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
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Montana State University
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Abstract:
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respectively. There was no hand stripping.

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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
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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
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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

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.

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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.
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 bloodstream (64).

After the fourth or fifth month of pregnancy, the udder begins to en-
large 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 secre-
tions 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 *Streptococcus 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 natural 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 technique 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 quarter was exposed to the test organism from 2 to 12 times, infection occurred 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 pinpoint 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 internal 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 termination 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 *Streptococcus*, *Staphylococcus*, and *Bacillus*. Therefore, only these types of organisms will be discussed.

**Streptococcic Mastitis**—Infection of the cow with *Str. agalactiae* 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 \textit{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 \textit{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 \textit{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 \textit{Str. agalactiae} under practical field conditions.

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

\textbf{Staphylococcal Mastitis}--Schalm and Woods (59) in 1953, concluded that over 50 per cent of all clinical mastitis cases were due to the
Staphylococcus aureus. Schalm (56) believes that the eradication of *Str. agalactiae* through the use of antibiotics has upset the normal udder flora and permitted *Staph. aureus* to become the predominant organism associated with mastitis. Resistance of the *Staph. aureus* 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 *Str. agalactiae* (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 *Staph. aureus* from apparently healthy udders may, upon injection into another normal udder, be responsible for acute mastitis (48).

*Staphylococcus aureus* 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 *Staph. aureus* invasion was very nearly the same as that of the *Str. agalactiae* invasion, namely multiplication of the organism within the milk ducts and invasion through the duct wall.

However, the second stage in *Staph. aureus* invasion differs from *Str. agalactiae* 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 *Staph. aureus*, the pathological changes are
much more marked than in mastitis resulting from *Str. agalactiae*. *Staph. aureus* 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 *Staph. aureus* 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 *Staphylococcus* 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 *Staph. aureus*, 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 *Staph. aureus* from one herd, were unsuccessful.

**Bacillic Mastitis**—The coliform organisms appear to be the group most involved in mastitis due to the genus *Bacillus*. Dairy cows are surrounded by these organisms. Mastitis due to *Escherischia coli* is not rare, but is insignificant in comparison to mastitis due to *Staph. aureus*. 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
cow being tested is not in early or late lactation, the presence of inflam-
mation is very likely. However, a negative test does not always mean the
absence of inflammation from the quarter (10).

Microscopic Methods—These methods are useful as an aid in the diag-
nosis of mastitis, but require a microscope, a technician, and preferably
a laboratory (26).

The direct microscopic examination of milk will show (a) leukocyte con-
tent and (b) the types of bacteria present (26). Incubation of the sample
may be helpful in determining bacterial types, and does not interfere with
the determination of the leukocyte content. Leukocyte counts over 500,000
per ml. are considered abnormal, and counts over 1,500,000 per ml. indi-
cate that infection is present.

Cultural Methods—The cultural method is perhaps the most valuable
method of diagnosis when precise information as to numbers and types of
bacteria present is desired (10). However, cultural methods must be con-
ducted in a properly equipped laboratory, and results are not available
for several days after samples are taken.

The Hotis test, a cultural method, is described by Murphy (30) as
a simple method for detecting mastitis in milk as a result of Strepto-
cocci. The blood agar plate is another method for growing mastitis
organisms. It is not confined to Streptococcus agalactiae, and reveals
valuable characteristics of the organisms, especially their hemolytic
characteristics (26).

Where repeated cultural tests are conducted, they are nearly 100
per cent accurate. It has been concluded that the combination of physi-
cal, chemical, and cultural tests will give the most accurate diagnosis (10, 26). The test used will depend upon the facilities and equipment available, as well as the skill and amount of time which the diagnosti-
cian can devote to these procedures.

Rate of Milk Decline as a Detection Method—Schalm and Noorlander (54) have found that a graph of a cow's production is actually a picture of her performance, and often proves to be useful as an aid in diagnosing mastitis. Milk losses due to mastitis as a result of malfunctioning milking equipment, poor milking practices, and even underfeeding, become very obvious when production graphs are maintained on all cows in a herd (54).

Correlating the C.M.T. tests with standard production graphs increases their effectiveness, and the examination of a large number of graphs of normal cows reveals certain typical findings (54). Normal curves of mature cows show a sharp rise after parturition up to approximately 50 days post-parturition. Following this period, there is a gradual decline in milk production at a fairly constant rate until the cow is dried up. Such a pattern is shown in Figure 2. With normal healthy udders, the highest point of production is greater with each succeeding lactation until the fifth or sixth lactation.

