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# An Attempt to Develop Resistance in the Udder Against Streptococcus Dysgalactiae and Escherichia Coil

Donald E. Otterby

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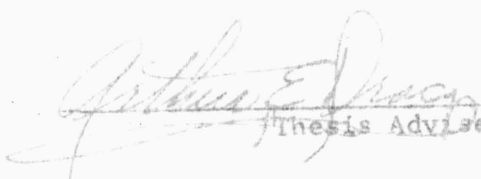
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**AN ATTEMPT TO DEVELOP RESISTANCE IN THE UDDER  
AGAINST STREPTOCOCCUS DYSGALACTIAE AND  
ESCHERICHIA COLI**

By

**Donald E. Otterby**

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

  
\_\_\_\_\_  
Thesis Adviser

  
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Head of the Major Department

A thesis submitted  
in partial fulfillment of the requirements for the  
degree Master of Science at South Dakota  
State College of Agriculture  
and Mechanic Arts

June, 1958

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Much of the emphasis when dealing with mastitis has been on cure rather than prevention. Despite the extensive investigations of antibiotics and other chemo-therapeutic agents as a cure for mastitis, a completely satisfactory answer to the mastitis problem has not been found.

Recent research has suggested that the mammary gland is capable of producing large amounts of antibodies when a foreign protein is introduced into the udder. Since antibody production of the udder has been demonstrated, there is reason to suspect that the udder of the cow might be injected with an antigen and produce an immunity to bacterial invasion. Thus this investigation deals with an attempt to immunize cows' udders with Streptococcus dysgalactiae and Escherichia coli.

## INTRODUCTION

The problem of mastitis in dairy animals has received much attention in recent years. As the business of dairying has developed and grown, mastitis has become more significant to the farmer and the processor. The economic loss from reduced production and sale of milk, loss of breeding animals, veterinary and medical fees is paramount.

Much of the emphasis when dealing with mastitis has been on cure rather than prevention. Despite the extensive investigations of antibiotics and other chemo-therapeutic agents as a cure for mastitis, a completely satisfactory answer to the mastitis problem has not been found.

Recent research has suggested that the mammary gland is capable of producing large amounts of antibodies when a foreign protein is introduced into the udder. Since antibody production of the udder has been demonstrated, there is reason to suspect that the udder of the cow might be injected with an antigen and produce an immunity to bacterial invasion. Thus this investigation deals with an attempt to immunize cows' udders with Streptococcus dysgalactiae and Escherichia coli.

Some cases of mastitis may develop into chronic mastitis. Swelling of the udder and altered milk are characteristic of this type. As the disease progresses, connective tissue develops in the udder and the udder becomes hard and at times atrophied (33).

West, (75) however, has classified mastitis in 5

## REVIEW OF LITERATURE

types which are as follows:

Mastitis had been defined as an inflammation of the mammary gland. The term refers to many maladies of the gland regardless of the fact that the causative agent may be bacterial, chemical, thermal or a resultant from injury.

Mastitis is usually classified into 3 major categories-- acute, subclinical and chronic. Acute mastitis is that which affects the parenchyma and the interstitial tissue of the udder. In addition many body reactions occur and particularly a rise in temperature is likely. Occasionally in this type of mastitis gangrene is manifested. Acute mastitis is sometimes fatal and in surviving animals the affected quarter is usually partially damaged so that the normal function of the gland is impaired.

Subclinical mastitis is usually undetected by the dairyman. The presence of mastitic organisms may be determined by the leucocyte count, chloride tests or other chemical means. Cases may arise in which the foremilk is thickened and the affected quarter is slightly swollen, but the major portion of the secretion may appear to be normal. Sometimes the acute and subclinical cases of mastitis may develop into chronic mastitis. Swelling of the udder and altered foremilk are characteristic of this type. As the disease progresses, connective tissue develops in the udder and the udder becomes hard and at times atrophied (33).

West, (75) however, has classified mastitis in 6



types which are as follows:

1. Mild chronic. In this type of mastitis there are no local or general symptoms which may be used in detecting the disease. Laboratory culturing of milk samples is the only method of determining the presence of mastitic organisms. Proper environmental conditions are necessary for this type of mastitis to become severe.
2. Severe chronic. A bromothymol blue test will readily indicate the presence of the infection. Palpation of the udder reveals fibrosis or scar tissue.
3. Subacute. In this type flakes appear in the milk. Laboratory tests show positive results and palpation of the udder shows induration which is an evidence of infection.
4. Acute. The infected quarter feels firm and hard and yields a stringy, ropy or cheesy secretion. The rectal temperature of the animal rises to  $103-105^{\circ}\text{F}$ . Impaired appetite, stiffness and lameness may also accompany acute mastitis.
5. Peracute. Indications of this type are a hot and swollen quarter with a watery, straw colored or reddish fluid. This is apt to be accompanied by a high fever, weak rapid pulse and rapid shallow breathing. The average decrease in yield was

6. Gangrenous. This is a final stage of acute mastitis. The secretion of the udder has an offensive odor and is red or yellow in color. The temperature of the animal is either normal or subnormal.

Incidence of Mastitis

The incidence of mastitis is high and therefore of great economic significance. A loss of 5 million tons of milk per year has been estimated for Europe (3). In the United States 1 cow in 4 can be expected to be afflicted with mastitis, while in a country such as Italy the incidence is 29 per cent (4).

Mastitis is chiefly caused by micro-organisms such as Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Micrococcus pyogenes, Escherichia coli, Aerobacter aerogenes and Pseudomonas aeruginosa. Other organisms such as Pasteurella miltocida, Corynebacterium pyogenes, yeasts and acid-fast bacilli have also caused mastitis (4). Murphy (46) reported that Streptococcus agalactiae, the other streptococci, the staphylococci and the bacilli account for more than 99 per cent of the mastitis although more than 20 causes are known. Little and Plastridge (33) also state that most mastitis is caused by the organisms listed above. Hale et al. (21) compared milk yield, based on butterfat production, with the presence or absence of Streptococcus agalactiae infections in 328 herds over a 5 year period. The average decrease in yield was

10.2 per cent per cow in herds which became infected, while an increase of 14.3 per cent was noted in herds which became negative to Streptococcus agalactiae.

The stage of lactation and age of the animal has been correlated with the susceptibility to mastitis infection. In a survey of the incidence of mastitis, Alberts and Bryan (1) reported that streptococci were isolated from 40 per cent of the samples from individual cows. The above mentioned authors found that the incidence of streptococci increased from 23.9 per cent in first lactation heifers to 85.7 per cent in cows in their twelfth lactation. Spencer and Kraft (67) contended that advancing age is not the primary explanation for increased infection of mastitis, although it may be a factor. Their contention was that the animals on a sound management program increased in incidence of mastitis from 5 to 35 per cent in the first 4 lactations; however, this was followed by a gradual decrease. Improper management led to an incidence of 60 to 72 per cent during the first 5 lactations with a sharp increase in successive lactations. According to Oliver et al. (50) the incidence of mastitis regressed as lactation progressed.

