood stream (64).

After the fourth or fifth month of pregnancy, the udder begins to enlarge due to the formation of the milk secreting tissue known as alveoli. This growth is brought about through estrogen-progestogen synergism. As pregnancy progresses, and parturition is approached, the hormonal secretions are sharply increased and alveolar growth is accelerated. Such activity is reflected by a tremendous increase in the size of the udder just prior to parturition. Following parturition as a heifer, the udder continues to grow until the animal reaches maturity (five to seven years of age) (26).

Modern-day cows produce up to 25,000 to 30,000 pounds of milk per year, or some 90 to 100 pounds per day. Little and Plastridge (26) state that to produce 100 pounds of milk per day requires some 30,000 to 40,000 pounds of blood to be circulated through the udder each 24 hours. Swett et al (66) have shown that the empty weight of an udder may be as much as 165 pounds, or up to 10 per cent of the weight of the cow, and have a holding capacity of 170 pounds of milk. Thus, a full udder may weigh as much as 335 pounds.

At the beginning of each lactation, then, this organ often becomes extremely large and subject to injury, a predisposing factor to mastitis. High-producing cows are generally recognized as being more subject to mastitis than low producers.

## ANATOMY OF THE UDDER

The anatomy of the udder may be broken down as follows: the teat

meatus, teat canal and its supporting structures, teat cistern, annular fold, gland cistern, gland duct system, and alveoli.

At the point of entrance to the teat, there is a small funnel-shaped depression which leads to the opening of the teat proper. This opening is known as the external meatus, or orifice, of the teat (67) (See Figure 1). Just above the teat meatus is a structure known as the teat canal or streak canal (15, 67). This canal, with its supporting structures, is designed to retain the milk in the udder against the pressure developed in the gland during the interval between milkings (67). The teat canal, beginning with the teat meatus, is lined with many layers of epithelial cells. This tissue is more commonly referred to as keratin tissue, and is not easily damaged even though it is quite soft. Surrounding the teat canal is the sphincter muscle which regulates the diameter of the teat canal (64). If this muscle is thick-walled, the canal will be small and the animal hard to milk (31).

