BOVINE MASTITIS: A REVIEW

W. N. PLASTRIDGE
Department of Animal Diseases, Storrs Agricultural Experiment Station, University of Connecticut, Storrs

SUMMARY

The principal organisms associated with mastitis are *Streptococcus agalactiae*, *Streptococcus uberis*, and *Micrococcus pyogenes*. These organisms usually cause a chronic mastitis and a loss in milk yield with or without the appearance of clinical symptoms. Mastitis due to other organisms is not common, but may be a serious problem in an occasional herd, especially following udder infusions improperly administered. When cultural tests are made on incubated milk, the leucocyte count must be used in interpreting the results, and when made on fresh milk, consideration must be given to the number and kinds of organisms present. *S. agalactiae* can be eradicated from most herds by good sanitation combined with frequent cultural tests and treatment of all infected quarters.

The sanitary measures now in general use are inadequate to prevent new and reinfection with *M. pyogenes* and *S. uberis*. Thus, treatment of infected quarters is of limited value.

Degree of exposure to infection, natural resistance of individuals, age, stage of lactation, and prolonged milking duration influence the rate of udder infections. Abusive machine milking, especially prolonged milking and use of worn or poorly designed teat cup liners, tends to increase the rate of clinical mastitis in infected quarters.

Mastitis reduces the milk yield and shortens the productive life of affected cows, causing an estimated loss of $225,504,000 annually in the United States (2). While it is generally agreed that the immediate cause of mastitis is infection, a difference of opinion exists regarding the relative importance of management (predisposing factors) and infection. Some believe that clinical mastitis occurs only when a phase of management reduces the resistance of the animal, and that the main emphasis in control should be placed on good management. Others believe that mastitis is caused by specific organisms and that control should be based on the prevention and elimination of infection.

There is no question that predisposing factors are important and should be minimized so far as practical conditions permit. It is also essential that consideration be given to specific ways of preventing infection and of eliminating infection when it occurs. To date, such measures have been developed for the control of mastitis due to *Streptococcus agalactiae*. Further research is needed to develop specific measures to aid in the control of other forms of the disease.

Information on predisposing factors, infection, diagnosis, control, and antibiotics in market milk will be presented.\(^1\)

\(^{1}\) A complete review is beyond the scope of this communication. The literature cited here has been limited to reports especially concerned with, or representative of findings on, several phases of the mastitis problem. More complete reviews have been published (87, 149). Literature up to 1938 was abstracted by Munch-Petersen (197), and during 1953 to 1955 by Murphy (114).
of others, and because individual animals under apparently identical conditions differ in susceptibility. Furthermore, most of the literature deals with clinical observations which are difficult to evaluate.

Chilling. Exposure of the udder to chilling probably increases inflammation in udders already infected; however, this has not been determined experimentally. Reid (169) observed an increased rate of clinical mastitis in the spring and in the fall in herds in which animals were left in pasture over night while the ground was cold, and in animals which were exposed to drafts in poorly ventilated barns. The relation of new S. agalactiae infection to the season of the year was determined in 14 herds (average 735 cows) in Scotland by Ineson and Cunningham (68). The number of new infections that occurred during the spring, summer, fall, and winter was 54, 45, 75, and 38, respectively. The peak months of the year were October and April. The stage of lactation appeared to have no effect on the occurrence of new infection. Oliver et al. (135) observed no seasonal trends in the incidence of udder infection and mastitis when allowance was made for the effect of stage of lactation and age differences of spring-autumn-calving cows.

Feed. Observations of Udall and Johnson (225) and conversations with dairymen suggest that reducing the amount of protein concentrates in the ration tends to reduce clinical evidence of mastitis in cows with a past history of mastitis and in cows with udder infections, as shown by laboratory tests. However, the limited experiments of Hotis and Woodward (59) and Moore et al. (104) failed to show a relation between the diets used and either the severity or rate of mastitis. Pounden and associates (164-166) found that the diet of cows affected the ability of their milk to resist acid formation by S. agalactiae in in vitro tests. The relation of this characteristic to susceptibility or resistance of the udder to infection was not determined. Their findings are of interest and would seem to warrant further studies on the influence of the ration on mastitis. However, definitive experiments would be difficult to arrange, due to other factors that affect the establishment of infection and rate of clinical mastitis in infected quarters.

Hypersensitivity. In the transmission experiments of Jones and Little (73,81), repeated exposure to small numbers of S. agalactiae organisms was required to produce infection. Exposure was accomplished by dipping the end of a small glass rod into dilutions of a 6-hr. broth culture, and passing the end through the teat canal. They suggested that the udder first must be sensitized before infection can result from exposure to small numbers of organisms. This explanation seems to be invalid in view of the findings of Murphy and Stuart (120), that all of 11 quarters of four first-calf heifers became infected following the introduction of 35 ± 31 S. agalactiae organisms into the teat cavity by means of a special pipette.

Spencer and Angevine (199) reviewed the literature on hypersensitivity and showed that cows may be sensitized to S. agalactiae antigens. Intradermal injections of formalinized cultures produced larger and more persistent swellings in infected than in normal cows. Injection of antigens into quarters in which
infection was previously removed by treatment caused the quarters to become swollen and the secretion abnormal 5 hr. later. Control quarters showed a less severe reaction. In a later report, Spencer and Simon (204) found that prior sensitization by subcutaneous injections of living organisms did not increase susceptibility to experimental exposure by intramammary infusion of 1,100-24,000 organisms.

While hypersensitivity may not be a prerequisite to infection with S. agalactiae, it appears to be involved in the pathogenesis of mastitis due to this organism (199).

Heredity. Data collected on individual herds have shown that selected cow families (dam and daughters) appear to have a relatively high or low incidence of mastitis (88, 118, 169). Milk yield is influenced by heredity, but the volume of milk produced does not appear to be related to susceptibility to mastitis (118, 169, 235). Milking rate is inherited (29), and Dodd and Neave (31) found that fast milkers tend to become infected more readily than slow milkers. However, they state that differences in milking rates can not account for the large differences in mastitis-incidence rates found between separate herds. Evidence that the sire is a factor in inherited resistance to infection was given by Legates and Grinnells (79) and by Reid (169).

Age. As would be expected, the rate of udder infection increases with age. For example, Seelemann (187) reported an infection rate among 5,834 cows of 9, 30, 42, 44, and 52% for animals in their first, second, third, fourth, and fifth lactations; Plastridge et al. (153) found that the rate of infection (mostly S. agalactiae) increased from 14 to 100%, and that the proportion of animals giving visibly abnormal milk intermittently increased from 8 to 86% from the first to eighth lactation when the animals in two herds were grouped according to lactation. With few exceptions, first-calf heifers are free of S. agalactiae at the time of parturition (87). The exceptions are due to infection acquired during calfhood, when calves are fed infected milk and allowed to suckle each other (172a).

An increase-with-age pattern for S. agalactiae commonly has been observed; however, different explanations have been proposed. The observations made by Murphy (112) on a large herd over a 7-yr. period indicate that an “age factor” independent of teat injury, milking rate, prior sensitization, and degree of exposure is involved. Support for this explanation is given by the findings of Lancaster and Stuart (76). They found that two of seven first-calf heifers, four of five second-calf heifers, and six of six older cows became infected within a period of 15 wk. when exposed by milking them with hands previously dipped in S. agalactiae-infected milk. While an age factor may be involved, the observations of Ormsbee and Schalm (136), Plastridge et al. (151), and Spencer and Kraft (200) show that the degree and extent of exposure are major factors affecting the rate of infection in heifers as well as in older cows.

An increase of infection with non-S. agalactiae streptococci and hemolytic staphylococci, and clinical mastitis, were considered to be due to persisting infections and reinfections, in a herd observed by Oliver et al. (132, 135) for
age incidence of infection. In a large *S. agalactiae*-free herd, Schalm and Woods (185) found that the incidence of *Micrococcus pyogenes* infection increased with age, from 20% for first-lactation animals to 74% for cows in their eighth lactation.

While the degree and extent of exposure, and persistence of infection, contribute to the relationship of age to incidence of infection, the findings of Murphy (112) and Lancaster and Stuart (76) indicate that unknown changes associated with age may be related to susceptibility to *S. agalactiae* infection.

*Stage of lactation.* *S. agalactiae* infection does not appear to be related to the stage of lactation, as indicated by the findings of Plastridge et al. (151). Of 157 heifers in six *S. agalactiae*-infected herds, seven, eight, and seven became infected during the first third, second third, and last third of their first lactation.

Oliver et al. (133, 135) reported that the rate of new infection with non-*S. agalactiae* streptococci and staphylococci was highest during the first month of lactation and during the early "dry period." About one-half of the latter infections persisted until calving (134). The rate of infection during the dry period appeared to be related to the milk yield at the last milking and to the method of stripping before the cows were dried off (133). The percentage of quarters that became infected increased from 18% for cows that yielded less than 7 lb. at their last milking to 43% for those that yielded 21 lb. The percentage of new infection during the dry period was 18% for machine-stripped cows and 37% for hand-stripped cows.

*Degree of exposure.* A major factor in determining the incidence of *S. agalactiae*, and probably other infections in a given herd, is the degree and extent of exposure. This is illustrated by results reported by Spencer and Kraft (200). In two herds with a low incidence of *S. agalactiae* infection (average of 21%), the rate of infection for first-, second-, third-, and fourth-lactation animals was 5.3, 15, 25, and 35%, respectively. In comparison, in ten herds with a high rate of infection (average of 71%), the rate of infection for the four lactations was 61, 68, 68 and 73%, respectively.

*Teat characteristics.* Injuries resulting from cows stepping on teats, cuts from barbed wire, teat surgery, and lesions at the end of the teat, destroy or impair a natural barrier to infection and may facilitate the entrance into the udder of various kinds of microorganisms present on the skin of the teat. The variety of organisms associated with teat injury was shown by the findings of Ferguson (43). Of 283 samples from cows with mastitis following teat injury, 24% contained *S. agalactiae*, 23% *Streptococcus dysgalactiae*, 15% *Streptococcus uberis*, 10% other streptococci, 15% staphylococci, 6% *Corynebacterium pyogenes*, 2% coliforms, and 7% mixed infections. *S. agalactiae* was not found after injury in herds known to be free from this organism.

The size and shape of the teat, length of the teat canal, and superficial sores on the teats do not appear to be related to the incidence of mastitis (75, 93, 125, 212).

