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The Reliability of Various Diagnostic Tests and the Efficiency of Certain Therapeutic Measures in Control of Mastitis

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INTRODUCTION

Bovine mastitis is perhaps one of the most important problems confronting the dairy industry at the present time. It is impossible to ascertain accurately the losses due to this disease. A general idea, however, may be obtained from the reports of the various investigators. Shaw and Beam (1935) concluded that mastitis infection reduced milk production in affected quarters by approximately 22 per cent. In 1940, Bryan reported a survey involving over 300 dairy herds in Michigan. Eighty-six per cent of the herds and 26.2 per cent of all lactating quarters were infected with streptococci. Palmer (1942) reported that surveys in the United States and Europe showed that 25 per cent of the dairy cattle are infected with mastitis. He concluded that the annual total monetary loss in the United States from mastitis at that time was at least $200,000,000. In addition to the actual monetary loss, there is the added labor and cost of caring for the infected animals and the loss of milk unfit for human consumption.

Clinical Symptoms Mastitis.—Mastitis is described by Miller (1942) as any inflammation of the udder. This descriptive term indicates widely varying disease changes in the udder which are expressed or characterized by changes in the tissues and its secretory products. The factors contributing to this condition may be of infectious, chemical, or thermal nature, or injuries.

Mastitis is generally divided into three main classifications: acute, subclinical, and chronic.

The acute form may involve the entire udder or only individual quarters, but in either event there may be a severe reaction within the udder accompanied by a general systematic disturbance. Death of the animal may result, but even in surviving animals the affected quarters are usually no longer serviceable, and there is often sloughing of udder tissue.

The subclinical form may be so mild as to escape detection by the dairy-

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man because no visible changes may be present in the udder or its secretion and only bacteriological and biochemical tests will detect the actual changes in the secretion.

The chronic type is by far the most common form of mastitis. Usually it develops slowly with only occasional flare-ups. As the infection or irritation increases there will be a gradual replacement of the secretory tissue by connective tissue and changes in the gland secretions as well as lowered milk production occurs.

Bacteriology and Transmission-Organisms Commonly Found.—Numerous factors are associated with the cause of mastitis, but most of these are related to a bacterial infection of the udder. The chronic form of mastitis seems to be more commonly caused by the *streptococci*, *staphylococci*, and the *coliform* group of organisms as contrasted to the *staphylococci*, *corynebacterium*, and *coliform* organisms usually found with the acute form of mastitis. Hucker (1937) made a bacteriological study of 24 udders, aseptically removed from cows known to be free of mastitis and having milked one or more lactation periods. All were found to contain mastitis streptococci. Bryan (1940) concluded that *Streptococcus agalactiae* was responsible for 95 per cent of infectious mastitis and that the streptococci group accounted for 98 per cent of all infectious mastitis. Palmer (1941) regards the streptococci as responsible for 90 per cent of all mastitis. This is in strong contrast to the report of Packer (1947) who gives evidence to show that 847 samples of mastitis in Iowa which were examined bacteriologically, *Staphylococcus aureus* constituted 74.4 per cent, streptococci 17.9 per cent, and other organisms 7.7 per cent.

Bean *et al.* (1943) found *Corynebacterium pyogenes* to be the cause of mastitis in 23 quarters of 15 cows in 3 herds. The infections usually produced a chronic suppurative type of mastitis which often resulted in permanent damage to the quarters. Lovell (1943) claims that *C. pyogenes* is probably the most frequent cause of suppuration in cattle.

Experimental Production of Mastitis.—Attempts to establish experimental mastitis have met with varying degrees of success. Carpenter (1922) established a transient infection by introducing a culture of *Streptococcus agalactiae* into the udders of lactating cows. Jones *et al.* (1934) and Little (1937) found that to cause mastitis by introducing *Streptococcus agalactiae* into the udder, it was necessary to use a large number of organisms or give repeated exposures with cultures containing fewer organisms. Peterson *et al.* (1939) were able to infect only 9 of 34 quarters by placing cultures of *Streptococcus agalactiae* upon the teat orifice, but by using glass inoculating rods they infected 15 of 17 quarters with a single inoculation.

Carpenter (1922) inoculated broth cultures of *Staphylococcus aureus* into udders of 3 heifers and 2 cows. Four animals reacted severely and one died with a septicemia. Parshall (1934) inoculated a combination of *Staphylococcus aureus* and *Clostridium perfringens* and obtained a severe gangrenous form of mastitis. The acute form did not occur when either culture was inoculated separately. However, other workers, Little *et al.* (1935), Minett (1936), and Schalm (1944) were able to produce mastitis

by the inoculation of pure broth cultures of *Staphylococcus aureus*.

Hadley *et al.* (1930), Minett (1936), Bendixen (1938), and Schalm (1944), report that various strains of *Streptococcus pyogenes* produce mastitis in the udder of the cow when introduced by way of the teat duct.

Carpenter (1922) and Hadley and Frost (1933) report production of mastitis by inoculation of *coliform* organisms into cows udders. Reactions varied from mild to severe, but in all cases, the bacteria disappeared from the milk within four to six days.

Davis (1935) showed that dyes can be introduced into the teat duct by applying pressure to the teat, immersing it into the dye, and then releasing the pressure. The so-called negative pressure was not found within the udder by Espe and Cannon (1942) through use of barium and X-ray photographs. However, a small amount of the barium was drawn into the lower part of the teat duct. Weight of the barium might have accounted for the failure to demonstrate negative pressure.

PREDISPOSING CAUSES OF MASTITIS

Effect of Injuries—Teat Patency.—Since the universal use of mechanical milkers many workers have studied correlation of injuries such as bruises, etc., and teat patency with the incidence of mastitis. Christiansen and Nielsen (1934) and Bendixen (1935) found that when the teat closing mechanism was impaired, infection could easily be established by rubbing a culture over the incised wound, but not after healing had taken place. Little (1937) using negative pressure in combination with broth cultures of *Streptococci agalactiae*, found it easier to infect old cows with patent teats than first calf heifers with intact sphincters. Murphy (1944) indicated that the trend for infection is toward patency rather than non-patency. Mattick (1942) observed that in herds infected by *Streptococcus agalactiae*, chronic mastitis usually followed injuries while in non-infected herds, chronic mastitis did not follow injuries. Ferguson (1944) reports an observation on 317 quarters of 284 cows which developed mastitis following an injury to teat or udder. Eighty-nine per cent of these quarters were found to contain infectious organisms, while in the remaining 11 per cent which did not contain infectious organisms, the injury did not involve the teat duct. Espe and Cannon (1942) noted that soreness at the end of the teat was frequently associated sooner or later with mastitis in that quarter and in many cases the teat sphincter tended to remain slightly everted and become eroded. Udall (1947) reported observations on 160 machine milked cows, 85 per cent of which showed erosion due to injury of the teat to some extent. The type of milking machine used on these cows was changed and a reduction in the number of cases of mastitis was noted, also, by the end of the year, teat erosions of any significance had almost completely disappeared.

