BOVINE MAMMARY INTRACISTERNAL TEMPERATURES

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ABSTRACT

BOVINE MAMMARY INTRACISTERNAL TEMPERATURES

by Harish Chandra Joshi

In this work, the mammary intracisternal temperatures of 1 non-lactating and 8 lactating cows were determined and evaluated as a possible adjunct to the diagnosis of mastitis.

Intracisternal temperatures of 109 clinically normal quarters were recorded with a Tri-R electronic thermometer^{*}. Rectal and room temperatures were also observed. Rectal temperatures were taken with a clinical thermometer (Cary)^{**}, and the room temperature with a mercury thermometer.

Experiments were performed to determine the most effective chemical for sterilization of the probe of the Tri-R electronic thermometer. Four disinfectants were tested: Liquid Germicidal Detergent***, Clenesco Liquid 75****, ethyl alcohol 70% and Novadine ****. Superior results were obtained with Novadine at a strength of 1:200 (1 part of Novadine and 199 parts of water), which killed <u>Staphylococcus</u>aureus as a test organism in less than 2 minutes. Various diagnostic tests were also performed to the milk; California Mastitis Test (CMT), leukocyte counts, and bacteriological examination.

The intracisternal temperatures were found to vary from 91.5 F to 96.6 F in different normal quarters of the cows.

Intracisternal temperatures were also recorded in a cow with a natural infection of staphylococcic mastitis, and the intracisternal temperature of that quarter was found to be significantly higher than the other quarters.

The quarters of a cow were infused with 2% NaCl solution to produce an acute inflammation of the udder. Preand post-infusion intracisternal temperatures were recorded. Twelve hours after 20 ml. of 2% NaCl solution were infused the intracisternal temperature raised to 5.1 F and the rectal temperature to 1.5 F above the pre-infusion level, and the leukocyte counts were also high. The rectal temperature returned to the normal pre-infusion level after 24 hours, however, the intracisternal temperature did not return until after 72 hours. The bacteriological examination of the milk sample of this cow revealed non-hemolytic <u>Staphylocoecus</u> <u>aureus</u> before the infusion, but 12 hours later a hemolytic <u>Staphylococcus aureus</u> was recovered. Within 24 hours this organism again became non-hemolytic. This change in the pattern of staphyloccic microorganisms in the production of hemolysin could not be explained. It might be possible that the severity of irritation produced this change.

Intracisternal temperatures were also registered when the quarters of a cow were infused with a 24 hour broth culture of non-hemolytic and coagulase-negative <u>Staphylococcus</u> <u>aureus</u>. Five hours after 5 ml. of this broth culture were infused the intracisternal temperature was elevated to 7.0 F and the rectal temperature to 4.9 F above the pre-infusion level. The rectal temperature returned to the pre-infusion level after 57 hours but the intracisternal temperature remained above normal for 12 days.

From the results it appears that determination of intracisternal temperatures might be a possible adjunct to the diagnosis of udder irritation in the early stage of infection.

Tri-R instruments, Jamaica 35, N. Y.
** Cary instruments Ltd., 44 Whitehall St., N. Y.
*** Parke, Davis & Company, Detroit, Mich.
**** Cowles & Company, Cleveland, Ohio.

BOVINE MAMMARY INTRACISTERNAL TEMPERATURES

By

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A THESIS

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Respectfully dedicated to--

C. S. Bryan, the late Dean of the College of Veterinary Medicine, Michigan State University, for his intense and vast work in the field of mastitis

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I. INTRODUCTION

For many years mastitis has been a serious economic problem to the dairy industry. Mastitis not only causes a loss in milk yield, but may be responsible for the loss of valuable animals either through death or complete cessation of production. Before 1939, mastitis caused by streptococcic microorganisms was the most predominent and troublesome type in the United States. Since that time staphylococcic microorganisms have become increasingly more prevalent as the etiological agents of mastitis. These organisms have an omnipresent distribution and include both pathogenic and nonpathogenic varieties.

One of the reasons that mastitis has continued to be prevalent is the lack of early and adequate diagnostic procedure. According to Merchant and Packer (1952) the diagnosis of mastitis caused by bacteria was made by Nocard and Mollereu in 1884 when they described a streptococci isolated from the milk of a sick cow. Prescott and Breed (1910) described a method for determination of leukocytes and epithelial cells in milk associated with an abnormal

milk. The basic principle of leukocyte determination is still used today although modifications of the original method continue to be made. One disadvantage to this test is that it is strictly a laboratory procedure. Schalm and Noorlander (1957) developed a macroscopic field test based on the presence of leukocytes. This test which they termed the California Mastitis Test (CMT) gives evidence of a precipitate or gel formation when the fore-milk has a cell count over 100,000 cells per ml. or when "strippings" have a cell count in excess of 200,000 cells per ml. Since this reaction only indicates the presence of leukocytes, bacteriological tests are necessary for a complete diagnosis.

Early diagnosis of bovine mastitis is essential to enable efficient curative treatment before there is permanent damage to the secreting tissues. In this work, an attempt was made to determine if intracisternal temperature might be of value in the early detection of mastitis. Intracisternal temperatures of bovine mammary glands were measured with a Tri-R electronic thermometer*. The intracisternal temperatures of cows with mastitis, some of which occurred naturally, and some of which was induced by the intramammary infusion of either 2% NaCl solution or <u>Staphylococcus aureus</u> organisms.

* Tri-R Instrument, Jamaica 35, N. Y.

Concurrent with the temperature studies, the following diagnostic tests were performed on milk samples: California Mastitis Test, hemolysin production on blood agar, coagulase production, leukocyte counts, and bacterial examination.

II. REVIEW OF LITERATURE

A survey of the literature available in the Michigan State University library failed to reveal any material on the determination of intracisternal temperatures in the diagnosis of udder infections.

In view of the complete lack of literature on intracisternal temperatures it was thought that a brief review of certain phases related to the carrying out of this experimental work would be helpful. The following subject areas are reviewed:

1. Evaluation of disinfectants.

2. Inflammation.

3. Staphylococcic mastitis.

1. Evaluation of disinfectants

Before attempts to take intracisternal temperatures were carried out a method of disinfecting the thermometer was necessary.

Reddish (1957) in his text book makes mention of several noteworthy facts pertaining to methods of testing and evaluating disinfectants and are included among the

following statements. Buchhaltz, as early as 1875 developed a method for evaluating the effectiveness of antiseptics. He used tobacco-infusion as a culture medium and observed the effectiveness of many antiseptics. However, Robert Koch (1881) compared the germicidal value of various disinfectants upon a culture of microorganisms. He extended his experimentation to include the impregnation of silk threads with anthrax spores and then exposed them to solutions of chemicals for different intervals of time. After the exposure, the silk threads were washed with water and placed into nutrient broth and observed for bacterial growth. Geppert in 1889 repeated the work of Koch, but he found that mercuric chloride solution (1:1,000) failed to kill anthrax spores within that period which was reported by Koch. In 1897, Kronig and Paul devised a method for determining the germicidal value of a disinfectant. They modified the method of Koch and instead of using silk threads substituted garnets. During the experimentation they observed that the relative value of disinfectants is dependent upon various conditions such as temperature, number and species of microorganisms, and also the absence of organic matter. Rideal and Walker reported a more reliable method, whereby the effect of a disinfectant was compared with phenol. This

method is generally spoken of as the "Phenol Coefficient Method" for testing the effectiveness of antiseptics and disinfectants.

Anderson and McClintic (1911) modified the method of Rideal and Walker, and the resulting procedures became known as the Hygienic Laboratory Method. This method was used widely in the United States for many years.

In the year 1931, an official method for evaluating antiseptics and disinfectants based on the method of Rideal and Walker was prescribed with very few changes, and is known today as the Food and Drug Administration (FDA) Method. This method is the standard phenol coefficient test by which many antiseptics and disinfectants are tested. At the present time the official method employed in the evaluation of disinfectants is known as the "A.O.A.C. Phenol Coefficient Method"*.

Mallmann and Hanes (1945) proposed a method for evaluating antiseptics and disinfectants under all practical applications which is called the "Use-Dilution Method". Leavit (1946) worked out the comparative study of the usedilution method and the FDA Method. In a subsequent study, Mallman and Leavit (1948) reported that the use-dilution method is more satisfactory than the older procedures.