In commercial herds with a high number of C.M.T. positive cows, the pattern as shown in Figure 2 is rarely found (54). A sharp production drop anywhere along the line, when accompanied by a positive C.M.T. reaction of the milk, indicates lost production due to the inflammatory reaction within the udder. (See Figure 3). It must be kept in mind that it is not necessary for a cow to have clinical mastitis to have an abnormal
Figure 2. A Normal Milk Production Curve With All Quarters Reacting Negatively to Each Monthly C.M.T. Test For Ten-Month Lactation.
Figure 3. Lost Milk Production Expressed in Pounds, Due to Inflammation Beginning During Third Month of Lactation.
decline in milk production (54). Any irritations in the udder which cause a rise in leukocytes in the milk in numbers sufficient to give a positive C.M.T. test will result in a drop in milk production. The decline in milk secretion may precede the first positive C.M.T. test if the test is applied to mixed samples from the entire udder of one animal. However, when the C.M.T. test is used on the foremilk of individual quarters of such cows, one or more positive reacting quarters may be found (54).

The C.M.T. test correlated with standard production graphs, is a valuable procedure to show reasons for lost production. Conditions contributing to these losses can be detected and corrected before permanent damage has been done to the teats and udder (54).

MASTITIS CONTROL PROGRAMS

As late as 1958 (48) mastitis was considered the most prevalent and difficult of all dairy cattle diseases to control. During the period from 1945 to 1955, many states initiated mastitis control programs (41) based on the factors listed by Plastridge and Hale (45) and Hodges (19). These factors included: (1) the periodic testing of individual milk samples, (2) systematic treatment of infected quarters, and (3) a good herd management program. Such a management program included (a) disposal or segregation of cows that did not respond to treatment, (b) dipping of teats in an antiseptic solution after each milking, (c) protection of animals from physical injuries of the udders and teats and (d) mastitis-free replacements.

Schalm and Lasmanis (52) reported, however, in 1957, that mastitis control programs had not been completely successful because in the eradication of Streptococcus agalactiae, two extremely pathogenic organisms,
namely, *Staphylococcus aureus* and *Escherichia coli* were becoming prevalent in many herds. As pointed out earlier, these organisms are more difficult to control and produce more extensive damage to the udder tissue than the *Str. agalactiae*.

During this same period (1945-1955), very little attention was given to the mechanics of the pipeline milking systems (41). In 1958, Noorlander and Schalm (41) theorized that there was a need for a method of measuring the physical forces and differential pressures in the mechanical milking systems. Their theory was based on the fact that whereas all that is needed for a milking machine to function is a vacuum at the teat end (40), a constant vacuum at the teat causes discomfort to the cow. Therefore, an improperly operating pulsator which is designed to interrupt this vacuum periodically, would cause pain due to undue stress on the teats and udder.

Before one can understand how an improperly operating pulsator might cause damage to the udder, some knowledge of how the milking machine removes the milk from the udder is necessary. In the United States, we use a two-chambered teat cup which consists of a metal cup case and a rubber inflation. The inflation fits inside the cup with the mouthpiece, or the end that fits over the teat, stretched tightly over the open end of the cup. There is a rubber air tube which connects the inflation and metal cup to a claw structure which is under vacuum and pulsator action.

In the area between the teat cup and the inflation, the pressure is alternated between the milking phase of the pulsation stroke, and the resting phase of the pulsation stroke, by the action of the pulsator. The area into which the teat is inserted is under a relatively constant vacuum at
all times.

Thus, when the pulsator goes into the milking phase, it exerts a vacuum between the shell and the inflation that causes the inflation to remain in the "open" or in the "milk" position. The constant vacuum at the teat end results in the milk's flowing from the high pressure area in the teat and gland cistern to a vacuum, or low pressure area, inside the inflation and milking system. As the pulsator goes into the resting phase of its pulsation, it permits atmospheric air to enter between the shell and inflation. This allows the constant vacuum at the teat end, and in the milking system in general, to draw the walls of the inflation together. The main purpose of the resting phase is to massage the teat and redistribute the blood drawn to the point of the teat during the milking phase back to the peripheral tissue of the teat. Unless the vacuum between the shell and inflation is periodically interrupted by pulsator action, and the inflation allowed to collapse, the teat will become congested and mastitis may result (49).