The rate of milk flow (teat patency) appears to be a factor in susceptibility to infection, especially with hemolytic staphylococci (31, 42, 65, 93, 111, 112, 212).
In observations on a herd of 180 cows, McEwen and Cooper (93) found that the percentage of animals yielding milk with clots associated with “bacterial infection” was 18% for “hard milkers”, 15% for “moderate milkers”, and 33% for “easy milkers”. Dodd and Neave (31) divided 94 first-lactation animals, in a herd with a low incidence of *S. agalactiae* and a high incidence of staphylococcal infection, into five groups on the basis of rate of milk flow. The mean peak flow (lb. per minute) was 2.4, 3.6, 4.5, 5.6, and 6.8. The rate of new infection with *S. agalactiae* in the five groups was 0.0, 6.6, 3.8, 25, and 30%, and for hemolytic staphylococci, 5, 33, 46, 63, and 67%, respectively. They suggested that either a large or slack teat sphincter, or leaving the machine on longer than necessary for the fast milkers, may facilitate passage of bacteria into the udder.

The soft keratin in the external portion of the teat canal appears to be a natural defense mechanism against infection. Fincher et al. (45) found that infection invariably followed swab exposure to *S. agalactiae*, when this material was removed from the teats of heifers that had previously resisted exposure.

**Incomplete milking.** Leaving 1 lb. or less of milk in the udder does not appear to be a factor in mastitis (30, 179). Schalm and Mead (179) found that leaving about 2 lb. of milk did not cause alterations in the milk of normal quarters; however, in *S. agalactiae*-infected quarters an increase in the leucocyte count and visible symptoms of mastitis occurred.

**Hand vs. machine milking.** Early reports (19) indicated a higher incidence of mastitis in machine-milked animals than in animals milked by hand. Recent findings, that the milkers’ hands can be a major factor in the spread of udder infections, tend to reverse this situation, providing machine milking is done properly.

Wilson (231) reviewed the work of Lancaster and Stuart, in which 12 of 18 cows became infected within a period of 15 wk. when milked with hands previously dipped in *S. agalactiae*-infected milk. The 12 animals were then freed of infection by treatment with penicillin. When milked by machine, with the teat cup clusters dipped in infected milk before being put on each cow, only six of the 18 animals became infected during a similar period. Wilson (231) reported that the percentage of herds with *S. agalactiae* infection before and after treatment of infected cows was as follows: for 273 hand-milked herds, 63% pre-treatment and 14% post-treatment; for 122 machine-milked and hand-stripped herds, 68 and 29.7%; and for 189 machine-milked and machine-stripped herds, 52.8 and 8%.

The rate of new infection (mostly non-*S. agalactiae* streptococci) during the early dry period was found by Oliver et al. (133) to be 37.2% for a group of hand-stripped cows and 18.3% for a group of cows that were machine-stripped.

**Type of milking machine.** The type of machine does not appear to be important in mastitis control. Data collected by Ward (225) on 1,099 New Zealand herds in which five types of machines were used, failed to show any significant relation between the type of machine and incidence of mastitis. Hodges et al. (57) state that “The writers have seen many dairymen in trouble with mastitis...
regardless of the type of machine used. Many of these same dairymen using the same machine have reduced mastitis to the point where it is not a serious economic problem. This has, in general, been accomplished through adequate and judicious diagnosis and treatment, coupled with strict attention to good management and sanitation that includes proper milking practices and proper vacuum and pulsation adjustments on the machine."

Type of teat cup liners. In England, "stretched" or "extended" liners and "moulded" liners have been compared in several S. agalactiae–free herds. Neave et al. (129) observed a reduction in the rate of clinical mastitis in three of four herds when the liners were changed from "moulded type 1" to "moulded type 2" and an increase in one herd after changing from "stretched liner type A" to "moulded type 1". Most of the clinical cases were associated with hemolytic staphylococci.

In 84 cows with a mean age of 3.8 lactations, Dodd et al. (32) found that the percentage of clinical mastitis in the quarters of all cows was 5.5% for an extended type liner and 12.7% for a moulded type; and in quarters infected with hemolytic staphylococci and non–S. agalactiae streptococci, 9.8 and 37%, respectively. The superiority of the extended liner was attributed to its greater elasticity. No significant difference between the two liners in respect to rate of infection, teat condition, and leucocyte count of normal quarters was observed. They suggest that while the action of a liner may not affect a normal quarter, it can irritate an infected quarter and thus precipitate an attack of mastitis.

Vacuum level. In a review of machine milking and mastitis by Burkey and Sanders (19), the data presented suggested that a vacuum level of over 15 in. of mercury tends to increase the incidence of mastitis. An example of mastitis due to an excessive vacuum was given by Neave et al. (130). In a S. agalactiae–free herd of 60 milking Shorthorns a severe outbreak of clinical mastitis involving 26 cows occurred in the spring of 1943. At the time of the outbreak, the vacuum gauge registered 15 in. when, in fact, the vacuum was 19½ in. After replacing the gauge and adjusting the vacuum to 15 in., no further cases were reported. The symptoms were accompanied by distinct lameness and swelling, induration and pain in the affected quarters, and in 12 cows the secretion was coffee-colored with viscid clots. The majority of the bacteria isolated were S. uberis and a few were Streptococcus fecalis, both of which commonly occur on the skin of teats. A few samples contained no significant organisms.

Evidence that vacuum levels per se within a range of 10, 12, and 17 in. of mercury do not cause mastitis was given in a series of papers by Mochrie and associates (101, 102, 103). No significant differences were seen in the leucocyte count, chloride content, pH values, and incidence of either clinical mastitis or infection, of first lactation heifers and cows milked at the three vacuum levels. All of the animals used were free from S. agalactiae.

Milking duration. Evidence that leaving the machine attached to the udder after milk flow has practically ceased contributes to mastitis was presented in the review by Burkey and Sanders (19) and in several subsequent reports.
In the observations of Mochrie et al. (103) the average leucocyte counts of the milk from cows milked for a normal, twice normal, and four times normal milking duration were 461, 761, and 1,130 thousand per milliliter, respectively; the average chloride values were 131.7, 147.1, and 163.9 mg.%. Although no clinical mastitis developed during the 6 wk. of observation, the increased chloride and leucocyte values are evidence that irritation occurred when the milking time was extended.

The effect of a 4-min. duration of milking and an 8-min. duration on heifers in four herds was observed by Dodd et al. (30). The heifers were milked for an entire lactation. Of 19 animals in the 4-min. group, four became infected and none showed clinical evidence of mastitis. In the 8-min. group of 19 animals, seven became infected and four showed clinical evidence of mastitis in one or more quarters on one or more occasions. Animals were classed as infected when quarter samples contained “pathogenic bacteria” on two or more consecutive tests and showed a reaction to the Whiteside test. Presumably, the infecting organisms were mostly hemolytic staphylococci, since three of the herds were free of S. agalactiae.

Evidence that leaving the machine on after milk flow has stopped facilitates passage of bacteria through the teat canal was presented by McEwen and Samuel (94).

Pier et al. (146) showed by radiographic means that tissues of the mammary gland can be injured when the machine is allowed to remain on the udder after milking has been completed.

Improvement in milking-machine design to provide visual milking, and vacuum reduction with each pulsation, is being tried by Schalm and Noorderlander (181).

Comment. The reports reviewed indicate that: (a) The degree and extent of exposure, and milking duration, can affect the rate of udder infections, (b) abusive milking practices, exposure of the udder to chilling, and excessive feeding of protein concentrates may increase the incidence of clinical mastitis in infected quarters, and (c) the rate of milk flow, stage of lactation (infection other than S. agalactiae), age, and heredity influence susceptibility to infection.

INFECTION

During 1874 to 1878, Roberts (170) and Lister (80) advanced the theory that milk within the healthy udder is germ-free. This was soon followed by the theory that the udder is inhabited by a “normal flora” consisting mainly of streptococci, micrococci, and diphtheroids which are always present in the environment of cattle. Early research also showed that streptococci and micrococci are associated with mastitis. Later, tests were developed for identifying the common mastitis streptococci (S. agalactiae, S. dysgalactiae, and S. uberis) and for separating micrococci into pathogenic (M. pyogenes) and nonpatho-
genic micrococci. Transmission experiments have shown that the streptococci listed, Lancefield’s Group C streptococci, *M. pyogenes*, coliform organisms isolated from cases of mastitis, and *C. pyogenes* are capable of causing mastitis when injected into the teat cistern (87).

Invasion of the udder by microorganisms is by way of the teat canal, with the exception of transfer of organisms by way of the blood stream in diseases in which septicemia occurs. Following invasion, the establishment of infection depends upon the ability of the organism to survive within the udder; and the development of mastitis upon the ability of the organism to produce substances that are toxic to the glandular tissue. The extent of growth of pathogenic organisms and subsequent evidence of mastitis may be influenced by predisposing factors previously mentioned, especially inefficient milking techniques.

Microorganisms may pass through the teat canal either as a result of suction during milking (82, 94), or by growing their way through, as suggested by Murphy and Stuart (124), and as indicated by the observations of Oliver et al. (133) that new infections may occur during the dry period. The ability of the teat canal to resist the passage of *S. agalactiae* seems to explain the differences observed in the susceptibility of individual animals to infection when exposed by the Hadley-Wisconsin swab technique (46, 121, 123, 124).

Murphy (112) proposed a three-phase concept of udder infection: *Invasion*—passage of organisms into the interior of the udder (may or may not become established); *infection*—organisms become established; and *inflammation*—reaction of the tissues to injury by the organisms or their products. As pointed out by Spencer and McNutt (203), in *S. agalactiae* infection differentiation between infection and inflammation is difficult, since inflammation is present if the invading organism survives. They found that survival of *S. agalactiae* within the udder is invariably accompanied by inflammation which, in the early stages, is usually subclinical. However, in attempting to visualize the genesis of udder infections in general, Murphy’s explanation is useful.

*Milk yield.* In an experiment on transmission of *S. agalactiae* by Davidson et al. (28), six (13 quarters) of 18 cows became infected within a period of 16 wk. The average decrease in yield of the six cows was 10%. Hale et al. (49) reported a drop of 10.2% in the average annual yield per cow in 24 herds following the introduction of *S. agalactiae* infection, and an increase of 14.8% in yield per cow in 91 herds following the elimination of this organism.

Crossman et al. (25) observed the effect of *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, and *M. pyogenes* on the milk yield of the individual quarters of the cow’s udder. Subclinical mastitis (organism present and Whiteside test positive) associated with either streptococci or *M. pyogenes* was found to result in a

---

4 In Bergey’s Manual of Determinative Bacteriology, 7th ed., hemolytic coagulase positive members of the family Micrococaceae are classed as *Staphylococcus aureus*, and the coagulase negative members either as *Staphylococcus epidermidis* or as species of the genus Micrococcus. However, because the abbreviations for Streptococcus and Staphylococcus are similar, the term *M. pyogenes* will be applied herein to pathogenic staphylococci (hemolytic and/or coagulase positive), and cultures regarded as nonpathogenic will be designated as non-hemolytic or coagulase negative micrococci.
gradual reduction in the proportionate yield of most quarters and in no measurable reduction in a few quarters. A decrease in yield was found to continue after clinical but not bacteriologic cures in infected quarters.