Numerous predisposing factors contribute toward the animal's susceptibility to mastitic infection. Recessed teat orifices, nodular swellings in the teat duct, teat lesions, weak sphincter muscles of the teat orifice and large pendulous udders are all factors for high incidence of

yards also contribute to infection of the udder. Chance of infection (4, 33, 40).

infection is enhanced by irregular and improper milking and unsanitary stable conditions also contribute to a favorable substantiate the relationship of heredity to the incidence environment for growth of infectious organisms. Mastitis; of mastitis; however, there are reports to suggest that an animal's genetic makeup has a possible bearing on the re- extreme temperature changes and wet weather, sistence to mastitis. Workers (33) have found that when

comparing 2 cow families, 1 family was more resistant to milking machine on the incidence of mastitis. When compar- infection than the other. When comparing dam-daughter pairs, ing routine-, severe- and mild-machine milking, and hand a positive correlation was found between heredity and sus- milking, workers at the United States Department of Ag- ceptibility to mastitis. The suggestion has been made that riculture found marked improvement in cows with mastitis when breeding for high producing cows, a larger udder has been developed which is more vulnerable to mastitis. Le- milking and almost complete recovery when hand-milking was gates and Grinnells (32) surmised, in a study of 956 animals, performed (39). Gembrel (16) observed less mastitis occur- that some progress toward the reduction of mastitis by ring in herds where suspended type milking machines were simple mass selection has been accomplished. These workers used. Porter et al. (15) showed but very little difference demonstrated the lack of phenotypic relationship between in the incidence of mastitis when cows were milked with high mastitis and production.

or low vacuum milking machines. Similar reports have been given by other workers (19, 42, 43). Leaving the milking creating an environment which is conducive for infective machine on for an extensive period of time has resulted in organisms to gain entrance and establish themselves in the a significantly higher incidence of mastitis than where a bovine udder (4, 8, 20, 33, 40). Factors such as stalls of normal period of time was allowed before the machine was the correct size and adequate bedding help to prevent udder removed (19, 42, 43). Deed and Oliver (11) studied the injury and reduce mastitis. Loose housing has been found to effect of teat-cup liner design on the incidence of gas- offer more freedom from udder injury than stanchion barns (7, titis. They compared the molded liner with the extruded 33). Removal of barbed wire and other material from yards liner and found the incidence of mastitis to be 12.7 per cent and pastures reduces the possibility of udder injury. Muddy

yards also contribute to infection of the udder. Chance of infection is enhanced by irregular and improper milking and unsanitary stable conditions also contribute to a favorable environment for growth of infectious organisms. Mastitis infections are frequently observed during periods of extreme temperature change and wet weather.

Numerous studies have been made on the effect of the milking machine on the incidence of mastitis. When comparing routine-, severe- and mild-machine milking, and hand milking, workers at the United States Department of Agriculture found marked improvement in cows with mastitis when switched from routine- or severe- to mild-machine milking and almost complete recovery when hand-milking was performed (39). Gambrel (16) observed less mastitis occurring in herds where suspended type milking machines were used. Porter et al. (56) showed but very little difference in the incidence of mastitis when cows were milked with high or low vacuum milking machines. Similar reports have been given by other workers (19, 42, 43). Leaving the milking machine on for an extensive period of time has resulted in a significantly higher incidence of mastitis than where a normal period of time was allowed before the machine was removed (19, 42, 43). Dodd and Oliver (11) studied the effect of teat-cup liner design on the incidence of mastitis. They compared the molded liner with the extruded liner and found the incidence of mastitis to be 12.7 per cent

of new infection by the method of drying off cows at the end of the lactation period. Oliver et al. (46) who studied methods of drying off cows, compared the incidence of new infections in cows dried off by intermittent milking with the incidence of new infections in cows dried off by of feed fed has varied. Some workers (33) have reported that high protein feeding greatly aggravated the mastitic condition. Frost (15) found that the udder would not break down through high protein feeding. Pounden et al. (58) developed a method to determine the resistance of milk samples to Streptococcus agalactiae. Milk samples were taken from cows on 2 different rations--one of grass hay and heavy grain feeding and the other on alfalfa hay and meadow crop silage with little grain. Each milk sample was inoculated with Streptococcus agalactiae and the resistance of the milk to the organism was determined. After an 8 week period the milk from the cows on the heavy grain feed exhibited a significantly higher susceptibility to the organism than did the light grain feeding. Later experiments (59) showed that the addition of corn silage to a ration of mixed hay and grain caused a marked loss in resistance of milk to Streptococcus agalactiae. On the other hand other workers have observed no difference in low, medium or high protein feeding; therefore this would suggest little relationship between feeding and incidence of mastitis.

Conclusions as to the effect of feeding and the type of feed fed has varied. Some workers (33) have reported that high protein feeding greatly aggravated the mastitic condition. Frost (15) found that the udder would not break down through high protein feeding. Pounden et al. (58) developed a method to determine the resistance of milk samples to Streptococcus agalactiae. Milk samples were taken from cows on 2 different rations--one of grass hay and heavy grain feeding and the other on alfalfa hay and meadow crop silage with little grain. Each milk sample was inoculated with Streptococcus agalactiae and the resistance of the milk to the organism was determined. After an 8 week period the milk from the cows on the heavy grain feed exhibited a significantly higher susceptibility to the organism than did the light grain feeding. Later experiments (59) showed that the addition of corn silage to a ration of mixed hay and grain caused a marked loss in resistance of milk to Streptococcus agalactiae. On the other hand other workers have observed no difference in low, medium or high protein feeding; therefore this would suggest little relationship between feeding and incidence of mastitis.

Transmission of mastitis from cow to cow is often attributed to the teat cups of the milking machines. The transmission of mastitis from cow to cow was investigated by Davidson et al. (10) by using 9 sets of monozygous twins. There has been some postulation as to the incidence

of new infection by the method of drying off cows at the end of the lactation period. Oliver et al. (48) when studying methods of drying off cows, compared the incidence of new infections in cows dried off by intermittent milking with the incidence of new infections in cows dried off by cessation of milking. Differences in the incidence of new infection in cows dried off by either method was found to be insignificant. However, quarters which had been previously infected showed the disease again at the time of testing during the dry period. Other work done on infection during the dry period showed that about 50 per cent of the new infections during the dry period persist until freshening (51). The suggestion was made that natural sealing of the test sinus is of little importance in protecting against infection. Disinfection of the teats at the drying off time has been found to lower the infection rate during the dry period (49). Cows going dry were washed with a hypochlorite solution of 800 p.p.m., dried and then each teat was immersed in a tincture of iodine. This was done twice over a 24-hour period. The treatment reduced the infection caused by Streptococcus dysgalactiae and Streptococcus uberis into the teat canal. Rubbing Micrococcus pyogenes var. aureus. Rate of infection caused by Streptococcus uberis was not affected.

Transmission of mastitis from cow to cow is often attributed to the teat cups of the milking machine. The transmission of mastitis from cow to cow was investigated by Davidson et al. (10) by using 9 sets of monozygous twins. One twin was severely and died of septicemia 10

Four artificially infected donors were introduced into the herd. The organism used was Streptococcus agalactiae. The twins were divided into 2 groups, 1 animal of each set was assigned to each group. During the milking of 1 group, sterilized equipment was used for each cow while in the other group no disinfection of milking equipment was made between cows. In both groups the infected donors were milked first. In the group in which sterilized milking equipment was used, 2 quarters became infected, while in the other group 11 quarters became infected. This was found to be significant at the 4 per cent level. In other experiments where the teat cups had been smeared with infective organisms, infection was not induced. Furthermore no new mastitis cases developed when the teat cups were not disinfected between cows. Artificial inoculation of infective organisms into the udder has resulted in some severe cases of mastitis. Mastitis was produced by introducing 1 milliliter portions of a 24 hour serum broth culture of Streptococcus dysgalactiae, and Streptococcus uberis into the teat canal. Rubbing a bacterial culture over the teat meatus produced no infection whatsoever; introduction of the organism into the teat was necessary before infection could be effected experimentally (33). In an experiment 5 animals were injected with Staphylococcus aureus broth culture intramammarily. Four of the cows reacted severely and 1 died of septicemia 10



days later. Inoculating coliform organisms into the udder produced infection in the treated quarters, but after a few days the infection subsided and the bacteria disappeared from the secretion (33). Feeding of mastitic milk and drenching cows with broth cultures of Streptococcus agalactiae has failed to establish the organism in the udder.