With the use of a modified strain gauge amplifier, which measures negative and positive pressures applied to the teat by pulsator action, Noorlander and Schalm (41) demonstrated that there were significant differences between pulsators of different makes and also between models of the same make. Many pulsators showed varying degrees of wear; and a master pulsator, used to control a number of milking units, became increasingly inefficient as more units were added (40). It was concluded that poorly functioning pulsators undoubtedly interfere with the proper function of the liners and exert undue stress on the teats.

Pulsation ratios, i.e., the percentage of the total pulsation stroke,
"open" as related to "closed," regulate the speed of milking. If the inflation is open for a longer period of time than it is closed, faster milking will result; but if the inflation remains open too long, insufficient massage of the teat will occur (40). Pulsator speed also regulates speed of milking; but speeds above 50 to 60 pulsations per minute are of no advantage because the speed is so fast that the inflations never fully collapse. Pulsator ratios that are "open" too long, and pulsators that operate too fast, do not allow sufficient massage of the teat to redistribute the blood and, as a result, cause stress on the teat ends. Pulsator speeds of 40 to 60 per minute with a ratio not to exceed 60:40 are efficient, yet do not cause undue stress (40). Noorlander and Schalm (41) report up to 50 per cent of the pulsators checked in California were malfunctioning. They believe that pulsators must operate effectively if tissue stress is to be avoided (42).

Another factor in mastitis control programs is the teat cup and inflation design (42). In large-bore inflations, small teats must stretch or balloon to fill the inflation. At the end of the milking period, and particularly if cows are overmilked, the teats shrink and the teat cups tend to climb upward toward the gland cistern. This area of the udder is made up of tissue that is more delicate than the teat. If the teat is pulled into the teat cup, the gland cistern (see Figure 1) at the base of the teat may be closed—or shut off—thus placing the full force of the inflation, as it closes, on the tissue of the teat. Heever (17) points out that this condition can be avoided by using narrow-bore inflations in good condition and under tension at all times. In large-bore inflations, teats may be damaged
by slapping the sides of the inflations (55). Teat slap may also be eliminated and the stress on the teat reduced by the use of narrow-bore, stretched liners which produce a snug fit on the teat (50).

Willson (71) found that narrow-bore liners caused two cases of mastitis compared to 32 cases with large-bore liners; and McFarlane (28) states that such liners more nearly meet the requirement of a properly designed liner.

The pulsator and teat cups will not function properly if the vacuum pump does not remove sufficient amounts of air from the system (40). The amount of air a pump will move can be measured by an air flow meter (40). The pipeline system should be designed, however, so that the milk flow occupies the lower portion of the pipe, permitting air to flow uninterrupted above the milk (50). An interruption of the milk flow, which causes the pipe to fill with milk, will cause a vacuum block and uneven vacuum at the teat end (40, 50). A small quantity of air entering the system at the milking machine claw, and a gravity flow of milk in the pipeline helps keep an even milk flow (50).

If the vacuum pump does not remove enough air, or the air flow is interrupted, a constant vacuum at the teat will not be maintained. Such conditions exert undue stress on the teat and gland cistern tissues due to the incomplete collapse of the inflation and results in poor blood redistribution (55). A field survey of 252 installations (12) showed 65 per cent with vacuum pumps of insufficient size.

An important feature of a vacuum pump is its vacuum reserve. Vacuum reserve is the additional air-moving capacity of a vacuum pump after the requirements of the milking units, bleeder holes, operating accessories and
air leaks have been met. Such a reserve is equal to the amount of air entering the controller (45). Pipeline milkers generally require twice the vacuum reserve as do bucket milkers to maintain vacuum stability at the teat end. This is because there is a built-in reserve in the buckets (40). Methods of measuring vacuum reserve are outlined by Noorlander (40).

Improperly set, or dirty, malfunctioning vacuum controllers can result in too high a vacuum in the system and may be injurious to the teat (40).

In summary, it has been demonstrated that improperly operated and malfunctioning milking equipment may cause injury to the teats and udders and become predisposing factors to the development of mastitis (2, 40, 41, 42, 44, 49, 50, 53, 55, 60, 70, 71, 72). Those factors which may cause injury, but which are directly related to the decisions of management are: (1) size of inflation, (2) level of vacuum, and (3) incomplete inflation collapse (40). Size of inflations is a simple management choice between large- and small-bore inflations and the level of vacuum is set by the operator upon the recommendation of the manufacturer. Incomplete inflation collapse, on the other hand, may be due to malfunctioning pulsators, milk blocks in the inflations, claws, hoses, and pipelines, or to too small a vacuum pump (50). Some of these factors require some day-to-day attention in addition to the proper selection and installation of equipment.