Brucella Abortus in Mastitis.—Considerable question has risen as to the role of *Brucella abortus* infection upon the incidence of mastitis. Gill (1933) and Simms and Muth (1934) observed that streptococcal udder infections were more prevalent in herds infected with *Brucella abortus*. Bryan and

Meyer (1936) and Hofstad (1942) found that *Brucella abortus* udder infections results in changes of the secretory tissue which simulate mild mastitis. Minett and Martin (1936) compared milk yields in herds containing both non-infected animals and animals which were infected with *Brucella abortus*. Lower production was found in the infected group. This, they concluded, was due to some abnormality of the secretory tissues of the udder.

Role of Milking Machines.—The relation of milking machines to the rate of mastitis infection has been a controversial subject for a number of years. Miller (1934) was not able to induce mastitis in the udders of young cows by machine milking using teat cups which had not been sterilized after having been in contact with infected animals. In direct contrast to this, is a report of Hay (1941) who believes that improperly sterilized teat cups may harbor infectious bacteria and serve to spread mastitis in a herd. Minett (1936) presented evidence that milking machines were responsible for many cases of mastitis. Reduction of the milking machine vacuum lowered the rate of infection. Hucker (1942) and Cone (1944) found that machine milking caused an increase in the leucocyte count of milk. Cone, also, found that the chloride content of the milk was increased by machine milking when compared to hand milking. In 1943, Kennedy suggested that mechanical milkers injure the teats, thus, providing new routes for bacterial infection. Ferguson (1944) indicated that some cases of mastitis may be traced to milking machines without bacterial invasion of the udder.

Complete vs. Incomplete Milking.—In 1936, Woodward *et al.* in an experiment with 15 cows, 11 of which were infected with *Streptococcus agalactiae*, failed to find that incomplete milking caused a more severe infection. In contrast to this is the report of Schalm and Mead (1943). In their first experimental series, approximately 2 pounds of milk was left in the udders after milking for a period of 13 weeks. Seven non-infected quarters continued to give normal milk, four of five Staphylococcus infected quarters often showed visible particles in the fore milk. All eight quarters infected with *Streptococci agalactiae* developed visible mastitis symptoms. In the second experimental series, animals were not stripped after normal machine milking, except twice weekly to determine the amount of milk left in the quarters (.03 to .4 pounds per quarter per milking) 12 non-infected quarters continued to produce normal milk. Four of six Staphylococcus infected quarters sometimes showed visible particles in the fore milk. Variable response was obtained from the quarters infected with *Streptococcus agalactiae*.

Mastitis in heifers.—Extensive observations upon the incidence of mastitis in heifers have been reported. Hucker (1937) found that 47 per cent of 21 udders removed asceptically from virgin heifers and calves contained mastitis producing streptococci in the tissue. Little (1940) found *Streptococcus agalactiae* at parturition in three quarters of one heifer that was in a herd of 33 cows, 15 of which had chronic mastitis. Mattick *et al.* (1941) reported a 4.5 per cent infection during the first 20 days of lactation in 377 heifers that were raised apart from adult cows. Plastridge *et al.* (1942)

found that the rate of infection among heifers during their first lactation was related to the total herd infection with *Streptococcus agalactiae* Schalm (1942) found similar results in a study of four herds. As the rate of infection of mature cows increased so did the incidence of infection among the heifers except when precautions against calfhood exposure were taken. Palmer *et al.* (1941) observed that *Staphylococcus aureus* was responsible for mastitis in 22 of 26 cases in heifers in three herds.

Miller and Heishman (1944) produced experimental *Streptococcus agalactiae* infection in two of 15 quarters of five yearling heifers that had been raised on *Streptococcus agalactiae* free milk. They, also, found 31.2 per cent of 77 heifers shedding streptococcus other than *Streptococcus agalactiae.*

DIAGNOSIS OF MASTITIS—LABORATORY TESTS

Chlorides.—Many investigators regard the chloride test as the most accurate indicator of fibrosis in the udder. Numerous tests have been developed for measuring the chlorides in milk; such as the field test developed by Hayden (1932) and many laboratory tests. Several workers have found that mastitis causes an increase in the chloride content of milk. (Bryan and Trout (1935), Sharp and Struble (1935), Turner (1936), Hastings and Peterson (1940), and Van Landingham *et al.* (1941)). It is the general decision of a large number of workers to regard a chloride content in excess of .14 per cent as abnormal.

Catalase.—Prouty (1934) found that a rough relationship exists between the number of leucocytes per ml. of milk and the catalase content of the milk. The catalase test has been used to some extent in the diagnosis of mastitis but it is generally agreed that it is not too accurate for this purpose.

Bromothymol blue.—The bromothymol blue-test is based upon changes in the pH value of milk. The normal pH value is approximately 6.6 (Roadhouse and Henderson, 1941) and the bromothymol blue indicator has a zone of color change ranging from yellow at pH 6.0 to blue at pH 7.6 (Harrow, 1946). It is generally recognized that cows with chronic mastitis will not give consistently positive reactions with this test. Tompkins *et al.* (1946) reports work in which individual quarters of 4905 cows were tested by bromothymol blue and cultural methods. Of 786 cows which were positive with the bromothymol blue test, 2.1 per cent were negative by cultural methods, 17.6 per cent contained staphylococcus, 10.3 per cent contained streptococci other than *Streptococcus agalactiae* while 70.0 per cent contained *Streptococcus agalactiae*. Fifty-two per cent of 520 cows which gave suspicious reactions to the bromothymol blue test were infected with *Streptococcus agalactiae.*