Infective organisms are present almost everywhere; their primary requirement is a means of entry into the udder before they are able to induce mastitis. Chodkowski (9) has studied the distribution and survival of Streptococcus agalactiae at various locations other than the bovine udder.

Of the animals examined 38 per cent showed bacteria on the teats; about 5 per cent of the hides exhibited the presence of the organism. Bacteria isolation samples were secured from the milkers' clothing 20 per cent of the time during the experimental period. Isolated bacteria samples were also taken from 81 per cent of the teat lesions during this same time interval. Survival of the organism was determined to be 60 days in manure, 43 days in soil, 30 to 38 days on cloth, 29 days on hair, 10 days on straw, 4 days on floors, 5 days on windows, and 8 days in a bucket. Staphylococci were found on the skin of the cow and particularly on the udder, while other workers found coliforms to be present in numerous quantities in manure and bedding (33).

Diagnosis. Bromothymo blue test is used to detect changes in pH. Diagnosis of mastitis is important in the treatment

of the infection. Various physical, cultural and chemical means have been developed to determine the disease. The examination of the udder secretion has been one which has been resorted to by most farmers and has been used as an indication of the disease (33). The secretion in advanced cases of mastitis is usually abnormal and the secretion often contains flakes or clots and yellow or watery fluid in early stages of the disease. Macroscopic examination is not altogether reliable in that the animal may be affected for weeks or months without giving evidence to visibly altered milk.

Udall and Johnson (71) developed a practical method for classifying the udder. This system was based on the amount of tissue change within the udder. To determine the amount of fibrosis, each quarter of the udder was palpated and graded from no fibrosis to marked fibrosis. A number 1 udder was considered to be normal in all respects, while a number 2 udder had one or more quarters with slight fibrosis. An udder with 2 or more quarters with distinct induration was graded number 3. A number 4 udder was 1 with 2 or more quarters with extensive induration replacing most of the glandular tissue. The final classification included the history, production, strip cup, bromothymol blue reaction and bacteriological results.

The bromothymol blue test is used to detect changes in pH. When performing the test 0.5 milliliter of the

bromothymol blue was added to a test tube containing 2.5 milliliter of milk. If the solution turned a greenish-yellow color, the sample was considered normal. A light-green color was classed as suspicious while a greenish-blue to blue was regarded as positive. The darker colors of the reaction indicated the greater alkalinity (33).

A test using bromocresol purple has also been used to indicate pH changes in milk. A blue-gray color resulting from the addition of the reagent to the milk indicated a normal milk. Bromocresol purple has a yellow range below a pH of 5.2 so acidity of milk can also be determined. Varying degrees toward alkalinity are brought out by shades of blue to deep purple (33, 40). Jones and Helwig (25) instilled bromocresol purple solution into capillary tubes and allowed the reagent to dry. When testing an animal, the samples of milk were drawn directly from the teat into the tube. Incubation of the tubes for 18 to 24 hours and the presence of a yellow color indicated the presence of Streptococcus agalactiae while a blue-purple color indicated absence of the bacteria. Leucocyte count may not occur until several. The Whiteside test (33) employs the use of normal sodium-hydroxide mixed with milk. One drop of the sodium-hydroxide is mixed with 5 drops of milk for about 20 seconds. If the sample turns viscous or a precipitation occurs, the milk is positive. This test is based upon the cell count of the milk. If the count is abnormally high,

the cells are ruptured by the sodium-hydroxide releasing the protein of the cell. This in turn reacts with the trace sodium-hydroxide to form a gel or precipitate.

The salty taste of mastitic milk has led to the formation of tests for chlorides. A method has been described to test for the chlorides in milk in which a mixture of 2 milliliters of 10 per cent potassium chromate solution in 18 grams of milk was titrated with silver nitrate to a slight reddish-orange color. One milliliter of the silver nitrate corresponded to 0.01 per cent of chlorides. An excess of 0.15 per cent chlorides in the milk was considered abnormal (33).

The leucocyte count has been used quite extensively in the determination of mastitis. A cell count above 500,000 per milliliter of milk is regarded as abnormal and indicative of udder infection. Little (33) believes that the foremilk of heifers should be under 300,000 leucocytes per milliliter while normal milk of older cows may be expected to have counts from 500,000 to 1,000,000 per milliliter. An increase in leucocyte count may not occur until several days after infection and the count may vary greatly from day to day. However, Malcolm and Campbell (35) report that a rise in cell count is the first indication of the presence of the infection. Pathogenic bacteria such as streptococci and staphylococci were not always present in milk with high cell counts although the cows showed clinical

evidence of mastitis. In these cases a conclusion was reached that bacteria are only secondary invaders which gain entrance and become established in the udder because of some unknown cause which may be either specific or non-specific.

Large numbers of leucocytes are found in the secretion of the recently fresh cow (33, 71). Explanation for the large number of polymorphonuclear leucocytes is that the mucinous state of the milk calls forth large numbers of the cells. As lactation progresses both the mucin and the leucocytes disappear, but when the gland is being dried off, the leucocyte count again rises.

The leucocyte count of samples of herd milk may suggest the degree of infection within a herd. MacLeod et al. (24) compared the leucocyte count of herd milk to the incidence of mastitis and found that if the percentage of infected animals in the herd was over 40 per cent, the leucocyte count could be expected to be 1,000,000 or more per milliliter of milk.

A study has been made of the comparative value of cell counts and cultural tests. McFarlane and Blackburn (38) took ante-mortem milk samples and post-mortem udder tissue samples and from these made cell counts and culture tests. The cell counts were in 92 per cent agreement with the post-mortem culture tests. In 39 per cent of the histologically positive quarters, no mastitic organisms were found. They therefore concluded that the culture tests were not as

reliable in diagnostic work as the cell count.

Leucocytes of milk are high in the enzyme catalase (10). Quantitatively the amount of catalase indicates the approximate number of leucocytes in the milk. The catalase test involves the incubation of a mixture of milk and hydrogen-peroxide. The production of more than 1.5 milliliters of free gas suggests a positive reaction. Monlux (44) revealed a high positive correlation between low catalase values and low leucocyte counts. Increased quantities of catalase in milk were noted during the first 4 weeks of lactation and during periods of low production.

The "California test" has been developed by Schalm and Noorlander (62). The test reagents are a detergent, sodium-hydroxide and cresol red mixed with milk to determine the presence of an increased number of leucocytes and an abnormality in pH. To perform the test quarter samples of milk are drawn into a 4 compartment plastic paddle. To each compartment an equal quantity of the reagent is added. The paddle is rotated and the formation of a precipitate or gel with contrasting shades of purple is considered to be positive. The principle of the reaction is this: the reagent ruptures the cell releasing the cellular proteins; the freed proteins combine with the reagent and if sufficient quantity of protein is present, a gel or precipitate is formed. A deep purple colored mixture indicates a pH greater than 6.5. If the color of the mixture is yellow,

acidity is indicated. A gray color and liquid mixture with no viscosity visible is considered negative. The tests are scored as negative, trace, weak positive, distinct positive and strong positive.