Rate of infection. Numerous organisms have been reported as associated with mastitis. While an uncommon organism may be primarily responsible for mastitis in a given herd, S. agalactiae, other streptococci, and M. pyogenes are by far the most important causative agents in the cattle population as a whole. This has been shown by surveys made in England (1, 63, 65, 205, 231) and in the United States.

In Connecticut, of the 1,191 herds tested during 1957, 650 were S. agalactiae-free, either on initial test or as the result of treatment. In the S. agalactiae-negative herds (15,407 cows), 14% of the cows were classed as infected with non-S. agalactiae streptococci, 18% with M. pyogenes, and less than 1% with other organisms. In the 541 S. agalactiae-infected herds, 23% were classed as infected with this organism, 8% with other streptococci, 18% with M. pyogenes, and less than 1% with other organisms. The higher incidence of non-S. agalactiae infection in the S. agalactiae-free herds is believed to be apparent rather than real, because an animal with S. agalactiae in one quarter and another organism in some other quarter was classed as S. agalactiae-infected.

In New York State (44, 106), in “first A surveys” on 53,493 cows, the percentage of cows with abnormal secretion was 16.6%, the percentage of quarters infected with S. agalactiae was 10%, other streptococci 6%, M. pyogenes 8%, and other organisms, less than 1% (excluding nonhemolytic micrococci and diphtheroids other than C. pyogenes). In the “last A survey” on 39,043 cows, the percentage of cows with abnormal secretion was 9%, and the percentage of quarters infected with S. agalactiae was 5%, other streptococci 7%, M. pyogenes 8%, and other organisms, less than 1%.

Assuming that an average of two quarters per cow are infected, the results obtained in both states show that in S. agalactiae-infected herds about 50% of the cows and 25% of the quarters are infected with one of the three principal causative agents in mastitis, and that reduction of S. agalactiae infection through treatment does not necessarily result in a significant increase in other types of infection. The New York surveys also show that a reduction in S. agalactiae infection was accompanied by a reduction in clinical mastitis.

S. agalactiae belongs to Lancefield’s Group B (77). Mastitis due to this organism is contagious. Its habitat is the bovine udder. Apart from surfaces contaminated by infected milk, such as the skin of the teats, milkers’ hands, and teat cups it is rarely found outside of the bovine udder (1). The organism occurs occasionally in the human throat; however, for practical purposes this source of possible infection for the cow appears to be of little importance. S. agalactiae is highly susceptible to the antibacterial action of penicillin and chlorotetracycline. The development of penicillin-resistant strains following treatment has not been observed (6, 45, 138, 213).

The development of infection is usually slow, with clots appearing intermittently in the fore milk and increasing in amount, followed by a gradual in-
duration of the quarter, which may progress until milk secretion ceases (203). As few as 35 ± 31 S. agalactiae organisms may produce infection when introduced into the teat cavity (120). Following the entrance of an infective dose into the teat cavity, clinical mastitis may develop within a few days or may be delayed for weeks (76, 120, 121, 203). Once the organism becomes established, the infection tends to go through a cycle of an increased S. agalactiae count, followed by an increased leucocyte count, followed by a decreased S. agalactiae count, and repetition of the cycle at irregular intervals (81, 120, 121, 125). Clinical symptoms may or may not appear at, or immediately following, the peak of growth activity in the cycle.

Histopathologic findings on S. agalactiae-infected glands, producing either clinically normal or abnormal milk, show that acute inflammatory foci occur in from a few alveoli to one or more lobules. These areas later become fibrotic and new areas of inflammation develop, with a corresponding gradual decrease in milk yield (54, 81, 141, 203, 231).

*Streptococci other than S. agalactiae.* The majority are either S. uberis or S. dysgalactiae. While these organisms have been isolated from bovine tonsils and vaginae (87), the main reservoir of infection appears to be the infected udder. The literature on biochemical characteristics which differentiate between S. agalactiae, S. uberis, and S. dysgalactiae, and between these organisms and saprophytic streptococci found in the environment of cattle, was reviewed by Little and Plastridge (87). S. uberis belongs to none of Lancefield’s serologic groups (77, 162), and infected quarters usually show subclinical mastitis unless associated with teat injury (43) or with abusive machine milking (32, 129, 130, 231). S. dysgalactiae belongs to Lancefield’s serologic Group C (77), although the colonies on blood agar are gamma or alpha hemolytic. Mastitis due to this organism may be mild or severe, is usually of short duration, and is not common in most herds. Wide-zone beta hemolytic streptococci belonging to Lancefield’s Groups A, C, E, G, and K are rarely associated with mastitis. Group C (Streptococcus zooepidemicus) has been found more frequently than Groups A, E, G, and K (87).

*Micrococci.* The relation of hemolytic and plasma coagulating ability of udder micrococci to evidence of mastitis was first reported by Plastridge et al. (155, 160, 161). Some of the results (155) were as follows: The average leucocyte count of 2,125 bacteriologically negative samples was 73,000 per ml.; of 298 samples which contained nonhemolytic micrococci, 220,000, and of 398 samples which contained M. pyogenes, 1,361,000. The percentage of samples in the three groups with cell counts in excess of 500,000 per milliliter was 2, 12, and 63, and with cell counts in excess of 1,000,000 per milliliter, the percentage was 0.3, 3, and 43. None of the samples in the first two groups, and 5.2% of those in the latter group, contained visible clots. A close correlation has been found between hemolysis on ox or sheeps’ blood agar and ability to coagulate either human or rabbit’s blood plasma, and between ability to coagulate plasma and pathogenicity (93, 155, 178, 185, 197, 198). In general, the coagulase test is preferable to hemolysis, or lack of hemolysis, on blood agar for separating micro-
Mastitis

M. pyogenes. Most of the cultures of bovine origin produce beta toxin only, or beta and alpha toxin (16, 97, 98, 197). The rate of infection was found to be second to that of S. agalactiae, in surveys made in Connecticut and New York State. In S. agalactiae–free herds they are usually the most common organism associated with both clinical and subclinical mastitis. In general, the degree of inflammation tends to be milder, and the occurrence of clots in the milk from infected quarters less frequent, than in S. agalactiae infection (76, 93, 110, 153, 182, 185). However, misuse of the milking machine may increase the rate of clinical mastitis in infected udders (32, 129, 130, 186). First-half heifers are highly susceptible to infection (182, 206), and infection during the dry period may account for mastitis at calving time (133, 134, 135). Evidence that M. pyogenes mastitis should be regarded as contagious has been reported (129, 136).

Severe acute mastitis accompanied by a systemic reaction due to infection with highly toxigenic strains of M. pyogenes (85, 173) is not common. Such cases are usually sporadic, may result in gangrenous mastitis, and do not respond to intramammary infusions.

The principal source of M. pyogenes is essentially other infected glands (185), and the principal extramammary reservoirs are the skin of the teats and teat cups of the milking machine (202). Most cultures are sensitive to penicillin, chlorotetracycline, and oxytetracycline (3, 36); however, individual strains may show considerable resistance to one or more of the antibiotics listed (36, 186).

Corynebacteria. Diphtheroids, or members of the genus Corynebacterium other than C. pyogenes, are often found in freshly drawn milk. They are seldom associated with clinical mastitis. C. pyogenes causes a severe acute mastitis which may become gangrenous (10, 173). This form of mastitis is not common in the United States. The disease may occur in pregnant heifers and dry cows as well as in lactating animals (99). C. pyogenes is differentiated from other corynebacteria by producing weakly hemolytic colonies on ox blood agar, producing little, if any, growth on nutrient agar, and by producing a soft curd and partial peptonization of litmus milk in five days.

Miscellaneous organisms. A wide variety of microorganisms have been found to be associated with mastitis outbreaks in certain herds. Coliform organisms,3 Pseudomonas aeruginosa, and yeasts are the principal organisms involved. These organisms are resistant to penicillin and there is evidence to indicate that infec-

3 Bacteria which occur as gram negative, nonmotile rods, and which ferment lactose with acid and gas production, and produce moist, white, raised colonies on agar. The majority of cultures isolated from cases of mastitis belong to the genus Aerobacter, as described in Bergey's Manual of Determinative Bacteriology, 7th ed., 1957.
tion in some animals has resulted from lack of aseptic techniques in administering udder infusions.

Coliform organisms may cause either a mild mastitis or a severe acute mastitis with systemic symptoms (7, 17, 20, 35, 117, 149, 151, 183, 229). Coliform organisms responsible for acute mastitis differ from ordinary strains by possessing a distinct capsule (35, 149). The onset of acute coliform mastitis is sudden. Affected cows usually appear normal at one milking and show the following symptoms by the next milking: nearly complete cessation of udder secretion, loss of appetite, depression, a temperature of 104–108°F, and one or more swollen quarters. Usually, one or two cows in a herd are affected; however, in a few herds the disease has spread, within a few months, to 25% of the milking animals (87). It is of interest that death of calves due to capsulated coliform organisms has been reported as occurring simultaneously with an outbreak of mastitis due to these organisms (229).

Mastitis due to \textit{P. aeruginosa} is usually sporadic, but in a few herds may reach serious proportions (18, 21, 214). The onset is usually sudden and accompanied by clinical symptoms of relatively short duration. Infection may persist in a given quarter for several months, with occasional attacks of clinical mastitis. Pseudomonads are common in the environment of cattle. They are highly resistant to disinfectants (209) and to penicillin (214), streptomycin (175), chlortetracycline (176), and oxytetracycline (8).

Yeast-like fungi associated with mastitis were first reported by Murphy and Drake (115). Their culture was regarded as a member of the genus \textit{Trichosporon}. Ten infected quarters were seen during a 6-yr. period in a herd of 120 cows. The milk from nine quarters was macroscopically abnormal for a period of from six to 12 days, and the leucocyte count remained near 1,000,000 per milliliter for the duration of the 69-day period of observation. The cultures grew poorly on blood agar. The disease was reproduced by inoculation of the culture into a quarter of a normal udder. These organisms are resistant to penicillin and streptomycin, and in some herds infection has followed infusions of udders with penicillin preparations (5, 67, 210, 217).

\textit{Cryptococcus neoformans} mastitis has been reported in two herds (163, 191). The majority of clinically affected animals suffered severe attacks with marked swelling of the udder and decreased milk production. None of the antibiotics tried were effective, and reasonable success in reducing infection was achieved by segregating infected animals, improved sanitation, and care in milking-machine operation. The only detected source of infection observed by Pounden \textit{et al.} (163) was infected udders. Simon \textit{et al.} (191) reported that the administration of an antibiotic preparation without regard to aseptic techniques appeared to be the source of infection. The organism grew on blood agar and Sabouraud's maltose agar and was found to be pathogenic for mice, usually causing focal liver necrosis within 15 days following intraperitoneal inoculation.

