REPEATED ARTIFICIAL EXPOSURE OF BOVINE UDDERS TO COAGULASE POSITIVE HEMOLYTIC STAPHYLOCOCCI

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ABSTRACT

REPEATED ARTIFICIAL EXPOSURE OF BOVINE UDDERS
TO COAGULASE POSITIVE HEMOLYTIC STAPHYLOCOCCI

by Harbhajan Singh Nat

Udders of three young, healthy cows were repeatedly exposed to variable numbers of coagulase positive hemolytic staphylococci (strain 71). During the second series of exposures, mild trauma was applied to the injected quarter. After every exposure, the number of staphylococcic organisms shed in milk were counted. Their pathogenic nature was determined on alternate days. Multiplication of staphylococcic organisms was studied in fresh raw milk.

Except for the right rear quarter of the first cow, which was exposed four times, all other quarters were exposed three times after an interval of 10 days to two months with subsequent increasing doses of staphylococci. Differences appeared in the susceptibility of the quarters to invasion by these organisms. The right rear quarter of cow 1, when exposed to 13.5×10^8 staphylococci developed a mild form of clinical mastitis when the cow was in lactation, but did not show any clinical reaction with nearly the same number of organisms a month later when the cow had become dry.

With small numbers of organisms ranging from 16 x 10^2 to 28 x 10^2, clinical mastitis was seen in only one quarter (right fore) of cow 1. During the second series of exposures with 38 x 10^3 to 57 x 10^4 staphylococci, only two quarters (left fore and left rear) of cow 2 developed a mild form of clinical mastitis which lasted for four days. The remaining quarters did not show clinical symptoms of mastitis, but in most cases the milk was abnormal for four to six days and contained many flocculi. The third series of exposures to a large number of organisms (1 x 10^{10}) created a mild form of clinical mastitis in three quarters of lactating cow 191, while the two dry cows did not show any clinical reaction. This mild form of mastitis was overcome without treatment by the fifth day. Rather less variable staphylococcic milk counts were observed from cow 1, whereas in the other two cows, counts were greatly variable.

Staphylococcic organisms (strain 71) were added to fresh raw milk from cows 191 and 3. During the first three hours of inoculation a sharp decline in the number of staphylococcic organisms was observed which was followed by a rapid growth phase.

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Dedicated to my ever beloved father--

Sardar Basant Singh

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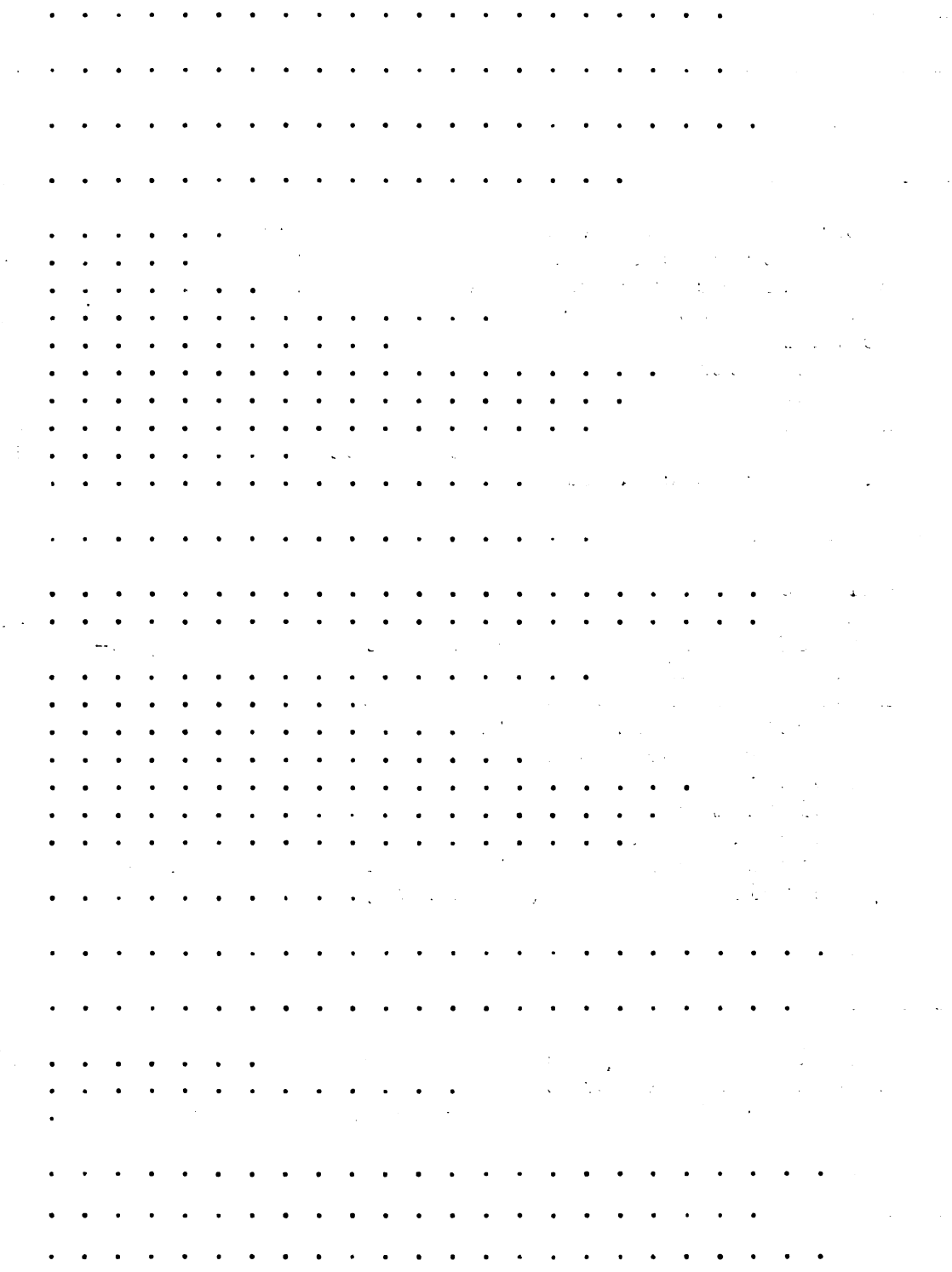

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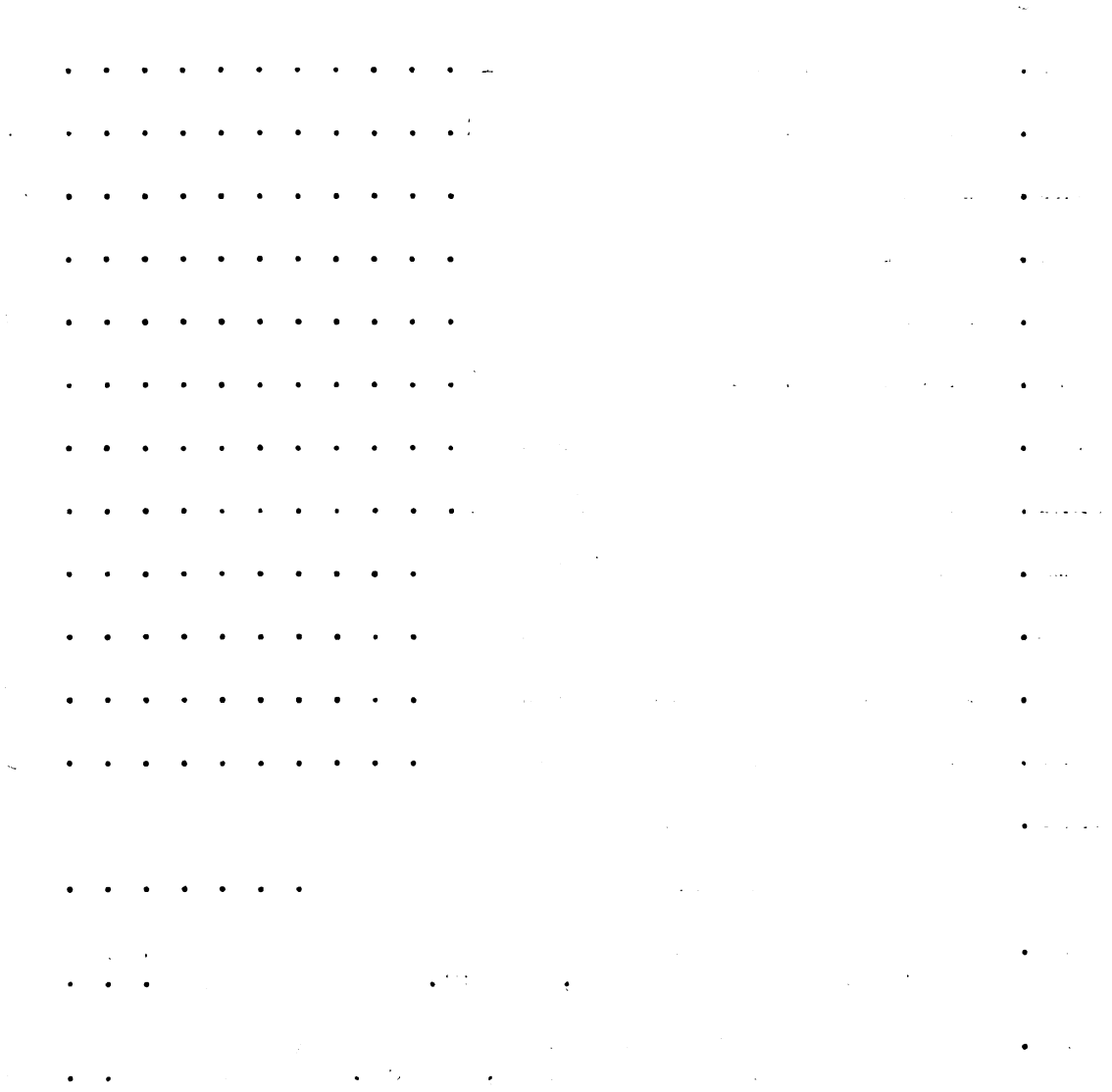


INTRODUCTION

From an economic standpoint, mastitis is one of the most important diseases affecting dairy cattle. It has wide distribution and a high incidence. It lowers the quantity as well as the quality of milk produced by the affected animals.

Streptococcus agalactiae has long been recognized as a causative organism in mastitis. Until recently little attention has been paid to staphylococcic organisms as a possible cause of this disease. Most research workers have considered this organism as a normal udder inhabitant or only responsible for a mild udder irritation. When udder flora is dominated by streptococci, the staphylococci do not seem to be a serious problem. Moreover, the media used for the isolation of streptococci has an inhibitory action towards the staphylococcic organisms. Thus, little attention was paid to the staphylococci before World War II. Since then the use of antibiotics has made it possible to eradicate Streptococcus agalactiae; consequently, for various reasons, staphylococcic organisms have become more prevalent in the udder flora and have acquired greater significance for the dairy industry and research workers.

Jordan (1930) and Litterer and Crabtree (1934) found

that staphylococci present in milk could cause food poisoning in man and skin carbuncles in man and various animals. MacDonald and White (1961) expressed doubt that coagulase positive hemolytic staphylococci could cause a mastitis outbreak in a dairy herd. These organisms were found in abundance even after the animals had been rid of the disease.

This work has been undertaken to study the possibility of causing an acute or gangrenous form of mastitis with coagulase positive hemolytic staphylococcic organisms. An attempt was made to find out the approximate number of staphylococcic organisms that a cow may carry in her udder without showing any clinical manifestation of disease. Factors of stress and trauma, as they occur naturally, were applied to the udders to determine their effect. Multiplication of staphylococcic organisms (strain 71) in freshly drawn milk from two cows was studied.

REVIEW OF LITERATURE

Burrows (1959) stated that the presence of staphylococcic organisms in pus was observed by Pasteur in 1880 and by Ogston the following year. The organisms were obtained in pure culture by Becker in 1883 and their relationship to suppurative wounds was described by Rosenback in 1884.

According to Elek (1959), Garre, in 1885, produced a large orange colored carbuncle in a man by rubbing his skin with a virulent culture of staphylococci.

Hucker (1924 B) stated that the term micrococcus was used by Cohn in 1872 prior to the use of staphylococcus by Rosenback in 1884. Hucker (1924 A) further reported that Rosenback originally used trinomial names, such as Staphylococcus pyogenes aureus for the golden pigmented pathogenic cocci and Staphylococcus albus pyogenes for nonpigmented pathogenic strains.

Extramammary reservoir of staphylococci:

Staphylococci are usually found on the skin and mucous membranes of the animal body, especially of the nose and mouth (Burrows, 1959). Evans (1916) found staphylococci in milk which culturally resembled those from bovine skin and other body sources. Mastitis was produced by Little

and Folly (1935) by injecting staphylococcic organisms isolated from skin lesions. McDiarmid (1947) found that Staphylococcus aureus was commonly present in milk and on the surface of teats of dairy cows.

Hemolytic coagulase positive micrococci were found by Spencer and Lasmanis (1952) on the skin of the teats of dairy cows and on the teat cups of milking machines. Newbould (1960) was of the opinion that Staphylococcus aureus was very widely distributed and could be found in every barn. Hemolytic coagulase positive staphylococci were isolated from the ventral surface of the udder and teats by Davidson (1961). He also found these organisms in external orifices such as eyes, nostrils, mouth, vagina and anus. Certain skin areas such as the chest, poll, and caudal folds were also sites of their presence.

Staphylococcic organisms in milk and udder:

Miller and Heishman (1943 A) has stated that the occurrence of micrococci in the udder has been recognized ever since the inception of bacteriological study of bovine mastitis. He himself noticed a high incidence of staphylococcic mastitis in several herds.

On culture, Ward (1900) isolated micrococci from 19 apparently normal udders of cows slaughtered as reactors to the tuberculin test. Strippings from 78 cows, when examined by Harding and Wilson (1913), showed micrococci in

70%. Evans (1916) examined 192 milk samples from 161 cows and isolated micrococci from 58% of the samples. Two years later Jones (1918) isolated micrococci from inflamed udders, having acute mastitis. On examination, Bean et al. (1943) found that 21% of the animals were infected with Staphylococcus aureus in a herd of dairy cattle.

Minett (1937 A), from his study of milk samples from separate quarters of 415 cows, concluded that 145 were shedders of Staphylococcus aureus. In a six-year study, Packer (1952) examined 15,693 samples of milk submitted for a laboratory diagnosis of mastitis and found that 70% of the samples contained micrococci. He revealed that the increase in incidence was associated with the progressive number of lactations. A high infection rate accompanied a low incidence of clinical mastitis. The milk samples with staphylococcic organisms ranged from 37 to 58%, according to Newberene and Mayer (1960). Their survey included five major regions of the United States.

Staphylococci associated with mastitis:

Rossel and Miller (1933) and Hucker and Udall (1933) reported the absence of streptococci in abnormal secretions from abnormal udders, as determined by microscopic examination of milk and physical palpation of udders. Similar conditions were noted by Hastings and Beach (1937), Peterson and Hastings (1938), and Hastings and Johns (1939).

They also observed that the incidence of abnormal milk increased from lactation to lactation. They named this condition nonspecific mastitis.