In the Hotis test (33, 40) milk samples containing bromocresol purple are incubated for 24 hours at 37°C. Tubes in which yellow flakes occur are positive and indicate the presence of Streptococcus agalactiae. When comparing the Hotis test with microscopic readings on positive, negative and questionable samples, Van Der Huver (72) observed that the 2 tests were in agreement 94.5 per cent of the time on negative samples. However, one-third of the samples which were questionable to the microscopic readings gave positive reactions to the Hotis test. The Hotis test was considered valuable in clarifying doubtful microscopic findings.

Various selective media such as blood agar have been used to differentiate the streptococci from other organisms (33). Edwards' esculin crystal violet blood agar has been used to determine Streptococcus agalactiae while azide crystal violet blood agar and other media have been used to differentiate between streptococci and other organisms. Van Oyen and Willems (73) describe the determination of pathogenic bacteria, Streptococcus agalactiae, dysgalactiae, uberis or pyogenes occurring in udder secretions by the use of various media, broths and solutions of different carbohydrates. They report a positive correlation between these

bacteriological determinations and clinical observations.

Detection of coliforms has been done by several methods (14). Liquid mediums such as brilliant green lactose peptone bile broth may be used in the test. If coliforms are present in the sample, gas will be produced in the incubated broth. Dilutions of the sample may be plated on violet red bile agar and incubated at 37°C. for 20 to 24 hours. The presence of large dark red colonies constitutes a positive presumptive test. A gram stain of the organism followed by examination under a microscope for gram negative nonspore forming rods together with gas formation by the organism indicates coliform bacteria.

#### Treatment of Mastitis

Mastitis has long been recognized as a disease of common occurrence and through the years many treatments have evolved. Guenon (22) when describing the treatment of the disease in 1850, recommended that the "best remedy" was to allow the calf to nurse the cow. If this did not eliminate the infection, blood letting and a drench of 1 pound of epsom salts, 1 ounce of powdered aniseed and 3 pints of warm water were recommended. The udder was to be bathed in hot water 3 times daily and after each bathing an ointment of yellow basilicon, camphor and spirits of wine was to be rubbed into the udder. In stubborn cases an ointment of hydriodate of potash and spermaceti ointment was to be applied. In 1887 an injection of boric acid into the



affected quarter was the applied treatment (33). Since that time many other chemical agents have been used in the treatment of mastitis. The milk became abnormal with clots and a gray color. In cows slaughtered 2 and 3 months after treatment, small deposits of silver were found throughout the udder tissue. Later studies reveal the use of silver oxide early in the dry period yielded the best results with little damage to mammary tissue (31, 33).

Entozon, an acridine, was used experimentally in the treatment of mastitis. Cures as high as 90 per cent were reported, however later experiments do not show results as favorable as these. Trypaflavin or neutral acriflavine in sterile water was used as an udder rinse in the United States and Europe. The method required that single quarters be infused with 500 to 2,400 milliliters of the solution. The liquid was allowed to remain in the quarter for a few minutes and then removed. The method was time consuming and involved sterilization of large quantities of solutions, but the results from this treatment were favorable.

Gramacidin, tyrocidin--crystalline products of Bacillus brevis called tyrothricin--have also been reported to effect cures as high as 90 per cent. Tyrocidin was found to be extremely toxic and has not been used commercially. Cost of producing gramacidin was very high. When treating quarters of affected animals with tyrothricin and gramacidin, production often dropped as much as 50 per cent (31).

The infusion of suspended silver oxide in mineral oil into the mammary system of infected animals during the dry period gave some favorable results. Treatment during lactation resulted in a severe udder reaction with the quarters becoming swollen and firm. The animals showed

... Reports (31) have been given of approximately a 35 per cent cure when sulphanylamide was administered to cows infected with Streptococcus agalactiae. Because some cows displayed toxic effects, the researchers concluded that oral administration of this drug could not be used in routine practice but was of some value in the treatment of the associated clinical symptoms. The drug

extreme discomfort and uneasiness. Subcutaneous edema also appeared along the ventral portion of the udder. Body temperatures rose 2.0 to 3.2 degrees. The milk became abnormal with clots and a gray color. In cows slaughtered 1 and 3 months after treatment, small deposits of silver were found throughout the udder tissue. Later studies reveal the use of silver oxide early in the dry period yielded the best results with little damage to mammary tissue (31, 33).

Much research has been done and reported on the therapeutic value of sulphanilamide. Allot (3) administered sulphanilamide orally to cows with advanced streptococcal mastitis. The condition of the udder improved and the organism disappeared; however, on discontinuation of the treatment, the organisms reappeared in the secretion and the udder returned to its previous state. Langer et al. (30) gave sulfamethazine to cows parenterally, orally and by infusion. Blood and milk determinations showed concentrations of 5 milligrams of the drug per 100 milliliters of milk and more than 10 milligrams in the blood. All 3 methods were satisfactorily effective against bacteria. Reports (33) have been given of approximately a 35 per cent cure when sulphanilamide was administered to cows infected with Streptococcus agalactiae. Because some cows displayed toxic effects, the researchers concluded that oral administration of this drug could not be used in routine practice but was of some value in the treatment of the associated clinical symptoms. The drug

was found to be eliminated rapidly by the kidneys.

In order for sulfanilamide to be most effective, the concentration in the udder must be 100 milligrams per cent or more. This is almost impossible to do with oral administration; therefore researchers deemed it advisable to introduce the drug intramammarily. When 265 quarters which were shedding Streptococcus agalactiae were treated with sulfanilamide in oil, 251 were effectively cured. Bacteriological examinations were not continued long after the pronounced cure so the bacteria could have reduced to undetectable numbers and established themselves again later. Other reports (31) present results with as low as 36 per cent cures. These same reports suggest that sulfanilamide greatly reduced the number of organisms in some quarters although it did not entirely eliminate the bacteria. The therapeutic value of oil has been investigated (33). Oil injected alone into the udder retarded the growth of streptococci for several days. The oil was reported to remain in the udder for several days adhering to the membrane lining, the cistern and the teat sinus. These investigators concluded that some of the effectiveness of sulfanilamide may be due to the effect of the oil on the growth of the streptococci (33). Knott and Petersen (29) injected sulfanilamide suspended in sterile-distilled water into quarters of cows infected with coliform organisms. Recovery was noted in all cases and no evidence of the organism was reported the first few weeks after

UNO reported that 100,000 units of penicillin treatment.

Much work has been done and reported on the antibiotic, penicillin, in the treatment of mastitis. Penicillin is produced by a mold, Penicillium notatum, and is effective principally against the gram-positive pathogens (31). Kakavas (26) reported first on investigations on the effectiveness of penicillin against mastitis. He found that penicillin was quite effective for eliminating the organisms Streptococcus agalactiae and the staphylococci from the udder.

Many investigators have worked on the effectiveness of penicillin against streptococcal infections and have reported 33 to 100 per cent elimination of the bacteria from the udder (6, 7, 24, 33, 69). It should be pointed out that many of these workers used different methods of injection and that the amount of penicillin used varied a great deal.

In the Bureau of Dairy Industry Herd one-third of the infections caused by staphylococci were eliminated. Later trials showed that 85 per cent of the staphylococcal, 93 per cent of the coliform, and 76 per cent of the pseudomonal infections were cured by the administration of penicillin (69).

