A COMPARATIVE STUDY BETWEEN THE NORMAL BACTERIAL FLORA OF THE UDDER AND THAT OF THE GENITAL ORGANS OF THE COW.

THESIS

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CONTENTS.

Introduction.

There are few agricultural products which have attracted more interest in both science and every day life than milk. This is by no means strange when one considers its widespread use, its high rank as one of the most complete foods, the comparative ease of production - qualities with which are associated important inconveniences, such as the readiness with which it lends itself to adulteration, and the dangers that may lurk in it in the form of pathogenic organisms.

The last mentioned consideration has given to the bacteriological study of milk an overwhelming importance which has been repeatedly emphasized through countless instances of local epidemics of typhoid, sore throat, infant mortality, and other outbreaks, traced directly or indirectly to the milk supply.

Naturally, the first task to which science subjected itself was the improvising of practical methods to render impotent any possible contamination. This partially accomplished, it devolved upon the scientific observer to investigate by means of laborious experiments the most important and at the same time the most intricate source of contamination - the udder of the cow.

I Does milk within the udder of the cow contain germs?

II How do these gain access to the udder?

I.

Robert Hall, in 1874, formulated the theory that milk, immediately upon being drawn from the udder, is germ free. In this assumption he was supported by Crotenfelt (19), who asserted that milk drawn from the udder of a healthy cow is germ free or sterile. The original sterility of normal milk, he believed, is due to the fact that bacteria cannot gain access to the milk glands from without as long as the udder is not injured in any way.

Freudenreich (14) holds a similar view: "In the udder milk is germ free except when the milk glands are diseased, as with tuberculosis or mastitis. In such cases the tubercle bacilli or those organisms causing the inflammation are present in the milk when drawn." He added, however, that immediately after being drawn it always contains bacteria due to its contaminated surroundings.

These views were shared by Lister (26), and as late as 1895 by Conn (6), and others.

The downfall of the theory began as early as 1892, when Schulz (37) made the first quantitative study of bacteria in the udder. While he found that the first milk drawn contained large numbers of germs, as the milking process continued their numbers decreased. This determination was afterward amplified by Lehman (24) who says: "The bacteria in the milk cistern will be largely washed out by the first milk drawn but not all will be removed until milking has progressed for some time." Bolley and Hall (5) state that "germs nearly

always gain admission to the interior of the teats and often, perhaps, the milk cistern proper, where some types multiply in great numbers".

Moore (29) corroborated Schulz's work by finding that there is a widely ranging bacterial content comprising several species in the freshly drawn fore milk, while the last milk drawn at a regular milking contains very few micro-organisms, comparatively. Seldom, however, is a sample of milk entirely free from germs, unless drawn from a single quarter well near the end of the milking process. This work of Moore succeeded that of Ward (38) who concluded from his first studies of the bacterial flora of the udder that: 1st. Certain species of bacteria normally persist in particular quarters of the udder for considerable periods of time, and, 2nd, that it is possible for bacteria to remain in the normal udder and not be ejected with the milk.

The well founded results of these investigators, coupled with those of Harding and Wilson (20), Evans (13), and others, have furnished us with entirely new viewpoints concerning the initial content and subsequent variations of micro-organisms in milk and their significance upon the proper interpretation of its sanitary bacteriological examinations.

The present paper is the result of some studies by the writer bearing upon the groundwork laid by these investigators. It is based upon a number of personally conducted studies on fifty-five samples of milk freshly drawn from the normal, healthy udders of twenty-five cows. The organisms isolated from these samples of milk were studied and compared

with a similar number isolated from the normal genital organs, (especially the vagina), of the cow.

The results of those studies which are here summarized comprise two parts: first, the normal flora of the udder and, second, the comparison of the normal flora of the udder and that of the genital organs.

The Normal Flora of the Udder.

By the normal flora of the udder is meant those organisms which, under the apparently normal condition of the animal, are able to grow and multiply in what is supposed to be their normal habitat. They should be found in all individuals under the same conditions of health, although their numbers may vary; they do not alter by their activities the normal functions of the organs in which they may be found.

Deviation from the normal condition of the udder may be due to physiological disturbances, but more generally to the incorporation of microorganisms from without whose activities are very often the cause of serious ailments and diseases.

Methods of Study.