Leucocytes.—In 1910, Prescott and Breed, described a direct microscopic technique for estimating the number of leucocytes per ml. of milk. Hucker *et al.* (1932) examined milk from 221 cows and concluded that 3,000,000 or more leucocytes per ml. of milk was indicative of a past or present infection with streptococci. Little and Jones (1933) found that

quarters infected with streptococci or hemolytic staphylococci had a leucocyte count exceeding 1,000,000 per ml. of milk. Hucker (1933) reported that 500,000 or more leucocytes per ml. of milk indicated an abnormal udder condition, while Halverson *et al.* (1934) and Prouty (1934) regarded a leucocyte count of over 100,000 per ml. of milk as indicating an udder infection. In 1939, Plastridge *et al.* examined 2983 milk samples which were free of streptococci. Two thousand one hundred twenty-five of the samples contained no mastitis organisms as shown by microscopic and cultural examinations, but 80.2 per cent had leucocyte counts of under 100,000 per ml. of milk, 17.7 per cent had counts ranging from 100,000 to 500,000 per ml., 2.0 per cent had 500,000 to 1,000,000 leucocytes per ml. and only 0.1 per cent had counts over 1,000,000. Two hundred ninety-eight samples containing non-hemolytic staphylococci had average leucocyte counts of 222,000 per ml. of milk while 554 samples containing hemolytic staphylococci averaged 1,250,000 leucocytes per ml. of milk.

Hotis Test and Microscopic Examination of Milk.—The Hotis test for detecting mastitis streptococci in milk was first described by Hotis and Miller (1936). They reported 95 per cent agreement with blood agar plate cultural methods. Bryan and Devereux (1937) were of the opinion that the Hotis test did not give consistent results. Murphy (1939) found 95 per cent agreement with the Hotis test, on individual quarters, with blood agar plate cultures. Mattick *et al.* (1941) cultured 447 Hotis negative samples on Edwards medium and recovered *Streptococcus agalactiae* from 8.5 per cent of these samples. Extensive work by Miller (1943) involving 10,000 to 15,000 samples of milk, showed that the Hotis test was 85 to 90 per cent as accurate as blood agar plates for detecting *Streptococcus agalactiae* in milk. Schalm (1944) likewise found that the Hotis test detected 84.3 per cent of 594 samples known to contain *Streptococcus agalactiae*.

The Hotis test was modified by Bryan *et al.* (1939) by the addition of 1:50,000 brilliant green, 1:15,000 sodium azide, and 1:1,000 dextrose to the samples. This modification was reported to have increased the efficiency on the 24-hour reading from 60 to 81 per cent and the 48-hour reading from 68 to 87.6 per cent. Little (1941) reported that the addition of sodium azide delayed the development of positive reactions and that a 48-hour period might be required for the development of positive reactions.

Several workers Little (1940), Cone and Grant (1940) and Schalm (1944) have suggested using the Hotis test in combination with the microscopic examination for the detection of mastitis streptococci. Schalm, using a combination of these two tests, reported that on 4132 milk samples, 83 per cent of the samples known to contain *Streptococcus agalactiae* were positive, 12.1 per cent were suspects and 4.9 per cent were negative.

Microscopic Test.—Baker and Breed (1920) suggested the use of incubated milk in the microscopic detection of udder streptococci. In 1933, Hucker suggested that only chains containing 10 or more cocci be regarded as indicative of streptococcal infection. However, Bryan (1935) classified cows as infected with streptococcus when chains of 6 or more units were present in incubated milk.

Shaw *et al.* (1937) found that Bryan's method of microscopic examination detected 92.5 per cent of the cases of severe mastitis and 36.6 per cent of chronic cases while giving 7.1 per cent false positives on non-infected samples. In 1938, Fay reported that Bryan's microscopic examination method detected 96 per cent of the positive samples and 96 per cent of the true negative samples of mastitis.

THERAPEUTIC MEASURES FOR CONTROL OF MASTITIS

Various drugs administered orally and in later years various udder infusion processes have been employed in combating mastitis. The sulfonamides and penicillin have come into heavy usage in recent years and offer some promise. A brief review of the relative efficiency of each type of treatment follows:

Sulfanilamide.—In 1942, Kakavas *et al.* reported the use of homogenized sulfanilamide in oil for the treatment of cows infected with *Streptococcus agalactiae* . Ninety-four per cent of the positive quarters were cleared of infection. Sanders (1943 and 1943a) observed that iodized mineral oil may be applied to the mammary tissues with safety and it was used in combination with sulfanilamide for treating 50 lactating quarters infected with streptococcus. Microscopic examination of the milk indicated that the streptococcus had been eliminated in 90 per cent of the quarters. Swett *et al.* (1944) studied the effectiveness of sulfanilamide in mineral oil and sulfadiazine-in-oil for treating various types of mastitis infections. The average rate of recovery was 77.6 per cent. In the acute cases 64.3 per cent were cleared, but the effectiveness of sulfonamide treatment was particularly low in the treatment of acute Pseudomonas infections. As a result of these treatments there was a decline of 4.21 per cent in milk production for the post-treatment period compared to the pre-treatment levels.

Christian (1948) reported on the use of sulfamethazine and penicillin in treating 154 chronic and 9 acute cases of mastitis. Of 119 infected quarters, 113 (94.9 per cent) were freed of infection in 2 to 5 treatments. Seventy-one per cent of 35 infected quarters were clinically free of mastitis after 6 to 10 treatments. Normal milk was produced by 8 of 9 treated quarters infected with acute mastitis within 3 to 6 days.

Penicillin.—In recent years, penicillin has been widely recommended and used in the treatment of mastitis. In 1945, Bryan *et al.* reported that the infusion of the sodium salt of penicillin was found to be relatively non-irritating to mammary tissue even when 400,000 units or more were injected. Murphy and Pfau (1945) reported that penicillin sodium was only slightly toxic. There was no direct sign of tissue damage and the milk yield was not adversely affected; however, there was a rise in the leucocyte count of the milk during the first stages of the treatment.

Bryan *et al.* (1945) also reported that after treatment of 32 cows with one or more infusions of 1,000 to 20,000 units of penicillin, all animals became free of *Streptococcus agalactiae* . The U. S. Bureau of Animal Industry in cooperation with the University of Maryland (abstract JAVMA, 1945), treated 46 cows showing streptococcal mastitis with penicillin con-

taining 68 to 95 oxford units per ml. and about 59 per cent of 76 infected quarters were cleared of *Streptococcus agalactiae*. Other streptococci were eliminated from 48.5 per cent of 19 infected quarters. Murphy and Pfau (1945) used 3 different dosage levels of penicillin in the treatment of *Streptococcus agalactiae* mastitis. One group of cows was treated with a single infusion of 5000 to 200,000 units of penicillin and was freed of infection. Another group received 10,000 units at 5 successive milkings and 6 of 10 treated quarters were cleared by this treatment. The third group received 20,000 units of penicillin at 5 successive milkings. All became free of infection.