Weirether et al. (74) report that penicillin was not very effective against coliforms. Breazeale et al. (7) reported that penicillin was more effective in eliminating streptococci than staphylococci. They also found that penicillin was somewhat less effective for reinfections and advanced cases of streptococcal mastitis. Swett et al. (69) and

and Tucker (70) reported that 100,000 units of penicillin were sufficient in curing most cases of mastitis. Packer (52) studied the threshold levels of penicillin in the bovine udder. He suggests that penicillin is eliminated from the udder of the low producing cow at a much more rapid rate than from the udder of the high producing cow, thus disputing the idea that larger dosages of penicillin must be given to cows of higher production. Weirether et al. (74) and Packer (53) found that penicillin was resorbed from the udder and eliminated in the urine. Wires (41) used a solution of Growth inhibition and bactericidal activity of penicillin have been investigated. Streptococci require about 100,000 times the amount of penicillin to kill as is needed to inhibit growth. The amount needed for killing of the staphylococci was 5000 times that for inhibition of growth (52). The pathogenic staphylococci and Pseudomonas aeruginosa.

Aureomycin, another antibiotic, has been used extensively for the treatment of mastitis. Packer (53) and McCulloch et al. (37) succeeded in eliminating 50 to 85 per cent of the infections due to Staphylococcus aureus by the use of aureomycin. The drug was ineffective in the treatment of Escherichia coli and streptococcal mastitis (53). In quarter samples taken from 350 animals, micrococci were found to be over 98 per cent sensitive to aureomycin in vitro (2). Klatt and Westermarck (28) reported that in vitro tests aureomycin was effective against Escherichia

coli in inhibiting growth. Observations on the use of terramycin in the treatment of mastitis have been most encouraging. Barnes (5) treated infected animals with oxytetracycline (terramycin). Cures were successful in streptococcal and coliform infections. Effectiveness against Staphylococcus aureus was about 25 per cent, while no effect was found on Pseudomonas aeruginosa.

Furacin used independently or in combination with penicillin has been investigated. Mires (41) used a solution of furacin in treating 10,000 cases of acute clinical mastitis. Good results were obtained in 78 per cent of the cases. Kakavas et al. (27) suggests that a penicillin and furacin mixture was an effective chemotherapeutic agent in acute and chronic cases of mastitis caused by Escherichia coli, the pathogenic staphylococci and Pseudomonas aeruginosa.

Some bacteria are able to resist certain antibiotics. Heishman (23) reports that Streptococcus agalactiae were resistant to subtilin and bacitracin. A combination of bacitracin and penicillin was more effective against Streptococcus agalactiae, Streptococcus uberis and hemolytic staphylococci than either one of these substances alone.

Production of hyaluronidase, an enzyme, by bacteria in the udder has been studied. The enzyme was thought to be beneficial in the absorption by the udder tissue. Gochanauer and Wilson (18) were unable to find either adverse

or favorable effects when using antibiotics in udders where hyaluronidase was present. On the other hand Sansoe (61) later reported that hyaluronidase hydrolyzes hyaluronic acid which inhibits the absorption of liquids in the mammary gland. He suggests that treatment with hyaluronidase favors the absorption of antibiotics and sulfonamides by the mammary parenchyma. Smith et al. (65) by injecting 10

Antibodies have long been known to be present in colostrum to provide protection against disease of the newborn. Ehrlich (13) demonstrated the presence of immune bodies in milk by allowing non-immune young to suckle an immune nurse. The immunity was passed onto the young through the milk. In order to further prove the presence of antibody production in milk, Ehrlich injected rabbit serum immunized against tetanus into a normal nursing mouse. The young were allowed to nurse and were later challenged with live tetanus organisms. They did not contract the disease, while the control animals died within 24 hours. Another experiment with hoof and mouth disease gave similar results. These series of experiments were the first to suggest that the secretion of the mammary gland was a carrier of immune bodies.

The production of specific antibodies by the mammary gland has been demonstrated by several workers.

Giltner et al. (17) showed the production of agglutinins by introducing Brucella abortus antigen into the quarter of a

cow's udder. Agglutinins were first noted in the treated quarter and later in the other 3. Nelson (47) demonstrated that anti-bacillus coli agglutinins were not present in the blood serum of the newborn calf until the calf had been allowed to nurse. Mason et al. (36) showed that immunity against lamb dysentery was passed from the ewe to the lamb through the milk. Smith et al. (65) by injecting 18 milliliters of killed Brucella abortus antigen into 1 quarter of a cow's udder observed that agglutinins were present in the milk within 2 days. The titer rose to 1:640 but after 20 days declined to 1:320. Unpublished work by Dracy (12) also confirms that injection of killed Brucella organisms into the udder results in a positive titer both in the blood and milk. Porter (55), Petersen and Campbell (54) and Porterfield (57) have demonstrated that intramammary injections of Salmonella pullorum, typhoid paratyphoid organisms, pneumococci, egg white and horse serum caused production of specific antibodies.

Conflicting reports appear in the literature as to the value of immunization of animals against mastitis. Through the use of bacterins several workers (33) have attempted to immunize cows against mastitis resulting from streptococci. Comparison of incidence of mastitis between the control and treated animals showed very little difference. died within 2 days. The vaccinated cows showed infection

Use of bacterins from coliform cultures appear to



have some value in the prevention of mastitis caused by this organism. Animals given 3 injections of the bacterin appeared to be more resistant than the control animals when both groups were exposed to infective organisms under normal herd conditions for several months (33).

Intravenous injections of vaccine against mastitis caused by Corynebacterium pyogenes have been reported to be effective in the prevention of mastitis as early as 1910. After use of the vaccine no new cases of mastitis appeared. Later experiments in which a toxoid prepared from cultures of Corynebacterium pyogenes was employed seemed to check the disease, however infected quarters were not saved.

Spencer and Angevine (66) gave intradermal injections of Streptococcus agalactiae antigen to normal cows and cows with mastitis due to Streptococcus agalactiae. Cows with the mastitis were more sensitive and gave greater reactions than cows without mastitis. Those cows with clinical symptoms manifested greater reactions than did the infected cows which showed no clinical symptoms.

Spencer et al. (68) isolated a strain of Micrococcus pyogenes from the udder of a cow. Vaccines were prepared and injected into 4 animals. Later these animals and 2 controls were challenged with the live organism injected into the quarters. The control cows developed gangrene and 1 died within 2 days. The vaccinated cows showed infection in 9 of 16 quarters. These became chronically infected.

infected. Milk from the infected quarters failed to support the growth of Micrococcus pyogenes.

Richou and Thieulin (60) report that mastitis incidence did not decrease in spite of the use of many chemotherapeutic agents. In order to combat the disease they contended that emphasis should be placed on prevention rather than cure. Throughout a 5 year period, these researchers observed the effect of a mastitis polyvalent autogenous vaccine composed of streptococci, staphylococci and coliform organisms on the incidence of mastitis. The vaccine, concentrated to 1,000,000 cells per cubic centimeter, was injected subcutaneously in 3 dosages 5 days apart, the first dose being 3 cubic centimeters and each of the following 5 cubic centimeters. They concluded that such vaccines were effective in the prevention of mastitis.

In view of the conflicting evidence on vaccines in the prevention of mastitis and the demonstrable antibody production of the mammary gland, it appears that more work should be carried out on this phase of mastitis therapy. Thus, the following experiments were conducted to determine the possibility of inhibiting mastitis in an immunized udder. Both antigens were prepared from bacterial cultures which had been propagated from organisms isolated from the udders of cows suffering from mastitis. The 2 organisms used were Streptococcus dysgalactiae and Escherichia coli. The bacteria were grown in a tryptose-phosphate broth.

Liter flasks of EXPERIMENTAL PROCEDURE inoculated with the General Management was at 37°C. for 48 hours for the antigen.