Above the teat canal, the cavity of the teat widens out to form the teat cistern. At the point where the enlargement begins, there are a number of small folds which radiate in all directions. This structure is called Furstenberg's Rosette (67). These rosettes vary in the number of folds and wrinkles they contain, from two to eight. They tend to fold over the teat canal and assist it and the sphincter muscle in retaining the milk in the udder (64, 67). The teat cistern is located above Furstenberg's Rosette and is the area where the milk collects inside the teat (69). This area has a number of longitudinal and oblique muscles which partly overlap each other, and as such, constitute a many-folded formation. These two
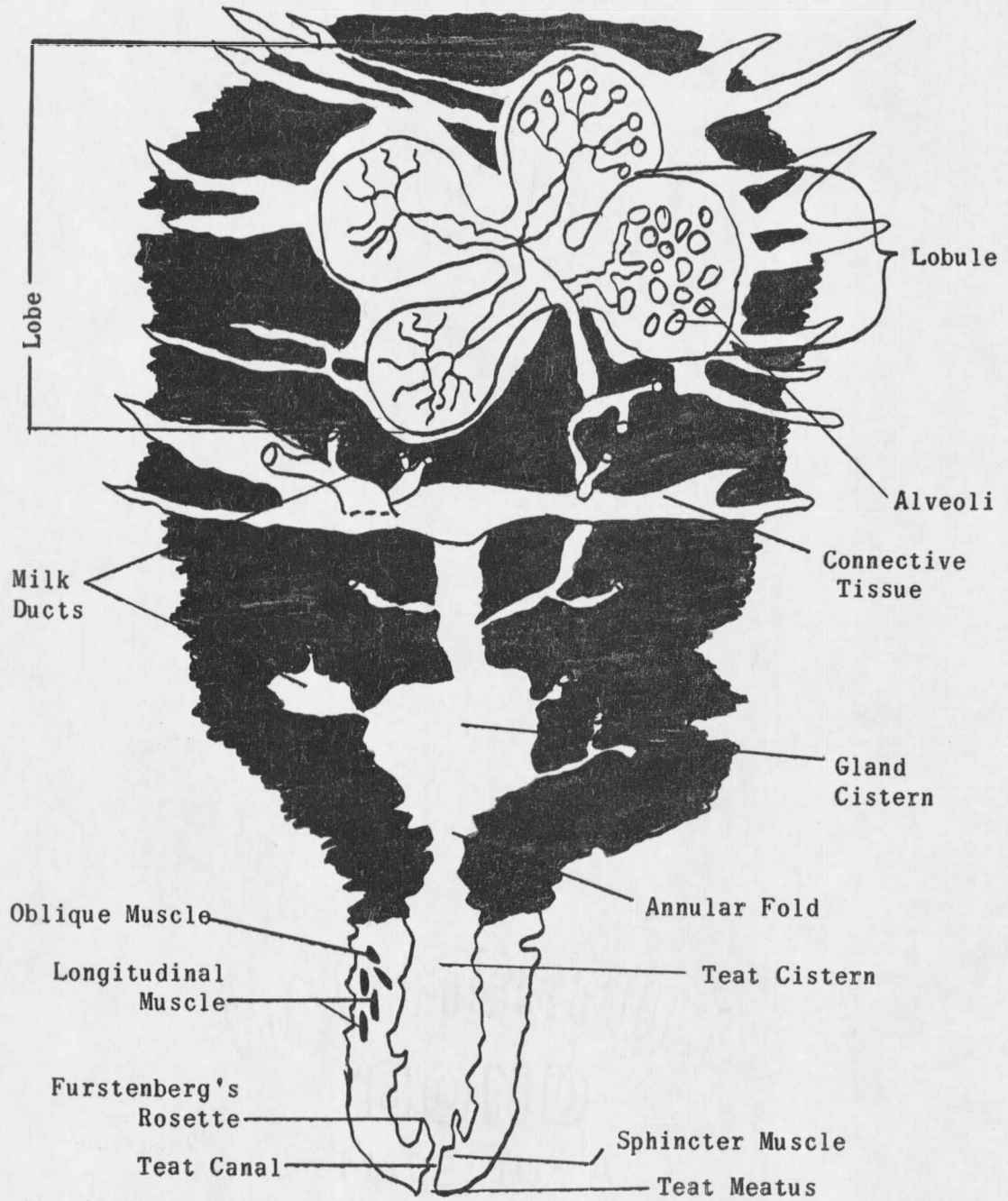
Figure 1. Sagittal Section of a Bovine Milk Secreting Gland
Showing Major Structures.

types of muscles within the wall of the teat give the teat cistern lining an increased firmness (64).

The upper end of the teat cistern opens dorsally into the gland cistern (67). The teat cistern is sharply distinguished from the gland cistern by a constriction in the form of an annular (cricoid) fold, 2 to 6 mm. in thickness, which has a central opening of connective tissue (67). This opening varies in size and may restrict the flow of milk into the teat cistern if it is too small. This may place undue stress on the teat due to insufficient milk in the teat cistern. The function of the gland cistern is to accommodate the milk as it is being secreted and store it during the interval between milkings (67).

Above the gland cistern there are usually eight to 12, or more, large milk ducts which open into the gland cistern. Each of these ducts branch and rebranch many times. The final branches end in alveoli, which contain the active milk secreting cells. A group of alveoli form a lobule which is surrounded by distinct connective tissue. A group of lobules are united by a broad connective tissue into a lobe (67).

## THE UDDER AND MASTITIS

The teat meatus, the point of entrance to the udder from the outside, has been observed to erode or flake off (15). High vacuum has been thought to cause this condition (15). More recently, Udall (68) has shown additional changes in the form of a very prominent meatus which is caused by malfunctioning or badly-worn milking equipment. Both conditions indicate abnormal stress and may be predisposing factors to mastitis.