Slanetz and Allen (1945) reported 93.2 per cent recovery of 55 *Streptococcus agalactiae* infected quarters by use of penicillin treatments. Fourteen quarters infected with staphylococci were treated with penicillin and 11 or 78.5 per cent recovered. Klein *et al.* (1945) used penicillin for treatment of 7 staphylococcus infected quarters and reported an apparent recovery in only 2 quarters.

Porter *et al.* (1946) used 7 different dosage levels of penicillin in treating 240 quarters of 120 cows infected with *Streptococcus agalactiae*. The recovery rates varied from 47 per cent for the 1000 unit level to 73 per cent recovery at the 100,000 unit level. Breazeale *et al.* (1947) treated 73 quarters for *Streptococcus agalactiae* infection, using penicillin (100,000 units per quarter per treatment) and 77.7 per cent of the treated quarters recovered. Of 37 non-treated quarters only 43.3 per cent became negative for *Streptococcus agalactiae*.

EXPERIMENTAL PROCEDURE

The Missouri Station dairy herd consisting of approximately 100 milking cows was used in these studies. This herd consists of Jerseys, Guernseys and Holsteins. The animals are housed in a stanchion type barn and milked by machine twice daily. Cows showing mastitis infections are milked last. Prior to milking, each cow's udder is cleaned with cloths soaked in chlorine water (250 parts per million) and at a temperature of 130°F.

The milking machines are sterilized by washing with a detergent and then rinsed in water containing 400 parts chlorine per million. Teat cups and inflaters are kept in a dilute lye solution during the interval between milking periods. Just prior to milking, all equipment is rinsed in chlorine water and then water only. The teat cups are rinsed in chlorine water and then water only prior to the milking of individual cows.

Diagnostic Tests Used

Leucocytes—The Breed Smear Method was used to determine the leucocyte count of the milk. In this method 0.01 ml. of milk was evenly spread over an area of 1 sq. cm. on a slide. When dry the slides were treated to remove fat, stained, and examined under the microscope using the oil immersion objective. If one or less leucocyte was found per field, the average of 100 fields was taken. If there were 5 to 4 leucocytes per field the average of 50 fields was used. If 5 leucocytes or more were present in each field, the average of 30 fields was used. The average count per field was

multiplied by the microscope factor, which expresses the area of each field, to determine the count/ml. A count of 500,000 or more leucocytes/ml. was considered as indicative of mastitis.

Chlorides—Two ml. of a 10% potassium chromate solution was added to an 18-gram sample of milk and this solution titrated to a reddish orange color with silver nitrate containing 8.63 gm. per liter. One ml. of this reagent corresponds to 0.01 per cent chlorides. Chloride values of over 0.14 per cent were regarded as positive.

Catalase—Fifteen ml. of milk was placed in a Smith fermentation tube. Then 5 ml. of 1 per cent hydrogen peroxide solution was added and the tube incubated for 3 hrs. at 37°C. The production of more than 1.5 ml. of gas in the closed arm was regarded as positive.

Bromothymol Blue—Five-tenths ml. of 0.1 per cent bromothymol blue dye solution was mixed with 2.5 ml. of milk in a small test tube. The samples showing a greenish-yellow color were regarded as normal while those showing a blue-green to blue or a bright yellow were classed as positive.

Potentiometer—This test was made using a Beckman glass electrode potentiometer. A pH of 6.5-6.8 was considered as normal. Samples showing pH values above and below that range were regarded as positive.

Hotis Test—The procedure used for this test was to add 9.5 cc of asceptically drawn fore-milk to sterile test tubes containing 0.5 cc of 0.5 per cent Bromcresol purple and incubate for 36 hours at 37°C. The tubes were examined at 24- and 36-hour intervals. Yellow flakes on the side of the tube, with or without acid production, were considered to be positive. Yellow sediment with no flakes on the sides of the tube and with or without acid production was considered to be questionable or suspicious. Tubes showing no acid production, no yellow sediment, and no yellow flakes were classed as negative.

Microscopic Test—A gram smear was made of the 24-hour Hotis samples and examined under the microscope using the oil immersion objective. The presence of several long chains (more than 8 cells per chain) was considered as indicative of *Streptococcus agalactiae* providing the Hotis tube was positive or even questionable.

PRESENTATION OF EXPERIMENTAL RESULTS

The Relative Efficiency of Diagnostic Tests.—One of the major problems for the prevention and control of mastitis is the early diagnosis of this disease. Early treatment affords the greatest possibilities for the elimination of the abnormal condition. Therefore, it is imperative that an accurate diagnosis of the condition be made as soon as possible. With this in mind, a study was made of the merits of various diagnostic tests commonly used to detect mastitis as compared to the findings obtained by the Hotis test and microscopic examination of the milk.

The tests studied were: **Leucocyte count, chlorides, catalase, bromothymol blue, and pH values.**