The samples of milk studied were obtained and handled in accord with the following procedure:

Most of the samples were collected from the afternoon milkings. The udders of the cows from which the samples were taken were first groomed, washed with a piece of cloth moistened in water and toward the middle of the milking streams

of milk were directed into separate sterile bottles or test tubes corresponding with the different quarters of the udder. The samples thus collected were immediately taken to the laboratory where plating was done within an hour after being drawn.

In plating, plain meat infusion agar, containing 1% lactose and with a reaction 1.2 - 1.5% normal acid to phenophthalein, was used. One cubic centimeter of a 1 to 50 dilution of milk which had been shaken 20 times was pipetted in each petri dish and to each a tube containing from 8 to 10 cubic centimeters of lactose agar at a temperature from 40 to 42° Centigrade was added. The plates were incubated either at 37.5° Centigrade for 48 hours or at room temperature (20 to 23° C.) for five days, and at the end of this period of incubation the sum of the colonies developing on the plates were taken as representing the germ content of the milk.

Occasionally, plates were found in which no colonies developed, these were taken as coming from a sterile quarter of the udder, or as having a number of organisms less than 50 which was the dilution used for plating.

From the colonies appearing on the plate some were isolated and studied under the following headings:

1. Morphological characteristics.

The morphology of each culture was studied from smears made from the growth on the agar streak. The streaks used were never more than four days old and the morphology of the individual cells was observed after staining lightly with an aqueous solution of methylene blue or fuchsin. Too heavy staining is objectionable

since it introduces a serious error by grouping the cells into masses which make difficult the observation of the individual cells. By means of an ocular micrometer the dimensions of the cells were recorded.

2. Staining characteristics.

These were observed by staining the smear of one agar growth, not over four days old, by the Gram method and with aqueous solution of methylene blue and fuchsin.

3. Cultural characteristics.

In this study the general character of growth on the agar slant was recorded. In nutrient broth the cultures were examined for the growth and the formation of pellicle, turbidity and sediment in the liquid in the tube. In the gelatin tubes the rate of liquefaction of all the cultures has been recorded.

4. Biochemical reactions.

a. Action upon milk. Both plain and litmus milk was prepared according to the standard methods and the actions of the cultures observed after three days of incubation.

b. Action upon carbohydrates. The characteristics observed in the media were the gas production and acid production.

The media for this fermentation reaction was made by adding to 1000 c.c. of the ordinary meat infusion broth 10 grams of the test substances. They were dextrose, lactose, saccharose, maltose, raffinose, mannite and glycerine. The reaction was made neutral for these fermentation tests in the various sugar broths above mentioned. A twenty-four hour culture of each organism

in plain bouillon was used and to each tube three loop-fulls of culture were added so that approximately the same amount of organisms were present in each tube of media. The cultures were incubated for fourteen days at room temperature and five c.c. of the culture titrated in the cold against twentieth-normal sodium hydrate with phenophthalein as an indicator. From the results so obtained is subtracted the titration of a blank and the results are expressed as the percentage of normal acid. The production of 1% normal acid marks the line between fermentation and no fermentation.

This method of recording the acid fermentations of any organism which is the one more generally adopted meets several inconveniences such as the use of a definite and uniform quantity of the culture growth in titration, the use of well prepared sodium hydrate used for the test, the time consumed in the procedure of titration, etc. While the study of the fermentations of the different organisms was being carried on as here outlined, the attention of the writer was struck by the work of Hiss on this matter.

Hiss devised the following media, in which the cleavage of any carbohydrate is indicated by the production of an acid reaction and by the coagulation of the serum (A Text-book of Bacteriology, by Hiss and Zinsser, 1914): "Clear beef serum is obtained by pipetting from clotted blood. To the clear serum is added three times its bulk of distilled water. The mixture of serum and water is heated in the Arnold Sterilizer for fifteen minutes at

100°C. to destroy the diastatic ferments in the serum. After cooling the mixture, a concentrated aqueous solution of litmus is added until a deep transparent blue is obtained. The mixture is then divided into as many parts as there are carbohydrates to be used. To each of these parts of the mixture is added one percent respectively of the sugar to be used.

For the preparation of inulin medium it is necessary to sterilize the inulin dissolved in water in an autoclave under 15 pounds pressure for fifteen minutes in order to kill spores before adding it to the serum. The serum water media is sterilized by the fractional method in the Arnold Sterilizer at 100°C. for twenty minutes on three successive days in order not to coagulate the serum."