In the very beginning of the twentieth century, Guillebeau and Svenwall, as described by Minett (1929), were the earliest workers to associate staphylococci with mastitis. They called them galactococci. Among 81 cases of mastitis, Jones (1918) found 24 in which micrococci were the cause. Clinically the cases were mild but occasionally there was acute inflammation which became chronic. Carpenter (1925) examined abnormal udder secretions from 150 cases of mastitis and isolated staphylococci in nine of them.

In periodic examinations of approximately 200 cows, Plastridge and Anderson (1934) found that 10% of the cases of abnormal udder secretions were due to staphylococci. Parshall (1934) described an acute gangrenous mastitis in California. Out of 22 cases, mostly in young lactating cows, 15 occurred during the cold, rainy season. The cases occurred sporadically and were often accompanied by subcutaneous edema over the abdomen. Nine acute cases of staphylococcic mastitis were described by Little and Folly (1935). Minett (1937 A) reported nine cases of staphylococcic mastitis. Studies made on two herds have been reported by Schalm (1944) where, in one herd, he found six cases of gangrenous mastitis within a six-weeks period and three cases in another herd of 115 milking cows.

Pathogenic staphylococci

The criteria for judging pathogenicity of staphylococci are the ability to secrete toxins which have the capacity to lyse the red blood cells of certain animal species and the capacity to coagulate blood plasma of some species.

Jones (1918) stated that Savage was the first to describe the biochemical characteristics of micrococci associated with mammitis, but credit went to Glenny and Stevenes (1935) who described two strains on the basis of toxins called alpha and beta for which there are corresponding antibodies. The alpha toxin causes hemolysis in sheep and rabbit blood, while the beta causes hemolysis of sheep blood, but no hemolysis of rabbit blood. The beta toxin is a weak hemolytic agent at 37° C but becomes more hemolytic at lower temperatures. This phenomenon is called "hot cold." The above findings were confirmed by Bryce and Rountree (1936) and Minett (1936).

Smith and Price (1938) described a gamma toxin, while a delta toxin was described by William and Herper (1947) in addition to the above toxins. The findings of William and Herper were confirmed by Marks and Vaughan (1950) and Slanetz and Bartley (1953).

Another hemotoxin called epsilon has been shown by Elek and Levy (1950). It is capable of causing lysis of sheep and rabbit erythrocytes and is secreted by coagulase

negative organisms.

Bryce and Rountree (1936) and Minett (1936) suggested that human strains usually produce alpha hemolysis while the majority of animal strains produce beta hemolysis. Similar findings were reported by Elek and Levy (1950).

Chapman and Berens (1934, 1938) and Cruickshank (1937) attributed pathogenicity of staphylococci to the presence of the enzyme coagulase. Pathogenicity of staphylococci to the udder, as reported by Plastridge and William (1936), was related to their hemolytic character on ox blood, to the formation of coagulase, and to the fermentation of mannitol. Smith, Hale and Smith (1944) postulated that the pathogenicity of staphylococci for a given species depends upon its ability to clot plasma of that species. Pathogenicity of udder micrococci as described by Schalm and Woods (1953 B) was related to the production of beta toxin and coagulation of rabbit plasma. In the same year Slantez and Bartley (1953) found that staphylococcic organisms having the capacity to produce alpha, alpha-beta or beta hemolytic toxins were mostly coagulase positive and capable of producing bovine mastitis.

The coagulase test is the one most reliable for differentiation between pathogenic and non-pathogenic staphylococci as reported by Chapman and Berens (1934, 1938), Cruickshank (1937), and Slantez and Bartley (1945). They further stated that no relationship existed between degree

of coagulating power and pathogenicity of a strain. Veilla, Delia and Faber (1947) showed that there was little relationship between blood hemolysis, pigment production and coagulase formation.

In spite of the fact that coagulase activity is considered a very important criterion for judging pathogenicity of staphylococci, Dubeler and Cole (1956) claim to have isolated coagulase negative non-hemolytic micrococci in eight out of twelve acute cases of bovine mastitis.

From this review of literature, it is apparent that the coagulase test is the most practical test to judge pathogenicity of staphylococcic organisms. This, combined with hemolytic ability and the mannitol fermentation test, leave little doubt as to the pathogenicity of a staphylococcic strain.

Possible mode of transmission

There are different theories put forward by various workers, but the exact mode of transmission is not known. According to Jones (1918), the infection was conveyed from animal to animal during the process of grooming and washing before milking.

Miller and Heishman (1943 A), on the other hand, thought that the hands of the milker or the teat cups of a milking machine were responsible for infection from an infected animal to a healthy animal. Source of pathogenic

staphylococci for invasion of the mammary glands was primarily from other infected glands, according to Schalm and Woods (1953 A).

Newbould (1960) was of the opinion that the constant use of milking machine inflations laden with bacteria contributed to the spread of infection. The most important source of infection was the cow's udder, as revealed by Davidson (1961), who also stated that pathogenic staphylococci commonly multiply outside as well as inside the udder.

Mode of action

Carpenter (1922), Minett (1936), Schalm (1944), and Miller and Heishman (1943) suggested that the main entrance of infection was through the streak canal. The exact mode of action is not known, but various hypotheses have been put forward.

Jones and Little (1922) and Little and Folly (1935) thought the micrococci enter the mammary glands by the way of the streak canal. Jones and Little (1927) hypothesized that after entry into the teat cistern the organisms remain confined there because of the presence of an inhibitory substance in the milk. When the concentration of this inhibitory substance in the affected quarter decreased the multiplication of the organisms no longer was confined to the teat canal but extended rapidly to the secretory tissue. Jones further suggested that, after parturition,

serum infiltration of the udder may be responsible for the change in character of the colostrum or milk, thus enhancing the establishment of the organisms.

Stagnation of blood flow in blood vessels followed by stabilization of staphylococci in the tissues, was reported by Cruickshank (1937). The concentration of staphylocoagulase formed local thromboses in the small capillaries, causing infarction and then necrosis or gangrene of the affected part.

Spink and John (1940) believed that leucocidin was the important factor present in the products elaborated by staphylococci which prevented the phagocytosis of staphylococci by fixed tissues and circulating leukocytes. This factor helped in stabilization of infection.

Coagulase prevented phagocytosis of micrococci, thus permitting stabilization of the organisms in the tissues was the view expressed by Smith, Hale and Smith (1944). Staphylococcic tissue invasion was followed by a local inflammatory reaction which served to wall off the infected area. The infiltration of polymorphonuclear leukocytes and microphages prevented further spread of the infection (Ibid.).

Schalm (1959) thought that the production of toxins and the coagulase enzyme favored the tissue invasion and localization of micrococci. Stress might enhance the action of the infective organisms, and in the absence of circulating

antitoxins a fulminating mastitis with gangrene of the udder is more likely to develop.

Changes in milk

Staphylococcic infected milk as reported by Jones (1918) was yellowish white, containing many large pus flocculi. On standing, many of the large particles coalesced to form a large viscid mass on the bottom of the vial. Sometimes the secretions were purulent in nature. Minett (1936) stated that milk from staphylococcic infected quarters was not grossly changed in appearance except that occasionally it was watery. Frequently the reaction was alkaline and there was an increased amount of sediment following centrifugation.

Leukocyte counts in excess of 500,000 cells per ml. of milk were seen by Plastridge et al. (1939) in milk which has staphylococci present. Gould (1942) reported that following infection, the milk secretion was a dirty greyish thin syrup with clots. Murphy (1943) described staphylococcic infected milk as being high in pH, chloride concentration, and leukocyte counts.

Alder and Migaki (1951) found that in staphylococcic infection of udders, there was a marked increase in polymorphonuclear leukocytes in the milk. Schalm and Woods (1953 B) reported that milk secretion was reduced and turned to straw color in cases of gangrenous mastitis. Edds and

Bieter (1953) reported that the milk secretion became scanty, odorless, and resembled clear serum following staphylococcic infection.

Pathology of udder

Gross pathology: Jones (1918) stated that in acute cases the affected quarters were swollen, firm and painful to manipulation. After the acute inflammation subsided a more chronic condition continued and the quarters became atrophied and secreted a smaller quantity of milk. Jones was of the opinion that some staphylococcic organisms were able to produce only a mild catarrh while others gave rise to severe parenchymatous inflammation.

Actinomycotic-like lesions were described by Albiston (1930), Smith (1934), and Davis (1935) in cases of chronic staphylococcic mastitis. Minett (1937 A) described histopathological changes in six udders affected with chronic micrococcic mastitis. In five of the udders he did not find gross pathological changes but in the sixth, one quarter was indurated and much of the tissue was brownish in color.

Typical induration and cording within the udder tissue were reported by Gould (1942). Parshall (1934) and Schalm (1944) reported postpartum cases of gangrenous mastitis in dairy cows due to a mixed infection of Clostridium welchi and staphylococci. They found the udders tense and

swollen. Within 24 to 48 hours the teats turned black or blue and this coloration extended upwards for a variable distance to involve the udder proper. In some cases there was gas formation and subcutaneous edema anterior to the udder.

Microscopic pathology: Minett (1937 A) found that in certain areas the acini were filled with solid masses of neutrophils and that these cells also were infiltrating the interaciner septa, which was thickened as a result of increased fibroblastic activity. In other parts where the disease process was advanced, there was a large increase of connective tissue and atrophy of the secreting gland tissue. The histopathology of staphylococcic mastitis was described by Edds and Bieter (1953) as follows:

In acute cases there was an active congestion with destruction of glandular tissue epithelium. The lumina of the alveoli were filled with coagulated masses of exudate and clotted milk. Many of the alveoli contained desquamated cells and polymorphonuclear leukocytes. The congestion was accompanied by lymphocytic infiltration into the area. Interlobular edema developed and the congested areas turned hard and were pink in color. In chronic cases there was an increase in the intralobular connective tissue which led to the ultimate disappearance of most of the alveoli.

Antitoxins in blood and milk of cows

Minett (1937 B) found that in cattle and goats there existed a direct relationship between the age of an animal and the antitoxin titer of the blood. He thought that the presence of micrococcic antitoxin in the blood of cowswas the result of previous infection of the udder with Micrococcoccus pyogenes. The incidence of the udder infections also increased with age. In milk whey from normal quarters, the amount of antitoxin found was 1/80th to 1/40th of that in the blood serum. In colostrum the antitoxin titer was higher than in the blood. The increase of antitoxin in the colostrum over that in the milk was due to an increase in the globulin.

That staphylococcic antitoxin in blood serum was the result of staphylococcic infection past or present, was reported by Miller and Heishman (1943 B). The normal milk contained one unit or less of antitoxin per cc, whereas the glands with acute mastitis showed 16 to 32 units of antitoxin per cc. in the diseased quarters. During artificial exposure of the udder to infection no antitoxin was found in the blood but when in those cases the infection was stabilized, four to eight units of antitoxin developed in the blood within five weeks.

Spencer and Lasmanis (1954) found that most cows showing mammary gland infection with Micrococcus pyogenes had two or more units of antitoxin per cc. of serum, whereas

noninfected cows had less than two units of antitoxin per cc.

Staphylococcic toxoids

Boak (1956) demonstrated that rabbits actively or passively immunized against staphylococcic toxins were more resistant than were normal animals to injections of living staphylococci. Immunity lasted for three months when Minett (1939) injected staphylococcic toxoid into the udder of sheep. Derbyshire (1960) vaccinated goats and cows with an adjuvent cell toxoid prepared from Staphylococcus aureus strain 201. A high level of immunity was conferred against experimental challenge with strain 201 and 106 but not against strain BB, N 90 and S 20 T, although the production of gangrene by these strains was checked in vaccinated goats.

Goats showed evidence of immunity to a massive intramammary test dose of strain BB after they were exposed to living staphylococci of the same strain by Derbyshire (1961). This immunity appeared to be local in nature, since it was not apparent when the other half of the udder was challenged by a similar dose of staphylococcic organisms.

Porterfield and Petersen (1959) reported that the bovine udder was a potent source of antibody production and that antibodies appeared in milk within two hours after lactating cows were infused with an antigen. The antibody contents of the blood serum and milk were higher 215 days following immunization.

Derbyshire (1961) has suggested the use of killed or living attenuated staphylococcic organisms in vaccines for use in the field. The use of toxoids for the treatment of subacute staphylococcic mastitis under field conditions was reported favorably by Gould (1942), Wilson (1942) and Roach (1945).

Research workers from the University of New Hampshire (Anonymous, 1959) reported favorably on the use of a staphylococcic toxoid. Its use decreased the number of acute mastitis flare-ups in infected cows. The vaccine used by Fincher, Hodges, Johnson and Reed (1960) neither produced change in the incidence of mastitis nor reduced the severity and damage to the udder. They also reported the failure of immunity to develop in another herd where the vaccinated animals suffered a severe type of mastitis from hemolytic staphylococci.

MATERIALS AND METHODS

Cultures

A staphylococci, strain 71, used in this study was recovered from a cow showing acute clinical mastitis. This strain has been maintained by monthly transfers on tryptose agar slants and stored in a refrigerator at 5° C. The subcultures used were always alpha-beta hemolytic on bovine blood agar and did coagulate rabbit plasma. Actively growing 22 to 24 hour broth cultures that previously had been transferred for several consecutive days were used. Transfer of the organism tends to stimulate a constant, uniform, rapid and more vigorous growth.

The cultures were incubated for 22 hours at 37° C, then diluted in 0.85% sodium chloride solution and infused immediately. At the time of dilution, poured blood agar plates were made for colony counts and 0.1 ml. of the diluted culture that was injected was spread on the surface of a blood agar plate to give an approximation of the number of organisms injected. Concurrent with injecting the cultures, tests of the hemolytic and rabbit plasma coagulation abilities were made. Samples of milk were obtained for culture after carefully washing the udder with a bactericidal agent. The milk samples were taken to the laboratory

and plated for counts in less than five minutes after collection.

Animals

This study was carried out on four clinically healthy cows. All cows were five to six years old. Cows 1 and 2 were nearing completion of their lactation period, whereas cows 191 and 3 were in the middle of their lactation. Cows 1 and 2 were of the Guernsey breed, while the breed of cows 191 and 3 was Holstein. These animals were obtained from the Michigan State University Dairy Department.

A detailed study was made on three cows, 1, 2 and 191. Cow 3 was used only for the study of multiplication of staphylococci in fresh raw milk. Upon palpation the udders of all cows were found clinically normal, without any swelling or hard indurated areas. The milk secretions from all quarters were normal in appearance when examined on a strip cup. Prior to experimentation numerous examinations were made for the presence of mastitis-producing organisms from their udders. Two cows (2 and 191) were found shedding coagulase negative nonhemolytic staphylococci in the milk from all quarters. Coagulase negative nonhemolytic staphylococci were also found in milk from the right rear quarter of cow 1.

Each individual quarter was considered as a separate unit (Turner, 1948) for artificial exposure to staphylococci.

Great care was taken to avoid contamination of other quarters, when another one was being exposed to infection. The animals were kept in separate stalls and fed a ration of good quality hay and grain. Infected and noninfected quarters were hand milked twice a day.