Figure 1 shows the various tests arranged in decreasing order of ability

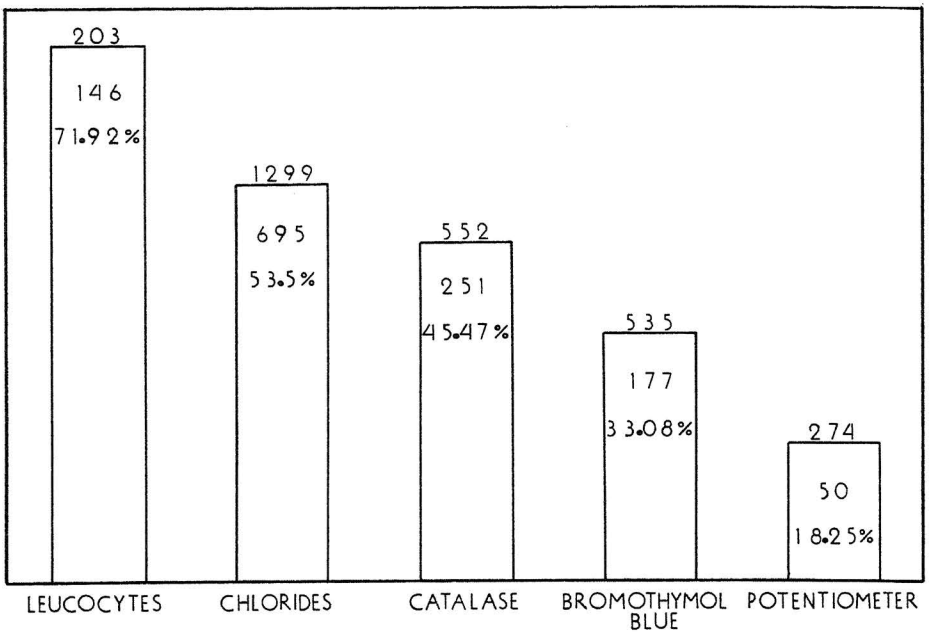

Figure 1.—A comparison of some of the diagnostic tests in detecting mastitis on quarters found to be positive by a combination of the Hotis test and microscopic examination of the milk.

to detect quarters shown to be positive by the Hotis test and microscopic examination.

Table 1 indicates the agreement found between the simple diagnostic tests and the Hotis test and microscopic examination on all samples studied during the course of the investigation. Many of these samples were taken at different intervals from the same quarter.

Leucocyte Count—Of 278 samples examined by both the Hotis and microscopic tests and the leucocyte count, 203 were declared positive by the Hotis and microscopic examination with the remaining 75 being classified as negative. The tests were in agreement on 146 (71.92 per cent) of the positive samples and 63 (84 per cent) of the negative samples. In the remaining samples where the tests disagreed, there was a tendency for the leucocyte count to show more false negatives than false positives, because 57 (28.08 per cent) samples were negative by the leucocyte count, but positive by Hotis and microscopic tests. Only 12 (16 per cent) leucocyte counts gave positives.

Chloride Test—Comparisons were made between the chloride test and the Hotis and microscopic examination on 3870 samples. Six hundred ninety-five (53.5 per cent) of the 1299 samples positive by the Hotis and microscopic examination were also positive by the chloride test. However, the chloride test showed 604 (46.5 per cent) false negatives. Two thousand

TABLE 1. - RELATIONSHIP BETWEEN VARIOUS DIAGNOSTIC TESTS FOR MASTITIS AND FINDINGS OF THE HOTIS TEST AND MICROSCOPIC EXAMINATION

Type of Test and Number of Samples									
Leucocytes		Chlorides		Catalase		Bromothymol blue		Potentiometer	
278		3870		1482		1852		940	
+	-	+	-	+	-	+	-	+	-
146 (203+)*	57	695 (1244+)	604	251 (552+)	301	177 (535+)	358	50 (274+)	224
12 (75-)	63	555 (2571-)	2016	105 (930-)	825	117 (1317-)	1200	54 (666-)	612

Figures in parentheses indicate the number of samples positive and the number negative according to the Hotis test and microscopic examination.

*Indicates number of positive quarters according to Hotis test and microscopic examination.

five hundred seventy-one samples were classified as negative by the Hotis and microscopic tests. By the chloride test 2016 (78.4 per cent) of these samples were also negative, but the remaining 555 (21.6 per cent) were false positives by the chloride test.

Catalase—Of 1482 milk samples examined for catalase activity and the presence of mastitis organisms by the Hotis and microscopic tests there were 552 samples positive by the Hotis and microscopic examinations. Of these samples, 251 (45.47 per cent) were also positive by the catalase test while the remaining 301 (54.53 per cent) were false negatives according to the catalase activity. The catalase test agreed with the Hotis and microscopic tests on 825 (88.73 per cent) of the 930 negative samples, however, 105 (11.27 per cent) were falsely declared positive.

Bromothymol Blue Test—A comparison was made between bromothymol blue test and the Hotis and microscopic examinations on 1852 samples. Of the 535 samples classified as positive by the Hotis and microscopic tests, only 177 (33.08 per cent) were also positive by the bromothymol blue test. This test indicated that 358 of the positive samples were negative. Of the 1317 negative samples 1200 (91.11 per cent) were also negative by the bromothymol blue test, with only 117 (8.89 per cent) being falsely classed as positive.

Potentiometer—The pH values of 940 milk samples were compared with the Hotis and microscopic findings on these samples. Two hundred seventy-four samples were classified as positive by the Hotis and microcopic tests, while the remaining 666 were negative. The pH value of the milk agreed with the Hotis and microscopic tests in only 50 (18.25 per cent) of the 274 positive samples. Two hundred twenty-four of these positive samples were falsely classified as negative by the pH determinations. A total of 612 (91.89 per cent) of the 666 negative samples were also negative according to pH determinations on the milk. The remaining 54 (8.11 per cent) negative samples were falsely classed as positive according to the pH values.

NATURAL RECOVERY DURING LACTATION AND THE DRY PERIOD

It has long been observed that many cases of mastitis seem to recover without treatment. Natural recovery must be taken into consideration when attempting to evaluate the relative merits of any treatment. Comparisons were therefore made on the rate of recovery in treated and non-treated cows during the dry period as well as during lactation.

As shown in Table 2, 32 (39.02 per cent) of the 82 quarters (37 animals) recovered without treatment in an average of 161.84 days which

TABLE 2. - RECOVERY OF TREATED AND NON-TREATED COWS FROM MASTITIS DURING THE DRY PERIOD

Group	No. animals	No. qtrs.	Qtrs. clear at 1st test after freshening		Treatment to test after freshening
			No.	%	Days
Treated	9	21	12	57.14	147
Non-Treated	37	82	32	39.02	162

TABLE 3. - RECOVERY OF TREATED AND NON-TREATED COWS FROM MASTITIS DURING LACTATION

Group	No. of animals	No. qtrs.	Qtrs. Clear				Avg. Interval	
			1st test		2nd test		Treatment to 1st test	1st to 2nd test
			No.	%	No.	%	days	days
Treated	140	289	205	70.9	145	50.19	23.0	54.0
No treatment	65	118	44	39.3	47	39.8	47.0	40.0

elapsed between the last test prior to the dry period and the first test after freshening. Contrasted with these figures are the results of various forms of treatment* given preceding the dry period. The number of animals in this group is limited however, 57.14 per cent or 12 of 21 quarters (21 animals) recovered in an average of 147 days between treatment and the first test after calving.