\textit{Pasteurella multocida} mastitis has occurred in a few herds (9, 140, 218). Treatment with chlortetracycline, oxytetracycline, and streptomycin was generally unsatisfactory (9).
Udder infections with acid-fast organisms, other than *Mycobacterium tuberculosis*, following intramammary infusions with antibiotics in oily suspensions, have been observed (211, 216).

Comment. Herd surveys, pathologic changes seen in infected glands, and results of transmission experiments leave no doubt that infection is the primary cause of mastitis. Predisposing factors are considered secondary because, as indicated in the preceding section, they contribute to mastitis mainly by either increasing the chances for infection to occur or by causing clinical attacks of mastitis in infected quarters. In the Connecticut and New York State surveys, about 50% of the cows and 25% of the quarters were found to be infected, and the rate of infection among the cows on initial test was found to be about 22% for *S. agalactiae*, 11% for non-*S. agalactiae* streptococci, 16% for *M. pyogenes*, and less than 1% for other organisms. Infection with streptococci and with *M. pyogenes* may cause either subclinical or clinical mastitis. A decrease in milk yield of quarters infected with these organisms has been shown to occur in the absence of clinical evidence of mastitis.

DIAGNOSIS

Depending on the degree of inflammation at the time of examination, mastitis may be classed as subclinical, mild clinical, and severe clinical. Subclinical mastitis is characterized by changes in the milk, such as, an increased pH value, chloride content, or leucocyte count, in the absence of obvious swelling of the udder or clots in the milk. Mild clinical mastitis is characterized by a slight or moderate swelling or induration of one or more quarters and/or a visibly abnormal secretion, including clots revealed by use of the strip-cup. In severe mastitis, the udder is swollen, the secretion is grossly abnormal, and the animal may show an increase in body temperature, loss of appetite, depression, and nearly complete cessation of milk secretion. The latter form of mastitis is not common and is usually caused by capsulated coliform organisms, *C. pyogenes*, or highly toxigenic strains of *M. pyogenes*.

The term “mastitis” is similar to the term “abortion.” Both terms describe an abnormal condition, but fail to indicate the cause. The organisms most commonly associated with mastitis are *S. agalactiae*, non-*S. agalactiae*-streptococci, and *M. pyogenes*. Infection with any of these organisms is usually chronic, with clinical flare-ups occurring at irregular intervals. For this reason, diagnosis should be based on bacteriologic tests combined with tests to detect evidence of inflammation. In the diagnosis of *S. agalactiae* mastitis for control purposes, tests which approach 100% efficiency are required. In the other forms of mastitis, laboratory tests are useful in (a) selecting the antibiotic most likely to be effective in cases of clinical mastitis, (b) culling chronically infected animals, and (c) indicating the level of udder health in a herd.

Tests for evidence of inflammation. Barn tests that may be used to estimate the extent of mastitis in a herd are: physical examination of the milked-out udder for abnormalities, strip-cup test for clots, bromthymol blue test for altered pH, Whiteside test (230) as modified for field use (109, 116, 177), and the
"California Mastitis Test" developed by Sehalm and Noorlander (180). In the Whiteside and C.M.T test, the degree of precipitation or gel formation tends to be related to the leucocyte count (116, 145, 177). Schalm and Lasmanis (178) reported the following percentages of positive reactions to the Whiteside test: in S. agalactiae or M. pyogenes infection 50, coliform infection 41, coagulase negative micrococci and non-S. agalactiae streptococci infection 23, and "no significant" bacteria, 14. Of the barn tests, the strip cup is most commonly used; however, on the day of examination only about one-half of the S. agalactiae-infected quarters (231) and about one-fourth of the quarters infected with M. pyogenes are detected (205). Of the laboratory tests for evidence of mastitis, the leucocyte count is generally regarded as superior to other procedures. All tests for abnormality of milk give variable results from time to time when applied to chronically infected quarters, may be positive when applied to milk from uninfected cows in early or late lactation, and none indicates the nature of the causative agent.

Leucocyte count. Milk from normal quarters rarely contains more than 500,000 leucocytes per milliliter (4, 34, 89, 93, 110, 120, 121, 155) and the milk from infected quarters usually exceeds this number. Hucker (62) regarded a count of 500,000 per milliliter as indicative of an abnormal udder condition. Anderson (4) reported that the average leucocyte count of milk from quarters infected with M. pyogenes, non-S. agalactiae streptococci, and S. agalactiae was 1,400,000, 1,900,000, and 2,100,000 per milliliter, respectively. In comparison, the average count of 3,789 samples from uninfected quarters was 180,000 per milliliter. McEwen and Cooper (93) collected quarter samples at intervals of 3 wk. over a period of 14 mo. from a herd of 180 cows. They found that the percentage of samples with counts of 1,000,000 or more per milliliter was: for quarters infected with S. agalactiae, 82.8, S. dysgalactiae, 42.9, S. uberis, 55.5, M. pyogenes, 52.9, nonhemolytic coagulase negative micrococci, 7, corynebacteria (not C. pyogenes), 5.8, and no bacteria, 1.2. In the transmission experiments of Little (81, 82, 83), Murphy (120, 121), and Pattison and Holman (141), leucocyte counts of 1,000,000 or more per milliliter invariably followed the establishment of infection with S. agalactiae.

After the elimination of infection with streptococci and M. pyogenes by treatment, a period of from 1 to 5 wk. is usually required for the leucocyte count to return to normal (14, 195).

Noninfectious factors which affect the leucocyte count. Age, milking practices, and stage of lactation may affect the leucocyte count.

Counts of up to 300,000 per milliliter for heifers and counts of 500,000 to 1,000,000 per milliliter for older cows were considered normal by Little (83). Of 192 samples collected from normal heifers by Mochrie et al. (102), 94% contained less than 300,000 leucocytes per milliliter. In comparison, the average count of milk from uninfected cows with an average of 2.4 lactations was found to be 446,000 per milliliter (103).

Machine-milked normal cows gave milk with an average count of 368,000 per milliliter as compared with a count of 112,000 per milliliter for milk from
hand-milked cows, in observations reported by Cone (24). Although Mochrie et al. (102) observed no difference in the average count of milk from heifers that were machine-milked at 10 and 17 in. of vacuum, they (103) found that the average count for milk from older cows milked for normal, twice normal, and four times the normal milking duration, was 461,000, 761,000, and 1,130,000 per milliliter, respectively. It seems very unlikely that the latter abuse would occur under practical conditions. The animals used by Mochrie et al. were free of infection with streptococci, *M. pyogenes*, and gram-negative bacteria.

From a few days to 2 wk. following calving (varies for individuals), the milk from uninfected cows usually contains from 500,000 to 2,000,000 leucocytes per milliliter. As explained by Hadwen and Gwatkin (47), “The mucinous state of the milk in newly calved cows may call forth large numbers of polymorphonuclear leucocytes which will be found gorged with the mucin; later, both mucin and leucocytes disappear.” Murphy’s (110) observations on 64 uninfected quarters showed that the leucocyte count exceeded 500,000 per milliliter in only 0.5% of 770 samples collected from 1 wk. after freshening for as long as milking was done. McEwen and Cooper (93) considered that increased leucocyte counts obtained within 2 wk. after calving and for a period of 4 wk. before drying off were not significant.

*Leucocyte count of herd milk.* In tests made by MacLeod et al. (90) on milk from 31 herds, counts of 1,000,000 or more per milliliter were usually obtained when the percentage of udder infection was 40% or more. Cows were classed as infected when the milk from one or more quarters contained either *S. agalactiae* and 500,000 or more leucocytes per milliliter, or other organisms and 1,000,000 or more leucocytes per milliliter. Factors other than infection apparently affected the count sufficiently to prevent a close correlation between the cell count of herd milk and the rate of infection, when the rate of infection was under 40%. It appears that occurrence of cell counts in excess of 1,000,000 per milliliter in herd milk on more than one occasion would justify an investigation of the possible cause of the high count, including a herd survey to determine the incidence of udder infection and a check of the milking machine and milking practices.

*Cultural tests.* These are necessary in determining the identity of organisms associated with both subclinical and clinical mastitis. However, organisms found in milk samples, including *S. agalactiae*, may originate from the skin of the teat (1,76,234) and teat canal (73,121), as well as from within the udder. Contamination of the sample can be reduced by careful disinfection of the skin of the teat, especially the teat orifice. Leucocyte and colony counts have been used to differentiate between organisms associated with infection and those from the skin of the teat or teat canal. Milk from quarters infected with known mastitis organisms usually contains leucocytes in excess of 1,000,000 per milliliter, as previously shown, and usually more than 200 per milliliter of the infecting organism, as shown by numerous workers (4, 81, 93, 110, 120, 121, 129, 132,141,197). Although neither criterion of infection is infallible, both have been shown to be useful in the practical application of cultural tests.
Incubated milk. In some laboratory procedures, milk samples are incubated before culturing, primarily for the purpose of detecting *S. agalactiae*. From the start, it was found necessary to use chemicals in the incubated milk and in the blood agar medium to prevent or retard the growth of bacteria other than *S. agalactiae*. The chemicals most commonly used, alone or in combination, are sodium azide or thallium acetate to retard growth of gram-negative bacteria, and crystal violet to prevent the growth of micrococci (26, 37, 95, 137, 150, 159, 198, 234). None of these agents will inhibit growth of fecal streptococci. To separate *S. agalactiae* from these organisms and *S. uberis*, either esculin (37) or carbohydrates which are fermented by non-—*S. agalactiae* streptococci but not by *S. agalactiae* (156, 159) are included in the blood agar medium, in addition to crystal violet. Each lot of crystal violet should be tested before use to determine the concentration necessary to prevent growth of micrococci without preventing growth of *S. agalactiae*.

Interpretation of the probable significance of organisms isolated from incubated samples requires use of the leucocyte count. In routine tests made in Connecticut (150, 156, 158, 159), incubated samples are screened by microscopic examination of ten fields of stained films (0.01 ml. on 1 cm.²). Samples found to contain less than 1,000,000 leucocytes per milliliter and no streptococci are classed as negative; those containing streptococci are cultured on a selective medium for *S. agalactiae*, and those containing no streptococci and 1,000,000 or more leucocytes per milliliter are cultured on blood agar. Weakly hemolytic streptococcus colonies on the selective medium are classed as *S. agalactiae*, colonies surrounded by green or brown zones as non-—*S. agalactiae*, and gamma colonies are identified as *S. agalactiae* or non-—*S. agalactiae* by the modified CAMP test (126). Micrococci colonies producing hemolytic zones (over 1 mm. in diameter) on blood agar are classed as *M. pyogenes* and others as non-pathogenic. Quarters yielding samples that contain *S. agalactiae* and have a leucocyte count of 500,000 or more per milliliter are classed as *S. agalactiae*-infected. Quarters yielding samples that contain 1,000,000 or more leucocytes per milliliter are classed according to the type of culture obtained. Quarters yielding samples that contain organisms other than *S. agalactiae* and have a leucocyte count under 1,000,000 per milliliter are classed as negative. Samples from cows in early or late lactation, which contain 1,000,000 or more leucocytes per milliliter and which are culturally negative for streptococci and *M. pyogenes*, are classed as negative.