Plan of the intramammary injections of staphylococcic organisms

Three series of intramammary inoculations of staphylococcic organisms were made. During the first series of injections a low number of organisms varying from 16×10^2 to 28×10^2 were used. In the second series (moderate range) the number of organisms varied from 38×10^3 to 57×10^4. High number of staphylococcic organisms (72×10^7 to 27×10^9) were injected in the third series of inoculations.

Each quarter was injected three times except the right rear quarter of cow 1 which was infused four times. All the quarters were successively injected with low, moderate and high numbers of staphylococcic organisms at intervals varying from ten days to two months. The right rear quarter of cow 1 was infused twice with a dose of staphylococcic organisms in the high range. The first of the two doses was administered when the cow was lactating and the second injection was repeated one month later when the cow was dry.

Details regarding the numbers of the organisms injected into each quarter and the interval between the different

injections are listed in the appendix, table XIII.

Intramammary injection technique

The technique of intramammary injection was the same in each instance. The external surfaces of the quarters were thoroughly washed with warm aqueous chlorine solution containing 200 parts per million of available chlorine and dried completely with muslin cloth. The end of the teat was disinfected with a cotton swab saturated with 70% alcohol. The experimental amount of diluted or undiluted culture was injected through the teat canal, just beyond the sphincter, with a sterile five or ten cc. syringe with a blunt pointed 24 gauge needle. The injections were usually made in the morning and cows were kept under constant observation throughout the day. Before injection, the rectal temperature of the cow was recorded and a milk sample was taken from the quarter. After injection, milk samples were taken at three-hour intervals for the first nine hours and thereafter once daily for 10 to 15 days.

Collection of milk samples

Before collecting a milk sample the udder and the teats were cleaned as described above. The workers' hands were also washed and then rinsed with 70% alcohol before each milk sample was collected. About 20 ml. of milk was drawn from the desired quarter into a sterile vial. No milk was discarded before the sample was collected. The

milk samples were promptly taken to the laboratory where the routine tests described below were performed.

Agar pour plate method

This test was carried out to find the approximate number of organisms infused or being shed in the milk. The basic media for the agar plate consisted of:

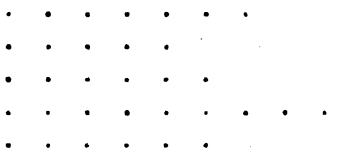
(1) Bacto-Tryptose. . . . . 10 grams
(2) Bacto-Beef Extract. . . . . 3 grams
(3) Sodium chloride . . . . . 5 grams
(4) Bacto-Agar. . . . . 17 grams
(5) Distilled water . . . . . 1000 cc.

Autoclaved at 15 lbs. pressure for 15 minutes.

Just prior to plating, the agar media were liquified by heating and cooled to 42° C and then poured over a measured quantity of milk on the petri dishes. When the agar became solidified, the petri dishes were kept inverted in the incubator for 24 to 48 hours. At these times the colonies were counted. Serial dilutions were made of cultures and milk so as to have count ranges from 30 to 300 colonies per plate.

Culture test

This test was used to find out the hemolytic character of staphylococci injected in the quarter or shed in milk. One loopful (0.01 ml.) of each milk sample was streaked over one quarter of a blood agar plate. In the blood agar there was bovine blood in the concentration of 5% and agar in the concentration of 2%. Blood was obtained from a cow



of known hemolytic susceptibility. The blood plates were dried in the incubator overnight.

This test was made by placing a loopful of the milk near the edge of the plate and drawing the loop over the agar for a distance of two to three centimeters towards the center of the plate. One plate was used for the milk from four quarters from one cow. The inoculated plates were incubated at 37° C for 24 to 48 hours and the results were recorded.

Coagulase test

Bacto-coagulase plasma purchased from Difco Laboratories, Detroit, Michigan, was reconstituted with three ml. of distilled water. In each small sterile vial supported in a rack, 0.5 ml. of the plasma solution was placed. Colonies were transferred with a sterile wire loop from the blood agar plate to the plasma solution. The vials were thoroughly shaken, incubated for 22 to 24 hours at 37° C and then the degree of clotting of the blood plasma was recorded in the following terms:

0	=	No clot
+	=	Floccules
++	=	50% clot
+++	=	Full clot

Microscopic test

The milk samples were incubated at 37° C for 24 hours before microscopic examination. Then 0.01 ml. of the incubated milk sample was smeared over a glass slide.

The smear was then dried in the air and stained by Newman's method. The microscopic test was undertaken to provide additional evidence of the presence of staphylococci and to ascertain the degree of leukocyte response.

Test to determine in vitro multiplication of staphylococcic organisms (strain 71)

This test was performed to find out the rate of multiplication of staphylococcic organisms in freshly drawn milk. Ten cc. of fresh raw milk were drawn from each quarter into a separate sterile small tube containing a glass bead. These tubes were inoculated with one loopful of an 18-hour broth culture of staphylococci, strain 71, which was diluted 100 times with 0.85% sodium chloride solution. Each tube was shaken before incubation, and at half-hourly intervals throughout the experiment. Contents of the tubes were withdrawn at various intervals and plated as described for the agar pour plate method. The number of colonies was counted after 24 hours of incubation at 37° C.

RESULTS

A. Challenge of bovine udders with a low range of staphylococcic organisms:

The right rear quarter of cow 1 and all the four quarters of cows 2 and 191 were shedding non-hemolytic coagulase negative staphylococcus aureus in their milk before they were challenged with staphylococcic organisms, strain 71. Except for the right fore quarter of cow 1, all the other quarters (11 quarters) failed to show any clinical evidence of infection following exposure to staphylococcic organisms. No gross abnormality was observed in the milk from these quarters; however, in every instance the milk did contain non-hemolytic coagulase negative staphylococcic organisms.

The injection of approximately 24×10^2 staphylococcic organisms resulted in acute clinical infection of the right fore quarter of cow 1 (appendix, table II). The infection later became chronic and lasted for about 10 days. Three hours following inoculation, congestion at the base of the teat was seen. During the next three hours, the quarter was swollen, firm and warm. The swelling of the quarter disappeared by the third day. Nine hours after the time of inoculation, the body temperature was elevated to 105.9° F but returned to 102.5° F within the next two hours.

Coagulase positive hemolytic staphylococcic organisms were cultured from the milk. No medicinal treatment was given to the cow.

Summary

All the quarters of three cows were challenged with a low range of staphylococcic organisms (16 x 10^2 to 28 x 10^2). These numbers of organisms failed to cause any clinical evidence of persistent infection except in the right fore quarter of cow 1 (appendix, table II).

B. Moderate range of staphylococcic organisms:

When 38 x 10^3 to 57 x 10^4 staphylococcic organisms were infused into the quarters of cows 1 and 191, only the right rear quarter of cow 1 failed to show abnormal milk. At the time the exposures were made, mild trauma was administered to the udder by a kick. The milk from these quarters (seven quarters) showed shreds and flocculi on the strip cup screen. It was yellowish or dirty grey in color. This abnormality in milk lasted for four to six days. The quarters returned to clinical normality. Hemolytic coagulase positive staphylococci were found in the milk from these quarters.

Two quarters of cow 2 (right fore and right rear) and one quarter (right rear) of cow 1 failed to show any clinical evidence of infection (appendix, tables IV, VI and VIII).

Acute clinical mastitis was seen in the two quarters (left fore and left rear) of cow 2 which lasted for four days (appendix, tables V and VII).

Three hours after intramammary inoculation, swelling was observed in the left fore quarter of cow 2 extending upwards from the base of the teat. Six hours after injection the body temperature was 104.0° F but returned to 102.1° F during the next three hours. On the second day following injection the swelling disappeared from the quarter. The milk continued to appear abnormal for four days and contained coagulase positive hemolytic staphylococcic organisms.

The left rear quarter of cow 2 developed acute clinical mastitis which subsided without treatment after five days (appendix, table VII). The inflamed quarter felt firm and tense until the end of the third day. Six hours after inoculation the body temperature was raised to 105.2° F but returned to normal during the next three hours. Coagulase positive hemolytic staphylococci were cultured from the milk of this quarter.

Summary

All three cows were reexposed to approximately 38×10^3 to 57×10^4 staphylococcic organisms. Mild physical trauma by kicks was applied to the injected quarters. Only two quarters showed clinical response to this moderate range of staphylococcic organisms.

C. High range of staphylococcic organisms

When cow 1 was in lactation, her right rear quarter was infused with 13.5 x 10^8 staphylococcic organisms. She developed acute clinical mastitis (appendix, table IV). There was swelling of the quarter for two days and the milk was abnormal for five days. Six hours after inoculation, the body temperature was 105.4° F. It returned to normal within the next three hours. Coagulase positive hemolytic staphylococci were cultured from the milk of this quarter. Approximately the same number of staphylococcic organisms did not produce any clinical evidence of infection when injected 25 days previously (appendix, table IV).

Except for the right rear quarter of cow 191, all quarters developed an acute clinical mastitis lasting for about five days (appendix, tables IX to XII). The right rear quarter of this cow secreted milk for three days which was yellowish in color, and contained many flocculi. The left fore quarter of cow 191 developed an acute clinical mastitis (appendix, table IX). Milk from this quarter was yellowish in color and contained many pus flocculi. The swollen quarter felt quite firm and warm upon palpation. Four hours after inoculation the body temperature was 104° F but returned to 102.5° F within the next three hours. Hemolytic coagulase positive staphylococcic organisms were cultured from the milk.

Approximately 11.2 x 10^8 staphylococcic organisms

caused an acute mastitis in the right fore quarter of cow 191. Five days following exposure there was no clinical evidence of infection even though treatment was not used (appendix, table X).

The left rear quarter of cow 191 developed clinical mastitis which lasted for five days (appendix, table XI). The quarter was hard, swollen and sensitive to the touch. Six hours after inoculation the body temperature was 104.0° F and then raised to 106° F during the next three hours. The swelling of the quarter disappeared next day. The milk was yellowish white and contained many flocculi. The abnormality lasted for five days.

The dry cows (1 and 2) did not show any clinical response to these numbers of staphylococcic organisms (appendix, tables 1 to VIII).

Summary

Three cows were challenged with intramammary injections of staphylococcic organisms ranging in number from 72×10^7 to 27×10^9. One quarter of one cow was inoculated twice with approximately the same numbers of organisms at an interval of 25 days. Four of the 13 quarters gave clinical evidence of acute infection.

DISCUSSION

Comparatively few references to the artificial introduction of small numbers of staphylococcic organisms into the udder have been found in the literature. Most of the work deals principally with the infusion of a relatively large number of organisms in contrast to the small number of organisms used in this work. Severe mastitis with systemic involvement followed injection of Staphylococcic aureus into the udders of two cows and three heifers by Carpenter (1922). He introduced two cc. of 24-hour broth culture of organisms recovered from a case of clinical mastitis. Four animals reacted severely showing high temperature with loss of appetite for 36 hours following the inoculation. Ten days later, one of the cows died from staphylococcic septicemia and at necropsy staphylococcic organisms were isolated from all the tissues of the body. In the other three animals abscesses appeared in the udders. A two ml. suspension of fresh culture isolated from a natural case of gangrenous mastitis was injected in two cows by Parshall (1934). The quarters became swollen and hot and the milk contained many clots. The reaction was very mild and gradually subsided.

Little and Folly (1935) injected from 200 to 2500

staphylococcic organisms through the teat meatus on the small, beaded end of a glass rod on nine different occasions over a period of about five weeks. Acute mastitis was seen on the second day after the last injection. The above staphylococcic organisms for the intramammary infusion were isolated from an abscess found on the skin of the udder. The quarter was swollen, congested and painful to manipulation. The secretion was scanty, yellowish and thick.

Minett (1936) was able to establish Staphylococcus aureus in one quarter with two exposures nine days apart, using 1 ml. of a 24-hour broth culture for each exposure. The infection persisted for some months and into the next lactation, after which time it disappeared.

Twenty-six quarters of seven lactating cows and one dry cow were injected by Miller and Heishman (1943 A) with a small number of organisms varying from 700 to about 30,000 on 55 occasions. Twenty-five attempts out of 55 resulted in infection of 22 quarters. Some variation was found in the ability of the different strains of staphylococci to establish and maintain themselves in the udder. Differences also appeared in the susceptibility of the quarters to invasion by these organisms. With some strains repeated exposures were necessary to bring about infection and in these cases, a mild type of mastitis of a rather short duration developed. With other strains, infection occurred after one or two exposures and chronic mastitis of a persistent

nature was established.

Schalm (1944) was able to produce gangrenous mastitis in two lactating cows following the injection of five ml. of pure broth culture of Staphylococcus aureus, while two dry cows receiving similar amounts developed a transitory mastitis. He was of the opinion that lactating udders were more vascular and the gangrenous process probably was the result of injury to the vascular system of the udder.

Slantez and Bartley (1953) found that 5×10^7 staphylococci were required to set up an acute infection. One cow was able to eliminate the infection after nine days.

Drury (1962) injected large numbers of staphylococcic organisms of strain 80/81 into the udder and demonstrated the continual shedding of staphylococci in milk for four months following exposure.

In this work with small numbers of organisms none of the quarters, except the right fore quarter of cow 1 showed any clinical evidence of mastitis. This particular quarter developed acute mastitis when injected with 24×10^2 staphylococcic organisms. The ability to establish a mastitis might not be solely due to the organisms but might be due to unknown factors which play an important part in increasing the mastitis-producing potential of the bacteria. This may be further evidence that there are differences in the susceptibility or resistance of an individual quarter to infection with staphylococci. Little and Folly (1935)

and Miller and Heishman (1943 A) were also unable to produce clinical mastitis with small numbers of organisms. They maintained that repeated exposures with large numbers of organisms were necessary to produce clinical evidence of mastitis.

In this project, the second series of injections (12 quarters) with staphylococci in the range of 38×10^3 to 57×10^4, two quarters (left fore and left rear of cow 2) developed a mild clinical form of mastitis which lasted for four days. In seven quarters the milk secretion was of abnormal color containing many flocculi. The abnormal color of milk lasted four to six days.

Stress and trauma might have played some significant part in causing clinical symptoms of mastitis in the two quarters of cow 2. However, the effect of stress or trauma is difficult to evaluate.

During lactation, cow 1 developed acute mastitis following exposure to 13.5×10^8 staphylococcic organisms but failed to do so when injected with nearly the same numbers of organisms during the dry period. This failure may be explained by increased resistance, or decreased vascularity of the udder, as suggested by Schalm (1944).

In this work the first exposure, using a small number of organisms, might have produced some immunity against the next higher dose of staphylococci. It is possible that the two cows (2 and 191) which were shedding nonhemolytic

coagulase negative staphylococci before inoculation might have developed some immunity.

From udders considered clinically normal, staphylococci may be shed without showing clinical infection. Coagulase positive hemolytic staphylococci may be responsible for subclinical infection in these udders. Due to the antigenic nature of staphylococci, they may possibly cause formations of antibodies which protect against further attacks of staphylococci. Whenever the resistance of the udder is lowered by unknown factors or if overwhelming numbers of staphylococcic organisms are introduced, clinical mastitis may develop.

Shedding of staphylococci in the milk

Staphylococci may constitute an important inhabitant of the apparently normal udder and may be shed in the milk from these udders. The numbers of staphylococcic organisms in each quarter may vary from the fore milk to the strippings and there may also be a variation in the daily counts.