The animals used in this experiment were grade Holstein and Guernsey cows and heifers. None of the animals were lactating at the time the experiment was begun. The general health and condition of the cattle was good. All of the cattle were similarly managed throughout the experiment. During early fall the animals were kept in a lot except when they were stanchioned for treatment. After November 1, 1957 all of the animals were kept in stanchions except for periods during the day when they were allowed to exercise. When the antigen had been declared free of live organisms a grain mixture, alfalfa hay and corn silage made up the ration fed to these cattle during the experiment. Before parturition each animal was fed 4 pounds of the grain mixture daily. After freshening, grain was fed at the rate of 1 pound for each 3 pounds of milk produced. The animals received alfalfa hay (ad lib.) and corn silage at the rate of approximately 30 pounds per head.

Preparation of the Antigen

For the conduct of this experiment, 2 antigens were prepared. Both antigens were prepared from bacterial cultures which had been propagated from organisms isolated from the udders of cows suffering from mastitis. The 2 organisms used were Streptococcus dysgalactiae and Escherichia coli. The bacteria were grown in a tryptose-phosphate broth portion of the udder. Two injections, each 5 days apart, were given.

Liter flasks of the sterile broth were inoculated with the bacteria and incubated at 37°C. for 48 hours for the Escherichia coli and 72 hours for the Streptococcus dysgalactiae. After incubation formaldehyde was added to the culture to kill the bacteria. Following treatment with formaldehyde, a bacterial plate count was run on the antigen to determine if the kill was complete. A direct microscopic count was made on each antigen to ascertain numbers. The Streptococcus dysgalactiae antigen contained approximately 22 billion cells per milliliter. The count on the Escherichia coli antigen numbered approximately 2 billion cells per milliliter. When the antigen had been declared free of live organisms, it was stored in sterile bottles under refrigeration until used.

#### Treatment

Challenge Two heifers were treated with the Streptococcus dysgalactiae antigen. In addition 1 animal served as a control; she received no treatment. One milliliter of the antigen was administered to each quarter through a sterile teat canula and syringe. Before injection the udder was washed with a chlorine solution to remove all dirt and extraneous matter. The teat was then carefully washed with a pledget of cotton soaked with 70 per cent alcohol. Extreme caution was taken to insure that the teat orifice was free from all foreign matter. After injection the udder was massaged to help transport the antigen well into the upper portion of the udder. Two infusions, each 5 days apart, were given.

Three cows were injected with the Escherichia coli antigen. All of these animals were dry when brought into the herd. Their udders were palpated for any signs of scar tissue which is an indication of previous mastitis. They were treated in a manner similar to those animals receiving the Streptococcus dysgalactiae antigen. The udders were washed with chlorine solution and then the teat was cleaned with 70 per cent alcohol. Ten milliliters of the antigen were injected into each quarter of the udder. The udder was massaged after infusion to distribute the antigen throughout the udder. Three administrations of the antigen at 5 day intervals were given.

Table 1 presents the information concerning the administration of the antigen and the calving dates of the animals.

### Challenging

Following calving the animals were allowed to lactate normally until such time as they were challenged with the corresponding organism with which they had been immunized. Challenging was done by injecting 1 milliliter of a tryptose-phosphate broth culture of the bacteria into each quarter of the udder. The Streptococcus dysgalactiae was allowed to incubate for 48 hours while the Escherichia coli culture was incubated for 24 hours. A count made on the coliform culture indicated that the broth contained approximately 50 million cells per milliliter. In the case of the cows immunized with

the streptococcal organism and the control cow, 3 challenges 5 days apart were made. Five days after the last challenge, 10 milliliters of the antigen was again infused into each quarter of all 3 cows. The cows injected with the coliform organism were challenged only once.

#### Sampling and Testing

Before and during the period of challenging a series of tests were made to ascertain the presence or absence of udder abnormalities and secretion which might indicate mastitis. The tests were as follows: a physical examination of the udder, a strip cup test, the Whiteside test, the California test, pH, a bacterial plate count and a leucocyte count.

Daily milk samples were drawn from the animals each day for a period of at least 5 days prior to challenging. Test samples were also taken during challenging. Samples were drawn in a manner to exclude as much contamination as possible. A stream or 2 of milk was first drawn to clear the teat canal and meatus of all foreign matter. The udder was then washed with a chlorine solution after which 70 per cent alcohol was employed to further clean the teats and reduce bacterial contamination. Composite samples of milk were drawn into sterile glass sample bottles for further examination at the laboratory.

Before taking the milk samples the udder was grossly examined, and milk from each quarter was examined by the

strip cup and California test. The udder was palpated to detect any swelling or inflammation which indicates infection. Two or 3 streams of milk from each quarter were drawn into a strip cup to note abnormalities of the secretion such as clots, blood andropy or stringy milk. The California test (42) was also conducted at the time of sampling. Approximately 2 or 3 milliliters of foremilk were drawn into a 4 compartment plastic paddle. To each compartment of the paddle was added a like amount of the reagent. This was stirred and the appearance of a gel, a deep purple color or a yellow precipitate was regarded as positive. If the mixture of the reagent and milk did not gel or precipitate and remained a blue-gray color, the test was considered negative. During the pre-challenge period, a series of plates were made immediately following sampling and the barn tests, further testing was done at the laboratory. The tests included the Whiteside test, a bacterial plate count, pH and a leucocyte count. Testing was completed as quickly as possible to reduce changes which might take place in the milk during storage. The pH of the samples was measured by the use of a pH meter. The meter was standardized each time it was used with a buffer solution of pH 7. Samples were adjusted to room temperature before testing. The electrodes of the meter were rinsed with distilled water and dried with absorbent tissue between samples. (14). The glass slides used in the

The Whiteside test (33), based on the leucocyte content of the milk, was made using 5 drops of milk and 1 drop of Normal sodium hydroxide mixed for about 20 seconds on a glass plate. The appearance of a gel or precipitate was regarded as positive.

A bacterial plate count was made of each composite sample of milk. Tryptose glucose extract agar was the media used for the count. Twenty-four grams of the agar were dissolved in 1 liter of cold distilled water and heated to boiling to dissolve all of the agar. The agar was poured into smaller receptacles and sterilized in an autoclave for 15 minutes at  $248^{\circ}\text{F}$ . and 15 pounds of pressure, after which it was cooled and stored until needed. On samples drawn during the pre-challenge period, a series of plates were made at dilutions of 1:10, 1:100, 1:1000 and 1:1,000,000. Plates were selected for counting on the basis of number of colonies per plate. Those plates with between 30 and 300 colonies were counted. If more than 300 colonies appeared in all dilutions, the plates were regarded as too numerous to count and were recorded as such. If less than 30 colonies appeared on the plates, the plates of the lowest dilution were counted. Recordings of such counts were recorded as "less than" the number of colonies appearing on the plate multiplied by the dilution factor.

When making the leucocyte count, a stained slide of the sample was prepared (14). The glass slides used in the



preparation were thoroughly cleaned with an abrasive, rinsed and dried with a paper towel. A 0.01 milliliter pipette was used to deposit the measured quantity of milk onto a slide which had been placed over a ruled square centimeter. The milk was spread over the area with a clean straight needle. Two films of each sample were prepared. The pipette was rinsed in warm water between samples and then the bore was again rinsed with milk of the next sample. The films were allowed to dry after which they were placed in xylene for 1 minute. Following fixation in xylene, the slides were again allowed to air dry and were covered with ethyl alcohol for 1 minute. Removal from the alcohol was followed by drying and staining with methylene blue for 10 to 15 seconds. The slides were washed, dried and examined under the oil immersion objective of a microscope. The leucocytes appeared as large, deeply stained, irregular shaped cells. The number of fields counted depended upon the average number of leucocytes found in each field. The following guide was used for determining the number of fields to be counted:

- a. 0.5 cells per field.....50 fields counted
- b. 0.5-1 cells per field....25 fields counted
- c. 1-10 cells per field.....10 fields counted
- d. 10-30 cells per field.... 5 fields counted
- e. Over 30 cells per field..recorded as uncountable.