By using the procedure of Hiss, fermenting power of an organism on a certain carbohydrate is observed more readily, more easily, and probably with the same degree of accuracy.

c. Action upon nitrates. Nitrate reduction was determined in the ordinary peptone broth after seven days growth of the culture at 20 C.

d. Pigment formation. Pigment formation was studied macroscopically after fourteen days growth of the cultures on agar slopes at room temperature (20°C.).

e. Liquefaction of Gelatin. For determining the amount of liquefaction a drop of broth culture was spread on the surface of a tube of infusion gelatin, the surface of the gelatin is marked around the side of the tube and

the cotton plug covered with paraffin to prevent evaporation. If liquefaction takes place it proceeds in a stratiform fashion and its amount is read in millimeters of liquefaction after thirty days incubation of the tubes at 20°c.

Hemolysis. To a tube of ordinary infusion agar at 50°C., 10% of defibrinated cow's blood was added and after carefully rolling the tube between the hands to mix the blood uniformly with the agar they were poured into each plate. When the agar had solidified, streaks of the cultures were made on it and the plates incubated at 37°C. After twenty-four hours the first observation of the plates was made and the streaks of the culture surrounded by a distinct, clear zone of hemolysis were designated as having a positive hemolytic power; those not showing any white zone of hemolysis were designated as having a negative hemolytic power, but since the rapidity with which the blood is hemolysed by various organisms is variable it seemed more convenient to record the observations on the plate for a longer period of time, four or five days being sufficient.

Morphology of the Bacteria from the Udder.

According to their morphology the bacteria of the udder will be here considered separately under three groups,- the streptococci, the micrococci, and the bacilli.

The Streptococcus Group.

According to Migula's classification of bacteria, to the genus Streptococcus belong all those microorganisms in which the cells are spherical, dividing in one plane, and having no flagella. Wilson describes some of the most salient characteristics of this genus as follows:

"The genus Streptococcus has been defined by its parasitic life, the occurrence of the cell in chains, positive reaction to the Gram stain, faint effused growth on media, faint fermentative power for most carbohydrates, but not for inulin, and the general failure to liquefy gelatin or reduce nitrates. They may appear in long chains of a hundred or more elements, or in pairs and short chains of four to six cells. Chains are best developed in broth cultures; but some strains display the tendency to chain formation much more markedly than others".

The different strains of streptococci differ so widely in morphology, cultural characteristics, and virulence that it has been difficult to classify them satisfactorily. Notwithstanding this fact, classificatory examinations of numerous strains have eventuated in the discovery of definite groups which possess conspicuous physiologic and pathogenic peculiarities; even among the members of one of these groups there have been found types which are differentiated by further refinement of laboratory technic.

Significant physiological dissimilarities among closely related streptococci have been determined by studying their reactions on special laboratory media. Among those

laboratory media those which have been more greatly used are the different sugar broths.

The question may thus be formulated: What relation does this group of microorganisms, which has exerted so intense an interest on the part of many workers in all lines of bacteriological research, bear to the milk problem?

Numerous investigators have demonstrated the presence of streptococci in milk coming from udders affected by mastitis. Streptococci have also been found in milk coming from apparently healthy udders. Various local outbreaks of "septic sore throat" due apparently to a streptococcus infection have been traced occasionally to a certain milk supply. This milk supply in question, which seems to have been the chief vehicle of infection, may have become infected under the two possibilities: Either that the pathogenic organisms were introduced into the milk supply by some infected person who had to handle the milk, or that they were derived from the cows which produced the milk.

Von Lingelsheim (23) mentions the fact that streptococci are found in market milk, and suggests that these bacteria may be the cause of many infantile intestinal diseases. This suggestion by Von Lingelsheim was supported and amplified by Petruscky (34) in 1904, who announced as the results of his investigations that the high infant mortality in summer was caused by streptococci which he found in milk in large numbers.

The presence of streptococci in milk has also been reported by Conn and Esten (7), Heinemann (21), Davis (11), Muller (31), Frost (15), Reed and Ward (35), and others, but despite their labor a satisfactory interpretation of their

significance to the wholesomeness of the milk has not yet been achieved.