In addition to the above, other factors, which are under the direct supervision of the milker or machine operator, may also cause injury to the teats and udders and become predisposing factors to the development of mastitis. These include: (a) attaching the machines before milk let-down is complete, (b) not hanging the machine properly on the udder, and (c) not re-
moving the machine when the udder is milked out (2).

Pier et al (44) in 1956 demonstrated that vacuum will enter the teats and gland cisterns when milk is not present. Noorlander (40) reports this results in pinpoint breakage of the blood vessels on the surface and ends of the teats, and also presumably on the inside of the teat canal, and is a predisposing factor to mastitis.

Since cows differ in their rate of milk ejection, temperament, and udder characteristics, milking machine operators play an essential role in mastitis control programs (2). If they do not understand the importance of milk let-down, proper adjustment of the machines to fit the characteristics of the udder, and removal of the machine when the udder is milked out, they may be a major contributor to udder injuries and mastitis (72).

Maffey (27) reported that out of 600 dairy herds studied in England, all herds with mastitis problems were machine milked. He reports that if an outbreak of mastitis occurs, and does not subside immediately, the milking machines are not being removed from the udders as they are milked out. He concluded that it is the individual milking speed of the cows and the stages of lactation that determine the number of machines with which an operator can keep pace. Cows late in lactation milk out faster and require more attention.

Schalm (49, 50) concludes that a good mastitis control program should include: (1) the routine observations of the cow at each milking, (2) a herd management program designed to prevent injury to the teats and udders, removal of shedder cows, and mastitis-free replacements, (3) periodic checking of the milking system, i.e., pulsation rate, air flow, etc., (4) a
training program for machine operators, and (5) the monthly use of the C.M.T. test. Procedure for scoring and interpretation of the C.M.T. test are given in Table I.
### TABLE I. GRADING AND INTERPRETATION OF C.M.T. REACTIONS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Suggested Meaning</th>
<th>Description of Visible Reaction</th>
<th>Interpretation of Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Negative</td>
<td>Mixture remains liquid with no evidence of formation of a precipitate.</td>
<td>0-200,000</td>
</tr>
<tr>
<td>T</td>
<td>Trace</td>
<td>A slight precipitate forms and is seen by tipping the paddle back and forth and observing mixture as it flows over bottom of cup.</td>
<td>150,000 - 500,000</td>
</tr>
<tr>
<td>1</td>
<td>Weak Positive</td>
<td>A distinct precipitate but no tendency toward gel formation.</td>
<td>400,000 - 1,500,000</td>
</tr>
<tr>
<td>2</td>
<td>Distinct Positive</td>
<td>The mixture thickens immediately with some suggestion of gel formation.</td>
<td>800,000 - 5,000,000</td>
</tr>
<tr>
<td>3</td>
<td>Strong Positive</td>
<td>A gel is formed which causes the surface of the mixture to become convex. Usually there is a central peak which remains projecting above the main mass after the motion of the paddle has been stopped.</td>
<td>Generally over 5,000,000</td>
</tr>
<tr>
<td>+</td>
<td>Alkaline Milk</td>
<td>Mixture turns deep purple color, which indicates a depression of secretory activity. This may occur either as a result of inflammation or in drying-off gland.</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>Acid Milk</td>
<td>Mixture turns yellow, which indicates fermentation of lactose by bacterial action within the gland. This reaction is very rare.</td>
<td></td>
</tr>
</tbody>
</table>
EXPERIMENTAL PROCEDURE

This study was conducted to determine if there was a greater incidence of mastitis and lowered milk production resulting from overmilking when properly designed and operated milking units were used.

The animals used in this experiment were selected on the basis of no positive C.M.T. tests or anatomical abnormalities during any preceding lactation or during a seven-day preliminary period. During this seven-day preliminary period each quarter of each cow was scored daily by the C.M.T. test, palpated for abnormalities, and the total milking time determined.

Using the above criteria, 12 animals were selected for the experiment. 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. All of the cows were healthy with normal udders except one cow each in Groups II and III. Each of these cows had three normal quarters and one dry quarter.