^{*} Association of Official Agricultural Chemists.

Literature concerning disinfectants used in this study was not available in the case of Novadine and Clenesco Liquid 75.

Liquid Germicidal Detergent (LGD) was demonstrated by Bryan et al. (1948) to be effective as an udder disinfectant.

Gershenfeld et al. (1951) reported on the sterilization of clinical thermometers. During experimentation they contaminated segments of the thermometers (about two inches in length) with bacterial cultures and reported that 70% ethyl alcohol killed staphylococci in 10 minutes.

2. Inflammation

Menkin (1950) has given us a classical report on the basic concepts of inflammation. The following information is taken from his textbook. A rise of temperature is one of the most dramatic manifestations of inflammation of living tissue, and has been known for a long time. Even Hippocrates frequently used this observation in the diagnosis of diseases, and regarded inflammation as being closely associated with fever. Galen discussed the subject of inflammation and thought it to be a common disease. For the first time the phenomenon of inflammation was described as a local fever. This idea of Galen was expanded by Hunter who reported that

the phenomenon of inflammation was the reaction to any injury caused therein and regarded the inflammatory process as a defensive reaction. Virchow reported that there is a swelling of parenchymatous structures when injured. Cohnheim also analyzed that the process of inflammation was due to some disturbed local physiology, and he reported that in inflammatory reactions there was increased permeability of the capillary wall, and an outward migration of leukocytes.

Wolf (1923) studied the phenomenon of chemotaxis and reported that histamine was strongly chemotactic. Dixon and McCutcheon (1935) reported that polymorphonuclear leukocytes were strongly attracted by staphylococcic organisms. Moon (1935) reported that the cells of normal tissues contained histamine in a non-diffusible form, and when the cells of the body were injured histamine was liberated locally in a diffusible form. Adjacent capillaries then become dilated and there was an increase in the permeability of the capillary endothelium resulting in transudation of plasma which produced edema. Grant and Wood (1928) reported that histamine had no appreciable power to cause emigration of leukocytes from the vessels.

Menkin (1937) concluded that the migration of polymorphonuclear leukocytes in inflammation was related to the

liberation of an active substance "leukotaxine" which was chemotactic to leukocytes. He further reported in the year 1943 that the important aspect of the inflammatory reaction was to localize or to fix the irritating material.

Extensive work has been done on the subject of inflammation, but this literature will be limited to the extent to which it is pertinent. Runnels (1946) reported the etiological agents of inflammation to be irritants such as physical injuries, chemical irritants, bacteria, and other parasites of lower animal life.

Menkin et al. (1937) reported that the cytological picture in an inflamed area was dependent upon the pH of the injured part. They further reported that hydrogen ion concentration was the factor conditioning the cellular pattern. The polymorphonuclear leukocytes were most predominent in an acute inflammation when the pH of the medium was seven or more. When the macrophages were predominent then this stage was called a chronic inflammation. The polymorphonuclear leukocytes contain an intracellular enzyme which was active in an alkaline medium. They have also shown that the polymorphonuclear leukocytes were incapable of surviving in an acid medium and the survival of leukocytes was determined by the hydrogen ion concentration.

3. Staphylococcic mastitis

Staphylococcic microorganisms are widespread in nature and found on the skin of animals and particularly on the mucous membranes of the nose and mouth, and become a part of the udder flora in the bovine.

Evans (1916) obtained "micrococci" from 58.8% of the milk samples drawn from the udders which they regarded as normal. Klimmer (1930) in Germany investigated the occurrence of mastitis of an acute type due to staphylococci in dairy Little and Foley (1935) found 9 acute cases of bovine COWS. mastitis due to staphylococci. Gwatkin et al. (1936) observed 143 cows affected with mastitis, and found that 30 cases were due to Staphylococcus aureus. Minett (1937) worked on the incidence of Staphylococcus aureus mastitis in five dairy herds. He demonstrated that out of 415 cows, 145 were found to shed staphylococcic microorganisms in the milk and the percentage of infection due to staphylococcic microorganisms was 34.9%. Little and Plastridge (1946) observed that 5.9% of the milk samples contained staphylococci associated with 1,000,000 or more leukocytes per ml. of milk, and an additional 4.8% contained staphylococci associated with leukocytes from 500,000 to 1,000,000 per ml. of milk. Packer (1952) reported, during his six years of study, that 70% of the milk samples

contained staphylococci. Schalm (1953) reported that from 25.6 to 75% of the dairy cows he explained had micrococci (25.6% of the herd in July 1949, to 75% in June 1950). During six years of study (1947 to 1952) on one dairy herd the average incidence (percentage of cows) of shedding of staphylococci increased from year to year as follows: 43, 49, 47, 64, 57, and 62. Both Schalm (1953) and Packer (1952) concluded that the incidence was correlated with increasing lactation age.

Carpenter (1922) introduced a 24 hour broth culture of <u>Staphylococcus aureus</u> organisms isolated from a case of mastitis into the udders of 2 cows and 3 heifers. For 4 cows he reported a severe type of mastitis with systemic involvement. In 1 cow there was no external evidence of inflammation of the udder. Parshel (1934) introduced <u>Staphylococcus</u> <u>aureus</u> into the quarters of 2 cows, and reported that the quarters became swollen and hot. The reaction was of a mild nature and subsided after a few days. Little and Foley (1935) injected staphylococcic organisms through the teat meatus on nine different occasions. They used approximately 800 staphylococci for the first injection and 2,500 staphylococci were injected later. Acute mastitis was produced after the last injection of 2,500 organisms. They further observed

that there was swelling and congestion of the quarter, and the quarter was painful on manipulation. Minett (1937) exposed quarters two times with 1 ml. of a 24 hour broth culture of Staphylococcus aureus and established infection which lasted for a few days. Miller and Heishman (1943) infused each of the udders of 8 cows with increasing numbers of staphylococcic organisms ranging from 700 to 30,000. Observations after 25 exposures revealed that 22 quarters were infected. Schalm (1944) produced gangrenous mastitis in two lactating cows by the intramammary infusion of 5 ml. of a pure broth culture of Staphylococcus aureus. Using the exudate from natural cases of mastitis or the Staphylococcus aureus organisms isolated therefrom, he produced transistory mastitis in two dry cows. Slanetz and Bartley (1953) produced an acute type of mastitis with large numbers of organisms and observed that there was a wide variation in response of mammary glands to inoculation of strains of staphylococcic organisms. After the injection of large numbers of phage type 80/81 (staphylococcic organisms) into the udders of two cows Drury et al. (1961) demonstrated the continual shedding of staphylococcic organisms in milk for four months. Nat (1962) introduced 16 x 10^2 to 28 x 10^2 staphylococcic organisms into 12 quarters and observed acute mastitis in one

quarter. When he infused 38×10^3 to 57×10^4 staphyloccic organisms in addition to mild trauma, he observed an acute mastitis in 2 of 12 quarters. For the third experiment he injected 1 x 10^{10} staphylococcic organisms into 13 quarters and observed acute mastitis in 4 quarters.

III. MATERIALS AND METHODS

1. Evaluation of disinfectants

<u>Staphylococcus</u> <u>aureus</u> was the test organism used to determine the efficacy of the products that might be used to disinfect the thermometers. The organism was taken from a stock culture of <u>Staphylococcus</u> <u>aureus</u>, transferred to a nutrient agar slant, incubated for 48 hours at 37 C., and then streaked on blood agar plates (5% of bovine blood), and again incubated for 24 hours. Suitable colonies were transferred to nutrient broth tubes and, after 24 hours incubation, were used for the testing procedure. Six glass rods (3 x 1/4inches in size) were used to simulate thermometers. Metal rods of the same length and size as the glass rods in the form of nails were also used.

Disinfectants tested

The following four disinfectants were tested: Novadine* which is a suspension of nonyl phenoxy ethanol-. iodine complex (providing 1.75% available iodine; Liquid

^{*} Cowles and Co., Cleveland, Ohio

Germicidal Detergent* which is a suspension of high molecular weight alkylamine hydrochlorides containing phemerol chloride as its active ingredient; Clenesco Liquid 75** is specifically designed for all food plant cleaning operations where the solution is applied by hand. It is a high foaming, mildly alkaline cleaner; and 70% ethyl alcohol.