Minett (1937 A) reported that there was an enormous variation in the count from time to time even when the milk samples were obtained at short intervals. On occasion he found less than ten colonies of staphylococci on the first plate, while from the same cow two days later there were innumerable colonies.

Little and Folly (1935) counted staphylococci from a clinically sound udder for a period of 31 days. They

found that staphylococcic counts varied between 10,000 to 20,000 per ml. of milk.

In this work higher and more constant counts were observed in cow 1 than in cows 2 and 191. Minimum and maximum staphylococcic counts were in the range of 1×10^3 to 1.7×10^6 per ml. of fore milk. The counts varied from 1×10^3 to 5×10^4 per ml. of fore milk. The number of staphylococcic colonies from the milk of cow 1 varied slightly from each quarter each day. In the other two cows (2 and 191) more variable counts were found from each quarter each day. This may be explained as biological phenomena. There appeared to be no definite pattern to the daily variations in numbers of staphylococcic organisms shed in the milk.

Effect of stress or trauma

According to McDonald and White (1961), hemolytic coagulase positive staphylococcic organisms were found in abundance even after the animals had recovered from clinical mastitis. Many workers doubt whether staphylococci are really the cause of infection or whether some unknown factor is responsible. Factors such as stress and trauma were suggested by Schalm (1959) as having the effect of increasing the susceptibility of the udder to infection. With this in mind, mild kicks were given to the quarters when they were injected for the second time. Only two quarters (left fore and left rear) of cow 2 developed a mild form of clinical mastitis. It is not clear whether

trauma made the udder more susceptible to infection or whether the infection was due to the greater number of staphylococci injected.

Multiplication of staphylococci in fresh raw milk

Many workers have commented on the bactericidal property of raw milk. It is well known that when certain bacteria are introduced into fresh raw milk their number declines for a time and subsequently increases. Hunziker (1901) was apparently the first investigator who showed a marked decrease in numbers of bacteria when they were introduced into the milk from some cows, whereas in others, the bactericidal effect was not noted. He observed only the action of bovine milk on its own milk flora and used no pure culture. It was demonstrated by Chambers (1920) that milk contained a definite bactericidal constituent which is destroyed by heating at 80° C or 90° C for two minutes. He found that the inhibitory action was specific and depended on both the cow and species of the bacteria employed. He also pointed out that lactic acid type of organisms were not inhibited in milk. Hassen (1924) found that fresh raw milk would inhibit the growth of Bacillus typhosus and Bacillus paratyphosus for one to four hours at 37° C. He further showed that there were periodic variations in the ability of a cow's milk to inhibit bacterial growth. He attributed this variation to the ration, since he thought

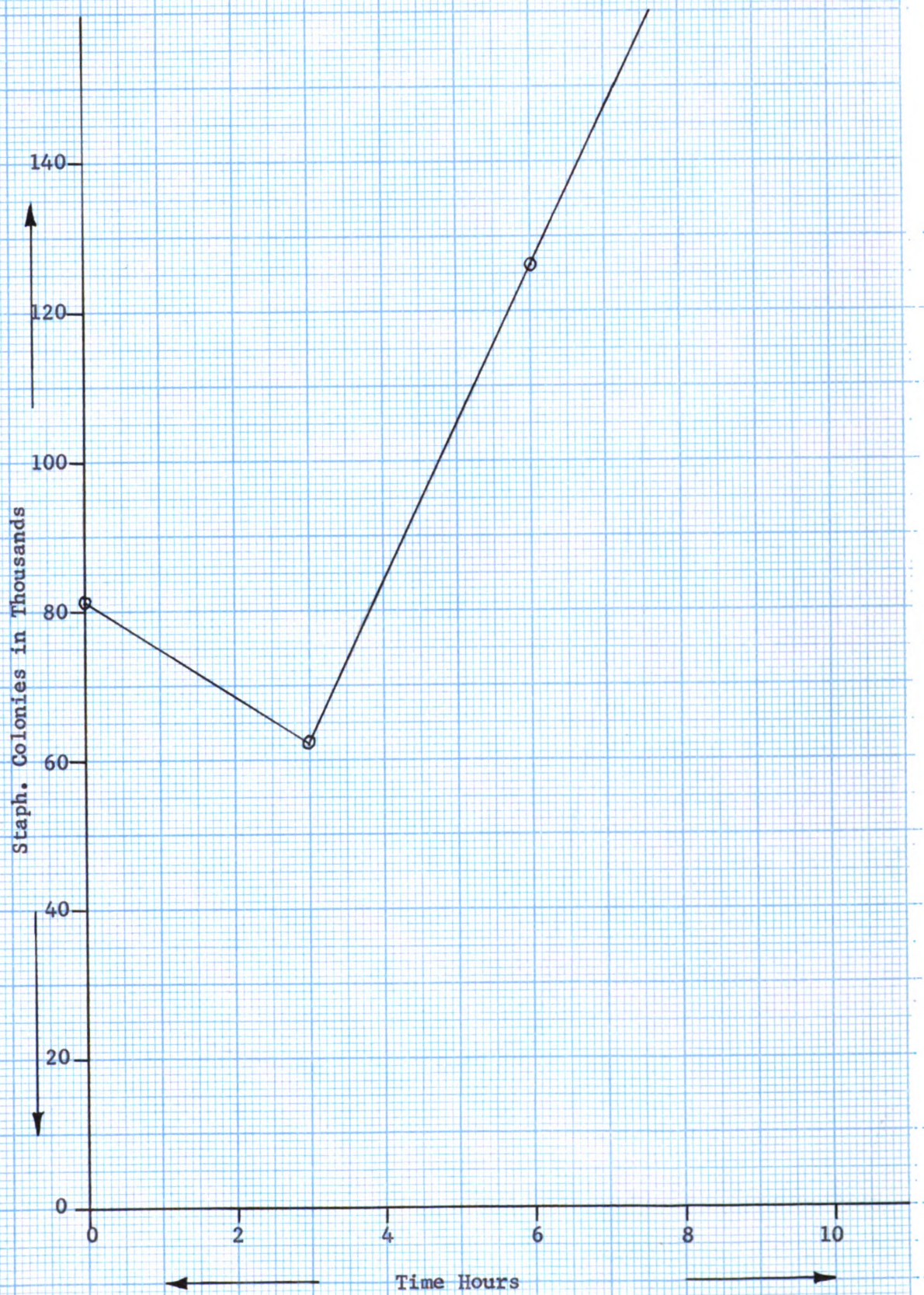
the bactericidal agent was an oxidizing enzyme which supposedly originated in the food and that its concentration in the milk was dependent upon the concentration in the ration.

According to Jones and Little (1927), the concentration of the inhibitory substance varied in the secretions of different animals. It might be as concentrated in a young cow early in her first lactation period as in an older or more resistant animal which has been repeatedly exposed to infection with streptococci. The bactericidal activity of fresh raw milk from a number of cows was tested with nonhemolytic mastitis streptococci. It was reported that there was a sharp decline in the number of organisms in the first four hours of incubation. After this, there was a rapid multiplication particularly in cases of young cows and cows susceptible to mastitis. In cases of resistant cows, there was no multiplication during the first eight hours of incubation. They added that this substance did not originate in food, but was in the udder, and that it did not increase when the cows were artificially immunized or repeatedly exposed to natural infection.

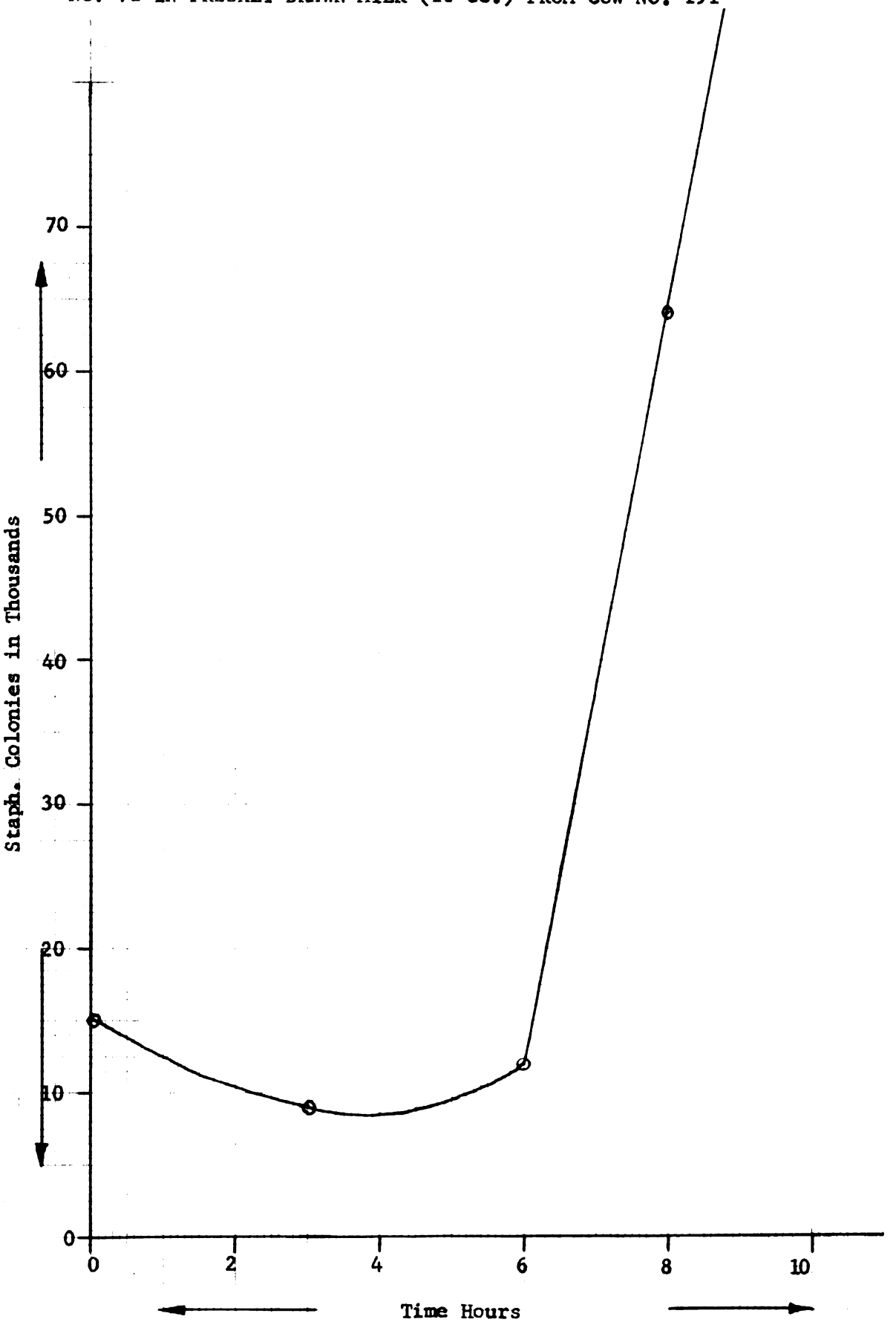
In this work the multiplication of hemolytic coagulase positive staphylococcic organisms (strain 71) was studied in the fresh raw milk from cows 191 and 3. It was observed that in milk from cow 191 there was a sharp decline in the numbers of staphylococcic organisms after the first

three hours of incubation. After this, there was a slight increase for two hours, followed by rapid multiplication at 24 hours. In cow 3 there was a definite decrease in the numbers of staphylococcic organisms for the first three hours, followed by a phase of rapid growth (see graphs on pages 39 and 40). These results are quite similar to those that have been reported by Jones and Little (1927) for streptococci.

GRAPH SHOWING GROWTH OF STAPHYLOCOCCIC ORGANISMS STRAIN NO: 71
IN FRESHLY DRAWN MILK (10 cc.) FROM COW NO: 3



GRAPH SHOWING GROWTH OF STAPHYLOCOCCIC ORGANISMS STRAIN NO: 71 IN FRESHLY DRAWN MILK (10 cc.) FROM COW NO. 191



SUMMARY

Coagulase positive hemolytic staphylococci of strain 71, recovered from a clinical case of mastitis, were injected into all quarters of three cows at various intervals. Except for one quarter, which was injected four times, all the other quarters were infused three times with increasingly greater numbers of organisms at intervals of ten days to two months.

For the first series of injections, small numbers of organisms (16×10^2 to 28×10^2) were infused into each quarter. Only one of the 12 quarters developed an acute clinical mastitis. This subsided in 12 days without treatment.

For the second series of intramammary injections, 38×10^3 to 57×10^4 staphylococcic organisms were introduced into each quarter. In addition, each quarter was mildly traumatized by physical force at the time of the injection. Two of the 12 quarters developed acute clinical mastitis. From seven additional quarters abnormal milk was obtained over periods ranging from four to five days. The remaining three quarters did not show any evidence of clinical mastitis.

For the third series of intramammary injections,

approximately 1 x 10^{10} staphylococcic organisms were used. Four of the 13 quarters gave clinical evidence of acute infection.

Staphylococci counts from milk were made for 10 to 15 days after each intramammary injection of staphylococcic organisms. In one cow the counts were high (5 x 10^4) and less variable, while in other cows the counts varied from 1 x 10^3 to 5 x 10^3.

The staphylococcic strain 71 used in this work was incubated in fresh raw milk. Milk from all four quarters of one cow inhibited growth of the organisms for eight hours, after which time they multiplied rapidly. Fresh raw milk from another cow inhibited multiplication for three hours only.

Conclusion

1. The response of bovine udders to the injections of approximately the same numbers of coagulase positive staphylococci will vary from quarter to quarter and from cow to cow. Clinically, the response ranged from no evidence of mastitis to an acute mastitis of short duration.
2. Coagulase positive hemolytic staphylococci may be apparently harmless inhabitants of bovine udders and may be shed in milk which grossly appears to be normal.
3. There appears to be no definite pattern to the daily variations in numbers of staphylococcic organisms shed in the milk.