It has been demonstrated many times that the teat canal, located

immediately above the meatus, plays a significant role in preventing foreign matter and bacteria from entering the udder. (1, 31, 34, 35, 37, 38, 39, 46).

Murphy and Stuart (37) discovered that a highly effective method of challenging the teat canal was to determine its infectibility with Strepto-coccus agalactiae using a swab technique. Their results show that the teat canal is a barrier which can and does prevent organisms from invading the gland. Murphy (35) further demonstrated that when a portion of the keratin lining was removed from the teat canal by reaming with a soft plastic canula, the resistance of the teat canal was broken and mastitis followed as a natu-ral occurrence in all cases.

Murphy and Stuart (38) have shown that the teat canal's susceptibility to infection by Str. agalactiae when challenged by means of a swab tech-nique did not appear to be related to its length.

In another study (39) the relationship of maximum rate of milk flow and infectibility by the Str. agalactiae was determined. When each quar-ter was exposed to the test organism from 2 to 12 times, infection oc-curred in only 14.0% of the exposures, and showed no general relationship to the maximum rate of milk flow. In addition, passage of cotton swabs once daily for 5 consecutive days and the subjection of the same animals to 10 minutes of overmilking at a high (17 inches) vacuum, after a normal milking at 13 inches of vacuum, produced no clinical mastitis, gross changes in the teat canal, or histological changes.

Though the milking machine may predispose the udder to mastitis through traumatic injury to the structures in the teat, little has been done to show that the milking machine injures the tissue inside the mammary

gland. Pier et al (44) have demonstrated, however, through the use of a
normal udder which was removed intact from a cow after slaughter, that
vacuum not only enters the teat cistern after the milk has been removed
from the gland, but that it extends on through the gland cistern and into
the large ducts throughout the udder. They also showed that a teat cup
under a vacuum of 12 inches when attached to the human arm for a period of
45 seconds, resulted in a circular area of redness punctuated with pin-
point hemorrhages. Massaging the area with a finger caused the redness
to disappear, but the pinpoint hemorrhages remained, some for two days.
They concluded that, "A similar effect might be anticipated on the inter-
nal tissues at the base of the teat when the machine is left in operation
after milk flow has ceased." Murphy (34), however, believes that the teat
canal is where traumatic injury occurs and the stage is set for mastitis.
In 1957, he stated, "Unfortunately, it is still true that no one, anywhere,
as yet knows how an udder infection takes place in nature."

## THE INITIATION AND MAINTENANCE OF MILK SECRETION

It is desirable to be familiar with the physiology of a normal udder if
we are to understand how overmilking with a poorly designed or malfunctioning
milking system may damage the udder tissue.

Research has shown that hormones produced by the endocrine glands play
a more important role in the removal of milk from the udder with a milking
machine (40) than when hand milked.

Since the purpose of the mammary gland is to provide nourishment for the
newborn calf, there must be mechanisms which initiate lactation at the termi-
nation of pregnancy. Oxytocin is considered by some authorities to be the

initiator of lactation, but these mechanisms are not fully understood (64).

Oxytocin, presumably liberated during parturition, not only initiates lactation, but also is responsible for the expulsion of milk, by activating the mechanism that expels milk from the alveoli (64).

It is well-known that, in the cow, the amount of milk present in the cisterns and larger ducts when milking begins is only a small fraction of the total quantity which can ultimately be collected at any given milking (64). The stimulation of the teat, after a short interval, produces a sudden rise in milk pressure in the udder; and only after this has occurred, can the full milk yield be obtained. Such a rise of pressure in the ducts also occurs in women in response to the stimulus of suckling (25). Medical research has shown that the discharge of milk from the breast depends not only on the suction exerted by the infant, but also on a contractile mechanism in the breast which expresses milk from the alveoli into the ducts (25).