Employing the use-dilution method (Mallmann 1945) the glass and metal rods were dipped into the broth culture for a period of 15 minutes and then removed from the broth and carefully laid on sterile filter paper in a closed petri dish for a 30 minutes drying period at room temperature. Care was taken not to roll while drying. At the end of the drying period these rods were dipped into the test disinfectant for the following periods of time: 0.5, 1.0 and 1.5 minutes. In the event that 1.5 minutes did not kill the organisms, the test was repeated using 2, 5, and 10 minute periods of time. After removal from the disinfectant the rods were rinsed in sterile water for one minute to remove excess disinfectant thus preventing further bacteriostatic Rods were then dipped into tubes containing sterile effect. nutrient broth and were shaken vigorously to remove organisms

^{*} Parke, Davis and Co., Detroit 1, Mich.

^{**} Cowles and Co., Cleveland, Ohio

adhering to the rods. Suitable dilutions were then plated on nutrient agar to measure quantitatively the extent of kill. The count for the bacterial growth was performed after 24 hours of incubation at 37 C.

Controls were run in the same manner, except that they were not dipped into the tubes containing disinfectant.

Glass and metal rods were also dipped in milk from a cow known to be shedding staphylococci in her milk. The testing with milk was performed with the disinfectant that gave superior results with the test organisms as had been determined previously. The presence or the absence of bacterial growth was determined by the use of blood agar media (5% of bovine blood).

2. Thermometers used

The following thermometers were obtained and used according to the manufacturer's instructions. Before use, the thermometers were checked for accuracy by partial immersion in a hot water bath. The "Cary" thermometer that compared most favorably with the readings on the Tri-R and mercury thermometers was used for determining rectal temperatures.

a. "Cary" thermometer *

The "Cary" thermometer (Fig. 1) is an unbreakable

* Cary Instruments, Limited, 44 Whitehall St., New York

metal dial thermometer, which is free from glass and mercury and can be used with complete safety. This thermometer was used only for the measurement of rectal temperatures since it was found to be too large to insert into the teat meati of some cows used in this experiment.

b. Tri-R thermometer**

This thermometer (Fig. 2) is a battery operated model supplied with a built-in long life mercury cell designed to provide a useful operating life exceeding 4,000 hours.

c. A standard laboratory mercury thermometer (centigrade)

This was used to determine room temperatures.

3. Measurement of intracisternal temperature.

Eight lactating and one non-lactating Holstein cows, ranging in age from 4 to 8 years were used for the recording of intracisternal temperatures. Some of these cows had a history of mastitis. All of the cows had teats of approximately equal diameter and length. Two of the cows were housed and fed in the MSU clinic; the others were housed and fed at the University Dairy barn. Animals were milked twice a day with milking machines.

The intracisternal temperatures were taken between 9 and 10 A.M., after the morning milking, and between 2 and

Tri-R Instruments Co., Jamaica 35, New York.

3 P.M., just before the evening milking. Since the temperatures were taken after and before the regular milking periods, the effect of the let-down phenomenon could be evaluated. At the time of measuring the intracisternal temperatures, cows were not provided with any kind of feed, nor were they excited by the presence of visitors. A line etched on the electronic thermometer 5 cm. from the point served as a marker to insure uniformity in depth of each insertion. The probe was disinfected for 2 minutes by immersing in Novadine solution (1:200) before each insertion.

At the time intracisternal temperatures were taken, the room temperatures were determined.

4. Tests applied to milk.

a. California Mastitis Test (CMT)

The procedure followed was that of Schalm and Noorlander (1957) as modified by Drury, et al. (1961).

b. Direct leukocyte count

Prescott and Breed (1910) investigated a method for determining the number of leukocytes in milk. This technique was used with the modification (Bryan, 1941) that instead of measuring the milk by a graduated pipette, a closed platinum loop which delivered a known amount of milk was used for spreading the milk on the slide.

c. Hemolysis test

Hemolysis was observed on blood (5% bovine) agar plates and was scored according to the method of Slanetz and Bartley (1953).

d. Coagualse test.

This test was performed according to the procedures outlined in the Difco Manual*.

5. Artificially induced mastitis.

Intracisternal and rectal temperatures were taken prior to and following the intramammary infusions of agents to induce mastitis in a cow. Temperatures were also determined at the time of sample collections. The following agents were used to produce mastitis:

a. 2% NaCl solution

Ten ml. of the saline solution were infused into the right rear quarter and twenty ml. into the left rear quarter. Milk samples were aseptically collected for bacteriological examination after each 4 hours on the first day and at 12 hour intervals for the next day and again after 24 hours.

b. Staphylococcus aureus

After an interval of 13 days, the above mentioned cow was used for this phase of the work. Four ml. of a 24

Difco Manual: Difco Laboratories, Inc., Detroit 1, Michigan.
 9th edition. 1953.

hour broth culture of non-hemolytic and coagulase-negative <u>Staphylococcus aureus</u> organisms were infused into the left rear quarter and 5 ml. of the same culture were infused into the right rear quarter. Milk samples were taken at hourly intervals for the first 5 hours of the first day and at 4 hour intervals on the second and third day. Subsequently one sample was collected on each of the next 9 days.

IV. RESULTS

1. Evaluation of disinfectants

The results on the evaluation of the four disinfectants tested are presented in Tables 1 through 8. As may be noted, Novadine was the most efficient disinfectant. Using test organisms it was found to be effective at a dilution of 1:200 in two minutes (Table 2) but not effective at 1:500 in 5 minutes (Table 1). In Table 3 results indicate that Novadine (1:200) was also effective in two minutes when infected (<u>Staphylococcus aureus</u>) milk was the test sample. Practical application of this use of Novadine (1:200) indicated that <u>Staphylococcus aureus</u> were killed on the thermometer probe following its use in taking intracisternal temperatures.

The remaining disinfectants (LGD, 70% ethyl alcohol, and Clenesco Liquid 75) tested were ineffective in two minutes as indicated in Tables 4 through 8.

2. Thermometers used

The comparison of the readings of the thermometers placed in hot water is shown in Table 9 and indicates that the Tri-R and mercury thermometer agreed closely. The

variation was 0.0 to 0.3 F. Of the four Cary thermometers tested, number 97517 was in closest agreement to the other thermometers used. Here the variation was 0.1 to 0.3 F.

3. Intracisternal temperatures

a. Clinically normal cows

The intracisternal temperatures of 5 clinically normal cows, each of which were determined on three or more days, are presented in Table 10. In this table milk examination data, body and room temperatures are also found, for each day the intracisternal temperatures were taken.

For cow 573 (Table 10) the greatest variation among the four intracisternal temperatures determined for a single day was 5.0 F (RF 95.0 F to RR 100.0 F). During the five days that this cow's intracisternal temperatures were taken, the highest temperature was 100.0 F and the lowest was 93.0 F.

The cow named Daya (Table 10) showed an intracisternal temperature variation among the four quarters on a single day of 2.0 F (95 F to 97 F). The highest intracisternal temperature throughout the seven days on which temperatures were determined was 97 F and the lowest was 94.2 F.

Cow 600 (Table 10) was not lactating during the time of this experiment. For a single day, the greatest variation among the four intracisternal temperatures was 2.1 F. The highest and lowest intracisternal temperature noted in three days was 98.0 F and 92.5 F respectively.

Cow 158 (Table 10), on a given day, had a maximum intracisternal temperature variation of 3.0 F among the four quarters. The highest and lowest intracisternal temperatures determined in three days was 95.0 F and 92.0 F respectively.

For cow 591 (Table 10) the greatest variation among the four intracisternal temperatures determined for a single day was 6 F. During the four days that observations were made the highest intracisternal temperature was 98.0 F and the lowest was 92.0 F.

Intracisternal temperature data on four cows that were not examined for more than two days is presented in the appendix. These intracisternal temperatures compare favorably with those in Table 10.

b. Cows with mastitis

The intracisternal temperature of the RF quarter of cow 591 (Table 11) during an acute flare-up of non-systemic mastitis was from two to three degrees higher than the highest temperature of the non-mastitic quarters. Milk from the RF quarter also had leukocyte counts appreciably higher than milk from the other three quarters. The CMT score for the
milk from the RF quarter was likewise higher than for the other quarters.