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APPENDIX

TABLE I

LEFT FORE QUARTER OF COW 1

After intramammary infusion of 1600 staphylococcic (strain 71) organisms

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml. Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
7/10	0	102.5	--	0	-	Normal	Normal
	3	101.6	1.5*	0	-	"	"
	6	101.3	0.8			"	"
	9	100.9	--			"	"
7/11		101.0	1.7	0	-	"	"
7/12		101.3	4.8			"	"
7/13		100.6	20			"	"
7/14		101.4	5.6	0	-	"	"
7/15		101.7	60			"	"
7/17		101.2	165			"	"
7/18		100.0	80	0	-	"	"
7/19		101.6	830			"	"
7/20		101.3	80			"	"
7/22		100.4	72.5	0	-	"	"
7/24		101.0	43			"	"
7/25		101.5	62	0	-	"	"
7/26		101.3	67			"	"
7/28		100.9	75			"	"
40×10^3 staph. and trauma							
8/25	0	100.5	560	+++	+	Flocculi	Normal
	3	101.4	480			"	"
	6	101.6	720			"	"
	9	100.9	1760**	++	+	"	"
8/26		101.4	612			"	"
8/27		101.3	830			"	"
8/29		100.8	240	++	+	Normal	"
8/30		101.1	580			"	"
9/1		100.6	120			"	"
9/2		101.2	720	+	+	"	"
9/3		101.5	450			"	"
9/4		101.4	700			"	"
9/6		100.7	440	++	+	"	"
27.6×10^8 staph.							
10/15	0	101.5	No count***			Normal	Normal
	6	100.7				"	"
	9	101.4				"	"

*Recorded in thousands
**Highest count
***Dry cow

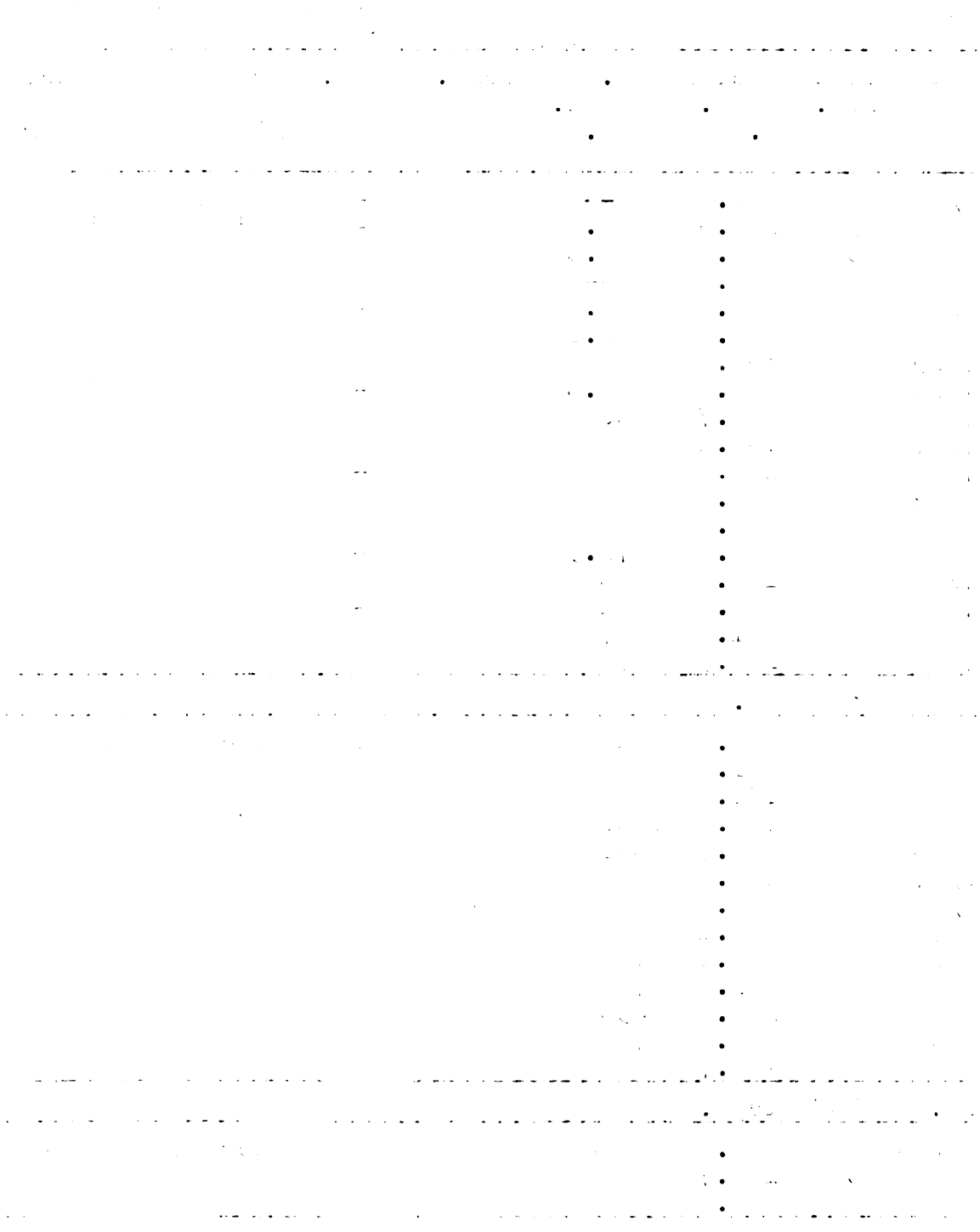

TABLE II

RIGHT FORE QUARTER OF COW 1

After infusion of 2400 staphylococcic (strain 71) organisms

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml. Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
7/15	0	101.6	--	0	-	Normal	Normal
	3	101.5	4.8*	+	+	Yellow	Congestion,
	6	103.5	6.5			white	swollen,
	9	105.9	10			with	firm and
	11	102.5	8			flocculi.	ademato us.
7/16		102.0	5.4	+	+	Yellow	Slightly
7/17		101.4	25			serum-	swollen.
7/18		110.9	35			like	Normal
7/19		101.7	4	+	+	with	"
7/20		100.6	32			flocculi.	"
7/21		101.4	150	+	+	"	"
7/22		102.3	61			"	"
7/23		100.9	71			"	"
7/25		100.5	60			"	"
7/27		100.6	52	+	+	Normal	"
7/28		101.5	130			"	"
7/29		101.3	145			"	"
7/30		100.7	240	0	+	"	"
8/1		101.5	880			"	"
8/2		101.1	120			"	"
8/4		101.4	105	+	+	"	"
8/5		101.6	96			"	"
8/7		100.8	147	+	+	"	"
8/10		101.4	165			"	"
8/12		101.2	88			"	"
8/15		100.6	105			"	"
54 x 10^3 staph. and trauma							
9/10	0	100.5	50	++	+	Dirty	Normal
	3	100.8	640			grey	"
	6	101.3	80			with	"
	9	101.9	720	++	+	flocculi.	"
9/11		100.9	150			"	"
9/12		101.2	56	+	+	"	"
9/14		100.7	176			"	"
9/15		101.5	140	+	+	"	"
9/17		101.3	191			"	"
9/19		101.7	192	+	+	Normal	"
9/20		101.1	112			"	"
9/22		100.8	173	+	+	"	"
9/23		101.4	115			"	"
9/25		101.2	450	+	+	"	"

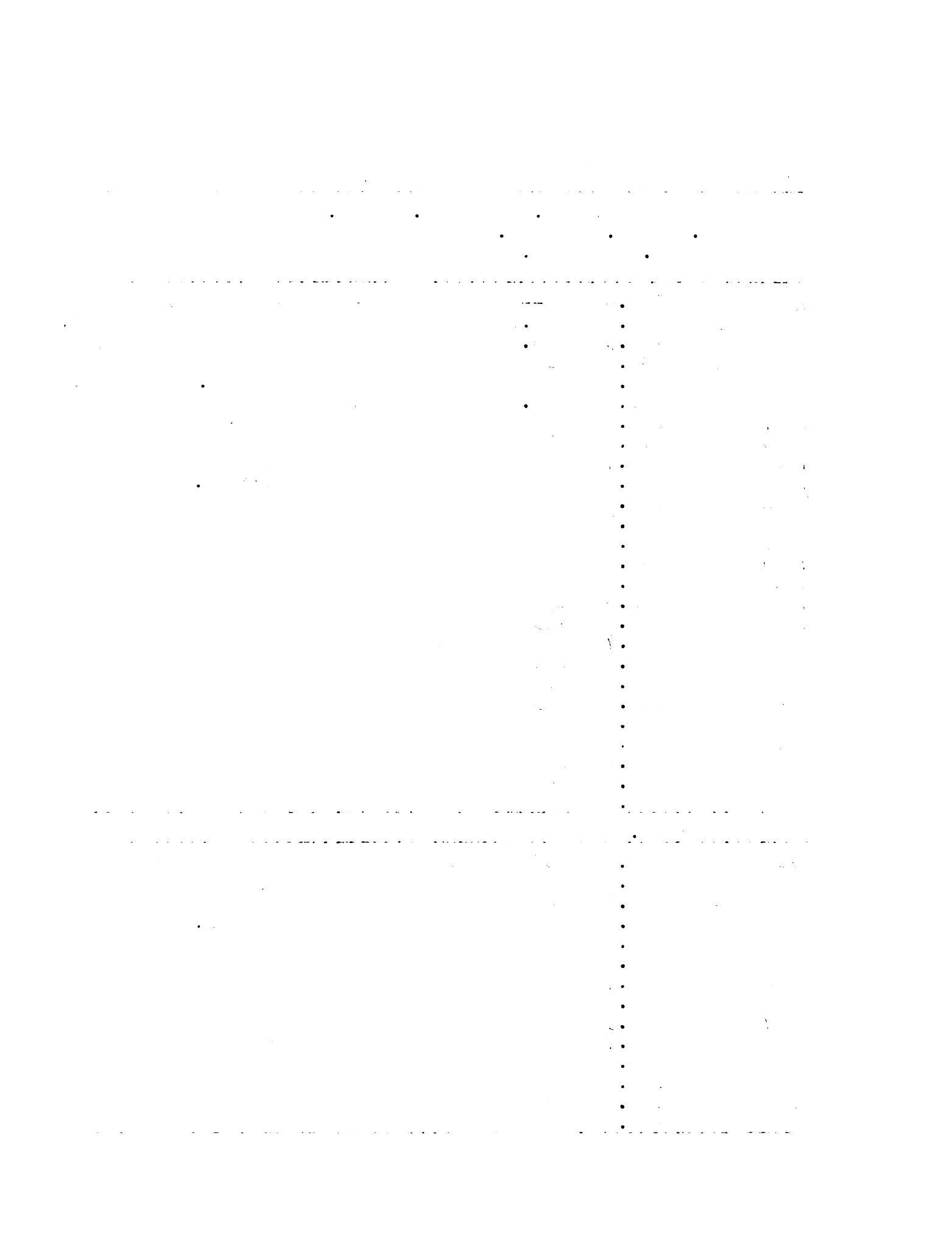

TABLE II--Continued

72 x 10^7 staph. organisms				
10/28	3	101.3	Cow was dry	Normal
	6	100.9	"	"
	9	101.2	"	"

*Recorded in thousands

TABLE III

LEFT REAR QUARTER OF COW 1

After infusion of 2600 staphylococcic (strain 71) organisms

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml. Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
7/25	0	101.2	--	-	-	Normal	Normal
	3	101.2	3.4*	+	+	"	"
	6	102.2	1.4				
	9	101.2	13				
7/27		100.8	80	0	+	"	"
7/28		101.2	9.6				
7/29		101.5	12	0	-	"	"
7/30		100.9	58				
7/31		101.5	56	0	-	"	"
8/2		100.7	87				
8/4		101.4	4.8	0	-	"	"
8/5		101.1	19				
8/6		101.2	7.8	0	-	"	"
8/8		100.9	180				
8/10		101.3	67	0	-	"	"
50×10^3 staph. and mild trauma							
9/20	0	101.5	3.2	0	-	Normal	Normal
	3	101.4	46	++	+	Straw color	
	6	101.7	80			Flakes and	"
	9	101.2	425	++	+	clots.	"
9/21		100.6	120				
9/22		101.3	456	++	+	"	"
9/24		100.9	396				
9/25		101.2	126	+	+	"	"
9/26		100.7	422				
9/27		101.6	675	+	+	Normal	"
9/28		101.2	322				
9/30		101.3	13	+	+	"	"
10/1		101.2	590				
10/2		100.9	386	+	+	"	"
10/3		101.2	425				
10/5		100.9	130	+	+	"	"
17.5×10^8 staph. organisms							
11/2	0	101.5	-	No counts**			"
	3	100.9					"
	6	101.2					"
	9	101.4					"

*Recorded in thousands

**Cow was dry

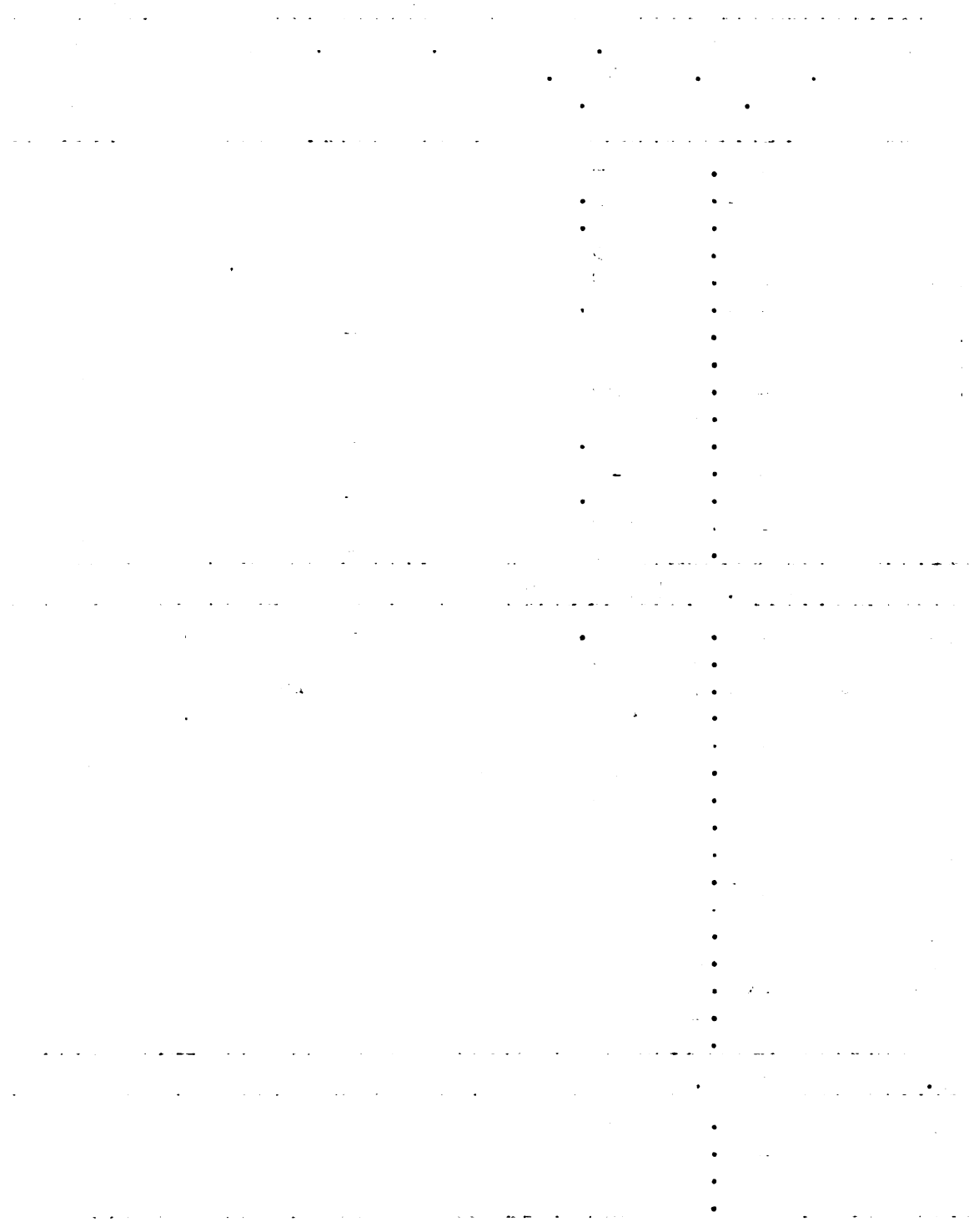

TABLE IV

RIGHT REAR QUARTER OF COW 1

After infusion of 2600 staphylococcic (strain 71) organisms

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml. Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
7/30	0	101.3	2.8*	0	-	Normal	Normal
	3	101.2	7.8				
	6	101.5	12	+	+	"	"
	9	100.9	9.6				
7/31		101.4	14	+	+	"	"
8/1		100.8	52				
8/2		101.3	180	0	-	"	"
8/3		101.4	70				
8/5		100.6	12	0	-	"	"
8/6		100.9	24				
8/7		101.2	9.4	0	-	"	"
8/8		101.5	12				
8/10		101.2	8.9				
8/12		101.3	72.7	0	-	"	"
8/13		101.2	44				
8/15		100.9	22.1	0	-	"	"
40×10^3 staph. organisms and mild trauma							
10/1	0	101.2	-				
	3	101.5	44	++	+	Normal	Normal
	6	100.6	68				
	9	100.9	120				
10/2		101.5	86	++	+	"	"
10/3		100.5	240				
10/4		101.4	58				
10/5		101.3	146	++	+	"	"
10/6		100.7	88				
10/8		101.3	57	++	+	"	"
10/10		101.4	54				
13.5×10^8 staph. organisms							
10/11	0	101.4	97	+	+	Normal	Normal
	3	101.2	184	+++	+	Yellowish	Swollen,
	6	105.4	780			white with	firm.
	9	102.5	1130	+++	+	flocculi.	"
10/12		101.8	540				
10/13		100.9	772	+++	+	"	Normal
10/15		101.2	1270				
10/16		102.1	662	++	+	"	"
10/17		100.5	780	+	+	Normal	"
10/18		100.9	449				
10/20		101.5	768	++	+	"	"