The group of animals in Table 3 were given various forms of treatment as shown in more detail (Table 4). Seventy and nine-tenths per cent or 205 of the 289 quarters treated (140 animals) recovered in an average of 23 days between treatment and the first post-treatment test. The second test was taken in an average of 54 days later and only 50.19 per cent or 145 of the original 289 treated quarters were still free of infection. In the non-treated group there were 65 animals with 118 infected quarters. With-

*Form of treatment shown in Table 4.

in an average of 47 days, 44 of these quarters (37.3 per cent) had recovered. A second test, 40 days later, showed that 47 or 39.8 per cent of the non-treated quarters had recovered and were free of mastitis infection.

RESULTS OF VARIOUS TREATMENTS FOR MASTITIS CAUSED BY STREPTOCOCCUS AGALACTIAE

An attempt has been made to evaluate the merits of some of the various treatments used for mastitis caused by *Streptococcus agalactiae*. A highly accurate comparison cannot be made because of the variation in the average interval between treatment and the first post-treatment test. The data on the results of various treatments used in this study are summarized in Table 4.

Sulfanilamide-in-Iodized Mineral Oil.—Three levels of treatment were studied; 80, 60, and 50 ml. dosages, infused into the udder at 24-hour intervals on four successive days. The infected quarters were stripped free of milk and the infused material worked well into the upper half of the udder.

Comparing infusion levels of 50 ml. and 60 ml., both of which consisted of 4 infusions 24 hours apart, it appears that the use of 60 ml. of sulfanilamide-in-iodized mineral oil is more effective than 50 ml. Thirty-six (90.0 per cent) of 40 infected quarters were freed of infection within an average of 13 days by infusion at the 60 ml. level as compared with 19 of 37 quarters (51.4 per cent) cleared within 12 days by 4 infusions of 50 ml.

The second test period, 58 days after treatment, for the group treated with 50 ml. showed the number of quarters free of infection had decreased to 13 or 35.1 per cent of the original 37 infected and treated quarters. In the group treated at the 60 ml. level, the number of quarters free of infection decreased to 30 or 75 per cent of the original 40 treated quarters in an average of 33 days between treatment and second post-treatment tests.

The data on the 80 ml. level is insufficient to draw conclusions.

Penicillin.—Two types of penicillin were used in this study, the standard G penicillin and amorphus penicillin. A few animals received both series of treatments, but in each case, more than 8 weeks had elapsed between the treatments.

The results of various penicillin treatments as reported in Table 4, indicate that the frequency of infusion is more important than the dosage level (between 25,000 and 50,000 units). Also, there seems to be little difference in this study between the efficiency of standard G penicillin and amorphus penicillin.

4, 4-Diamino-Diphenyl-Sulfone and Amorphus Penicillin.—Only a limited amount of the sulfone compound could be obtained, therefore only three cows were treated by this means. One of the cows was removed from the herd before a second test could be obtained, thus the data for only two cows is given. Both cows had a long history of mastitis which had resisted various types of treatment. Thirteen days after treatment, 5 (62.5 per cent) of the 8 treated quarters were free of infection and at the second test which was made 156 days after the treatment, 50 per cent of the 8 quarters were still free of infection.

TABLE 4*. - RESULTS OF INFUSIONS OF SULFANILAMIDE IN IODIZED MINERAL OIL, PENICILLIN, TYROTHRICIN, SULFONE, AND "SULVETIL" IN MASTITIS INFECTED QUARTERS

Treatment	Infusion Amount	Number Infusions	Infusion Interval	No. Animals	Qtrs. treated	Treated qtrs. clear				Average Treat. to 1st test	Interval Treat. to 2nd test
						1st test		2nd test			
						No.	%	No.	%		
	ml.		Hrs.							days	days
Sulfanilamide in Iodized Mineral Oil	50	4	24	19	37	19	51.4	13	35.1	12	58
	60	4	24	18	40	36	90.0	30	75.0	13	33
	80	4	24	3	5	5	100.0	5	100.0	14	30
Penicillin (G)	25,000 Units	4	12	6	17	14	82.4	10	58.8	34	73
	40,000 Units	2	24	15	26	12	46.1	8	30.8	39	139
	50,000 Units	2	24	28	69	59	85.5	37	53.6	14	56
	50,000 Units	4	24	15	25	16	64.0	8	32.0	45	118
Penicillin (Amor.)	40,000 Units	2	24	19	34	18	52.9	15	44.1	40	125
	50,000 Units	2	24	7	15	10	66.7	5	33.4	5	33
Sulfone Amor. Pen.	10 gm. 40,000 Units	1		2	8	5	62.5	4	50.0	13	156
Tyrothricin	50 ml.	1		5	13	11	84.62	10	76.92	13	38
Sulvetil*	40 ml.	2	24	3	8	2	25.00			10	
Totals and percent				140	289	205	70.9	145	50.19	23	54

*Sulvetil not included in totals and percent clear.

Tyrothricin.—Five cows with 13 infected quarters were treated with 50 ml. of this agent. The first test was made an average of 13 days after the treatment and at this time, 11 or 84.62 per cent of the 13 quarters were clear. Thirty-eight days after treatment, a second test was made and 10 quarters or 76.92 per cent of the quarters were found to be free of infection.

Sulvetil.—Only 3 animals with 8 infected quarters were treated with "Sulvetil" (Sulfanilamide in infusable mixture) and only one test was made after treatment. This test, made 10 days after treatment, found that 2 or 25 per cent of the 8 treated quarters had become free of infection.

EFFECT OF INTRAMAMMARY TREATMENT UPON MILK PRODUCTION

A major consideration in the intramammary treatment of lactating cows is the effect such treatment will have upon current milk production.

The effect of 63 series of sulfanilamide-in-oil and 19 series of penicillin in water treatments upon milk production was studied and the results are given in detail in Table 5. The average milk production was computed for 3 days prior to treatment, during treatment, and for 7 days post-treatment.

Treatment with penicillin did not cause significant change in the milk production.

Sulfanilamide treatments were analyzed according to number of quarters treated, amount of milk produced, and amount of infusion. In all groups, a significant decrease in production was noted and pre-treatment level was not reached until approximately 7 days following treatment.