Intracisternal temperatures and supplementary data for cow 591 in which mastitis was artificially induced by infusing 2% NaCl solution are presented in Tables 12 and 13 and Figures 3, 4, 5, 6, and 7.

At the peak of the inflammatory reaction as judged by leukocyte counts (Table 12) of 20, 30, and 14 millions per ml. of milk at 8, 12, and 24 hours respectively, following infusion of 20 ml. of 2% NaCl solution, the intracisternal temperature ranged from 101.6 to 98.5 F. These temperatures were from 2.5 to 6.8 degrees higher than intracisternal temperatures of the control quarters. Accompanying this period the infused quarter was swollen and painful. The 8 and 12 hour rectal temperatures as taken from Table 12 are also at peak level.

When 10 ml. of 2% NaCl solution were infused into the RR quarter of cow 591 (concurrently with the infusion of 20 ml. of the same solution into the LR quarter) results (Table 13 and Figures 5, 6, and 7) were similar to those mentioned following the infusion of 20 ml. of NaCl solution (Table 12 and Figures 3, 4, and 7). The major difference is an indication of a less severe inflammatory reaction as

judged by lower leukocyte counts and not as much increase in the intracisternal temperature.

When mastitis was produced by the intramammary infusion of 5 ml. of a 24 hour broth culture of non-hemolytic <u>Staphylococcus aureus</u> organisms into the RR quarter of cow 591 (Table 14), there were marked increases in the intracisternal temperature of the infected quarter. From the second to the 45th post infusion hour the temperature was 100 F or higher and on two occasions went as high as 103.5 F. The highest intracisternal temperature of the control quarters during this time was 96.6 F. Concurrent with the high temperature of the infected quarter were an elevation in rectal temperature to 106.3 F, and increase in the milk leukocyte count to 6 million per ml., painful swelling of the infused quarter, and grossly abnormal milk.

When 4 ml. of the same broth culture as used in the RR quarter were concurrently injected into the LR quarter results (Table 15) were similar to those presented in Table 14. The increases in the intracisternal temperature and leukocyte count were of the same magnitude but the duration of temperatures over 100 F was 26 hours as compared to 43 hours for the RR quarter.

USE-DILUTION TEST ON NOVADINE (1:500) TEST ORGANISM - STAPHYLOCOCCUS AUREUS

Immersion time	Plate dilution	Bacterial growth		
of rods in disinfectant (minutes)	on nutrient agar	on nutrient agar plate		
12	1:10	+		
$\frac{1}{2}$	1:100	+		
1	1:10	+		
1	1:100	+		
$1\frac{1}{2}$	1:10	+		
$1\frac{1}{2}$	1:100	+		
2	1:10	+		
2	1:100	+		
5	1:10	+ +		
5	1:100	+		
10	1:10	-		
10	1:100	_		

USE-DILUTION TEST ON NOVADINE (1:200) TEST ORGANISM - STAPHYLOCOCCUS AUREUS

Immersion time of rods in disinfectant (minutes)	Plate dilution on nutrient agar	Bacterial growth on nutrient agar plate			
1	1:10	+			
1	1:100	+			
2	1:10	-			
2	1:100	-			
5	1:10	-			
5	1:100	-			

USE-DILUTION TEST ON NOVADINE (1:200) TEST MATERIAL - MILK SAMPLE*

.

Immersion time of rods in disinfectant (minutes)	Plate dilution on nutrient agar	Bacterial growt on nutrient agar plate				
1	1:10	+				
1	1:100	+				
2	1:10	-				
2	1:100	-				
5	1:10	-				
5	1:100	-				

* Milk from a cow shedding <u>Staphylococcus</u> aureus.

USE-DILUTION TEST ON LIQUID GERMICIDAL DETERGENT (1:300) TEST ORGANISM - STAPHYLOCOCCUS AUREUS

Immersion time of rods in disinfectant (minutes)	Plate dilution on nutrient agar	Bacterial growt on nutrient agar plate		
$\frac{1}{2}$	1:10	+		
$\frac{1}{2}$	1:100	 +		
1	1:10	+		
1	1:100	+		
$1\frac{1}{2}$	1:10	+		
$1\frac{1}{2}$	1:100	+		
2	1:10	• +		
2	1:100	+		
5	1:10	+		
5	1:100	+		
10	1:10	-		
10	1:100	-		

USE-DILUTION TEST ON LIQUID GERMICIDAL DETERGENT (1:200) TEST ORGANISM - STAPHYLOCOCCUS AUREUS

Immersion time of rods in disinfectant (minutes)	Plate dilution on nutrient agar	Bacterial growt on nutrient agar plate		
$\frac{1}{2}$	1:10	+		
$\frac{1}{2}$	1:100	+		
1	1:10	+		
1	1:100	+		
11/2	1:10	+		
11/2	1:100	+		
2	1:10	+		
2	1:100	+		
5	1:10	-		
5	1:100	-		
10	1:10	-		
10	1:100	-		

USE-DILUTION TEST ON LIQUID GERMICIDAL DETERGENT (1:150) TEST ORGANISM - <u>STAPHYLOCOCCUS</u> AUREUS

Immersion time of rods in disinfectant (minutes)	Plate dilution on nutrient agar	Bacterial growt on nutrient agar plate		
$\frac{1}{2}$	1:10	+		
$\frac{1}{2}$	1:100	+		
1	1:10	+		
1	1:100	+		
$1\frac{1}{2}$	1:10	+		
$1\frac{1}{2}$	1:100	+		
2	1:10	+		
2	1:100	+		
5	1:10	-		
5	1:100	-		
10	1:10	-		
10	1:100	-		

USE-DILUTION TEST ON 70% ETHYL ALCOHOL TEST ORGANISM - STAPHYLOCOCCUS AUREUS

Immersion time of rods in	Plate dilution on	Bacterial growth on nutrient
disinfectant (minutes)	nutrient agar	agar plate
$\frac{1}{2}$	1:10	+
$\frac{1}{2}$	1:100	+
1	1:10	+
1	1:100	+
$1\frac{1}{2}$	1:10	+
112	1:100	+
2	1:10	+
2	1:100	+
5	1:10	+
5	1:100	+
10	1:10	-
10	1:100	-

USE-DILUTION TEST ON CLENESCO LIQUID 75 CLEANER (1:300) TEST ORGANISM - STAPHYLOCOCCUS AUREUS

.

Immersion time of rods in disinfectant (minutes)	Plate dilution on nutrient agar	Bacterial growth on nutrient agar plate
1 2	1:10	+
12	1:100	+
1	1:10	+
1	1:100	+
$1\frac{1}{2}$	1:10	+
$1\frac{1}{2}$	1:100	+
2	1:10	+
2	1:100	+
5	1:10	+
5	1:100	+
10	1:10	+
10	1:100	+

TEMPERATURE READINGS OF DIFFERENT THERMOMETERS (F) RECORDED IN HOT WATER BATH

Thermometers

Date		Ca	Tri-R	Mercury		
	90754	14083	81699	97517		
2/ 16	99.8	99.4	100.1	99.2	99.2	99.3
2/16	102.0	101.7	102.1	101.4	101.5	101.5
2/16	103.6	103.2	103.8	103.2	103.2	103.5
2 /16	105.0	104.5	105.0	104.5	104.5	104.6
2/ 17	100.2	99.5	99.8	99.6	99.6	99.7
2 /17	101.7	100.9	101.7	101.1	101.0	101.3
2/18	99.4	98.9	98.8	98.9	99.0	99.0
2/18	102.0	101.8	102.5	101.7	101.7	102.0
2/18	104.2	103.0	104.7	104.1	104.1	104.2