Disadvantages in the culturing of incubated milk are that (a) the leucocyte count must be determined, (b) two mediums must be used, a selective medium for streptococci and blood agar for *M. pyogenes*, and (c) contaminating fecal streptococci and coliform organisms are more likely to prevent the detection of pathogens than when fresh milk is cultured. Advantages are that (a) *S. agalactiae*-infected quarters (leucocyte count of 500,000 or more per milliliter) which at times may yield milk containing fewer than 100 organisms per milliliter (73, 120, 121) may be detected; and (b) the amount of cultural work can be reduced by culturing only those samples which contain either streptococci or
MASTITIS

1,000,000 or more leucocytes per milliliter, as shown by microscopic examination. While *S. agalactiae* can be cultured from some samples in which streptococci are not seen on microscopic examination, the findings of Sethi *et al.* (188) indicate that these organisms usually do not originate from inside the udder.

**Hotis test.** The test was described by Hotis and Miller (58) and is made by mixing 0.5 ml. of a 0.5% solution of bromocresol purple with 9.5 ml. of milk in a test tube, incubating the mixture, and observing the color after 24–48 hr. The appearance of yellow flakes on the side of the tube (58) or a thick yellow deposit in the bottom of the tube (108) is indicative of the presence of *S. agalactiae*. However, other organisms may produce a similar reaction (92, 96, 108) and some samples which contain *S. agalactiae* are not detected (13, 174). In routine use of the test, a wide range of color reactions are obtained which are difficult to interpret.

**Fresh milk.** Strict fore milk should be used because it usually contains more organisms and leucocytes than milk drawn after several streams have been discarded. Blood agar is the medium of choice, because it supports the growth of practically all organisms associated with mastitis, and the colonial characteristics aid in identifying the organisms present. Bovine blood should be used for the detection of hemolysis by *M. pyogenes* (97, 98); however, the blood from the donor cow must be free from antitoxin.

Gravity cream, sediment from centrifuged samples, and whole milk have been streaked on the surface of blood agar plates, and 1-ml. amounts of diluted milk (1:5 to 1:20) have been used in preparing pour-plates. In addition, fresh milk samples have been cultured on esculin blood agar (129, 132), esculin crystal violet blood agar (37), and on this medium plus 1:3,000 of thallium acetate (234). While the culturing of sediment or the use of pour-plates may be slightly more effective in detecting *S. agalactiae* than culturing whole milk or gravity cream on the surface of blood agar, these procedures are impractical for use in routine tests.

In the New York State mastitis research and control program (127), 0.01 ml. of a fresh fore-milk sample is streaked onto one-fourth of the surface of a bovine blood agar plate. The plates are examined after 24 hr. of incubation and a representative streptococcus colony, when present, is inoculated onto an esculin (0.1%) ferric citrate (0.01%) blood agar plate. The streptococci are classed as *S. agalactiae*-type if they do not split esculin, and as non-*S. agalactiae* if they do split esculin. Micrococci are classed as pathogenic or nonpathogenic on the basis of hemolytic ability. Other organisms are tentatively identified by their colonial characteristics. The presence of three or more colonies of the same type are usually considered significant, although true streptococcal infections usually show hundreds of colonies. When contamination sufficient to show by this method occurs, it usually appears as small numbers of several kinds of bacteria. In this case, three or more colonies are not regarded as significant, and an attempt is made to establish whether *S. agalactiae* is present.

Disadvantages of culturing 0.01 ml. of fresh milk on blood agar are (a) a few *S. agalactiae*-infected quarters, in which the number of organisms in the sample
is less than 100 per milliliter, may be missed, (b) streptococcus colonies on the blood agar require further identification, and (c) in some instances, the identification of more than one streptococcus colony may be advisable. Advantages of culturing fresh milk over incubated milk are that (a) less interference results from contaminants, (b) fewer false positives for \textit{S. agalactiae} infection are obtained, and (c) an estimation of the number of organisms present in the sample is possible.

Esculin bovine blood agar also has been used for culturing fresh milk. Oliver \textit{et al.} (132) streaked 0.05 ml. of fresh milk on the surface of the medium, and regarded as positive, samples that yielded "large numbers" of streptococci or \textit{M. pyogenes} and gave a positive Whiteside test. Neave \textit{et al.} (129) inoculated the surface of esculin blood agar with 0.025 ml. of milk and considered the appearance of ten or more colonies of either streptococci or \textit{M. pyogenes} as evidence of infection. The counts of milk from persistently infected quarters were seldom less than 200 per milliliter. Although few of the samples examined by Oliver \textit{et al.} (132) and none of those examined by Neave \textit{et al.} (129) contained \textit{S. agalactiae}, it appears that esculin blood agar may have an advantage over blood agar for the routine culturing of fresh milk, since the esculin should aid in separating \textit{S. agalactiae} from other streptococci.

The method adopted by the Technical Committee of the Agricultural Research Council of England (213) for the examination of fresh milk samples was described by Wilson and Slavin (234). A layer of esculin crystal violet blood agar (37) plus 1:3,000 of thallium sulfate or thallium acetate is placed in jars of the cold cream type, 4 cm. deep and 6 cm. wide, and equipped with a screw type aluminium lid containing an absorbent pad. The jars are incubated before use to test for sterility and to dry the medium. Small amounts of fore milk from all four quarters are drawn into the jar, spread over the surface of the medium, and the excess discarded. The jars are inverted, incubated 48 hr., and the number and kinds of colonies recorded. Streptococcus colonies are identified by the CAMP test (23). In cows showing clinical mastitis, the sample is drawn as described, but the lid is replaced before discarding the excess milk, and the jar inverted so that the absorbent pad in the lid is soaked with milk and clots. The excess milk is then discarded. These jars are marked to identify cows with clinical mastitis. At the laboratory, material is removed from the pad with a loop and cultured on blood agar to detect mastitis due to organisms other than streptococci. Disadvantages of this procedure are that (a) in large-scale testing the size of the jars, as compared with test tubes, would present a problem in handling, refrigeration during transportation, and incubation, (b) cows with mastitis due to organisms other than \textit{S. agalactiae}, and which is subclinical at the time of test would not be detected, and (c) the infected quarters would not be identified. The advantages claimed for this method are (a) a reduction in the cost of examining samples, as compared with most other methods, and (b) more streptococcal infections are detected than when fresh milk is cultured on blood agar.

In Denmark, a modified CAMP test was devised by Hovmand (60) and
Hauge and Ellingsen (52) for screening herds for *S. agalactiae* by culturing can samples, and for testing samples from individual cows. The test is made either by culturing the sample on blood agar containing thallium sulfate (1:3,000), crystal violet (1:750,000), and a suitable amount of *M. pyogenes* beta toxin, or by placing drops of milk on a plate containing this medium minus the toxin and inoculating the seeded areas with a beta toxin—producing culture of *M. pyogenes*. For the latter purpose, a stainless steel stamp with radiating spokes is used; hence, the method is called the "SUN CAMP" test. The disadvantage of this procedure is that infection with *M. pyogenes* is not detected. An advantage is that *S. agalactiae* colonies may be identified from their appearance on the original plate.

"Sterile" cultural tests on mastitis milk. Samples from quarters which show evidence of mastitis may be culturally negative for several reasons: (a) in severe clinical mastitis the secretion may be sterile due to the action of leucocytes and bacteriolysins, (b) certain strains of *S. agalactiae* are micro-aerophilic, as shown by Jepsen and Szabo (70), (c) the quarters tested may recently have been infused with an antibiotic (48), (d) in rare instances unusual organisms, such as the acid-fast organisms described by Tucker (216), may fail to grow on the medium used, and (e) irritation from abusive machine-milking may increase the leucocyte count (103) and in extreme cases cause clinical evidence of mastitis (11, 130, 231).

Factors not primarily associated with infection of the udder may, in rare instances, cause the secretion to be abnormal. Berger and Francis (11) found that in certain cows clots may appear in the milk at the onset of estrus. Certain diseases, such as leptospirosis, may cause the milk to be abnormal in appearance.

Comment. In general, a leucocyte count in excess of 500,000 per milliliter for milk from cows in full milk and a colony count of more than 200 per milliliter for fresh samples of fore milk are indicative of infection. Both the leucocyte count and colony count have limitations which must be taken into consideration in the routine diagnosing of udder infections. However, the two basic procedures described—cultural tests on incubated milk when combined with the leucocyte count and direct culturing of fresh fore milk—have been effective in programs for the elimination of *S. agalactiae*. In the use of cultural tests, it is essential that (a) contamination be reduced to a minimum by thoroughly cleaning and disinfecting the teats and teat ends, (b) samples be refrigerated during transit, and (c) the person making the tests have had experience with the method used.

CONTROL

Of the three principal etiologic forms of mastitis (*S. agalactiae*, other streptococci, and *M. pyogenes*), *S. agalactiae* mastitis is at present the only one that can be eradicated. Until improved methods are developed for preventing other types of infection, it will be necessary to rely mainly on management practices to reduce the rate of clinical mastitis in infected udders. A combination of good management and sanitation, and treatment based on a laboratory diagnosis, is necessary for maximum reduction in the rate of mastitis in a herd.
Management. Practices which can be recommended on the basis of available information are (a) comfortable, dry, well-bedded stalls, (b) treatment of severe teat injuries by a veterinarian, (c) preventing exposure of the udder to chilling by draughts, cold concrete floors, and night pasturing during cold weather, (d) sanitation to reduce the degree and extent of exposure to infection, and (e) proper machine-milking. The latter involves operation of the machine at proper vacuum level and pulsation rate, use of teat cup liners that are in good condition and, of special importance, the removal of the teat cup assembly after milk flow ceases.

Sanitation. Extensive research in England (1, 64) on the modes of spread of *S. agalactiae* shows that the main vehicles of transmission are (a) udder washing cloths and udder washing water, (b) milking-machine teat cups, and (c) milkers’ hands. It seems reasonable to assume that the same applies to *M. pyogenes* and other pathogenic organisms, since infected udders are a main source of infection. To avoid transfer of infection by the milkers’ hands, the English workers suggest that hand-stripping be replaced by machine-stripping (38, 64, 206, 213, 231, 233).