The sequence of events that causes milk let-down by the hormone oxytocin, is as follows (64, 67). Milk let-down begins with the stimulation of the teat or nipple. This stimulation causes the release of oxytocin into the blood stream. The oxytocin is then carried via the blood stream to the mammary gland, or breast, where it produces contraction of the myoepithelium surrounding the alveoli. This causes the alveoli to expel their milk into the milk ducts. The milk ducts remain open by the contraction of their longitudinal myoepithelial layers. For these reasons, it is believed that milk let-down is a neurohumoral mechanism. Thus, nervous and psychological factors, can definitely influence lactation (64). It has

been concluded by the University of California mastitis workers (40) that since milk let-down is a neurohumoral mechanism, cows must be properly stimulated prior to attaching the milking machine to the udder. Failure to do so will result in the vacuum entering the teats and udder proper as milk is not yet present in the gland. This may damage the structural and secretory tissue therein and may result in clinical mastitis.

## PATHOGENESIS OF MASTITIS

Infection of the mammary gland always occurs via the teat canal. The development of inflammation can be explained in terms of three stages: invasion, infection, and inflammation (3). Invasion is the passing of the organism from the exterior to the interior of the teat via the teat canal. Infection is the multiplication of the organisms and the invasion of the udder tissue which results in inflammation and clinical mastitis.

Blood and Henderson (3) in 1960 reported that 99 per cent of all mastitis cases are due to <u>Streptococcus</u>, <u>Staphylococcus</u>, and <u>Bacillus</u>. Therefore, only these types of organisms will be discussed.

<u>Streptococcic Mastitis</u>--Infection of the cow with <u>Str. agalactiae</u> depends on the number of organisms and the resistance of each individual animal, the nature of which is not fully understood (34). Under certain circumstances, the organism multiplies rapidly within the milk ducts. The essential mechanism of streptococcic invasion is as follows: 1. There is a sudden multiplication of organisms within the milk ducts measured by a sharp rise in the bacterial count of the milk. 2. The organisms pass through the duct walls into the adjacent lymphatic vessels. This stimulates an immediate defensive outpouring of neutrophils to the milk ducts,

and is measured by a sharp rise in the neutrophil content of the blood. Often blood samples are drawn on acute mastitis cases as a diagnostic procedure (14, 33). 3. The animal's temperature rises rapidly, and there are the usual signs of fever. 4. Bacteriological culture of the supramammary lymph node at the time of invasion is frequently positive, in contrast to negative culture at all other times.

In most cases, tissue damage in streptococcic mastitis is patchy: one area may be unaffected, while an adjacent area is rendered completely void of functioning tissue (48).

Str. agalactiae is dependent upon the udder for its survival; and once it is introduced into a herd, the incidence of clinical mastitis appears to rise and fall according to the average age of the herd (33). These organisms have been isolated from the outside of the teats, udder or other parts of the cow's skin, as well as from the milker's hands, clothes, milking utensils, barn floors and loafing sheds (11). However, they apparently do not survive for long periods of time outside the udder. Schalm (50) has demonstrated that Str. agalactiae can be eradicated from herds by employing sanitary milking practices, strict segregation of all shedder cows, and the application of intramammary therapy to the infected glands. However, Australian researchers (29) feel that there is considerable doubt as to the practicability of eliminating the Str. agalactiae from the average Australian herd. A number of other countries have been quite successful, however (32).

Blood and Henderson (3) feel that if the following procedure is adhered to, approximately 80 per cent of the herds involved can be rid of

this organism within a one-year period:  first, the treatment of all quarters of all cows in all herds with intramammary infusions of penicillin after five successive evening milkings; second, the milkers' hands and the cows' teats must be disinfected after milking each cow; third, the thorough washing and cleaning of every udder in a disinfectant at each milking prior to attaching the machine to the udder; fourth, sterilized udder cloths and sterilized clothing for the milkers must be used on the last day the cows are under intramammary therapy.

D. G. Howell (20) conducted an experiment on the continuation of infection with Str. agalactiae through the dry period in the cow, and the results indicated that this organism can persist in the udder through a dry period of normal duration.