TABLE IV--Continued

16×10^8 staph. organisms					
11/5	0	100.6	-	**	Normal
	3	100.9			
	6	100.8			
	9	101.5			

*Recorded in thousands
**The cow was dry

TABLE V

LEFT FORE QUARTER OF COW 2

After infusion of 2800 staphylococcic organisms (strain 71)

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml. Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
8/22	0	101.5	1.8*	0	-	Normal	Normal
	3	101.2	6.0				
	6	101.3	4.2	0	-	"	"
	9	101.1	10.0				
8/23		100.9	2.8	0	-	"	"
8/26		101.3	0.9				
8/28		101.5	2.0	0	-	"	"
8/29		100.9	6.1				
8/30		101.2	3.5	0	-	"	"
9/1		101.5	0.8	0	-	"	"
240×10^3 staph. organisms and mild trauma							
9/20	0	101.2	-			Normal	Normal
	3	101.2	160	++	+	Yellowish	Inflam-
	6	104.5	80			white	mation
	9	102.1	95			with	extending
						flocculi.	upwards.
9/21		101.4	50	+++	+	"	Swelling disappeared.
9/22		100.9	70			"	Normal
9/23		101.2	4.0	++	+	"	"
9/25		102.1	3.0			Normal	
9/27		101.4	1.0	++	+	"	"
9/30		101.2	0.09				
10/1		100.9	10	+	+	"	"
10/2		101.5	11				
10/5		100.7	23	+	+	"	"
10/8		101.3	2.6			"	"
10/9		101.2	1.4	0	+	"	"
10/11		100.6	5.0	+	+	"	"
27×10^9 staph. organisms							
10/19	0	100.9	No count			Cow was dry	Normal
	3	101.0					
	6	101.3					
	9	100.7					

*Recorded in thousands

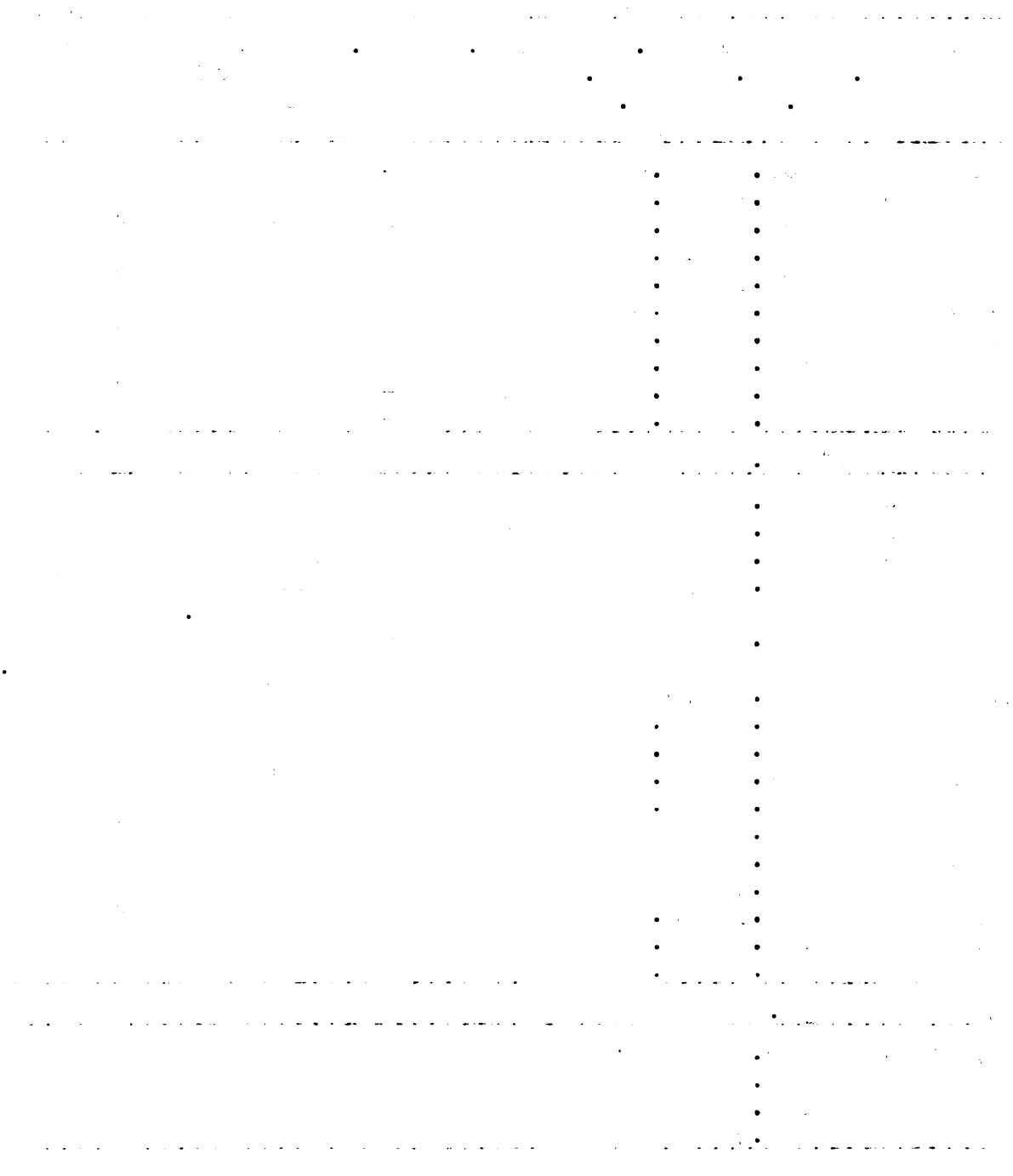

TABLE VI

RIGHT FORE QUARTER OF COW 2

After infusion of 2400 staphylococcic organisms (strain 71)

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml. Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
8/27	0	100.9	2.2*	-	0	Normal	Normal
	3	101.2	3.5			"	"
	6	101.5	2.7	0	+	"	"
	9	101.5	2.9				
8/28		100.7	8.8	0	+	"	"
8/29		101.3	12				
8/30		100.9	4	0	-	"	"
9/1		101.5	96				
9/2		100.9	1.2	0	-	"	"
9/4		101.4	12				
9/5		102.0	1.3	0	-	"	"
9/7		100.9	3.7				
44×10^3 staph. organisms and mild trauma							
9/27	0	101.3	1.2	0	-	Normal	Normal
	3	101.2	96.0	++	+	"	"
	6	102.2	56				
	9	101.5	112	++	+	"	"
9/28		101.2	86				
9/29		101.1	65	++	+	"	"
9/30		100.9	7.6				
10/1		101.4	3.7	+	+	"	"
10/2		100.6	9.6				
10/4		101.5	52	+	+	"	"
10/5		101.7	4.5				
10/6		100.9	18				
10/8		101.3	7.6	+	+	"	"
10/10		102.2	11.7				
18×10^8 staph. organisms							
10/25	0	101.3	No counts		**	Normal	Normal
	3	101.2					
	6	101.5					
	9	100.9					

*Recorded in thousands

**Cow was dry

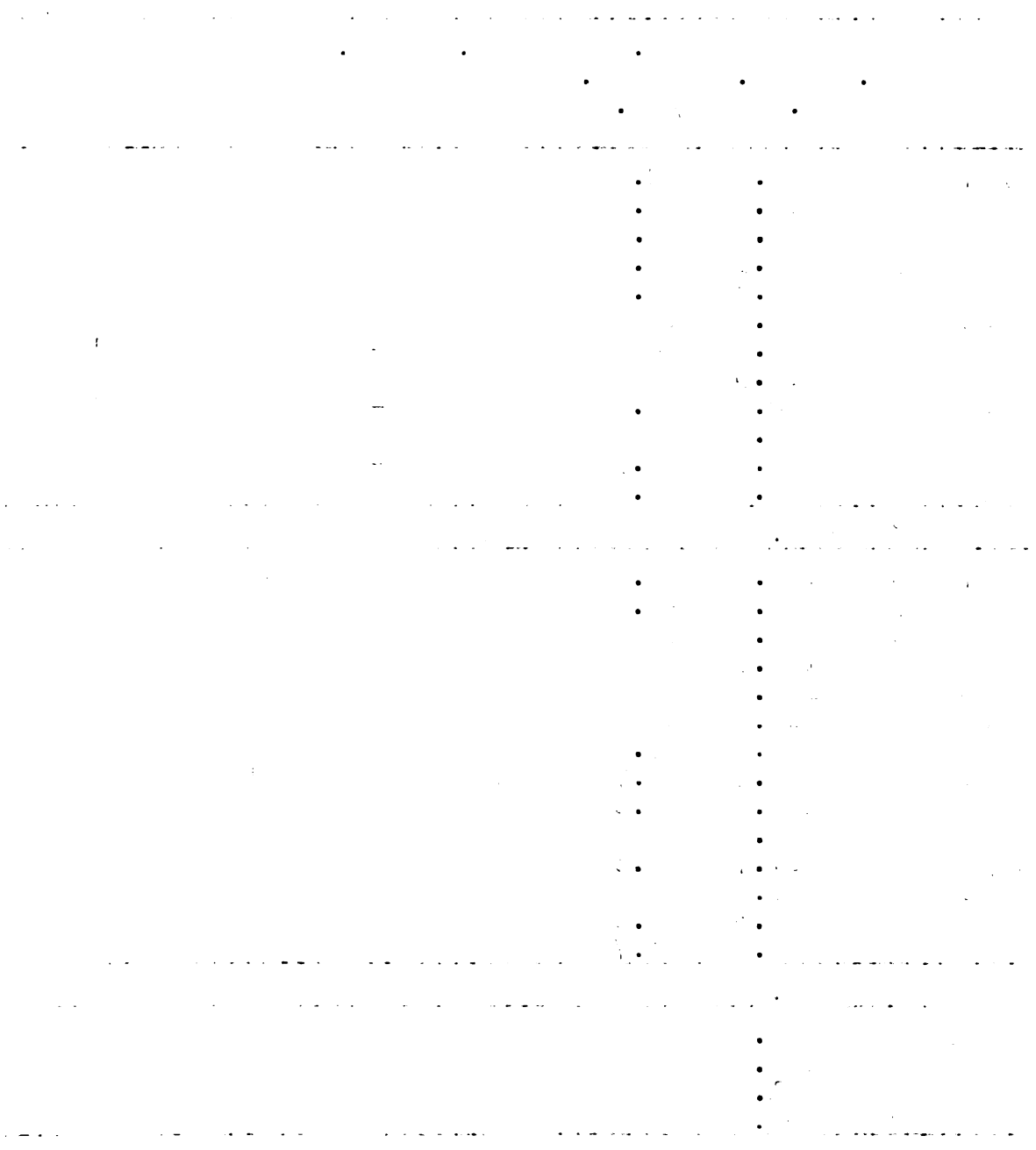

TABLE VII

LEFT REAR QUARTER OF COW 2

After infusion of 2100 staphylococcic (strain 71) organisms

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
9/4	0	100.9	1.4*	0	-	Normal	Normal
	3	101.3	2.7				
	6	101.2	1.8	0	+	"	"
	9	101.5	3.1				
9/5		100.9	1.6	0	+	"	"
9/6		102.0	2.9				
9/8		101.2	0.7	0	-	"	"
9/9		100.9	0.9				
9/10		100.6	7.6	0	-	"	"
9/12		101.5	5.6				
9/14		101.4	1.1	0	-	"	"
9/15		101.1	0.8				
9/17		101.4	8.7	0	-	"	"
545×10^2 staph. organisms and mild trauma							
10/5	0	101.4	9.6	0	-	Normal	Normal
	3	101.4	12			Yellowish	Congestion,
	6	105.2	4.7	++	+	white	hard and
	9	101.6	120			with	edematous
10/6		100.9	76	+	+	"	swelling.
10/7		101.3	6.7				Swelling
10/8		101.2	9.7	+	+	"	disappeared.
10/9		101.6	15.4			"	"
10/10		100.8	4.5	+	+	Normal	"
10/12		101.4	7.6				
10/13		101.3	18	+	+	"	"
10/15		100.6	28.5				
10/16		101.2	5.7	+	+	"	"
5×10^8 staph.							
11/7	0	102.0	No count			**	Normal
	3	101.4					
	6	101.3					
	9	100.9					

*Recorded in thousands

**Cow was dry

TABLE VIII

RIGHT REAR QUARTER OF COW 2

After infusion of 1800 staphylococcic (strain 71) organisms

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml. Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
9/10	0	101.3	0.9*	0	-	Normal	Normal
	3	101.2	1.1				
	6	101.2	3.6	+	+	"	"
	9	101.5	0.3				
9/10		100.9	3.9				
9/11		101.3	2.4	0	+	"	"
9/12		102.0	5.6				
9/14		101.1	1.4	0	-	"	"
9/15		100.8	4.4				
9/18		101.3	0.9	0	-	"	"
9/19		101.1	8.9				
9/20		100.6	0.8	0	-	"	"
9/21		101.2	7.8				
57×10^3 staph. and mild trauma							
10/15	0	100.7	2.4	0	-	Normal	Normal
	3	101.1	86.0			"	"
	6	101.5	5.4	++	+	"	"
	9	100.9	9.6			"	"
10/17		101.3	17.5				
10/18		101.4	8.1	++	+	"	"
10/19		100.9	71.0				
10/20		101.3	18.5	+	+	"	"
10/21		100.6	7.5				
10/22		101.4	19.5	+	+	"	"
10/24		101.3	8.8				
10/25		101.5	12.0	+	+	"	"
10/26		100.9	9.6				
10/28		101.2	14	+	+	"	"
5×10^9 staph.							
11/12	0	101.2	No count		**	Normal	Normal
	3	101.5					
	6	101.4					
	9	101.2					