Disinfectants. Reduction of bacterial counts and prevention of udder infection should be regarded as two separate problems. High bacterial counts are due primarily to fecal and soil bacteria. These organisms are rarely the cause of mastitis. While sanitary methods commonly used may reduce bacterial counts sufficiently to meet regulations, they are not adequate in preventing the spread of infection because: (a) in ordinary udder washing a separate towel for each cow is not used and little attention is given to disinfecting the ends of the teats, (b) in dipping teat cups, the cups are not immersed for a sufficient length of time in the disinfectant solution, and the solution is not changed often enough, to maintain an active concentration of disinfectant, (c) after milking, milk left on the skin of the teat may provide a medium for the growth of any pathogenic bacteria that may be present, and (d) certain types of mastitis organisms, especially *M. pyogenes*, are not easily killed by disinfectants.

The principal chemicals used are chlorine in the form of sodium or calcium hypochlorite, quaternary ammonium compounds, preparations containing chlorhexidine, and iodine compounds. In vitro tests with these preparations indicate that, in the presence of 0.5–1% of milk, quaternary ammonium compounds are superior to chlorine against *M. pyogenes* and streptococci (78, 209), chlorhexidine is equal to quaternary ammonium compounds against *M. pyogenes* and superior to the latter against streptococci and *P. aeruginosa* (22, 209). The newer iodine compounds, called “iodophors”, were reported as being superior to chlorine and quaternary ammonium compounds against *M. pyogenes* and *P. aeruginosa* by Johns (71).

Sterilization of teat cups. Davidson et al. (28) reported that in one group of nine *S. agalactiae*-free heifers and two infected donor cows, when each animal was milked with a steam-sterilized teat cup cluster, two quarters of one cow became infected during a period of 16 wk. and, that in a similar group in which the teat cup clusters were not washed or sterilized between cows, five of the nine
heifers (36 quarters) became infected. Dipping teat cups in solutions of hypochlorite containing from 50 to 700 p.p.m. per million of chlorine has been found to be relatively ineffective in removing \textit{M. pyogenes} (53, 131, 209), and in reducing the bacterial counts of the liners (131, 209). In the experiments of Stewart \textit{et al.} (209) with teat cups contaminated with different organisms, a 1:5,000 solution of a quaternary ammonium compound and a 1:5,000 solution of chlorhexidine were more effective than a solution of sodium hypochlorite containing 200 p.p.m. of chlorine in removing \textit{M. pyogenes} and streptococci; the chlorhexidine solution was more effective against \textit{P. aeruginosa} than the two other solutions, and all of the three preparations were relatively ineffective in removing \textit{Escherichia coli}. Chowdowski (22) found that dipping teat cups artificially contaminated with milk containing \textit{S. agalactiae} in a 1% solution of CTAB\(^4\) removed the organism, whereas a 2% solution of Chloros\(^5\) failed to do so.

\textbf{Application of disinfectants to the skin of the teat.} For removal of \textit{S. agalactiae} from the skin of the teat, Chowdowski (22) found that a 1% aqueous solution of CTAB, a 3% CTAB cream, and 3% iodine in either 70% alcohol or in a solution of potassium iodide (6%) were effective. Preparations found ineffective were a 2% solution of Chloros, a 4% iodine ointment, a 1% solution of liquid soap, and a 1% solution of Lysol. Oliver \textit{et al.} (134) reported that immersing the teats of cows after their last milking in a 5% iodine tincture reduced the rate of "dry" cow infection with \textit{M. pyogenes} but not with non-\textit{S. agalactiae} streptococci.

No literature has been found on the value of dipping teats in an antiseptic solution after milking, as a preventive measure against infection. However, Chowdowski (22) suggests that teats with persistent sores be washed after each milking, first with a solution of a quaternary ammonium compound and then with water and, when dry, rubbed with penicillin cream or 3% CTAB cream.

\textbf{Disinfection of udder cloths.} It is generally agreed that a separate sterile cloth or paper towel should be used for each cow. If a common cloth is used and rinsed in a disinfectant solution between cows, it appears that quaternary ammonium compounds and chlorhexidine are preferable to chlorine. Chowdowski (22) reported that a 1% solution of CTAB removed \textit{S. agalactiae} from cloths contaminated with infected milk, and that 2% solutions of either Chloros or liquid soap failed to do so. However, the quaternary ammonium compounds are not highly effective against \textit{P. aeruginosa} and coliform organisms, according to Stewart \textit{et al.} (209).

\textbf{Comparison of relative effectiveness of sanitary practices.} Hughes (64) compared the effect of different procedures in reducing the spread of \textit{S. agalactiae}. A herd of ten \textit{S. agalactiae}-free cows plus one donor cow (\textit{S. agalactiae}-infected) was used. At the completion of each experiment the test animals were freed of infection by treatment. The donor cow was milked first and observations

\(^4\) CTAB, or Cetavlon, is the trademark for a quaternary ammonium compound.

\(^5\) Chloros is the trademark for a sodium hypochlorite preparation containing 10% of available chlorine.
were made for a period of 20–27 wk. No hand-stripping was used. In trials in which the teat cups were disinfected, two sets of teat cup clusters were used. As a set was removed from a cow it was rinsed in water and immersed in a hypochlorite solution containing 800 p.p.m. of chlorine for 4–5 min. The results were as follows: Disinfection of teat cups, sterile cloth for each cow and no disinfectant in the wash water—no established infections but *S. agalactiae* was isolated from the teats of all cows one or more times; disinfection of teat cups, separate cloth for each cow, and disinfectant in the wash water—*S. agalactiae* was isolated once from the teats of one cow; no disinfection of teat cups, sterile cloth for each cow, and disinfectant in the wash water—two cows became infected and *S. agalactiae* was isolated once from the teats of three cows. Hughes (64) concluded that failure to sterilize teat cups between cows resulted in transfer of *S. agalactiae* from cow to cow, but that the rate of spread was not as rapid as when a poor udder-washing technique was used.

Comment. The results presented indicate that quaternary ammonium compounds and chlorhexidine are preferable to chlorine, for use in water for washing udders and dipping teat cups for the prevention of udder infection. At best, all that can be expected from the presently available chemicals, as ordinarily used, is a reduction in the number of pathogens on the skin of the teats, milkers’ hands, and teat cups. Further research is needed on the evaluation of available chemicals, including the newer iodine compounds, in preventing udder infection, and to develop more effective preparations than those now available.

Segregation. Segregation of clinically affected animals was first suggested by Frank in 1875 and later by others (149). After the establishment of *S. agalactiae* as the principal cause of contagious mastitis, segregation and gradual replacement of animals infected with this organism were suggested as a control measure. In the first reports on the use of this method (100, 152, 153), *S. agalactiae* infection was eliminated from several herds in from two to five years, and a herd started with first-calf heifers remained *S. agalactiae*-free during the 3-yr. period of observation (152). These findings were confirmed by others (27, 51, 82, 172, 207).

Application of the program on a larger scale showed that the average annual rate of new infection was about 10% for 75 segregated herds and 27% for 25 herds which were not segregated (151). The elimination of *S. agalactiae* by segregation and replacement of infected cows is a long and tedious process, and use of the program is impractical in many herds.

Since the discovery of effective agents for freeing udders from *S. agalactiae*, systematic testing and treatment have largely replaced segregation. However, since a few infected cows fail to respond to treatment, the milking of treated cows after the negative ones is advisable. Purchased replacements should either be treated or segregated, tested and treated if infected, before addition to the herd.

Segregation of animals infected with organisms other than *S. agalactiae* appears to be impractical, although Schalm and Woods (184) recommended
the segregation of all shedders of *M. pyogenes*, since the infected udder is the principal reservoir of this organism.

**Chemotherapy.** *S. agalactiae* mastitis can be eliminated by treatment of all infected quarters, provided the sanitary measures used are effective in preventing new and reinfection. In herds with sporadic clinical cases of mastitis due to non-*S. agalactiae* streptococci and *M. pyogenes*, treatment may be justified primarily for the purpose of alleviating clinical symptoms; however, quarters freed from infection often become reinfected (38, 232). A high rate of clinical mastitis due to *S. uberis* and *M. pyogenes* indicates that some phase of management, especially poor milking technique, is involved (30, 32, 130, 231), and that attempts to alleviate the condition by treatment alone will fail (232).

In general, penicillin (66, 149, 158, 192, 193, 205, 222, 231) and chlortetracycline (39, 139, 149, 157, 158, 196, 201) have been found to be more effective than other antibiotics against infections with streptococci and micrococci, and streptomycin more effective than other agents against coliform infection (34, 35, 175). Combinations of penicillin with Furacin® (193), neomycin sulfate (192), streptomycin (39, 157, 158), and sulfathiazine (201) were not found to be more effective against *S. agalactiae* and *M. pyogenes* infections than penicillin alone.

In *S. agalactiae* infection, this organism can be eliminated from 80 to 90% of infected quarters by maintaining an effective level of penicillin in the milk for a period of from three to five days. This can be done by daily infusions of 25,000–100,000 units of penicillin in saline, or by one or two infusions (72-hr. interval) of 100,000 units in a water-in-oil emulsion or in an ointment. Increasing the dose beyond this level does not significantly increase the effectiveness of penicillin against *S. agalactiae* (219, 231).

Intramuscular injections of large doses of penicillin have been used successfully by a few investigators. Sadek (171) reported that four of five cows were freed of streptococcic mastitis by three injections of 5,000 units per pound of body weight given at 24-hr. intervals. Murphy and Stuart (122) eliminated *S. agalactiae* from two cows by injecting a total of 42,000,000 units; 6,000,000 at the first injection and 3,000,000 units at 12-hr. intervals for six days. Tucker and Fuller (221) reported that several cows, involving 15 quarters with clinical *S. agalactiae* mastitis, were cured by two or three injections of 10,000,000 units.

Failure of penicillin or chlortetracycline to remove *S. agalactiae* from infected quarters is attributed to the extent and nature of the lesions, particularly clots in the milk ducts which prevent diffusion of the drug to all foci of infection (54, 69, 72, 138, 203, 213). There is no evidence that failure of treatment is due to the development of penicillin-resistant strains (6, 45, 213).

Streptococcic infections other than *S. agalactiae* are fairly susceptible to chlortetracycline (157), penicillin (205, 232), and oxytetracycline (8), and the percentage of quarters reported as freed from infection, at least temporarily, ranges from 48 to 90. *S. dysgalactiae*, which is relatively uncommon in most herds, has been eradicated by treatment (232); however, reinfection often follows successful treatment of *S. uberis*–infected quarters (232).
M. pyogenes infections are fairly resistant to penicillin therapy. Recent infections are more likely to respond than are those of long duration (14, 91, 158). The amounts of penicillin, chlortetracycline, and oxytetracycline ordinarily used have been reported as freeing from 20 to 80% of treated quarters from infection (39, 139, 149, 157, 176, 196). Due to inadequate sanitation, reinfection frequently occurs (38). Massive doses of penicillin (1,000,000 units), combined with streptomycin (1 g.), were reported as 70% effective by Schalm and Woods (184). Failure of intramammary treatment has been attributed as due primarily to long duration of infection rather than to development of resistant strains, although some strains of M. pyogenes are highly resistant to one or more of the antibiotics commonly used (3, 36, 39, 105, 185, 186). The histopathology of M. pyogenes–infected glands shows that the organisms may occur in foci which are surrounded by connective tissue which renders them inaccessible to infused antibiotics (54). Sadek (171) reported that five of six cows with M. pyogenes mastitis were cleared of infection by three intramuscular injections of 5,000 units of penicillin per pound of body weight given at 24-hr. intervals.