Howell, Pattison, Holman, and Smith (21) noticed that certain cows possessed some immunity to Str. agalactiae, and considered that such resistance should be the subject of further investigation.  Several researchers attempted to develop a vaccination program for the control of mastitis due to Str. agalactiae (4, 21, 22, 23).  However, these workers concluded that the measurable protection given by vaccine was too small to play a significant role in controlling mastitis due to Str. agalactiae under practical field conditions.

As early as 1953, Schalm and Woods (58) reported that with widespread eradication programs leveled at the Str. agalactiae, mastitis as a result of this organism had become rather insignificant.

Staphylococcic Mastitis--Schalm and Woods (59) in 1953, concluded that over 50 per cent of all clinical mastitis cases were due to the

<u>Staphylococcus aureus</u>. Schalm (58) believes that the eradication of <u>Str.</u> <u>agalactiae</u> through the use of antibiotics has upset the normal udder flora and permitted <u>Staph. aureus</u> to become the predominant organism associated with mastitis. Resistance of the <u>Staph. aureus</u> to antibiotics, particularly penicillin, has been reported (56). The incidence of infection by this organism increases with the age of the animal in much the same manner as infections due to <u>Str. agalactiae</u> (56). Those animals in a herd that resist treatment remain as a source of infection for the remainder of the animals (60). It has also been reported that <u>Staph. aureus</u> from apparently healthy udders may, upon injection into another normal udder, be responsible for acute mastitis (48).

<u>Staphylococcus aureus</u> is often responsible for both gangrenous as well as mild mastitis (48). It is a toxin producer, sometimes to a marked degree; and many strains excrete enzymes that add to their pathogenicity (47, 48). This organism is not dependent upon the udder for survival, and is commonly present on the skin and mucous membranes, especially of the nose and mouth.

Pattison (43) found that the first stage of the <u>Staph. aureus</u> invasion was very nearly the same as that of the <u>Str. agalactiae</u> invasion, namely multiplication of the organism within the milk ducts and invasion through the duct wall.

However, the second stage in <u>Staph. aureus</u> invasion differs from <u>Str.</u> <u>agalactiae</u> in that after invasion through the duct wall, it establishes itself in the udder tissue in one massive initial invasion. In severe cases of clinical mastitis due to the <u>Staph. aureus</u>, the pathological changes are

much more marked than in mastitis resulting from <u>Str</u>. <u>agalactiae</u>. <u>Staph</u>. <u>aureus</u> can live and multiply inside the udder tissue, and thus establish themselves in numerous areas of the udder where they cause extensive damage.

The progress of the disease from the acute stage will depend on the amount of udder tissue involved and the success of antibiotic therapy. If the infection is widespread, large areas of the gland may be destroyed.

Several researchers have studied the types of toxins produced by many strains of <u>Staph</u>. <u>aureus</u> isolated from the udders of cows with mastitis (61, 63). These studies concluded that 91.7 per cent of all cultures investigated produced alpha and beta toxin, 6.7 per cent formed only beta toxin, and 1.6 per cent formed only alpha toxin.

Since the <u>Staphylococcus</u> is a toxin producer, much work has been done to develop a vaccine to use in combating this organism (5, 6, 7, 8, 13, 52, 59, 60, 62, 65).

Vaccination programs using staphylococcic toxoid-bacterins, have shown some promise in reducing clinical mastitis (7). The eradication of the <u>Staph</u>. <u>aureus</u>, however, is proving to be a very difficult problem (52, 53). Schalm and Lasmanis (52), after working 12 years using vigorous control measures aimed at eradicating the <u>Staph</u>. <u>aureus</u> from one herd, were unsuccessful.

<u>Bacillic Mastitis</u>--The coliform organisms appear to be the group most involved in mastitis due to the genus <u>Bacillus</u>. Dairy cows are surrounded by these organisms. Mastitis due to <u>Escherischia</u> <u>coli</u> is not rare, but is insignificant in comparison to mastitis due to <u>Staph</u>. <u>aureus</u>. Coliform mastitis is on the increase, however, in many of the dairy herds that have

had eradication programs directed toward the Str. agalactiae and the Staph. aureus. Schalm and Woods (57) classified the infections that resulted from E. coli as latent, chronic, acute local, and acute systemic. The acute systemic form was found to be accompanied by a rise in body temperature from 103 to 108°F., and to show slight to very marked symptoms of toxemia. In many cases, if immediate action is not taken, the cow dies in a matter of hours.