At the end of the seven-day preliminary period, the cows in Group I were machine milked until the milk was completely evacuated. The machines were removed when milk ceased to flow. There was no hand stripping. The cows in Group II were machine milked for 1-1/2 times or 50 per cent more time than was required to evacuate the udder during the preliminary test period. The animals in Group III were machine milked for twice, or 100 per cent more than, the time required to evacuate the udder as established during the preliminary test period. At the end of each seven-day test period, the total milking time for each animal was re-established and used as a basis for determining the milking time for each cow for the next test.
At the evening milking each day, samples of milk from each quarter were scored by the C.M.T. test. Prior to withdrawing these samples, the ventral portion of the udder, including the floor and teats, was washed with a disinfectant solution, and three or four streams of milk were drawn into a strip cup. Following this, two or three milliliters of milk were drawn from each quarter into the C.M.T. paddle and the test completed. All of the tests were scored by the same person and the grading and interpretation of the C.M.T. results were as outlined by Schalm (53), with the exception that all quarters showing a trace (T) were scored as a C.M.T. number 1, rather than merely a trace.

After the C.M.T. tests had been performed, and milk let-down complete, as determined by the turgidity of the teats and udder, each quarter was palpated. The machines were then attached, and left on for the predetermined time. The time interval was measured in each case by a laboratory interval timer. At the end of the prescribed milking time, the machines were removed, the udder again palpated for anatomical abnormalities, and the teat ends dipped into a viscous disinfectant as an aid to preventing infection and freezing of the teats. The milk from each cow was weighed and recorded at each milking.

All of the cows were milked with the same high level pipeline milker, which, based on an analysis with the air flow meter and pulsation recorder, was mechanically nearly perfect. The pulsator action, pump capacity, and vacuum stability at the teat ends were within the tolerance level established by the California Mastitis Team (40). The machines were equipped
with narrow-bore pre-collapsed inflations.

The animals were housed in a loafing-type shed, fed a good quality alfalfa hay, ad libitum, and grass-legume silage at the rate of 30-40 pounds per animal per day. Concentrates were fed at the rate of one pound per four pounds of fat-corrected milk produced daily.

The average age of the cows on this experiment was four years and five days (range—two years and one month to five years). The stage of lactation ranged from two to three months. The daily milk production average was 46 pounds at the start of the trial. Management practices were uniform for all groups and the trial continued for eight weeks.

The criteria for determining the effect of overmilking were: (a) number of cases of clinical mastitis, as detected by flakes of milk in the strip cup, palpation, and/or a number 3 score on the C.M.T. test; (b) percent increase in positive C.M.T. tests; and (c) decline in milk production. Positive C.M.T. tests (1-2-3 scores) and percent decline in production were used to determine the damage and/or irritation resulting from overmilking.
The cows appeared to adjust rather quickly to overmilking. During the first two to three days cow number 91, in the 50 per cent overmilked group, kicked the machine off several times during each milking. After this period, she did not cause any further trouble.

No clinical mastitis cases developed. The data on the C.M.T. tests are presented in Table II. Since a C.M.T. test was performed on each lactating quarter daily, a total of 896 C.M.T.s were scored on the control group during the eight-week test period. However, since one animal in each of the overmilked groups had only three lactating quarters, only 840 C.M.T.s were scored on each of these groups. In the control group, 773 of the 896 C.M.T. tests conducted were graded as negative, 99 were graded as number 1, 23 as number 2, and one as number 3. In the 50 per cent overmilked group there were 785 C.M.T. tests graded negative, 47 were graded as number 1, 7 as number 2, and one as number 3. In the 100 per cent overmilked group, there were only 695 C.M.T. tests graded negative, 137 as number 1, 8 as number 2, and none as number 3. These differences were not statistically significant (P > 0.05).

Data on milk production are presented in Figure 4. During the eight-week experimental period, the control group averaged 49.0 pounds of milk per day per cow. The 50 per cent overmilked group averaged 43.0 pounds of milk per day per cow, while the 100 per cent overmilked group averaged 44.6 pounds of milk per day per cow. The average rate of decline in milk production for the entire period was 10.5% in the 100 per cent overmilked group, followed by 7.0% in the 50 per cent overmilked group. The control
TABLE II. RESPONSE OF LACTATING QUARTERS TO OVERMILKING AS MEASURED BY DAILY C.M.T. TESTS FOR AN EIGHT-WEEK PERIOD.