Decreases in production compared to the number of quarters treated were not significant. The decrease did not vary directly with production levels; however, high producing cows seemed to reach pre-treatment levels earlier than low producers. Production decreases from different dosage levels were not significant although 5 of 6 cows given 80 ml. infusions, declined more than when 60 ml. were infused into the udder.

DISCUSSION

The experimental work reported herein is primarily concerned with the determination of the causative organisms found in mastitis infected cows in the Missouri Station herd; a comparison of the various diagnostic tests for determining mastitis, and the efficacy of udder infusions in freeing the infected glands of what might be considered mastitis-producing organisms.

A survey of the herd from 1942 to 1948 indicated that the rate of infection was 24.13 per cent on a quarter basis. *Streptococcus agalactiae* organisms were found to be predominant in 75.6 per cent of the infected quarters. *Staphylococcus aureus* was predominant in only 15.3 per cent of the cases and other organisms including coliform and *Pseudomonas aeruginosa* were found in 9.1 per cent of the cases. Therefore most of the therapeutic measures followed were directed toward the elimination of *Streptococcus agalactiae*.

A comparison of the efficiency of the various diagnostic tests, used under field conditions by many veterinarians and dairymen, with the Hotis

TABLE 5. - THE EFFECT OF INTRAMAMMARY TREATMENT FOR MASTITIS UPON DAILY MILK PRODUCTION

Treatment Grouping	Iodine and Sulfanilamide in Mineral Oil Daily for Four Days											Penicillin in H_2O Twice Daily for two days
	Number of Quarters Treated				Quantity of Milk, lbs.			Size of Injection				
	One	Two	Three	Four	1-20	21-40	41-up	50 ml.	60 ml.	80 ml.	60 ml.	
Number of Cows	30	12	8	13	17	34	12	16	41	6	6*	19
3 days before inj.	26.11	26.62	42.34	29.48	11.99	28.64	53.92	20.81	32.17	28.80	31.73	29.22
2 days before inj.	26.32	26.69	42.05	30.08	12.02	29.02	53.86	20.36	32.40	30.53	31.83	29.76
1 day before inj.	26.27	26.93	41.99	30.10	11.94	29.01	54.11	20.33	32.46	30.38	32.03	29.16
Ave. preinj. milk	26.23	26.73	42.13	29.88	11.98	28.88	53.96	20.49	32.34	29.90	31.85	29.37
1st day of inj.	23.94	23.23	39.00	25.26	10.47	24.64	51.81	16.63	29.32	28.22	30.63	28.51
2nd day of inj.	23.85	23.23	38.53	26.59	10.28	25.26	51.21	17.59	29.27	27.78	30.25	27.08
3rd day of inj.	23.24	24.05	39.04	27.58	10.20	25.34	51.81	18.72	29.31	25.87	30.13	----
4th day of inj.	23.47	24.14	38.25	27.12	10.11	25.41	51.36	18.77	28.97	27.37	29.26	----
Avg. while treated	23.64	23.67	38.70	26.65	10.27	25.18	51.56	17.94	29.23	27.33	30.08	27.81
1 day after inj.	23.74	25.23	38.01	27.25	10.44	25.90	51.14	19.34	29.35	26.73	28.78	27.82
2 days after inj.	24.14	25.33	38.89	28.92	10.56	26.89	51.04	19.52	30.92	27.05	29.58	27.72
3 days after inj.	24.33	25.66	39.24	28.42	11.14	26.83	51.64	19.43	30.37	27.53	29.65	27.74
4 days after inj.	24.38	25.48	41.09	29.37	10.84	27.46	52.48	20.06	30.87	26.85	29.82	28.91
5 days after inj.	24.39	25.82	39.86	29.62	10.72	27.31	52.89	19.78	30.88	27.18	29.56	29.46
6 days after inj.	24.56	25.33	39.08	30.35	11.12	27.29	52.61	19.64	30.93	27.60	29.55	28.75
7 days after inj.	24.96	25.24	40.41	31.15	10.91	27.84	53.98	20.06	31.44	28.33	32.07	29.03
Change Mean	-2.59	-3.07	-3.43	-3.23	-1.71	-3.71	-2.40	-2.56	-3.11	-2.57	-1.77	-1.56
during Std.	± .84	±1.29	±1.25	± .95	± .41	± .85	± .39	± .85	± .69	± .50	± .67	± .99
treat- error	-25.5	-12.1	-10.1	- 9.7	- 4.4	-25.5	- 7.1	-12.1	-25.5	- 4.6	- 4.1	-13.1
ment Range	to	to	to	to	to	to	to	to	to	to	to	to
	+ 0.2	- 0.4	- 0.1	+ 1.1	- 0.3	+ 1.0	+ 1.1	- 0.3	+ 1.1	- 1.1	- 0.2	+ 4.1

*Same 6 cows which were later treated with 80 ml. of sulfanilamide in iodized mineral oil.

test and the microscopic examination of the milk for chain organisms does not indicate very close agreement. As indicated in Table 1 the commonly employed brom-thymol-blue test detected only 33.08 per cent of the positive quarters; the chloride test, using above 0.14 per cent chlorides as positive, 53.5 per cent; the catalase test 45.47 per cent; the pH values 18.25 per cent and the leucocyte count of the milk 71.92 per cent for the most favorable agreement with the Hotis test and microscopic examination.

It would appear that if satisfactory progress is to be made in the detection and treatment of mastitis under field conditions more facilities for the use of such diagnostic tests as the Hotis test and the microscopic examination of the suspected milk must be made available to dairymen and practitioners. The milk of many cows contained an excessively high percentage of chlorides without the presence of *Streptococcus agalactiae* or other mastitis producing organisms. In many cases this occurred in older cows which had a previous history of mastitis infection. In general the chloride test and the brom-thymol-blue test often give reactions not directly attributable to mastitis infections and can be relied upon much less than the leucocyte count as a significant test where facilities are not available for conduct of the Hotis test and microscopic examination of the milk. These two last named tests are by all odds the most reliable for diagnostic purposes and should be used more widely in mastitis control programs.

In several cows where *Streptococcus agalactiae* was found no other symptoms of mastitis were present and in all cases could not be used as evidence of an established infection. These observations bear out the prevailing opinion of many investigators that the presence of *Streptococcus agalactiae* does not always mean a positive case of mastitis, but that the conditions for the establishment of a foci of infection vary a great deal in different cows and perhaps between quarters. The failure of mastitis producing organisms to increase progressively and become established when present in the udder is not too well understood, but may be associated with the general body resistance of the animal, inhibiting factors in the milk, blood, or udder tissues, and perhaps to weakened virulence of the organisms present.