Aerobacter aerogenes (9, 36), another organism of this genus, is present in the feed and bedding of the cow, and is also responsible for acute and peracute mastitis even though it usually is not considered an animal pathogen.

Derbyshire (13) indicates that there appears to be little hope of success for the development of a vaccine to combat these infections.

Schalm and Woods (60) emphasize the dangers of improper therapy and indiscriminate use of antibiotics which is resulting in the shift in dominance from the Str. agalactiae to the Staph. aureus and finally to the coliform organisms.

## DIAGNOSIS OF MASTITIS

There are various methods used in diagnosing mastitis, but it appears that a combination of several diagnostic methods is most accurate. These various methods are divided into physical, chemical, microscopic, cultural, and the rate of milk decline. Some require very little equipment and technical know-how, while others are necessarily used only in the laboratory.

The methods required to make a diagnosis vary with different cases. Acute and peracute cases can be readily diagnosed by simple methods, but

chronic cases require laboratory procedure.

Physical Methods--For many years people concerned with the diagnosis of mastitis have used udder palpation as a means of detecting acute mastitis (16, 18, 26).

Detailed examination of the udder by palpation should begin at the teat meatus and be carefully extended to the entire gland (26). Any abnormalities such as abrasions, abnormally weak teat sphincters, and any increase in connective tissue in any portion of the teat should be recorded. Superficial, followed by deep palpation of the gland cisterns and tissue, should be practiced. As a general rule, chronic mastitis is a progressive, ascending infection; thus connective tissue increases begin in the gland cistern and gradually ascend as the infection continues upward. Careful palpation of the infected udder after each milking will give information as to the duration of the disease and the extent of the process (26).

Bacteriologists consider the physical examination of the udder a basis for the rejection of cows in a public health program where the principal aim is to prevent abnormal milk from entering the milk supply (26). These workers conclude, however, that the control of the chronic mastitis, without the aid of a cultural examination of the milk, would fail.

Chemical Methods--Chemical tests for mastitis either detect the presence of abnormal substances in the secretion or abnormal amounts of the normal components in the secretion (10). It appears that the degree of change is dependent upon the severity and extent of the infectious process (10).

Foremilk should always be used for such tests, as the first few milli-

meters of milk will usually, but not always, show the greatest change if the quarter is abnormal (53). The most popular chemical method in use today is the California Mastitis Test (C.M.T.). This test can be conducted at the side of the cow in a period of 30 seconds, without the presence of a skilled technician (53). Milk drawn from each teat into a paddle containing four receptacles is mixed with chemical reagent by a gentle circular motion of the paddles. The degree of precipitation, or gel formation, determines the degree of abnormalities (cell count) of the milk.

It can be used with the foremilk or with the strippings of the individual glands. It is also applicable to bucket milk, for the rapid screening of herds for mastitic cows, and to bulk milk as it is delivered to the creamery for the same purpose.

Other chemical tests include the Bromthymol blue, Bromcresol purple, and the Whiteside Test. The Bromthymol blue and the Bromcresol purple measure the pH of the milk, as milk from infected quarters is usually abnormally alkaline. The Whiteside Test, which was the basis for the development of the present C.M.T. test, is based on the agglomeration of cells in an alkaline solution (24). It has been discarded because both normal and abnormal milk tend to form a thick mass after a few minutes in contact with the chemical solution (51).

Repeated negative chemical tests at intervals of several days are worthwhile, not only in diagnosing mastitis, but also in determining the degree of irritation on individual quarters. Chemical tests, as such, are not direct evidence of the presence of inflammation, but can be of assistance if carefully interpreted. If these tests are positive and the