<table>
<thead>
<tr>
<th>Group</th>
<th>Negative</th>
<th>C.M.T. 1</th>
<th>C.M.T. 2</th>
<th>C.M.T. 3</th>
<th>Total Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>773</td>
<td>99</td>
<td>23</td>
<td>1</td>
<td>896</td>
</tr>
<tr>
<td>50 Per Cent Overmilked</td>
<td>785</td>
<td>47</td>
<td>7</td>
<td>1</td>
<td>840</td>
</tr>
<tr>
<td>100 Per Cent Overmilked</td>
<td>695</td>
<td>137</td>
<td>8</td>
<td>--</td>
<td>840</td>
</tr>
<tr>
<td>Total for all Groups</td>
<td>2253</td>
<td>283</td>
<td>38</td>
<td>2</td>
<td>2576</td>
</tr>
</tbody>
</table>
Figure 4. Average Decline in Milk Production for Three Different Milking Machine Time Levels.
group had the smallest average (5.0%) decline in milk production. However, these differences were not statistically significant (P > 0.05).

Data on milking machine time per cow for each of the three groups are presented in Figure 5. The time required to milk the animals in the control group ranged from 2.5 minutes for the fastest milking animal to 5 minutes for the slowest milking animal. The total milking machine time (the total time the machine was attached to the udder) in the 50 per cent overmilked group ranged from 4.75 minutes to 8.75 minutes, while in the 100 per cent overmilked group the range was from 3.75 minutes to 12.5 minutes per cow. In all cases the time required remained about the same throughout the experiment.

Since this experiment was conducted during the coldest period of the year, it was assumed that the incidence of frozen teats might influence the incidence of mastitis. There were no frozen teats in the control group. However, 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 only those animals in the overmilked groups had become quite hard and calloused. However, pinpoint breakage and hemorrhaging did not appear on any of the teats.
Figure 5. Average Milking Machine Time for Three Different Milking Machine Time Levels.
DISCUSSION AND CONCLUSIONS

It is evident that bovine mastitis is a very complex disease and that no single control program has yet been devised that will completely protect the udder from all causes of inflammation. Best results have been obtained when preventive measures have included, in addition to the control of bacterial infections, improvement in the efficiency of the mechanical milking systems.

Many workers in the field of mastitis (28, 31, 40, 42, 44, 49, 50, 53, 55, 60, 68, 70) point out that much of our mastitis today is the result of the milking machine causing stress to the tissues of the mammary gland either by using malfunctioning or badly worn equipment, or by leaving the machine attached to the udder after milk flow has ceased. However, from the results obtained in this experiment, it appears that overmilking may not be as injurious to teat and udder tissues as has previously been believed, if the milking equipment meets the standards established by the University of California (40).

From the experimental results obtained, it is the author's opinion that the milking system was in such a high state of efficiency in relation to the physical forces it presented to the teats and udders that there were no injuries to these tissues, with or without the presence of milk. It is well to point out once again that narrow-bore, pre-collapsed, inflations were used during this experiment so that they would ride low on the cow's teats, and not crawl up to the more delicate tissue at the base of the gland cistern after the milk had been removed from the gland.

If injury had occurred to the structural or secretory tissue within
the gland during this eight-week overmilking experiment, the C.M.T. tests (53) would have revealed such damage, even though clinical mastitis was not encountered.

The hypothesis that the milking time for the animals in the overmilked groups would increase throughout the trial as a natural body defense mechanism to offset the effects of overmilking by letting down their milk at a slower rate, was not confirmed. The animals in all groups maintained approximately the same rate of milk flow throughout the experimental period.

Even though there were no statistically significant differences in the rate of milk decline between groups, it is evident from Figure 4 that the rate of milk decline in per cent was greatest for the 100 per cent overmilked group (10.5 per cent) and least for the control group (5.1 per cent). The per cent decline in milk production for the 50 per cent overmilked group was 7.0 per cent. It is the author's opinion that even though the above differences, as well as the differences in C.M.T. scores between groups, were not statistically significant, that some pain was inflicted to the teats and udders of the overmilked groups during the process of overmilking. This pain inflicted to the empty udders may have resulted in the release of the hormone epinephrine (commonly released under stress) and, as such, may be responsible for overcoming the action of oxytocin. When epinephrine is released into the blood stream during the milking act, all of the milk is not removed from the udder (40), and the rate of milk decline under such conditions will exceed the normal rate.
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