TEMPERATURE AND MILK EXAMINATION DATA OF CLINICALLY NORMAL COWS *

ogy**	RR	+ 5	+ 0	+ ო	1	1 73	5	- 73	 5	- 5	1 73	- 7] –
terio]	LR	+	+ 0	5	5 +	2 -	2 -	-	2 -	1 5	1 5	1 13	1 -
and bac	LF	1+	+ 0	2 +	1+	4 +	2 -	1 5	2 -	7	1 5	1 5	1+
CMT 3	\mathbf{RF}	1+	1+	+ 1	1+	5	2 -	7	- 5	ti	ti	ti	- 0
	RR	0.10	0.15	1.20	0.30	0.45	0.60	06.0	06.0	0.30	06.00	1.20	0.70
es/ml. lion	LR	0.10	0.15	06.0	0.45	0.30	0.60	06.0	0.60	0.45	06.0	1.20	0.45
in mil:	LLF	0.30	0.15	0.20	0.30	1.00	06.0	0.90	06.0	0.30	06.0	1.20	0.60
Lei	RF	0.60	0.60	0.15	0.20	0.45	06.0	06.0	06.0	ti	ti	ti	0.30
cemp	RR	95.0	93.3	100.0	93.5	96.25	94.4	95.5	94.4	96.0	96.6	97.0	96.0
ernal 1 F	LR	93.6	93.4	96.5	96.0	96.0	94.3	95.0	94.3	95.6	96.0	96.4	95.0
racist	LF	94.0	93.0	0.66	93.5	98.0	95.5	94.8	94.2	96.5	96.0	95.0	95.0
Int	RF	94.0	93.5	95.0	93.6	95.5	96.0	95.4	95.2	ti	ti	ti	95.5
Room temp	I	53.6	61.0	61.0	76.0	68.0	66.2	66.2	66.2	77.0	62.6	76.0	68.0
Body temp	I	99.5	100.7	103.2	100.5	100.8	101.2	101.2	101.2	100.9	101.2	101.3	100.8
Name or	No.	573	=	E	=	:	Da ya	:	=	=	:	:	:

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Continued.

TABLE 10 (Continued)

*		I	1	1	1		1	ł	+	I	I	
gy	RR	0	0	0	0	н	Ч	H	0	n	e	
teriolc	LR	+ 1	- 0	+	-	1 -	-	1 -	+	ا ش	1 ന	fewer
nd bac	LF	+	1+	1 -	ti	ti	ti	ti	1	၊ က	: ო	se with
CMT 8	RF	I 0	1 -	- 7	4 -	3 3	5	-	I 0	ເ	၊ က	or thos
•	RR	0.30	0.20	0.45	0.15	0.30	0.45	0.45	0.45	1.20	1.20	s. (F
ces/ ml	LR	0.60	0.30	0.30	0.20	0.45	0.60	0.60	0.45	1.20	1.20	re day
ukocyt in mil	LLF	0.20	0.60	0.30	ti	ti	ti	ti	0.90	1.20	1.20	or mo
Le	RF	0.07	0.30	0.40	1.20	06.0	0.60	0.60	0.70	1.20	1.50	three
temp	RR	94.4	93.5	94.0	92.0	92.4	93.5	94.0	94.0	97.4	94.0	each of
ernal. F	LR	94.2	92.5	92.0	94.0	94.5	95.0	95.2	94.2	97.5	97.5	ned on
racist	LF	93.5	94.5	93.5	ti	ti	ti	ti	94.6	98.0	98.0	exami
Int	RF	93.0	94.2	95.0	98.0	97.0	96.6	96.5	92.5	96.0	97.6	those
Room temp	La 2	61.0	76.0	68.0	76.0	62.6	75.2	78.0	61.0	76.0	68.0	uded are
Body temp	la 1 2 2	101.2	101.5	100.8	101.4	101.8	101.5	101.4	100.6	100.4	100.5	ws inclu
Name or	No.	158	:	:	591	:	:	:	600 ^{&}	:	:	* Col

examinations see appendix lable to/

** += hemolytic staph.

-= non-hemolytic staph.

non-lactating cow. ഷ

teat injury - did not insert thermometer ti

TEMPERATURE AND MILK EXAMINATION DATA OF COW 591 AFFECTED WITH ACUTE NON-SYSTEMIC MASTITIS (RF QUARTER)

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log	RI	0	l	7	8	Г	I	Ч	Ч
erio	LR	•	I	I 	•	1	•	•	•
acte		•		•	•	•	•	•	•
d br	LF	8	•	•	1 8	- 7	- 7	- 7	8
T aı		I	I	ł	I	I	I	1	I
CM	RF	4	4	4	4	4	4	4	4
	RR	.30	.30	.45	•45	.45	.60	.60	.45
_:		0	0	0	0	0	0	0	0
s/m. ion	LR	0.3(0.45	0.3(0.45	0.45	0.6(0.6(0.45
cyte: mill	LF	.60	.60	.45	.60	06.	.60	.90	.60
auko in		0	0	0	0	0	0	0	0
Å	RF	1.20	1.20	1.20	1.30	1.50	1.40	1.50	1.20
		4	9	0	0	0	80	0	0
.dme	RF	92.	92.	94.	95.	95.	94.	95.	95.
l te	Я	• 5	.2	•	•	•	.2	•2	•5
erna	Π	94	94	93	94	94	94	94	94
tst. F	, Fi	i.	'n	i.	Ļ	Ţ,	ŗ	÷Ħ	i
trac	П	-	4	4	4	+	+	4	+
In	RF	98.0	97.8	97.6	97.4	97.7	97.5	97.5	97.0
	2	•	0.	•	•	•	•	.5	0.
Rot	5	63	68	11	75	75	78	78	78
d y	2	0.0	80	• 2	• 0	• 2	• £		8
Bo te		102	101	101	101	101	101	101	101
Da te		4/20	4/22	4/23	4/24	4/25	4/26	4/27	4/28

* - = non-hemolytic, coagulase negative staph.

ti - teat injury - did not insert thermometer.

			INFUSI	ON OF 2	O ML. OI	7 2% NaCl S	SOLUTION IN	LR QUARTER		
Da te	Body temp	Room temp	Intrac LR	cisterna F (RF Cont	l temp LF)* rol	Time in hours after infusion	Leukocytes/ ml. in million	Bacteriolog	/ Gross Milk	appearance of Quarter
4/30	101.5	76.0	96.5	(96.0	95.0)	0	06.0	NHS 8**	Norma l	Norma l
4/30	101.6	77.2	99.5	(96.2	94.8)	4	2.10	Slight hemolysis Sa	E	Swollen
4/30	102.9	77.0	101.5	(96.0	94.6)	œ	20.0	alpha hemo- lysis Sa	:	Swollen & painful
4/30	103.0	76.0	101.6	(95.8	94.8)	12	30.0	:	=	E
5/1	101.2	76.0	98.5	0.96)	95.2)	24	14.0	NHSa	=	Swellings & pain decreased
5/1	101.5	75.0	100.0	(96.2	95.4)	30	4.0	:	=	
5/2	100.8	75.0	97.5	(96,0	95.0)	48	1.50	:	=	:
5/3	100.7	77.0	96.6	(95.8	94.8)	72	1.00	:	Ξ	Normal
* RR	quarter	vas si	multane	ously i	nfused v	vith 10 ml.	of 2% NaCl	solution (Se	e Table	13)

** non-hemolytic staph.

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TABLE 12

TEMPERATURE, MILK AND CLINICAL EXAMINATION DATA FOR COW 591 FOLLOWING

	appearance of	Quarter	Norma l	Swollen	Swollen & painful	=	Swelling & pain decreased	•	:	Norma l	e 12)
	Gross	Milk	Norma 1	:	**	=	:	=	=	=	see Tabl
QUARTER	Bacteriology		NHSa **	Slight hemolysis Sa	alpha hem- lysis Sa	:	NHSa	=	=	E	l solution (
OLUTION IN RF	Leukocytes/ ml. in	million	0.40	1.80	10.0	25.0	13.0	3.0	1.0	0.90	ml. of 2% NaC
2% NaCl S	Time in hours	after infusion	0	4	ω	12	24	30	48	72	With 20
10 ML. OF	nal temp	ols) * LF	95.0)	94.8)	94.6)	94.8)	95.2)	95.4)	95.0)	94.8)	ly infused
ON OF	cister	(Contr RF	(96.0	(96.2	(96.0	(95.8	(96.0	(96.2	(96.0	(95.8	aneous
INFUSI	Intra	RR	96.0	0.09	100.5	100.8	98.5	99.66	97.0	96.0	simult
	Room temp	•	76.0	77.2	77.0	76.0	76.0	75.0	75.0	77.0	r was
	Body temp		101.5	101.6	102.9	103.0	101.2	101.5	100.8	100.7	R quarte
	Da te		4/30	4/30	4/30	4/30	5/1	5/1	5/2	5/3	• ·

non-hemolytic staph.