Coliform mastitis (not common) usually responds to treatment with streptomycin. In mild chronic cases, intramammary infusions of 0.5 g. daily for four days eliminated the organism from 17 of 21 quarters treated by Schalm (175). In severe, acute cases, Easterbrooks and associates (31, 35) found that intravenous injection of sulfamethazine (250 ml. of a 25% solution), followed by intramammary infusions of streptomycin as soon as the udder became pliable, resulted in recovery in about 50% of the animals treated, and that the remainder nearly always responded to intramuscular injections of 2–5 g. of dihydrostreptomycin daily, until 24 hr. after apparent return to normal.

Bacillary and yeast infections do not respond to penicillin therapy. In P. aeruginosa mastitis, Tucker (218) and Tucker and Johnson (222) have reported a recovery rate of 40% following intramammary infusion of neomycin sulfate. Prophylactic treatment of dry cows for the prevention of C. pyogenes mastitis was suggested by Pearson (143, 144). No antibiotic has been found to be effective in the treatment of yeast mastitis.

Effect of eliminating infection. Milk production has been found to increase following the elimination of S. agalactiae. Edwards and Brownlee (40) reported that the yield of eight cows, following the elimination of S. agalactiae, was restored in the treated quarters in the next lactation. Edwards (38) observed one herd in which the average yield per cow increased from 600 gal. (during the year of treatment) to 800 gal. 5 yr. later. During this period, the percentage of cows which were retained beyond their fourth lactation increased from about 20 to 45%. Plastridge and Hale (156) observed a herd of 120 cows in which the initial rate of S. agalactiae infection was 78%. Over a 6-yr. period this organism was eliminated and the average yield per cow increased from 8,116 to 11,657 lb. The increase in yield was attributed, in part, to the culling of low-producing heifers which otherwise would have been needed to replace cows with mastitis. An illustration of the effect of eliminating S. agalactiae by treatment was given by Wilson (231). In a herd of 20 cows, the aver-
age annual yield (gal.) per cow before, during, and 1 and 2 yr. after the elimination of this organism, was 630, 660, 970, and 1,080, respectively.

In quarters which were infected with non-\textit{S. agalactiae} streptococci and \textit{M. pyogenes}, and which were successfully treated, Crossman \textit{et al.} (25) reported a recovery in milk yield during the lactation following treatment.

\textit{Comment.} In the absence of a diagnostic laboratory, a reduction in clinical mastitis can be accomplished by good management and sanitation, and the use of first calf heifers to replace cows with a history of repeated attacks of clinical mastitis; however, \textit{S. agalactiae} can not be eliminated. In areas where laboratory tests are available, these facilities should be used as a basis for treatment in the eradication of \textit{S. agalactiae}, and as an aid in diagnosing other forms of mastitis. Any herd program for the reduction of mastitis by management, sanitation, and treatment should be based on the advice of an experienced veterinarian.

\textbf{CONTROL PROGRAMS}

Programs primarily for the control of \textit{S. agalactiae} mastitis have been established in Connecticut, New York, Massachusetts, England, and Denmark.

\textit{Connecticut.} Laboratory testing of milk samples on a state-wide basis was started in 1940. The samples are drawn by fieldmen employed by the State Department of Agriculture, tested at the Storrs Agricultural Experiment Station, and treatment done at the owner's expense. Individual quarter samples are collected from \textit{S. agalactiae}-infected herds and pooled cow samples from \textit{S. agalactiae}-free herds. The samples are refrigerated from the time of collection until delivered to the laboratory. The interval between retests is 2–3 wk. when \textit{S. agalactiae}-infected quarters are treated and from 3 to 6 mo. for \textit{S. agalactiae}-free herds.

At the start, segregation of \textit{S. agalactiae}-infected animals was the basis for the control program. During 1946 to 1948 (147, 148, 156), results obtained with penicillin therapy in 25 herds showed that by prompt treatment of all infected quarters, frequent retests, and disposal of a few animals that failed to respond to treatment, \textit{S. agalactiae} could be eliminated from most herds in a relatively short time. Since 1946 (154), treatment of all \textit{S. agalactiae}-infected quarters, frequent retests until the herd is free of this organism, and sanitary measures have been recommended. In 1957, 1,191 herds (31,684 cows) were tested and of these 650 were \textit{S. agalactiae}-free.

The time required to eliminate \textit{S. agalactiae} has varied in different herds depending, in part, on the rate of initial infection, age of the cows, frequency of retests, whether or not cows which failed to respond to treatment were removed, addition of infected purchased replacements, and the extent of hygienic precautions against spread. Of 20 herds selected at random, the time required varied from 2 to 20 mo. and averaged 7 mo. (158). The initial rate of \textit{S. agalactiae} infection in these herds varied from 10 to 80%, and the proportion of infected quarters freed from this organism (including those reinfected) by one treatment averaged 80%.
Records on DHIA herds have shown that the average production per cow increased about 14% in 91 herds following elimination of *S. agalactiae*, and decreased about 10% in 24 herds which were negative and later became infected (49).

**New York State.** A research and control program was started in 1946. The laboratory tests are made in five regional laboratories. Individual quarter samples of fore milk are collected by a veterinarian who, at the same time, examines the cows for clinical evidence of mastitis (127, 142, 220). The samples are refrigerated during transportation. A special effort is made to correct faulty management practices, particularly improper care and use of the milking machine, preferably as a prerequisite to entrance into the testing program.

Progress has been made in reducing both the rate of *S. agalactiae* infection and the incidence of clinical mastitis. As shown in a recent report on 854 herds (44), the percentage of *S. agalactiae*-infected cows was reduced from 22 to 8, the incidence of cows yielding an abnormal secretion from 13 to 7, and of cows with physically abnormal udders, from 22 to 12.

**England.** As summarized by the Technical Committee on Mastitis of the Agricultural Research Council in 1955 (213), control measures have passed through the following stages: (a) treatment of infected quarters only, (b) treatment of all quarters of all infected cows, (c) treatment of all cows, and (d) treatment of all cows with disinfection of hands, udders, cows, and premises.

At the time of this report, treatment of all infected cows and use of antiseptic cream on the milkers’ hands and udder were considered sufficient unless infection is widespread, when treatment of all cows may be advisable. Their recommended program consists of: (a) cultural examination of milk samples, (b) treatment of all quarters of infected and dry cows with two doses of 100,000 units of procaine penicillin in an oil base, with an interval of three days between infusions; (c) a retest 6 wk. after treatment and, after a herd has passed two negative tests, a test once yearly, and (d) removal of cows that resist two treatments. All purchased cows are regarded as infected and are treated immediately.

Their recommended sanitary measures are: (a) Use an approved disinfectant in proper strength, and change the solution as soon as efficiency is impaired by organic matter; (b) use a separate udder cloth for each cow; (c) apply an antiseptic cream to hands of milkers before each milking; (d) disinfect the teat cups before each cow; and (e) replace hand-stripping by machine-stripping. In herds with a high rate of teat sores, they suggest delaying treatment of udder infection until the sores are cured.

The three main ways by which herds have become reinfeeted are (a) purchase of an infected cow, (b) heifers, purchased or home-bred, which may calve with *S. agalactiae* infection (rare), and (c) hands of a milker who has come from an infected herd.

They report that *S. agalactiae* has been eradicated from hundreds of herds in one county and from large numbers of herds in various parts of Great Britain with considerable increase in milk yield.

**Denmark.** Following a series of “Sun CAMP” tests on can samples, tests
on individual cows are made and all infected cows are treated in all quarters with three doses of 25,000 units of sodium penicillin in an aluminum monostearate base, given at intervals of 48 hr. (61). Hygienic measures used are (a) Once a day the skin of all teats of cows in the herd are rubbed with a penicillin cream (1,000 units per g.); (b) separate udder cloths saturated with a solution of sodium hypoehlorite are used on each cow before milking, (c) during treatment, the floor of the stalls are cleaned and disinfected, and (d) treated cows are milked last.

Of 52 \textit{S. agalactiae}-infected herds, 20 were freed of this infection by one treatment; in 25 herds reoccurring and new infections developed and these herds required from 3 to 7 mo. for elimination of \textit{S. agalactiae}; and seven remained infected after 20 mo. Tests were made at intervals of about 4 mo. Failure to eradicate \textit{S. agalactiae} from the seven herds was attributed to failure to use hygienic measures and to eliminate cows with persistent infection.

\textbf{Comment.} Progress in eradication of \textit{S. agalactiae} by the use of organized control programs has been made in England, Denmark, and in the United States. Although the cultural methods used have differed, there is general agreement on the following procedures: (a) cultural tests on all cows, (b) treatment of all infected quarters, (c) frequent retests and treatment of infected quarters until \textit{S. agalactiae} is eliminated, (d) disposal of cows that fail to respond to treatment, and (e) sanitation to reduce spread of infection.

Programs for the elimination of \textit{S. agalactiae} require the full cooperation of the herd manager and his veterinarian, the director of the program, and the laboratory. The cultural tests used must approach 100\% efficiency in the detection of \textit{S. agalactiae}-infected quarters. This requires that the samples be collected carefully and refrigerated during transportation, and that the tests be made or supervised by a person with training in bacteriology plus experience with the procedures used.

\textbf{ANTIBIOTICS IN MILK}

Since 1948, it has been recognized that the milk from herds in which cows have been treated for mastitis by intramammary infusion may contain enough antibiotic to inhibit cheese-starter cultures. According to Welch et al. (227), the cheese industry has tried to control this difficulty by insisting that their suppliers discard the milk of treated cows for three or more days following treatment, by testing milk for the presence of antibiotics, and by developing starter cultures which are resistant to penicillin.

Milk for human consumption which contains antibiotics is considered to be adulterated and a potential health hazard, since it is estimated that 10\% of the human population is allergic to penicillin (226). While penicillin has been found more often than other antibiotics in market milk (228), by law there is no tolerance for the adulteration of milk with penicillin or other antibiotics (228). In surveys of market milk made during 1955, the percentage of 474 samples found to contain penicillin (0.003 to 0.08 unit per milliliter) was 11.6 and, in surveys conducted in 1956, of a total of 1,706 samples, 5.9\% contained
penicillin (228). Antibiotic residues in milk are largely the result of improper use of mastitis preparations, which includes failure to conform to the instructions (required by law) on packaged preparations, to the effect that milk from treated quarters should be discarded or used for purposes other than human consumption for at least 72 hr. after the last treatment (228).