Some of the characteristics of the organisms isolated from this source are as follows:

Strongly hemolytic power. Found in the same proportion in certified milk as in that which has been pasteurized, they are more resistant to heat than the human strains of hemolytic streptococci. They possess little or no virulence for rabbits, therefore in all probabilities not for man. They rapidly acidify and coagulate milk and grow well at 20° C. They may form short or long chains, but as seen in milk they often appear in pairs or a chain of few elements. These milk strains are different from those found in case of diseased udders in cows in that these latter resemble the strains of hemolytic streptococci from human sources and are virulent for rabbits. Mathers (27) found that hemolytic streptococci, with all the characteristics of the human type, may be highly virulent for cows when injected into the milk ducts, they produce a severe mastitis or may grow and multiply in the milk ducts without causing any visible changes in the udder, but the milk in this instance contained an increase of leukocytes as well as streptococci.

Mathers also says that hemolytic streptococci from milk and the Streptococcus lacticus may produce an acute inflammation of the milk ducts, but he observed it was of a transitory nature and the mammary glands regained their normal functions very rapidly. Contrary to the findings of these investigators are those of Baehr[2] who on examination of eighty-

one samples of milk, part from milk dealers and part from individual cows, found Streptococcus pyogenes in only two of the samples.

Harding and Wilson who made the first extensive study on the normal flora of the udder in this country on an examination of twelve hundred and thirty (1230) samples of milk direct from the udders of seventy-eight cows (78) found only two representatives of the genus Streptococcus and they say further: "The absence of this genus was noted early in the qualitative work and attention given to the detection of streptococci. Neither of the two forms here listed are typical members of the genus, their tendency to form chains being rather weak. It will also be noted that two strains of Streptococcus lacticus are among the named species with an interrogation point. These strains were not streptococci but agreed fairly closely with the descriptions of that species in other respects".

Quite in opposition to these results are those of Evans, described in her recent work. She begins by saying: "A most important matter in the study of the streptococci in milk is the differentiation between the long chained streptococci, which may be of a virulent type, and the saprophytic Streptococcus lacticus (Kruse). It is my opinion that such a differentiation can be made by the simple method here described, although there are undoubtedly variations which do not conform to either of the two types".

"Long-chain streptococci usually curdle the milk, and reduction of the litmus may take place after curdling, but the color is never completely reduced. With Streptococcus

lacticus cultures the reduction of litmus precedes the curdling and is complete beneath the sharply defined pink surface layer". "Streptococcus lacticus lacks the tendency to form long chains and some or all of the cells are elongated with tapering ends."

From the one hundred and ninety-two samples of milk studied by her, Streptococcus lacticus (Kruse) was isolated only once and was discarded as contamination, but there were found long-chained streptococci which failed to give the reduction of litmus, characteristic for Streptococcus lacticus, in 15.1 percent of the samples studied by her, and in numbers ranging from a very few to 264,000 per cubic centimeter. All the results of these investigators, with the exception of those of Harding and Wilson, point to the evidence that streptococci may be found in milk in numbers varying from a few hundreds to several thousands per cubic centimeters. But by a detailed analysis of the studies of these investigators, it is seen that their observations have been made largely on bacteriological examinations of market milk.

The work of Harding and Wilson and that of Evans deals principally on studies of freshly drawn milk, and yet their results are strictly in opposition. While Harding and Wilson found only two members of the streptococcus group in their observations on a large number of samples of freshly drawn milk, Evans claims to have isolated streptococci in 15.1% of the samples of milk studied by her.

Without attempting to criticise the results of these investigators, the results of Evans' work reporting the find-

ings of streptococci in freshly drawn milk in numbers ranging from a very few to 264,000 per cubic centimeters cannot restrain the writer from considering this high number of organisms as rather improbable in freshly drawn milk supposed to have been drawn under the best of aseptic precautions. The reliability of this assertion of Evans is again questioned by the results of the writer in finding that the germ content of freshly drawn milk from apparently healthy udders, calculated on the numbers of organisms developing on the agar plate, averages not higher than five hundred organisms per cubic centimeters. And her results are questioned again when one considers that it has been stated (by Committees on bacteriological studies of milk) that it is possible to produce certified milk, and one of the requirements of this milk is that it shall not contain more than 10,000 microorganisms per c.c. at any time after it has been drawn from the udder of the cow, and yet this milk containing so few a number of microorganisms comes in contact, although for a very short time, with the air of the surroundings where it is produced or prepared to be carried to the consumer.