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TABLE 13

TEMPERATURE, MILK AND CLINICAL EXAMINATION DATA FOR COW 591 FOLLOWING

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* LR quarter was simultaneously infused with 4 ml. of 24 hour broth culture of non-hemolytic Staphylococcus aureus • #

RR QUARTER	ogy Gross appearance of	Milk Quarter		Norma 1 Norma 1	11 11	11 11	" Swollen	Abnormal Swollen &	painful		11 11	11 11	11 44	14 14	••		11 11	**	11 11	" Swelling	decreased	Norma l Norma l	11 11	11 11	11 11	11 11	=
AUREUS IN	Bacteriol			** ash	:	=	=	:		:	:	:	:	=	:	:	:	:	=	:			:	:	=	:	•
APHYLOCOCCUS	Leukocytes/ ml. in	million		0.15	0.45	06.0	3.60	4.50		4.80	6.00	4.50	3.00	2.00	1.80	1.60	1.60	1.50	1.45	1.40		1.40	1.20	1.20	1.00	1.00	0.90
IOLYTIC ST	Time in hours	after	infusion	0	1	2	ი	4		ы С	6	21	25	29	41	45	57										
NON-HEW	temp	rols	L.F.) *	95.4)	95.4)	95.2)	95.4)	95.2)		95.4)	92.6)	95.2)	95.4)	92.6)	95.0)	95.2)	95.0)	95.0)	92.6)	95.4)		95.2)	95.4)	95.5)	94.8)	94.6)	95.2)
LTURE OF	cisterna] F	Cont	(RF	(96.2	(96.0	(96.2	(96.4	(96.5		(96.6	(96.5	(96.0	(96.2	(95.8	(96.0	(96.2	(96.6	(96.0	(96.5	(96.0		(95.8	(96.0	(96.3	(95.6	(96.0	(96.4
OTH CUI	Intra		RR	96.5	97.5	100.0	101.0	101.5		103.5	101.5	102.0	103.5	101.6	100.0	100.0	99.8	99.5	0.06	98.5		98.0	97.5	97.4	97.0	97.1	96.8
HOUR BR	Room temp			86.0	86.0	86.0	87.8	86.0		85.0	86.0	82.4	89.6	86.0	82.4	80.6	73.4	78.2	78.2	80.0		80.0	78.0	75.0	76.0	71.6	75.0
OF 24]	Body temp			101.4	102.0	101.8	103.6	105.1		106.3	103.8	105.6	105.6	104.4	102.1	102.0	101.8	101.1	100.8	101.2		101.2	101.0	100.8	100.6	100.8	101.0
	Da te			5/16	5/16	5/16	5/16	5/16		5/16	5/16	5/17	5/17	5/17	5/18	5/18	5/19	5/20	5/21	5/22		5/23	5/24	5/25	5/26	5/27	5/28

TABLE 14

TEMPERATURE, MILK AND CLINICAL EXAMINATION DATA FOR COW 591 FOLLOWING INFUSION OF 5 ML.

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* RR quarter was simultaneously infused with 5 ml. of 24 hour broth culture of non-hemolytic . <u>Staphylococcus</u> aureus * *

								చ												አስ	pe						
ER	a ppearance of	Quarter		Norma l	••		Swollen	Swollen	painful	84	••	••	•				:	:	••	Swelling	decrease	Norma l		•	••	••	=
QUARTEI	r Gross	Milk		Norma l	:	:	:	:		:	:	•	:	:	:	:	=	:	:	:		:	:	:	:	:	:
AUREUS IN LI	Bacteriology			* * shu	:	E	E	:		:	:	=	:	:	:	••	E	:	E	-		=	:	=	E	=	=
NPHYLOCOCCUS	Leukocytes/ ml. in	million		0.075	0.30	0.75	2.70	3.90		4.50	5.40	3.90	2.00	1.80	1.60	1.40	1.50	1.40	1.30	1.30		1.20	1.10	1.00	1.00	0.90	0.75
HOUR BROTH CULTURE OF NON-HEMOLYTIC ST	Time in hours	after	infusion	0	1	7	ო	4		5	6	21	25	29	41	45	57										
	al temp	rols	L.F.) +	95.4)	95.4)	95.2)	95.4)	95.2)		95.4)	92.6)	95.2)	95.4)	95.6)	95.0)	95.2)	95.0)	95.0)	92.6)	95.4)		95.2)	95.4)	95.5)	94.8)	94.6)	95.2)
	cistern F	Cont	(RF	(96.2	(96.0	(96.2	(96.4	(96.5		(96.6	(96.5	(96.0	(96.2	(95.8	(96.0	(96.2	(96.6	(96.0	(96.5	(96.0		(95.8	(96.0	(96.3	(92.6	(96.0	(96.4
	Intra		LR	96.0	97.5	99.8	100.5	101.5		103.5	101.0	101.5	102.0	101.0	99.5	99.2	0.06	98.5	98.0	97.8		97.0	96.6	96.5	96.4	96.2	95.8
	Room temp	•		86.0	86.0	86.0	87.8	86.0		85.0	86.0	82.4	89.6	86.0	82.4	80.0	73.4	78.2	78.2	80.0		80.0	78.0	75.0	76.0	71.6	75.0
OF 24]	Body temp	·		101.4	102.0	101.8	103.6	105.1		106.3	103.8	105.6	105.6	104.4	102.1	102.0	101.8	101.1	100.8	101.2		101.2	101.0	100.8	100.6	100.8	101.0
	Da te			5/16	5/16	5/16	5/16	5/16		5/16	5/16	5/17	5/17	5/17	5/18	5/18	5/19	5/20	5/21	5/22		5/23	5/24	5/25	5/26	5/27	5/28

, TABLE 15

TEMPERATURE, MILK AND CLINICAL EXAMINATION DATA FOR COW 591 FOLLOWING INFUSION OF 4 ML.

V. DISCUSSION

1. Selection of disinfectant

In considering the selection of the disinfectant for thermometer sterilization, the following list of properties were considered:

Bacterial activity

An ideal disinfectant or antiseptic should have a wide range of killing power. In this work <u>Staphylococcus</u> <u>aureus</u> was melected as the test organism because of its predominence in most cases of mastitis. However the final criterion on the effectiveness of a disinfectant was considered under conditions of practical application.

Availability and cost

The disinfectant chosen for this purpose should be readily available and relatively inexpensive.

Toxic effect on tissues

Toxic effect of the evaluated antiseptic should be considered when a selection is to be made. In the opinion of the author every chemical agent is somewhat toxic to animal tissue, however no work was done in this connection.

Only the gross appearance of the quarter was noted for any abnormality following insertion of chemically sterilized thermometer, and none could be detected.

Speed of action

The speed of action or rate of kill is also an important factor when selecting an antiseptic or the disinfectant. The action of the antiseptic or the disinfectant should be rapid, so that little time is required for effective sterilization.

Stability

A good disinfectant should not lose its effectiveness when stored for reasonable lengths of time at ordinary room or barn temperature.

a. Ethyl alcohol

Force and Kerr (1920) reported that clinical thermometers were best sterilized by chemical disinfectants and an exposure of 4 minutes was found to be necessary with 50% alcohol to secure adequate disinfection of oral thermometers. Price (1950) indicated that an exposure of 5 minutes to either 70 or 80% ethyl alcohol was necessary to kill the <u>M. pyogenes var. aureus</u>. Reddish (1956) reported that 70% alcohol was satisfactory for the disinfection of clinical thermometers. Immersion of the thermometers in 70% alcohol for 10 minutes was sufficient for this purpose.

During this work 70% ethyl alcohol was tested as one of the disinfectants. However, it would not kill <u>Staphylococcus aureus</u>, the test organism, in 5 minutes, but did kill effectively in 10 minutes (Table 7). Since economy of time was considered important in this work a ten minute sterilization time was considered impractical.

b. Clenesco Liquid 75 Cleaner

Actually this product is specifically designed for food plant cleaning operations. When tested for use in this work it did not give any promising results. It would not kill the test organism at the suitable dilution in 10 minutes (Table 8).

c. Liquid Germicidal Detergent (LGD)

Bryan and associates (1948) reported that this product was an efficient udder disinfectant and would destroy mastitis producing organisms that might be on the external surface of the teats and udder.