Levels of antibiotics following treatment. Following intramammary infusion of quarters of lactating cows with 100,000 units of penicillin in an oily base, the units of penicillin per milliliter of secretion after 12, 24, 48, and 72 hr. have been found to vary from 3 to 156, 1 to 25, 0.0 to 3, and 0.0 to 1, respectively (41, 56, 66, 168). When 300,000 units in an oily base were infused, Uvarov and Muggleton (224) found detectable amounts of penicillin (less than 0.5 unit) from four to eight days after treatment.

The penicillin content of the milk from 25 herds after infusion of the quarters of S. agalactiae-infected cows with 50,000 units of penicillin in an oily base was found by Hovmand et al. (61) to vary from 0.4 to 18 units (average 2.2 units) per milliliter on the first day following treatment. A total of 75 of the 193 cows in the 25 herds was treated. On the second day after treatment the average level was 0.04 unit per milliliter of herd milk.

Penicillin levels of the milk of cows following intramuscular injection of 5,000 units per pound of body weight were found to reach a maximum of 0.05 to 0.2 unit in from 5 to 12 hr. after treatment (41, 168, 171), then gradually to decrease to less than 0.008 unit by the fourth day (168). Similar amounts given intravenously by Edwards and Haskins (41) produced a level of 0.43 unit 2, 3, and 4 hr. after the injection. After 24 hr. the milk was negative.

Following intramammary infusion of 426 mg. of chlortetracycline, the concentration of the drug in micrograms per milliliter one, two, and three days after treatment, was found to be 50.4, 3.9, and 0.85 by Kanegis et al. (74). Similar findings were reported by Randall et al. (168); however, they found that traces of the drug persisted up to 3 wk. When 3 mg. per pound of body weight were injected intravenously, a maximum level of 1.6 µg. per milliliter of milk was found 8–12 hr. after treatment and none after three days (168).

Intramammary infusion of 426 mg. of oxytetracycline resulted in the presence of detectable amounts of the drug up to five days after treatment, in trials made by Randall et al. (168).

Test for antibiotics in herd milk. Tentative methods for the determination of antibiotics in milk were mimeographed in 1957 by the Food and Drug Administration, Washington, D. C. Although these methods provide an accurate estimate of the levels of different antibiotics in milk, they are relatively complex and require a minimum of 48 hr. for an analysis.

Simpler, more rapid, but less sensitive tests which may be of practical use to dairy plants have been described. The tests require from 2 to 4 hr. Several methods and the minimum concentrations of antibiotics that can be detected by their use have been reported as follows: Berridge (12), 0.015 unit of penicillin per milliliter; Neal and Calbert (128), 0.04 unit of penicillin, 0.3 µg. of chlortetracycline and oxytetracycline, and 4.0 µg. of streptomycin per milliliter;
Shahani and Badami (189), 0.1 p.p.m. of penicillin and chlortetracycline and 0.3 p.p.m. of streptomycin and oxytetracycline, and Shipper and Petersen (190), 0.05 μg. of chlortetracycline and 0.075 μg. of oxytetracycline per milliliter.

Suggestions for eliminating antibiotics in market milk. As a step in this direction, the penicillin content of single packaged doses of preparations for intramammary infusion was reduced to 100,000 units by order of the Deputy Commissioner of Food and Drugs on August 12, 1957. It seems doubtful that this measure alone will be highly effective. An educational program designed to inform dairymen of the need for withholding milk from treated quarters for a period of 72 hr. also has been proposed (226). Such a program should also include information on the limitations of antibiotics in mastitis control and the need for veterinary and laboratory assistance in their use. The use of tests for antibiotics by dairy plants to eliminate antibiotics from the milk supply was suggested recently by the USDA, Federal Extension Service.

Two other possibilities have been considered: 1. The restricting of the use of antibiotics to licensed veterinarians. 2. Requiring the addition of an innocuous dye, which would color the milk for a period up to 72 hr. after infusion, to any antibiotic that is packaged for intramammary use.

It appears that an educational program is preferable to other methods and that it may be necessary to supplement the program with tests for antibiotics at the dairy plant.

CONCLUSIONS

The view that infection is the primary cause of mastitis is supported by the findings that (a) mastitis can be produced experimentally by injection of udders with known etiologic agents; (b) infection is usually associated with mastitis, and (c) predisposing factors contribute to mastitis mainly by increasing the chances for infection to occur or by lowering the resistance of infected quarters, thereby increasing the rate of clinical mastitis.

Predisposing factors that influence the chances for infection to occur are degree of exposure, degree of inherited resistance to infection, age, ability of the teat canal to prevent passage of microorganisms, and prolonged milking. Factors that may lower the resistance of infected quarters are chilling, excessive feeding of high protein concentrates, stage of lactation, incomplete milking, prolonged milking, and the condition and type of teat cup liner. Recently, attention has been given to the effect of the ration on mastitis. The finding that certain rations increase or decrease to some extent the ability of milk to resist acid production by S. agalactiae probably has little to do with invasion of the udder by pathogenic bacteria, because evidence has been presented to show that invasion of the gland is controlled largely by the characteristics of the teat canal. Further research is needed to show that the ration may be a factor in determining the extent of clinical mastitis in infected quarters.

Infection usually follows passage of pathogenic organisms through the teat canal, either by growing their way through or by suction during milking. If infection is established, inflammation results in changes in the udder secretion.
and glandular tissue. These changes may or may not be associated with clinical symptoms of varying degrees of severity. Quarters infected with streptococci and *M. pyogenes* usually show mild clinical symptoms at irregular intervals, and a reduction in milk secretion with or without the appearance of clinical symptoms.

The principal organisms found in infected quarters are *S. agalactiae*, other streptococci (mostly *S. uberis*), and *M. pyogenes*. *S. agalactiae* infection is unquestionably contagious and there is some evidence that infection with *M. pyogenes* is contagious. Mastitis due to miscellaneous organisms, such as members of the coliform group, *P. aeruginosa*, *C. pyogenes*, and yeasts, is usually sporadic and is a herd problem in less than 1% of the herds surveyed in Connecticut and New York.

In diagnosis, tests that may be used in the barn are useful in estimating the extent of mastitis in a herd, and laboratory tests are essential when the control of *S. agalactiae* mastitis is attempted. In general, a leukocyte count of 1,000,000 or more per milliliter, excepting cows in early or late lactation, and a count of 200 or more per milliliter of a known pathogen, are indicative of infection. Routine laboratory procedures that have proven useful in diagnosis are (a) cultural tests on incubated milk when combined with a microscopic examination for the detection of streptococci and for the determination of the leukocyte count, and (b) cultural tests on fresh fore-milk samples from individual quarters, when the number and kinds of organisms found are used in interpreting the results. The use of any cultural method requires that skin contamination be reduced to a minimum by careful disinfection of the teat ends before drawing the samples, and that the samples be refrigerated during transit.

In control, prevention of infection is of first consideration and treatment is secondary. *S. agalactiae* can be eliminated by the use of frequent cultural tests and prompt treatment of all infected quarters, provided sanitary measures are reasonably effective in preventing new and reinfections. Programs organized for the control of *S. agalactiae* infection require the close cooperation of the dairyman and his veterinarian, the director of the program, and the laboratory. In most *S. agalactiae*–infected herds, the cost of eradicating this organism is more than compensated for by an increased milk yield, reduction in the rate of clinical mastitis, and prolonged productive life of cows in the herd. In quarters successfully treated, the increase in yield is most marked during the following lactation.

No adequate program has been devised for the elimination of udder infections with organisms other than *S. agalactiae*. This is primarily due to the ineffectiveness of present sanitary measures in eliminating pathogenic organisms, especially *M. pyogenes*, from the skin of the teat, teat cups, and milkers’ hands when hand-stripping is used. A reduction in clinical mastitis due to non-*S. agalactiae* streptococci and *M. pyogenes* usually can be accomplished by improved management, especially by the use of proper milking practices. These involve operation of the milking machine at the proper vacuum level and pulsation rate, removal of the teat cups as soon as milk flow ceases, and the use of
teat cup liners kept in good condition and replaced as soon as evidence of deterioration or loss of elasticity is seen. In herds in which management and sanitation are above average, a reduction in the rate of infection with *M. pyogenes* and non-*S. agalactiae* streptococci can be accomplished by the use of periodic tests and treatment of infected quarters, and by gradually culling cows with persistent infection.

To prevent adulteration of market milk with antibiotics, dairymen should be informed of the need for discarding milk from treated quarters for a period of 72 hr. after treatment and, if this fails, the next logical procedure would seem to be the testing of herd milk for antibiotics at the dairy plant.

For more efficient application of available knowledge on mastitis control, an educational program is needed. The program should emphasize (a) elimination of *S. agalactiae* by the use of treatment based on laboratory tests, (b) reduction in the spread of infection by good sanitation, (c) reduction in clinical mastitis by improved management, especially the use of proper milking techniques, (d) that treatment of clinical cases only will not reduce the rate of udder infections, and (e) that veterinary assistance is needed in planning and carrying out a herd program for the reduction of mastitis.

Research is needed to improve ways of preventing udder infection, especially with *M. pyogenes* and non-*S. agalactiae*. It appears that consideration should be given to (a) the development of more effective bactericidal agents and methods, for removing pathogenic organisms from the skin of the teat and teat-cups, and (b) continued improvement in milking machines.

The ultimate goal in mastitis control should be the establishment of herds that are free from all forms of udder infection, because infection results in pathologic changes in the mammary tissue and a reduction in milk yield, with or without the occurrence of clinical symptoms.

REFERENCES


(68) Ineson, P. J., and Cunningham, A. Investigations on Bovine Mastitis. 4. The Association of New Infections Caused by Streptococcus agalactiae with (a) Season and (b) Stage of Lactation. J. Dairy Research, 16: 139. 1949.


(106) Morse, G. E. Personal communication. 1957.


(137) PACKER, R. A. The Use of Sodium Azide (Na₃) and Crystal Violet in a Selective Medium for Streptococci and Erysipelothrix rhusiopathiae. J. Bacteriol., 46: 343. 1943.


(169) REID, J. J. Bovine Mastitis. II. A Study of Underlying Causes of Mastitis and Evalu-


(172) SCHALM, O. W. The Control of Streptococcic Mastitis in a Certified Herd. Certified Milk, 15: 11. 1940.


(197) Slanetz, L. W., and Bartley, C. H. The Diagnosis of Staphylococcal Mastitis, with Special Reference to the Characteristics of Mastitis Staphylococci. J. Infectious Diseases, 92: 139. 1953.


(210) Stuart, P. An Outbreak of Bovine Mastitis from which Yeasts were Isolated and Attempts to Reproduce the Conditions Experimentally. Vet. Record, 63: 314. 1951.