In his present studies the writer has failed to isolate streptococci from the samples of milk drawn under the best of aseptic precautions. These failures (should these organisms be found to prevail generally in the freshly drawn milk) may be ascribed: First, to the small number of samples studied; second, to the fact that, generally, the streptococci grow rather poorly in the laboratory media commonly employed in this work and they may even fail to form chains of cells, their distinguishing characteristic; and third, to the failure to separate the colonies supposed to represent the de-

sired type from among all the others developing upon the agar plate because their similarity is so striking as to require both experience and judgment for its success.

The Bacilli Common in Normal Milk.

The application of bacteriological research to the study of the sanitary conditions in milk has revealed to us, many years back, that the typhoid bacillus, the tubercle bacillus, and a few other pathogenic bacilli are frequently found in milk.

Milk in which these organisms have been found has been considered as unclean, unfit for human consumption, and therefore regarded as being contaminated. This contamination has been traced to the improper handling to which milk is often subjected before reaching the consumer, or to various infections of the udder.

But it is only during recent years, through the observations of Mohler and Traum (28), Schroeder and Cotton (36), Fabyan (16), Evans (12), and other investigators, that attention has been focused to the presence in milk coming from apparently normal udders, of an organism which agrees in its characteristics with a similar one isolated by Bang, a Danish bacteriologist, in 1895, from the uterine exudate and aborted fetus of an infected cow. The organism today bears his name and is denominated,- Bacterium abortus (Bang).

The failure of the common laboratory technique as applied to milk to reveal the presence of this organism has made it necessary to employ special methods for its isolation. One of these methods consists in incubating the plate from which

observations of this organism are to be made, under anaerobic conditions as devised by Nowak, and the other by confirming the presence of these organisms in milk by inoculating laboratory animals, especially guinea pigs, with the milk and waiting for the development of lesions in these animals.

The pathogenicity and deleterious effect of this organism upon man has been questioned by Larson and Sedgwick (25), Melvin (32), Nicoll and Pratt (33), Cooledge (9), Williams and Kolmer (4C), and others, and the significance of this organism (Bacterium abortus) in milk coming from apparently normal udders is one of the most keenly discussed questions that the Science of Dairy Bacteriology is called upon to solve.

In the light of the new researches on this problem, it is premature to assume whether the Bacterium abortus is to be regarded as a type of the normal udder, but the following facts are known:

First; The Bacterium abortus is not found in all udders or in the milk coming from them.

Second; Organisms to be considered a type of the normal udder should be found in all cases exposed to the same variability of conditions and environment.

Third; The latest researches on this problem have strengthened the conviction that this is the only type of bacillus that prevails in the apparently healthy udder, but it is not found in all apparently healthy udders.

If we rest our assumption on these well known facts, it is not unreasonable to conclude that Bacterium abortus cannot be regarded as a type present in all normal udders, but yet we

cannot make this conclusion for we lack definite methods of study to prove that this is the case.

The Micrococci.

Among the microorganisms inhabiting the normal flora of the udder, the micrococci are the ones which occur there more frequently and the accumulative evidences that are beginning to be derived from studies of the bacterial flora of the udder may prove, finally, that perhaps this is the only type that predominates in the normal udder of the cow.

The classification of the streptococci, closely related to the micrococci, carried to a very high degree of refinement and perfection in the hands of Gordon (17), Andrewes and Horder (1), Winslow (42), and other investigators, has laid the steps by which a systematic classification of the micrococci will soon be achieved.

Upon examination of an agar plate containing several colonies of micrococci, one readily perceives the close similarity in shape, color, and appearance among all the colonies developing upon the plate. The similarities are often so striking as to lead the observer to question whether all the colonies do not represent the same type of organism. When examined under the microscope the doubt is strengthened as to whether they all possess a morphological identity. However, by further study of some of these colonies it becomes certain that there exist sharp limits of variability among them.

Morphological differences among the bacteria are so slight, especially in the members of the family Coccaceae,

that physiological properties have to be relied upon for their classification, and yet such properties are so extremely variable - especially the fermentation reactions, the pathogenic power, etc. - that it has been necessary to find out whether they exhibit any degree of constancy.

On the morphological basis the micrococci have been divided into two types -- the staphylococci and the micrococci proper.