Table 1. Examination of Milk

Cow No.	E 101	E 102	E 123	E 150	E 151	E 152	E 153	E 165	E 164
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Table 1. Immunization and calving dates of experimental and control animals.

Cow No.	Date	Antigen	No. cc./ injection	Billions of cells/cc.	Calving Date
E 101	9-12-57	<u>S. dysgalactiae</u>	1	22	9-25-57
	9-16-57	<u>S. dysgalactiae</u>	1	22	
E 102	8-27-57	<u>S. dysgalactiae</u>	1	22	9-4-57
	9-2-57	<u>S. dysgalactiae</u>	1	22	
E 123		Control	-	-	8-21-57
E 180	10-21-57	<u>E. coli</u>	10	2	
	10-25-57	<u>E. coli</u>	10	2	11-28-57
	10-29-57	<u>E. coli</u>	10	2	
E 181	10-21-57	<u>E. coli</u>	10	2	
	10-25-57	<u>E. coli</u>	10	2	12-8-57
	10-29-57	<u>E. coli</u>	10	2	
E 182	10-21-57	<u>E. coli</u>	10	2	
	10-25-57	<u>E. coli</u>	10	2	1-1-57
	10-29-57	<u>E. coli</u>	10	2	
E 100		Control	-	-	10-2-57
E 165		Control	-	-	7-24-57
E 166		Control	-	-	8-27-57

of E102 was too numerous to count at a dilution of 1:1,000,000  
RESULTS  
Detailed data on the heifers immunized with the  
Streptococcus dysgalactiae antigen has been summarized and is  
presented in Table 2. Daily samples were taken from the  
animals 9 days prior to challenging. Three challenges were  
made at 5 day intervals. One week after the last challenge,  
the cows were immunized with 10 milliliters of the antigen.

Data gathered during the prechallenge period indicated  
animals to be free of infection in general. One animal,  
E102, did show a higher average leucocyte count than the other  
heifers and was positive at times to the California tests;  
however, the other tests on the milk of this animal showed  
negative results. Average leucocyte counts for the pre-  
challenge period for all animals was 251,000 cells per cubic  
centimeter. The bacterial plate counts were run at dilutions  
of 1:10, 1:100 and 1:1000. In most instances plates of the  
1:1000 dilution showed very little growth; therefore plates  
of the lower dilutions were counted. The bacterial count  
averaged 2952. The pH of all samples during the prechallenge  
period averaged 6.7. The California, Whiteside and strip  
cup tests were negative excluding the positive California test  
on E102. Each day during the sampling period, the udders of the

During the interval following Challenge I, the average  
leucocyte counts increased to 1,750,000. The plates were run  
at dilutions as high as 1:1,000,000 and for the most part  
were read at this dilution. The bacterial count of the milk

of E102 was too numerous to count at a dilution of 1:1,000,000 while the count of the milk of the other heifers raised significantly. The California tests in all cases were positive. One heifer, E101, was positive to the strip cup during the first challenge period; however, the other animals manifested no abnormal milk at this time. The control, E123, was negative to the Whiteside test during the first challenge.

Leucocyte counts averaged 3,453,000 during Challenge II. In general, the average plate counts were too numerous to count. All of the animals were positive to the California, Whiteside and strip cup tests except for a negative strip cup test recorded for E123.

In Challenge III leucocyte counts remained high at 3,558,000 cells per cubic centimeter. The plates could not be counted at dilutions of 1:1,000,000. E123 was negative to the strip cup and Whiteside tests, while E101 and E102 were positive to all tests.

Reimmunization resulted in a rise in leucocyte count to an average of 4,057,000. The bacterial plates could not be counted because of the presence of too many colonies.

The California, Whiteside and strip cup tests were all positive. Each day during the sampling period, the udders of the cows were examined for fibrosis, swelling and inflammation.

No abnormalities of the udders were noted during the experimental period. All through the trial period the pH averaged 6.7 to 6.8. bacterial count could not be made at dilutions

of 1:1. Table 3 presents the averages of data gathered during a 9 day prechallenge and challenge period on the cows injected with Escherichia coli broth culture. Leucocytes averaged 193,000 per cubic centimeter for all cows during the 2 week period. The bacterial count of the samples numbered 2631 for the animals during the prechallenge period. All animals with the exception of E181 were negative to the California, Whiteside and strip cup tests. Cow E181 was positive to the Whiteside and California tests. Her milk also had a higher bacterial count and leucocyte count than the other cows. E180 died within 2 days and E180 died 4 days after injection. When the animals were challenged with 1 milliliter of a 24 hour broth culture, violent body reactions occurred. Within 6 hours after challenging, the animals manifested an extremely abnormal udder secretion which was clotted and straw colored. All milk samples were distinctly positive to the California, Whiteside and strip cup tests. Alkalinity was indicated by an increase in pH from an average of 6.7 to an average of 7.0. The amount of milk given dropped 63.81 per cent on the first milking after injection. All cows exhibited extreme diarrhea and body discomfort. All temperatures were normal except for E182 which was 98°F. 10 hours after challenging. In addition to the drop in temperature, the skin around the udder, vulva and nose turned blue. The animal died within 19 hours after injection with the broth culture. A bacterial count could not be made at dilutions

of 1:1,000,000 because of excessive growth.

The remainder of the animals continued to show diarrhea and a general weakened condition. These animals refused feed, and the secretion of the udder dropped to virtually nothing. The challenged cows became so weak that it was impossible for some of them to get on their feet.

Thirty-six hours after injection, antibiotics were administered to the 5 remaining animals. Animals E100, E180 and E182 were given 150 milligrams of furacin and 100,000 units of penicillin. Dosages were given every 24 hours; however, E180 died within 2 days and E100 died 4 days after injections of furacin-penicillin had been initiated. E181, in addition to the penicillin-furacin treatment, received 3 infusions into the udder of 400 milligrams of Chlorotetracycline HCl. This treatment seemed to alleviate the condition somewhat, but she died 25 days after injection with the broth culture. Animals E165 and E166 were given 3 infusions of 30 milligrams of terramycin and 10,000 units of Polyxin B into each quarter of the udder. This treatment arrested the infection and the animals recovered and returned to seemingly normal health; however, normal production was not regained.

Table 2. Summary of data on experimental mastitis in dairy cattle.

Time of treatment	6:7	6:7	6:9	6:6	6:7	
Pre-challenge	6:7	6:7	6:9	6:6	6:7	
Challenge I Nov. 27.	6:7	6:7	6:9	6:6	6:7	
Challenge II Dec. 2.	6:7	6:7	6:9	6:6	6:7	
Challenge III Dec. 6.	6:7	6:7	6:9	6:6	6:7	
Re-inoculation Dec. 15.	6:7	6:7	6:9	6:6	6:7	
I Control.						

Table 2. Summary of data on cows immunized, challenged and reimmunized with Streptococcus dysgalactiae.