The results of the research conducted by the Parke, Davis and Company on Liquid Germicidal Detergent using <u>Staphylococcus aureus</u> as one of the test organisms are shown as follows:

Culture	Dilution bacte	ricidal at 20 C.	
	5 minutes	10 minutes	
Staphylococcus aureus	200	300	

1 part of sample 199 parts of water

1 part of sample 299 parts of water

Tables 4 and 5 show that LGD was effective in 5 minutes at a dilution of 1:200 and in 10 minutes at a dilution of 1:300, thus these results are in agreement with those of the manufacturer. Neither of these dilutions nor a dilution of 1:150 would kill the test organism in 2 minutes. Since it was considered important that 2 minutes be the maximum time allowed for disinfection of the thermometer, LGD did not qualify.

d. Novadine

The antibacterial efficiency tests of this compound showed that in a dilution of 1:500 test organisms were not killed in 2 minutes, that they were killed in 10 minutes (Table 1). Since this killing time was not satisfactory another dilution (1:200) was tested. The use-dilution test (Table 2) shows that in 2 minutes, there was a complete kill of the test organism. In the use-dilution test using infected (Staph.-aureus) milk as the test material (Table 3), Novadine

was also found to be effective in 2 minutes at a dilution of 1:200.

Novadine was the disinfectant of choice for thermometer sterilization because in addition to fulfilling the properties previously discussed, it was found to be effective within 2 minutes in a concentration that did not cause noticeable irritation.

2. Normal intracisternal temperature

The mammary intracisternal temperatures of 9 clinically mormal cows (Table 10 and 16) varied from 91.5 F to 100.0 F. A total of 109 intracisternal temperatures were determined on these cows throughout a period of two months. Intracisternal temperatures were taken on as many as seven different days in the case of the cow named Daya.

Room temperatures encountered during this study did not seem to have any notable effect on the intracisternal temperatures (Table 10).

With one exception rectal temperatures did not exceed 101.8 F. In the case of cow 573 (Table 10, line 3) the rectal temperature was 103.2 F. At this time there was also a leukocyte count of 1,200,000 per ml. of milk from the RR quarter and a CMT score of 3. In view of the fact that this cow's body temperature was slightly elevated and there

was a high leukocyte count in the RR quaater, it is felt that a subclinical case of mastitis was present and was possibly responsible for the high intracisternal temperature of 100.0 F in the RR quaater. If this cow was considered to have mastitis and if the determinations for this day are excluded from the table, the highest intracisternal temperature recorded for normal cows was 98.0 F (Table 10, lines 5, 14, 15, and 19).

Cow 600 was non-lactating at the time of these observations (Table 10). The first observations were made shortly after she had finished her lactation period (had not been milked for five days). When the second and third observations were made the animal had been non-lactating for 9 and 13 days respectively. According to Schalm et al. (1957) high milk leukocyte counts are present when cows are between consecutive lactation periods. Table 10 shows this to be true for cow 600. Accompanying the high milk body cell count CMT scores of 3. It is noteworthy that intracisternal temperatures tend to be slightly higher during non-lactating periods.

Cow 591 (Table 10), on the first day, had a milk leukocyte count of 1,200,000 per ml. of milk, in the RF quarter accompanied by a CMT score of 4. Although not

clinically evident, it is felt that mastitis was present in this quarter and that the intracisternal temperature of 98 F was the result of the inflammation.

If the two cases of probable mastitis (cow 573, line 3; and cow 591, line 19) and cow 600 during the non-lactating period excluded from Table 10, the intracisternal temperature for mastitis-free, lactating cows are found to vary from 91.5 F to 96.6 F.

3. Intracisternal temperatures of cows with mastitis

Cow 591 was observed during an acute attack of nonsystemic mastitis in the RF quarter. In Table 11 can be seen the intracisternal temperatures for this quarter in comparison to the other two quarters. The leukocyte counts of the milk from this quarter ranged from 1,200,000 to 1,500,000 per ml., the CMT score was consistently 4, and the intracisternal temperatures were from 2 to 3 degrees (F) higher than the noninflamed quarters.

When mastitis was artificially induced by the injection of 2% NaCl solution or <u>Staphylococcus aureus</u> organism, the inflamed quarters had intracisternal temperatures considerably higher than the control quarters: of the two amounts of 2% NaCl solution, the 20 ml. injection resulted in slightly higher intracisternal temperatures than the 10 ml. injection

(Tables 12 and 13). Both quarters had intracisternal temperatures 2 to 5 degrees F higher than temperatures of the control quarters. Following each of the NaCl infusions, it was noted that the non-hemolytic <u>Staphylococcus aureus</u> which was present in the quarters became hemolytic within four hours after the infusion (Tables 12 and 13). The organism continued to be hemolytic for 12 hours after which time it again became non-hemolytic.

Intracisternal temperatures of quarters injected with 4 ml. and 5 ml. of 24 hour broth culture of non-hemolytic <u>Staphylococcus aureus</u> organisms (Tables 14 and 15) followed similar patterns to that observed when NaCl solution was injected. However, in the case of the <u>Staphylococcus aureus</u> injections the intracisternal temperatures of the injected quarters became higher and there was more evidence of a systemic reaction evidenced by rectal temperatures up to 106.3 F. It is interesting to note that the injection of the Staphylococcus organisms did not result in milk leukocyte counts (Tables 14 and 15) as high as were recorded following injection of the NaCl solution (Tables 12 and 13; Figures 3 and 5). The 5 ml. injection of organisms (Table 14) resulted in elevated intracisternal temperatures for a longer

period (approximately 43 hours) than when a four ml. injection was given (approximately 26 hours; Table 15).

FIGURE 1

"Cary" thermometer



FIGURE 2

Tri-R electronic thermometer





* Infusion was made 0 hour after immediately making the leukocyte count

FIGURE 3



Intracisternal temperature after infusion * of 20 ml. of 2% NaCl solution in left rearquarter: cow 591



* Infusion was made at 0 hour immediately after taking the . intracisternal temperature.

FIGURE 5

Leukocyte count after infusion * of 10 ml. of 2% NaCl solution in right rear quarter: cow 591



Infusion was made at 0 hour after immediately making the leukocyte count.



Intracisternal temperature after infusion* of 10 ml. of 2% NaCl solution in right rear quarter: cow 591



* Infusion was made at 0 hour immediately after taking intracisternal temperature.

FIGURE 7

Rectal temperature following the intracisternal infusion * of 2% NaCl solution (20 and 10 ml. in left rear and right rear quarters respectively): cow 591



Infusion was made at 0 hour immediately after taking
the rectal temperature.

VI. SUMMARY

For this work, it was found that of the four disinfectants tested, Novadine was the most satisfactory for sterilization of the thermometer used for the determination of mammary intracisternal temperatures.

The mammary intracisternal temperatures of 9 clinically normal dairy cows were determined by the use of an electronic thermometer (Tri-R). From repeated observations over a period of four months it was found that intracisternal temperatures of mastitis-free, lactating cows varied from 91.5 to 96.6 F. In one lactating cow, intracisternal temperatures ranged from 92.5 to 98.0 F.

Collaborative data collected during this study include: rectal temperature, room temperature, California Mastitis Test, milk leukocyte counts, and bacteriological examination of milk samples.

The intracisternal temperatures of one cow with naturally occuring mastitis in the RF quarter were 2 to 3 degrees (F) higher than the non-inflamed quarters.

Acute mastitis was produced by intramammary injections of 2% NaCl solution and also by injections of non-hemolytic <u>Staphylococcus aureus</u> organisms. Pre- and post-injection intracisternal temperatures were taken. When the 2% NaCl solution was the mastitis inducing agent intracisternal temperatures of the inflamed quarters were 2 to 5 degrees (**P**) higher than the pre-injection or control quarter temperature. When <u>Staphylococcus aureus</u> was the mastitis inducing agent intracisternal temperatures of the inflamed quarters were 3 to 7 degrees F higher than pre-injection or control quarter temperatures.
VII. CONCLUSIONS

Determination of mammary intracisternal temperature
 was found to be possible in the cow.

2. The intracisternal temperatures of non-mastitic quarters of clinically normal, lactating cows vary from 91.5 to 96.6 F. In the non-lactating cow it varies from 92.5 to 98.0 F.