*Recorded in thousands

**Cow was dry

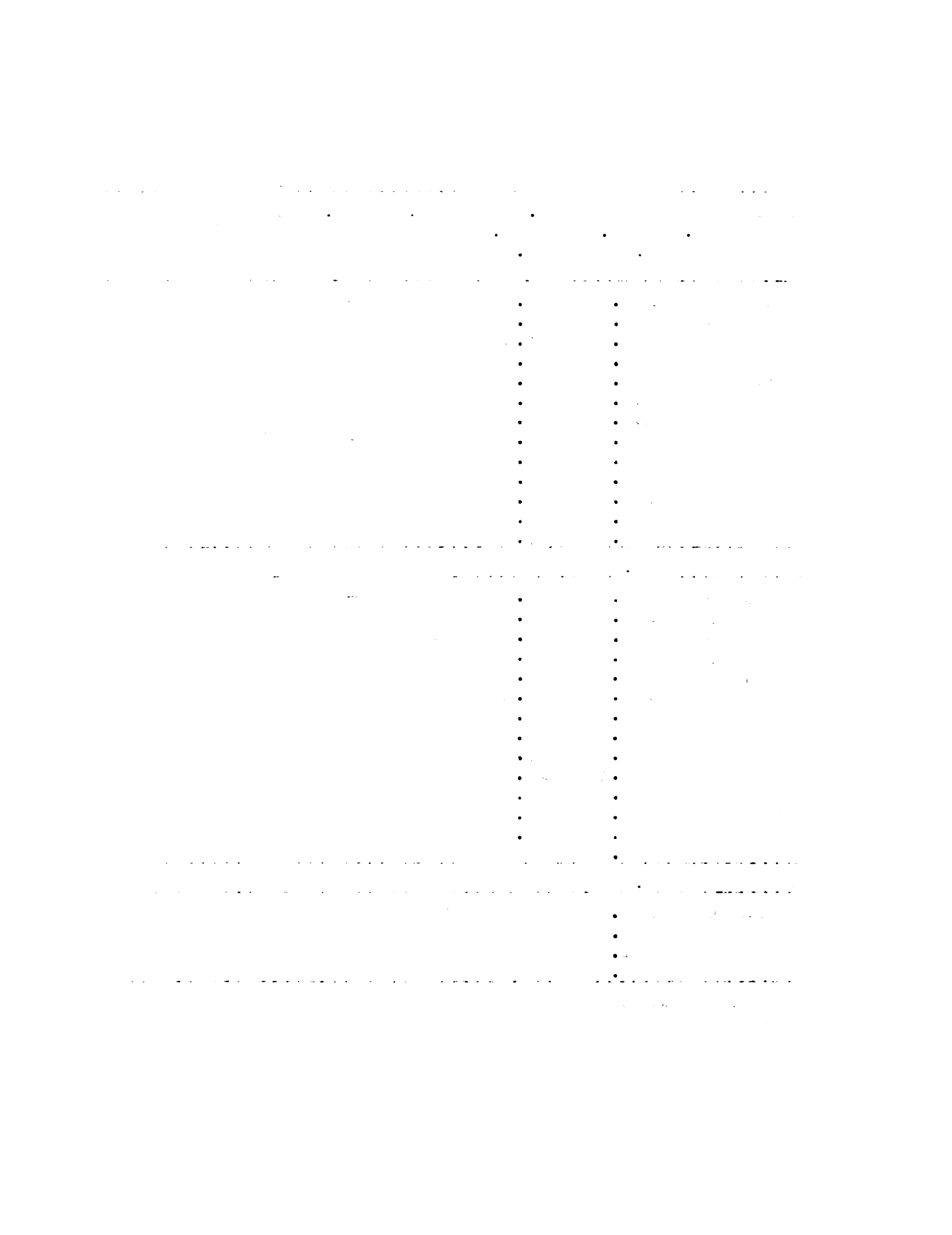

TABLE IX

LEFT FORE QUARTER OF COW 191

After infusion of 1800 staphylococcic (strain 71) organisms

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml. Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
10/3	0	101.4	1.2*	0	-	Normal	Normal
	3	101.4	2.0	+	+	"	"
	6	101.8	6.2				
	9	101.2	3.5				
10/4		100.5	9.0	0	+	"	"
10/5		101.2	5.5				
10/6		101.2	0.8	0	-	"	"
10/7		100.9	1.4				
10/8		101.2	3.0	0	-	"	"
10/10		101.4	96.0				
10/12		101.2	2.0	0	-	"	"
10/14		100.7	0.7				
10/16		101.6	3.9	0	-	"	"
44×10^3 staph. organisms and mild trauma							
10/17	0	101.2	4.4	0	-	Normal	Normal
	3	101.2	7.6				
	6	101.3	15.0	++	+	Yellowish	"
	9	101.4	8.7			white with	
10/18		101.1	1.2	+	+	flocculi.	"
10/19		100.9	14.5				
10/20		101.2	2.6	+	+	Normal	"
10/22		101.2	96				
10/24		100.9	5.8	+	+	"	"
10/26		101.3	0.9				
10/28		101.4	1.2	+	+	"	"
10.2×10^8 staph. organisms							
10/29	0	101.2					
	3	100.5	TNC**	++	+	Yellowish	Hard and
	6	104.0	24.0			serum	diffused
	9	102.5	7.8			like with	swelling.
10/30		101.4	5.6	++	+	pus floc-	Normal
10/31		100.9	4.2			culi.	
11/2		100.9	18.4	++	+	"	"
11/4		101.2	76				
11/5		101.4	3.3	++	+	"	"
11/6		101.5	1.6			Normal	"
11/8		100.9	2.6	+	+	"	"
11/9		101.2	3.5				

*Recorded in thousands

**Too numerous to count

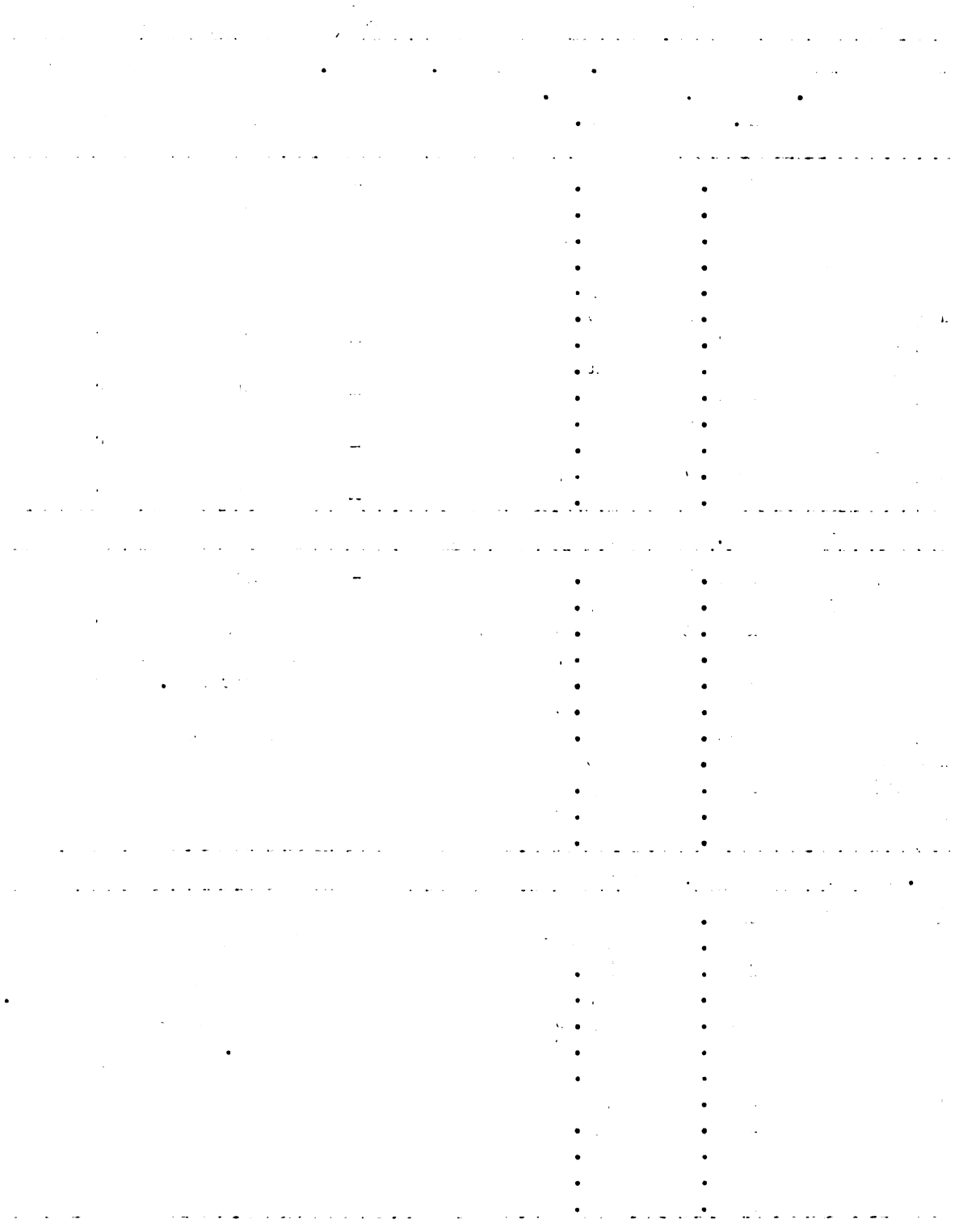

TABLE X

RIGHT FORE QUARTER OF COW 191

After infusion of 2400 staphylococcic (strain 71) organisms

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml. Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
10/15	0	101.7	2.2*	0	-	Normal	Normal
	3	101.5	4.5				
	6	101.3	6.6	0	+	"	"
	9	101.1	4.2				
10/16		100.7	5.0	0	-	"	"
10/18		101.2	2.1				
10/19		100.5	3.3	0	+	"	"
10/22		101.2	1.7				
10/24		101.1	2.3	0	-	"	"
38 x 10^3 staph. organisms and mild trauma							
10/25	0	100.9	19.5			Normal	Normal
	3	100.9	19.5	++	+	Yellowish	"
	6	101.2	7.2			with	
	9	101.1	12.5	++	+	flocculi.	"
10/26		100.5	9.8				
10/27		100.9	4.5	+	+	"	"
10/28		101.2	17.5				
10/30		101.2	5.0	+	+	Normal	"
11/2		100.7	94.0				
11/4		100.6	4.8	+	+	"	"
11/5		101.3	4.2				
11.2 x 10^8 staph.							
11/7	0	101.2	39.0	+	+	Normal	Normal
	3	101.5	225.0			Yellowish	Hard and
	6	102.0	144.0	++		white	diffused
	9	103.5	280.0			with	swelling.
11/8		101.2	25.0	++	+	flocculi.	"
11/10		101.3	186.0				Swelling
11/11		101.3	TNC**	++	+	"	disappeared.
11/12		100.9	TNC			Normal	"
11/13		101.2	15.0	+	+	"	"
11/15		101.5	9.6				
11/16		100.7	7.6	+	+	"	"
11/18		101.2	140.0				
11/20		101.3	5.4	++	+	"	"

*Recorded in thousands
**Too numerous to count

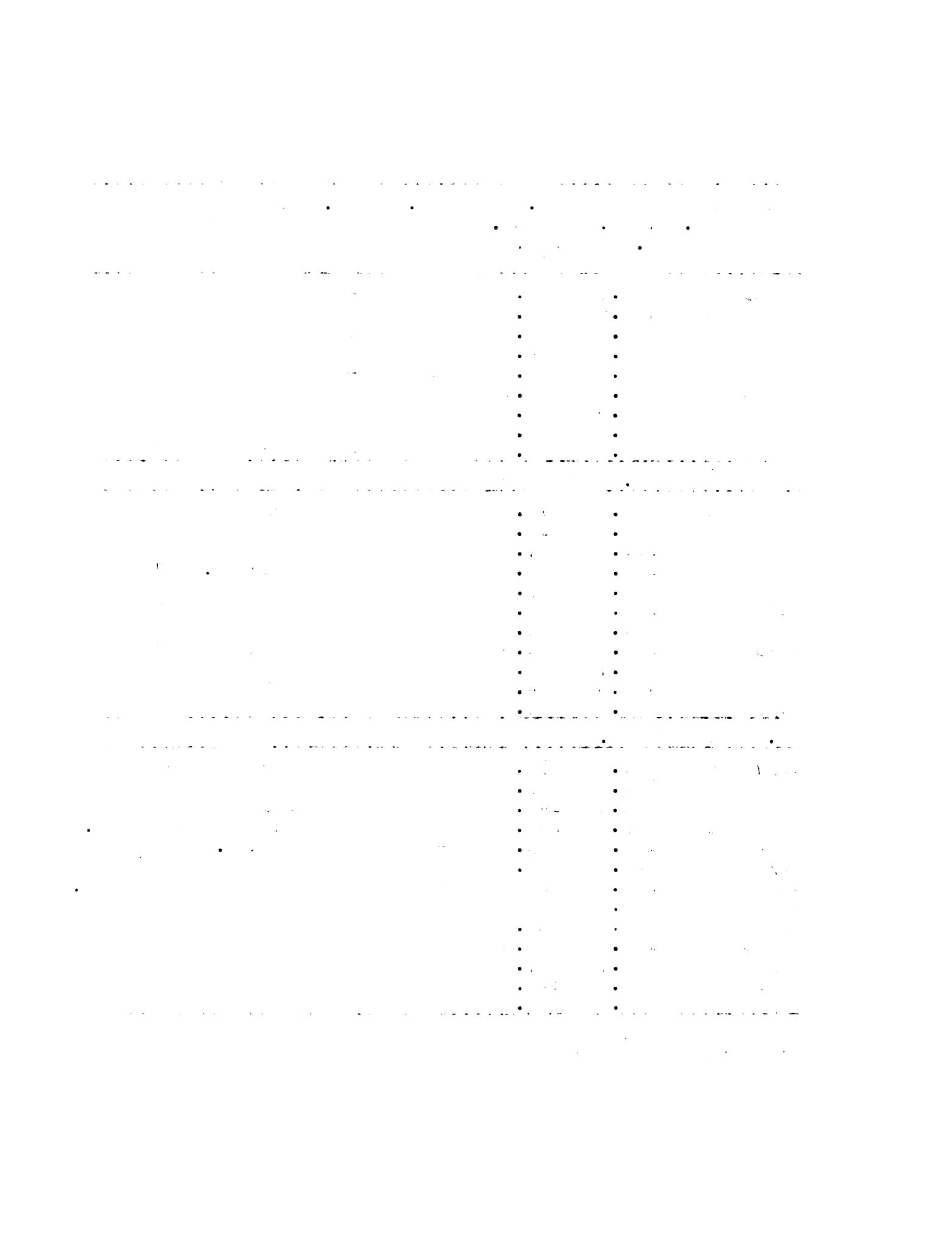

TABLE XI

LEFT REAR QUARTER OF COW 191

After infusion of 1800 staphylococcic (strain 71) organisms

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml. Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
10/10	0	101.2	2.2*	0	-	Normal	Normal
	3	101.4	3.5				
	6	101.4	4.2	0	+	"	"
	9	101.5	2.0				
10/11		101.2	2.5				
10/12		101.3	8.5	0	-	"	"
10/13		101.4	4.2				
10/15		100.7	2.4	0	-	"	"
10/16		101.3	4.0				
10/17		100.8	2.1				
10/18		101.3	0.9	0	-	"	"
444×10^3 staph. organisms and mild trauma							
10/20	0	100.5	1.2	0	-	Normal	Normal
	3	100.5	12.0				
	6	100.9	7.0	++	+	Yellowish	"
	9	101.0	9.5			serum like.	
10/21		100.6	13.0				
10/22		101.4	3.5	++	+	"	"
10/24		100.5	9.5				
10/26		100.5	150.0	++	+	Normal	"
10/27		101.4	75.0				
10/29		100.8	5.4	++	+	"	"
10/31		100.5	2.8				
11/1		100.2	40.0	+	+	"	"
29×10^8 staph. organisms							
11/2	0	101.8	5.6	+	+	Normal	Normal
	3	102.5	95.0				
	6	104.0	5.6	++	+	Yellowish white with	Hard and tense swelling.
	9	106.0	125.0			flocculi.	Swelling
11/3		101.2	50.0	++	+		disappeared.
11/4		101.2	4.9				
11/5		100.9	5.6	+	+	"	"
11/6		101.4	156.0				
11/7		101.5	37.0	+	+	Normal	"
11/8		100.9	3.4				
11/10		101.4	148.0	+	+	"	"
11/12		102.1	38.0				
11/14		101.3	7.6	+	+	"	"
11/15		100.9	4.5	++	+	"	"