Date and kind of treatment	Daily samples collected	Cow no.	Leucocyte count ave.	Plate count ave.	Calif. test	Strip cup	White-side test	pH ave.
Prechallenge								
Prechallenge	Jan. 20-28.	E180	160,000	400	Neg.	Neg.	Neg.	6.8
		E181	450,000	11,200	Neg.	Neg.	Neg.	6.7
	Nov. 23-27.	E123 <sup>1</sup>	1,140,000	73,500	Neg.	Neg.	Neg.	6.6.7
		E101	120,000	3,100	Neg.	Neg.	Neg.	6.7
Challenge I Nov. 27.	Nov. 28-29.	E102	1,500,000	22,200	Pos.	Neg.	Neg.	6.6.7
		E165	170,000	2,100	Neg.	Neg.	Neg.	6.7
		E166	130,000	1,100	Neg.	Neg.	Neg.	6.6
		E123	1,550,000	3,000,000	Pos.	Neg.	Neg.	6.7
Challenge II Dec. 2.	Dec. 3-8.	E101	1,950,000	37,000 <sup>2</sup>	Pos.	Pos.	Pos.	6.7
		E102	1,770,000	TNTC	Pos.	Pos.	Pos.	7.6.7
		E101	--	TNTC	Pos.	Pos.	Pos.	7.2
		E102	--	TNTC	Pos.	Pos.	Pos.	7.0
Challenge III Dec. 8.	Dec. 9-15.	E123	6,470,000	TNTC	Pos.	Neg.	Pos.	6.7
		E101	2,450,000	TNTC	Pos.	Pos.	Pos.	7.6.9
		E102	1,440,000	TNTC	Pos.	Pos.	Pos.	6.6.7
		E166	--	TNTC	Pos.	Pos.	Pos.	7.0
1 Controls. 2 Too numerous to	Dec. 9-15.	E123	2,080,000	TNTC	Pos.	Neg.	Neg.	6.7
		E101	5,650,000	TNTC	Pos.	Pos.	Pos.	6.9
		E102	2,940,000	TNTC	Pos.	Pos.	Pos.	6.8
Reimmunization Dec. 15.	Dec. 16-20.	E123	3,220,000	TNTC	Pos.	Pos.	Pos.	6.6
		E101	6,000,000	TNTC	Pos.	Pos.	Pos.	6.7
		E102	2,950,000	TNTC	Pos.	Pos.	Pos.	6.7

1 Control. 2. Too numerous to count.

Table 3. Summary of data on cows immunized and challenged with Escherichia coli.

Date and kind of treatment	Daily samples collected	Cow no.	Leucocyte count ave.	Plate count ave.	Calif. test	Strip cup	White-side test	pH ave.
Prechallenge	Jan. 20-28.	E180	160,000	400	Neg.	Neg.	Neg.	6.8
		E181	450,000	11,400	Neg.	Neg.	Neg.	6.7
		E182	110,000	700	Neg.	Neg.	Neg.	6.6
		E100 <sup>1</sup>	130,000	200	Neg.	Neg.	Neg.	6.7
		E165	170,000	2,100	Neg.	Neg.	Neg.	6.7
		E166	130,000	1,100	Neg.	Neg.	Neg.	6.6
		Challenge Jan. 28.	Jan. 29.	E180	--	TNTC <sup>2</sup>	Pos.	Pos.
E181	--			TNTC	Pos.	Pos.	Pos.	7.2
E182	--			TNTC	Pos.	Pos.	Pos.	7.0
E100	--			TNTC	Pos.	Pos.	Pos.	7.0
E165	--			TNTC	Pos.	Pos.	Pos.	6.9
E166	--			TNTC	Pos.	Pos.	Pos.	7.0

1 Controls. 2 Too numerous to count.



## DISCUSSION OF RESULTS

The results of these experiments deal with the possibility of immunizing the mammary gland against specific mastitic organisms. Before challenging, the heifers immunized with the Streptococcus dysgalactiae antigen seemed to be normal and free from mastitis. Except for the higher leucocyte count and a positive California test, E102 appeared to be normal. Animals with leucocyte counts under 500,000 are usually regarded as negative although some workers feel that leucocyte counts for heifers should be below 300,000. In any event the counts in all cases increased by at least 1,000,000 cells after the first challenge. The higher leucocyte count along with the increase in the bacteria count would appear to indicate the establishment of the organism in the udder. Although samples were drawn with extreme care so as to exclude bacterial contamination, there is the possibility that some of the samples became contaminated when sampling. However, the consistently lower counts during the prechallenge period compared with the much higher bacteria counts of the challenge periods would imply that contamination was less important. Subsequent challenges with the organism resulted in continued high leucocyte and bacterial counts along with positive California tests. The control, E123, did not exhibit positive results to the strip cup and Whiteside tests during the 3 challenges; however, the leucocyte and bacterial counts were high and

the California tests were positive. In this particular trial it would appear that there was not any great difference between the immunized and control animals. Perhaps greater resistance would have been attained had the animals been injected earlier in the dry period. During the challenge periods no fibrosis or swelling could be detected in the udder, even though stringy andropy milk was observed on the strip cup.

Observations during reimmunization suggest that this practice was of no apparent advantage in combating the infection. If anything the infections seemed to become more severe. Petersen and Campbell (54) demonstrated that a severe udder reaction occurred when immunizing a lactating udder which may also be a somewhat similar reaction to that observed in this experiment.

The introduction of the Escherichia coli organisms produced such a violent body and udder reaction that any definite conclusions as to the value of immunizing the udder against this particular organism could not be reached. That the number of organisms used in the challenge was far beyond the number which would enter into the udder in a normal infection should be pointed out. Perhaps more antigen should have been used during the period when the udder was actually making up. It appears that once established in the udder, Escherichia coli produced many body effects other than infection of the mammary gland.

Of interest is the effect of antibiotics on the infection of Escherichia coli in the udder. From the results of the treatment with the antibiotics, it would appear that the infusion of terramycin and Polyxin B was more effective than the other treatments since those animals treated with terramycin recovered. From all outward appearances before the antibiotic treatment, none of the animals were expected to survive. The animals given the terramycin with Polyxin B were not producing nearly as heavily as the animals which died. The question might be raised as to whether or not the rate of production had any bearing on their ability to recover from the disease.

3. The data presented herein suggest that immunizing the udder against Streptococcus dysgalactiae and Escherichia coli is of questionable value in the prevention of mastitis.
4. It should be recognized that the exposure to the infective organisms was not that which would occur under normal conditions; however, the fact remains that animals whether immunized or control did exhibit establishment of the infective organisms in the udder when challenged.
5. There apparently was not enough resistance built up through immunization of the udder to combat the organisms infused through the teat canal.
6. The results of these experiments may be somewhat misleading because of the virulence and the amount of material used. There is the possibility that a smaller dose of live organisms would have been more satisfactory as well as using a less virulent strain of organism. Further work is necessary to confirm this possibility.

## SUMMARY AND CONCLUSIONS

The experiments reported were conducted to determine the ability of an immunized udder to resist organisms introduced into the udder via the teat canal. Information summarizing the data and conclusions drawn therefrom are presented below.

- 1.3) Two animals were injected intramammarily with Streptococcus dysgalactiae antigen and 3 cows with Escherichia coli antigen during the latter dry period.
- 2.5) After calving the animals were challenged with the corresponding organisms after a prechallenge test period.
3. The animals challenged with Escherichia coli exhibited abnormal udder secretion and a general debilitating effect. One animal died 19 hours after challenging.
4. Five animals were treated with antibiotics. Terramycin with Polyxin B was the only one which seemed to arrest the infection enough so that the animals could recover.
5. There apparently was not enough resistance built up through immunization of the udder to combat the organisms infused through the teat canal.
6. The results of these experiments may be somewhat misleading because of the virulence and the amount of material used. There is the possibility that a smaller dose of live organisms would have been more satisfactory as well as using a less virulent strain of organism. Further work is necessary to confirm this possibility.

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