The staphylococci are cocci whose planes of division are not at right angles but divide at different intervals with a consequent irregular grouping of the cells much resembling grapes in clusters. The genus is frequently included under Micrococcus. It contains some of the more common pyogenic cocci. It may be separated by its Gram positive stain and the production of white or orange pigment.

The pyogenic staphylococci are found very widely distributed in nature and are found in the air, sometimes in water, in man on the skin and mucous membranes, in the alimentary canal, and in the mouth, also in suppurative conditions occurring in the mammalia and birds. In human pathological lesions they are frequently found in pus, especially in abscesses on various parts of the body. Occasionally, the staphylococci enter the blood stream giving rise to a purulent infection known as pyaemia.

Under the group of staphylococci, the following microorganisms are included: Staphylococcus aureus, citreus, albus, rosaceus, flavus, and griseus, the first three being the ones which occur more frequently. In their biological properties, these microorganisms are very similar, differing

from one another principally in the color of their growths. Aside from their difference in pigment production, attempts have been made to further differentiate them on the basis of gelatin liquefaction.

Gelatin is liquefied by staphylococcus aureus, citreus, rosaceus, and albus.

Gelatin is not liquefied by staphylococcus flavus, and griseus.

The Micrococci. This genus is frequently defined to include the genus Staphylococcus just discussed. It may be differentiated from this generally by its Gram-negative character. They exhibit a great variability of pigment production, although the majority of them produce the different shades of yellow pigment.

Before attempting to describe the characteristics of the Micrococci composing the flora of the normal udder, it is important to distinguish the different kinds of variability among them which contribute to the complexity of their classification.

The Micrococci from the udder exhibit these different variations:

First: Those due to conditions of environment, such as color. Thus, the intensity of color of the pigment of a good many of the Micrococci from this source varies with the age of the culture. At first, some cultures appear to possess a whitish color which, after several weeks, changes to yellow, orange, etc. The intensity of the color is also dependent upon the supply of oxygen, becoming more intense with the greater supply of oxygen.

Second: Certain physiological characteristics which are influenced by the age and the frequency with which the cultures are transferred to fresh media. Thus it is observed that some Micrococci, which at first do not ferment some carbohydrates, after they are grown for a considerable period of time seem to acquire the ability to ferment such substances.

Third: Certain laboratory media used as a means of differentiation between other closely related organisms seem to be of no avail as an index of differentiation when applied to the physiological variations of the Micrococci. For example -- milk. Milk is a favorable nutrient medium for bacterial growth because of its properties, and the changes produced in it by many organisms are important as diagnostic means.

Milk has no special diagnostic value for the Micrococci since all the changes it undergoes are correlated with those which occur in the various sugar broths and are dependent upon the general activity of the organisms. The changes produced in milk by these Micrococci are the production of acid or alkali, coagulation, and decolorization of the litmus.

Decolorization of the milk has no significance except as an indication of the activity of growth of an organism. When an organism is growing most actively it uses up oxygen and so reduces the litmus which is therefore decolorized, and the reverse is true when the activity is less. Oxygen is no longer used by the growing organism and consequently it diffuses down from

the surface, giving back some of the color to the litmus.

The coagulation of the milk is largely brought about by the amount of acid produced and this is more easily and accurately studied in a broth containing the different carbohydrates.

It is then understood at this point that the reactions of the Micrococci in different laboratory media used in the differentiation of other organisms do not furnish us with a means for their differentiation, but by studying their activities on broths containing the different carbohydrates and other related substances the progressive divergence from their apparently fundamental resemblances is observed.

The following protocol exemplifies the fermentation reactions of the Micrococci isolated from the milk drawn from the udders of normal cows. For the denomination of the color of the pigments in the following table, Ridgway's "Color Standards and Nomenclature" was used.

Characteristics of the Micrococci from the Udder.