*Recorded in thousands

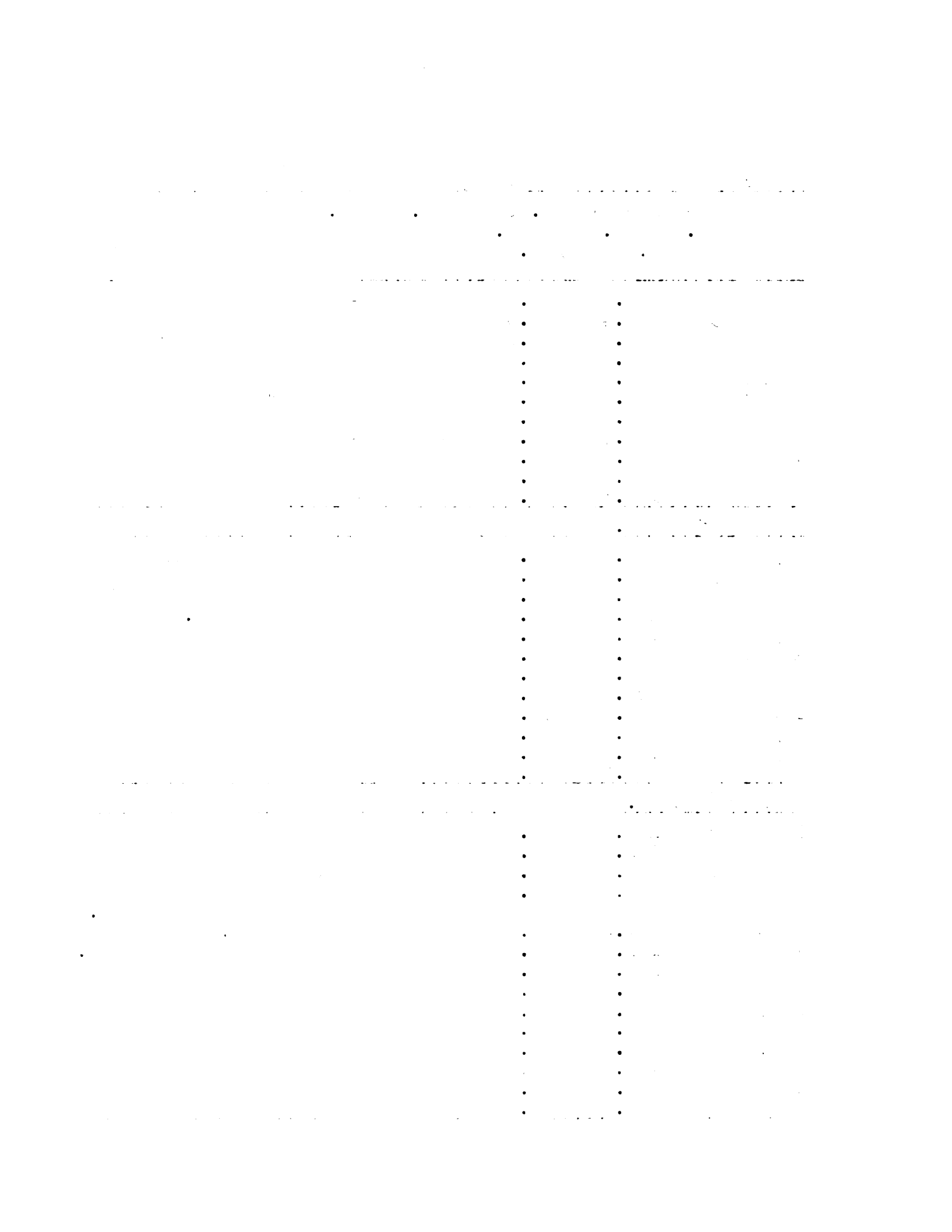

TABLE XII

RIGHT REAR QUARTER OF COW 191

After infusion of 2300 staphylococcic (strain 71) organisms

Date	Time hrs.	Rectal Temp. F.	No. of Staph. /ml. Milk	Coag.	Hemo.	Gross appearance of Milk	Gross appearance of Quarter
10/21	0	100.8	1.2*	-	-	Normal	Normal
	3	100.8	1.5	0	+	"	"
	6	100.9	6.4				
	9	101.1	4.3				
10/23		101.2	9.0	0	+	"	"
10/24		101.4	8.5				
10/25		100.6	0.9	0	-	"	"
10/26		101.3	1.1				
10/27		101.1	2.5	0	-	"	"
10/29		102.1	1.8				
10/30		100.6	0.8	0	-	"	"
57×10^4 staph. organisms and mild trauma							
10/31	0	101.3	1.7	0	-	Normal	Normal
	3	101.2	68.0	++	+	Yellowish white serum-like.	"
	6	100.9	12.0				
	9	100.7	5.6				
11/1		101.2	7.8	++	+	"	"
11/2		100.9	7.7				
11/4		101.2	26.0	+	+	"	"
11/6		101.4	51.2				
11/7		101.3	7.2	+	+	Normal	"
11/8		101.3	9.6				
11/10		100.9	6.7	+	+	"	"
11/11		101.1	4.5				
88×10^7 staph. organisms							
11/15	0	101.4	5.7	+	+	Normal	Normal
	3	101.2	166.0			Yellowish white with flocculi.	"
	6	100.9	34.0	++	+		
	9	100.8	9.0				
11/16		100.7	14.0				
11/17		101.3	5.6	++	+	"	"
11/18		101.5	1.6			Normal	
11/20		100.5	89.0				
11/21		101.5	36.0	+	+	"	"
11/22		100.9	9.8				
11/23		101.2	54.0	+	+	"	"
11/24		101.1	2.6				
11/26		100.9	87.0	+	+	"	"
11/27		101.3	62.0				
11/30		101.1	9.7	+	+	"	"

*Recorded in thousands

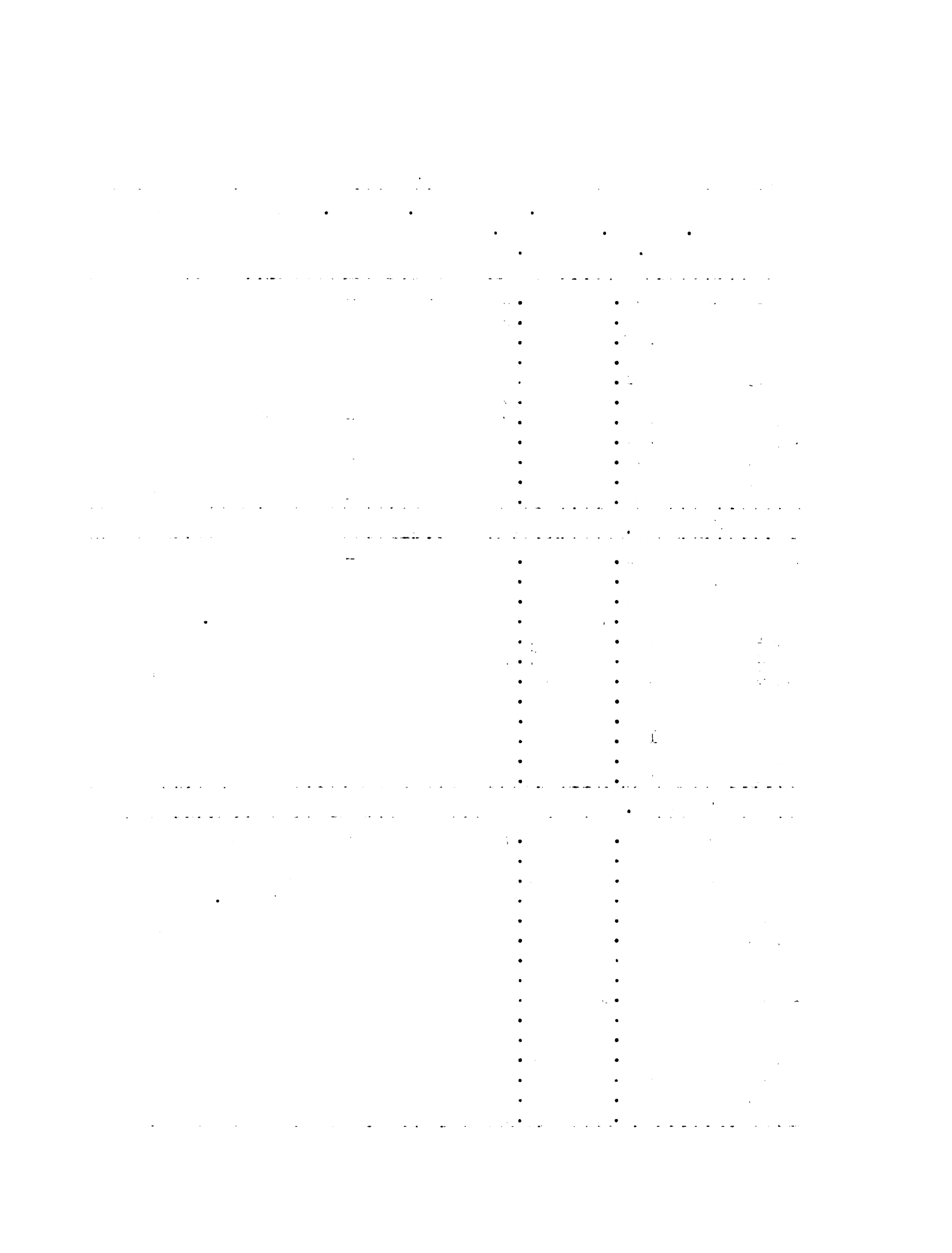

TABLE XIII

SUMMARY OF THE EXPERIMENTAL PROCEDURES FOR THE INTRAMAMMARY CHALLENGE OF THE THREE BOVINE UDDERS BY STAPHYLOCOCCIC ORGANISMS

Cow	Quarter	Staphylococci injected	Date of injection of staphylococcic organisms	Interval in days in between two consecutive injections
1	LF*	16×10^2	7/10	--
1	RF**	24×10^2	7/15	--
1	LR***	26×10^2	7/25	--
1	RR****	26×10^2	7/30	--
2	LF	28×10^2	8/22	--
1	LF	40×10^3	8/25	46
2	RF	24×10^2	8/27	--
2	LR	21×10^2	9/4	-
2	RR	18×10^2	9/10	--
1	RF	54×10^3	9/10	56
1	LR	50×10^3	9/20	56
2	LF	24×10^4	9/20	28
2	RF	44×10^3	9/27	30
1	RR	40×10^4	10/1	63
191	LF	18×10^2	10/3	--
2	LR	54.5×10^3	10/5	30
191	LR	18×10^2	10/10	--
1	RR	13.5×10^3	10/11	10
2	RR	67×10^3	10/15	34
1	LF	27.6×10^3	10/15	50
191	RF	24×10^2	10/15	--
191	LF	44×10^3	10/17	14
2	LF	27×10^9	10/19	28
191	LR	44.4×10^4	10/20	10
191	RR	23×10^2	10/21	--
191	RF	38×10^3	10/25	10
2	RF	18×10^8	10/25	27
1	RF	72×10^7	10/28	48
191	LF	10.2×10^8	10/29	12
191	RR	54×10^4	10/31	10
191	LR	24×10^8	11/2	11
1	LR	17.5×10^8	11/2	42
1	RR	16×10^8	11/5	25
191	RF	11.2×10^8	11/7	12
2	LR	5×10^8	11/7	32
2	RR	5×10^9	11/12	27
191	RR	88×10^7	11/15	14

* = Left fore
** = Right fore
*** = Left rear
**** = Right rear

TABLE XIV

GROWTH OF STAPHYLOCOCCIC ORGANISMS (STRAIN 71) IN FRESHLY DRAWN MILK, 10 cc. FROM COW 191

Quarter	0 Hrs.	3 Hrs.	6 Hrs.	8 Hrs.	24 Hrs.
Date: 9/25					
L.F.	66*	57*	69*	140*	TNC**
R.F.	78	72	86	125	TNC
L.R.	94	87	99	148	TNC
R.R.	40	29	42	98	TNC
Date: 9/27					
L.F.	30	27	32	75	TNC
R.F.	16	10	19	51	TNC
L.R.	15	14	9	56	TNC
R.R.	15	9	12	64	TNC***
Date 10/1					
L.F.	75	62	78	198	TNC
R.F.	82	67	96	215	TNC
L.R.	68	52	72	140	TNC
R.R.	96	80	95	186	TNC

*Staph. colonies counted in thousands in milk
**Too numerous to count
***For this reading see graph on page 40

TABLE XV

GROWTH OF STAPHYLOCOCCIC ORGANISMS (STRAIN 71) IN FRESHLY DRAWN RAW MILK, 10 cc. FROM COW 3

Quarter	0 Hrs.	3 Hrs.	6 Hrs.	8 Hrs.	24 Hrs.
Date: 8/25					
L.F.	840*	620*	1120*	TNC**	TNC**
R.F.	1420	1100	1490	TNC	TNC
L.R.	620	410	1240	TNC	TNC
R.R.	910	740	1530	TNC	TNC
Date 8/30					
L.F.	157	144	296	TNC	TNC
R.F.	143	139	260	TNC	TNC
L.R.	81	62	126	TNC	TNC***
R.R.	72	42	210	TNC	TNC

*Staph. colonies counted in thousands in milk
**Too numerous to count
***For this reading see graph on page 39