It is difficult to accurately evaluate the effect of therapeutic measures in mastitis treatment. Some cows tend to recover in the absence of treatment, and often there may be re-infection of quarters which appear as negative on the first tests following treatment. In these studies the intramammary infusion of sulfanilamide-in-iodized mineral oil resulted in about 75 to 80 per cent of the treated quarters appearing free of *S. agalactiae* on tests made 12 to 14 days after infusion. Diagnostic tests (Hotis and microscopic examination) made 30 to 58 days later, however, showed only about 70 per cent of these same quarters to be negative. The most successful treatment with sulfanilamide and iodized mineral oil involved 60 ml. infusions on 4 successive days. There seems to be no logical explanation for the improvement 60 ml. infusions gave over 50 ml. except possibly the virulency of the organisms differed. The two dosages were not compared simultaneously and the 50 ml. infusions followed the use of penicillin and "Sulve-

til" in some instances and only the more virulent organisms were surviving after these treatments.

Penicillin infusions resulted in 46 to 85 per cent of infected quarters showing negative on the first test (about 30 days) following treatment. Generally speaking the best results in freeing the infected quarters of *S. agalactiae* were obtained where 50,000 units of penicillin per infusion was administered in 2 to 4 doses over a 24-hour period.

Twenty-two staphylococci infected quarters were treated with penicillin using two 50,000-unit infusions at 24-hour intervals. Two successive tests 30 and 60 days apart showed 18 or (81 per cent) of the quarters free of these organisms.

The therapeutic measures employed were not particularly harmful in reducing milk production following their use. This is definitely one of the advantages of sulfanilamide and penicillin infusions. The cow may be treated during lactation without great loss in milk production thereby utilizing the advantages of early treatment, which are all important, particularly in an out-break of mastitis of herd size proportions with organisms of high virulency involved.

It is interesting to note that about 39 per cent of the infected quarters made what might be termed "natural recovery." This occurred during the dry period and during lactation. This phenomenon is not unusual and illustrates the role of the animals own system in combating infections. The role of "natural recovery" no doubt should be given consideration in evaluating the effectiveness of therapeutic treatment, but certainly should not diminish the necessity or use of such treatments.

SUMMARY

1. A study of the incidence of mastitis and its control was made on the Missouri Station dairy herd for the seven-year period of 1942 to 1948 inclusive. In 1942 a survey showed that 24.13 per cent of the lactating quarters were infected. *Streptococcus agalactiae* organisms were found predominating in 75.6 per cent, *Staphylococcus aureus* in 15.3 per cent, and other organisms including *Pseudomonas aeruginosa* in 9.1 per cent of infected quarters.
2. A comparison of the relative efficiency of various diagnostic tests to detect mastitis, with the Hotis test and the microscopic examination of the milk used as a standard, was made. The results are:
   (a) A leucocyte count of more than 500,000 per ml. was considered as positive. This test was in agreement with the Hotis and microscopic tests for 71.9 per cent of 203 infected quarters while giving 16 per cent false positives.
   (b) A chloride content of 0.14 per cent was found to detect 53.5 per cent of 1299 positive quarters; however, it showed 21.6 per cent false positives.
   (c) The production of 1.5 ml. of gas in the catalase test detected 45.47 per cent of 552 positive quarters, but 11.3 per cent of the negative quarters were falsely classed as positive.
   (d) The bromothymol-blue-test showed agreement with the Hotis and microscopic tests in 33.1 per cent of 535 positive quarters with only 8.9 per cent of the negative quarters being falsely classed as positive.
   (e) The potentiometer or pH values of the milk detected only 18.3 per cent of 274 quarters which were positive by the Hotis and microscopic test. Of the negative quarters, 8.1 per cent were classed as positive by the pH determinations on the milk samples.
3. Udder infusions using sulfanilamide-in-iodized mineral oil were made at 50 ml., 60 ml., and 80 ml. dosage levels on 82 quarters. An average of 75 to 80 per cent of the treated quarters were found to be free of *S. agalactiae* 12 to 14 days following treatment and about 70 per cent free 58 days after infusion.
4. Penicillin (Standard G and Amorphous forms), was used in the treatment of 186 quarters. Dosages of 25,000 to 50,000 units of penicillin injected in 2 doses over a 12- to 24-hour period resulted in 46 to 85 per cent recovery with an average of approximately 75 per cent and compared favorably with the sulfanilamide in oil. The frequency of penicillin infusion was more important than total dosages in obtaining recovery.
5. Thirteen quarters treated with Tyrothricin showed 84.6 per cent recovery based on tests conducted 13 days after treatment.
6. Eight quarters treated with a combination of sulfone and amorphous penicillin resulted in 62.5 per cent recovery and 8 quarters treated with "Sulvetil" 25 per cent 13 days after treatment. The effect of infusing

sulfanilamide-in-oil and penicillin in water into the udder upon daily milk production was studied. The penicillin treatment did not cause any significant change in the milk production.

7. In all groups of sulfanilamide treatments, a slight, but significant, decrease in production was noted. The pre-treatment level was not reached until approximately 7 days after treatment. The decrease in production did not vary directly with production levels and was not correlated with the number of quarters treated or the dosage level.
8. Many cows affected with mastitis undergo "natural recovery". A recovery rate of 39.02 per cent was noted for animals which had passed through a dry period between diagnostic tests but had not been treated. The average time elapsed between tests was 162 days. During lactation, non-treated animals showed a natural recovery rate of 37.3 per cent within 48 days and within 87 days after having been classed positive 39.8 per cent had undergone recovery.
9. Following treatment of the infected quarters and the "natural recovery" undergone by some cows the incidence of *Streptococcus agalactiae* infection dropped to approximately 10 per cent of the lactating cows in the herd. Several older cows with a long history of *S. agalactiae* infection in the udder were not freed of the organisms by any of the treatments given.
10. A practice of running diagnostic tests (Hotis and microscopic examination) on the milk of each cow in the herd; the dividing of the milking strings into groups based on negative, suspicious, and postive reactions, and milking the cows in that order has resulted in the reduction of mastitis to occasional flare-ups, even though all animals are not free of *Streptococcus agalactiae*.

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