3. The intracisternal temperatures of mastitic quarters are higher than temperatures of non-mastitic quarters of the same animal or of intracisternal temperatures of normal, non-mastitic cows. Intracisternal temperatures of inflamed quarters can go as high as 103.5 F.

4. The intracisternal temperature has a correlation with the California Mastitis Test, leukocyte counts, and rectal temperature in the acute stage of udder irritation.

5. Novadine (1:200) is a safe, effective disinfectant for use on the thermometer employed for intracisternal temperature determination.

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IX. APPENDIX

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TABLE 16

TEMPERATURE AND MILK EXAMINATION DATA OF CLINICALLY NORMAL COWS

Name tor tor 101.Body tom tom RRoom tom RIntracisternal tom in millionLeukocytes/ ml. in millionCMT and bacteriology activeNo.remp tom Rtemp tomrIntracisternal tom rin millionCMT and bacteriologyNo.remp rremp rremp rrIRIRIRIRIRNo.53.695.095.094.194.50.150.600.100.302-2-1-2''101.661.095.094.296.095.00.150.600.902-1-22''101.453.693.094.393.00.600.300.450.071-1-22''101.261.094.094.094.50.200.200.150.202-2-22''101.261.094.094.50.200.200.150.202-222''101.261.094.094.50.200.200.150.202-2222''101.261.094.094.592.30.200.200.150.301+1+1+1''100.876.094.492.492.00.450.450.300.601+1+1+1+1+1	+		+	+	1	1	+	+	+
Name bor tempBody tempRoom tempIntracisternal temp rIeukocytes/ml.CMT and bacterioNo.remp rempremp remprIRLFLRRRIrLFLRNo.RFLFLRRRRFLFLRRRRLFLR575101.053.695.094.194.50.150.600.100.302-2-1-"101.661.095.094.194.50.150.600.600.902-1-2+78101.453.693.094.393.00.600.600.902-1-2+78101.261.094.094.094.50.200.150.071-1-2+78101.261.094.094.094.50.200.150.150.071-1-2+78101.261.094.094.094.50.200.150.150.071-1-2+78101.261.094.094.094.50.200.150.150.071-1-2+78101.261.093.5094.094.50.200.150.150.161-1-2+79100.876.094.492.492.00.450.200.150.151+1+1+1+	logy	RR	3	8	1	5	T	H	0
Name bodyBodyRoom tempIntracisternal tempLeukocytes/ml.CMT and baor No.temp temptemp temp \mathbf{F} $\mathbf{L}\mathbf{F}$ $\mathbf{L}\mathbf{R}$ \mathbf{R} \mathbf{R} \mathbf{R}^{T} \mathbf{L}^{T} \mathbf{R} \mathbf{R}^{T} \mathbf{L}^{T} No. \mathbf{R} \mathbf{L} \mathbf{L} \mathbf{R} \mathbf{L} \mathbf{R} \mathbf{R} \mathbf{R}^{T} \mathbf{L}^{T} \mathbf{R} \mathbf{R}^{T} \mathbf{L}^{T} 575 101.0 53.6 95.0 94.1 94.5 0.15 0.60 0.30 2.0 2^{-} 2^{-} \mathbf{R} 101.6 61.0 95.0 94.2 96.0 95.0 0.15 0.60 0.90 2^{-} 1^{-} 78 101.4 53.6 93.0 94.3 93.0 0.60 0.30 0.45 0.07 1^{-} 1^{-} 78 101.4 53.6 93.0 94.0 94.5 0.20 0.20 0.15 0.07 1^{-} 1^{-} 78 101.4 53.6 93.0 94.0 94.5 0.20 0.20 0.15 0.07 1^{-} 1^{-} 78 101.2 61.0 94.0 94.0 94.5 0.20 0.20 0.15 0.07 1^{-} 1^{-} 78 100.8 61.0 94.4 92.4 92.4 92.0 0.45 0.45 0.30 0.60 1^{+} 1^{+} 79 100.8 76.0 94.4 92.4 <td< td=""><td>cterio</td><td>LR</td><td>1 -</td><td>1 10</td><td>+ 7</td><td>2 +</td><td>+</td><td>1+</td><td>+ 0</td></td<>	cterio	LR	1 -	1 10	+ 7	2 +	+	1+	+ 0
Name Body Room Intracisternal temp Leukocytes/ml. CMT or temp temp F LF LR RR F IR RR R R No. No. RF LF LR RR RF LF LR R	and ba	LF	2	1 -	1 -	7	1+	+ 1	+ 0
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Name Body Room Intracisternal temp Leukocytes/ml or temp F LF LR RR $Ieukocytes/ml$ 575 101.0 53.6 95.0 94.1 94.5 0.15 0.60 0.10 " 101.6 61.0 95.0 94.1 94.5 0.15 0.60 0.10 " 101.6 61.0 95.0 94.1 94.5 0.15 0.60 0.60 " 101.6 61.0 95.0 94.3 93.0 0.15 0.60 0.60 78 101.4 53.6 93.0 94.3 93.0 0.60 0.60 0.60 78 101.2 61.0 94.0 94.5 0.20 0.20 0.45 " 101.2 61.0 94.0 94.5 0.20 0.15 0.45 " 100.8 61.0 93.50 91.5 92.3 0.20 0.20 0.15 "	Leukocytes/ ml.	RR	0.30	06.0	0.07	0.20	0.30	0.60	0.45
NameBodyRoomIntracisternal tempLeukocyteortemptempFIntracisternal tempIeukocyteortemptempFIRIRNo.RFLFIRRRRFIR575101.053.695.094.194.50.150.60"101.661.095.094.296.095.00.150.60"101.453.693.094.294.393.00.600.3078101.453.693.094.094.094.50.200.20"101.261.094.094.094.094.50.200.20657100.861.093.5092.091.592.30.200.20"100.876.094.492.492.493.00.450.45		LR	0.10	0.60	0.45	0.15	0.15	0.30	0.30
Name Body Room Intracisternal temp Leup Leup Leup Ieup Ieu Ieu Ieu Ieu Ieup Ieu I		n mili	0.60	0.60	0.30	0.20	0.20	0.45	0.20
Name or tempBody tempRoom F Intracisternal Ftempor No.temp temp F F F No.temp temp F F F R 575101.053.695.094.194.5 r 101.661.095.094.296.095.0 r 101.453.693.094.294.393.0 r 101.261.094.094.094.594.5 r 101.261.093.5092.094.592.3 r 100.876.094.492.493.093.0		I RF	0.15	0.15	0.60	0.20	0.20	0.45	0.20
NameBodyRoomIntracisternaltoortemptemp F FNo.temptemp F F F S75101.053.695.094.194.1 r 101.661.095.094.296.0 r 101.453.693.094.394.3 r 101.261.094.094.094.0 r 101.261.094.094.094.0 r 100.861.093.5092.091.5 r 100.876.094.492.492.4	Intracisternal temp E	RR	94.5	95.0	93.0	94.5	92.3	93.0	96.0
NameBodyRoomIntracisteortemptemp F No. F F F No. F F F F F F F F 101.0 53.6 95.0 94.2 T 101.6 61.0 95.0 94.2 T 101.4 53.6 93.0 94.0 T 101.2 61.0 94.0 94.0 T 100.8 61.0 93.50 92.0 T 100.8 76.0 94.4 92.4		LR	94.1	96.0	94.3	94.0	91.5	92.4	94.6
NameBodyRoomIntrortemptempRFNo.575101.053.695.0575101.661.095.07878101.453.693.07878101.261.094.0657100.861.094.676.094.4		L.F.	95.0	94.2	93.0	94.0	92.0	92.4	95.0
NameBodyRoomortemptempNo.575101.053.6575101.661.078101.453.678101.261.0"100.861.0"100.876.0		RF	95.0	95.0	93.0	94.0	93.50	94.4	94.0
Name Body or temp No. [101.0 575 101.0 " 101.6 78 101.4 " 101.2 657 100.8 " 100.8	Room temp		53.6	61.0	53.6	61.0	61.0	76.0	68.0
Name or No. 575 78 78	Body temp		101.0	101.6	101.4	101.2	100.8	100.8	99.2
	Name	or No.	575	E	78	86	657	8	174

* + =hemolytic staph.

-- + non-hemolytic staph.