Culture	Coagulation of Milk.	Reduction of Litmus Milk.	Reduction of Nitrates.	Liquefaction of Gelatin.	Dextro
1	+	+	+	+	+
2	+	+	-	-	+
3	+	+	+	+	+
4	+	-	-	+	+
5	+	+	-	+	+
6	+	+	+	-	+
7	+	+	-	+	+
8	+	-	-	+	+
9	+	+			+
10	+	-	-	+	+
11	-	-	-	-	+
12	+	+	+	+	+
13	-	-	-	+	+
14	-	-	-	+	+
15	+	+	-	+	+
16	-	-	-	+	+
17	-	-	-	+	+
18	-	-	-	-	+
19	-	+	-	+	+
20	+	+	-	-	+
21	-	-	-	+	+
22	+	+	-	+	+
23	-	-	-	-	+
24	+	+	-	-	+
25	+	+	-	+	+
26	+	+	+	-	+
27	-	+	-	+	+
28	+	+	+	+	+
29	-	-	-	-	+
30	-	+	-	+	+
31	-	-	+	+	+
32	+	+	-	+	+
33	-	+	-	+	+
34	-	-	-	+	+
35	-	-	-	-	+
36	-	-	-	-	+
37	+	+	-	+	+

Maltose	Raffinose	Mannite	Glycerin	Hemolysis	Chromogenesis
+	-	+	-	+	pale orange yellow
+	-	-	-	-	white yellow
+	-	+	-	+	yellow
+	-	-	-	+	pale yellow orange
+	-	-	+	+++	white yellow
+	-	+	-	+++	pale orange yellow
+	-	+	-	-	white yellow
+	-	+	+	+++	orange buff
+	-	-	+	+	orange
+	-	+	-	+	light orange yellow
-	-	-	-	+	yellow
+	+	+	-	+++	light orange yellow
+	-	-	+	++	cream
+	-	+	-	+++	cadmium yellow
+	-	-	+	+	white yellow
+	-	+	-	+++	orange
+	-	-	-	+	buff yellow
-	-	-	-	+	slight pale yellow
-	+	+	+	+++	deep chrome
+	-	-	-	-	yellow
+	-	+	-	+++	cadmium
+	-	-	-	-	chrome
+	+	+	-	-	light cadmium
+	-	-	-	-	cream
+	-	+	-	++++	deep chrome
+	-	-	-	-	orange
+	-	-	-	-	yellow
+	+	+	+	++	yellow
+	-	+	-	+	chrome
+	-	+	-	+++	chrome
+	-	-	-	+	orange buff
+	-	-	+	-	orange
+	-	+	-	+++	cadmium yellow
+	+	-	-	-	orange
+	-	-	-	+	pale orange yellow
+	+	+	+	-	orange
+	-	+	-	+++	chrome

In conclusion, the following is a brief summary of the salient characteristics of the Micrococci studied in this paper.

The largest proportion of the Micrococci from the udder are aerobes, following the facultative anaerobes. It is possible that there may be in the udder some anaerobic Micrococci, but no special technique was employed to show whether they were present.

The character of the facultative anaerobism was observed especially on the agar plates on which colonies grown on the under side of the agar when transferred to the agar slant seemed to grow equally as well as those isolated from the upper surface of the solidified agar.

The reaction of litmus milk varies from complete coagulation and reduction of the litmus to no apparent change.

The majority of the organisms included in the foregoing list liquefy gelatin, varying in number of millimeters and rapidity of liquefaction. The larger percentage of organisms do not reduce nitrates.

Dextrose and lactose fermentations were positive for all the cultures.

No gas is formed when tested in the fermentation tube. No attempt was made to divide them into groups according to the substances acted upon.

Comparison Between the Organisms Isolated from the Udder and Those from the Genital Organs.

Realizing that the factor governing the fitness of the lacteal fluid for direct or indirect consumption lies largely in its germ content and that some of the microorganisms included in the latter, after the milk is drawn or yet within the udder, may have imperiled the wholesomeness of the milk by coming from the discharges of an infectious genital disease, the writer thought that a comparison showing whether any relation exists between the flora of the normal udder and that of the normal genital organs, (especially the vagina), might throw some light upon the question of the contamination of the milk while yet within the udder.

The result of these studies, showing that there exists some relation between the organisms found in the normal udder and those found in the normal genital organs, was necessary in order to measure successfully the degree to which the discharges of the genital organs of the cow prove to be a factor in the contamination of the milk.

In a study of this nature, the first question that commands our consideration is: How do the organisms gain access to the udder?

Scientific opinion concerning the answer to this question is divided between two factions of bacteriologists; on the one hand, those believing the theory that the organisms make their way into the udder through the circulatory system, and on the other hand, those supporting the assumption that they gain

entrance to the udder through the discharges from the genital organs.

Before stating the arguments by which each theory is upheld, let us turn our attention for a moment to the anatomy of the cow's udder to find what features in its anatomical structure will favor the incorporation into the udder of organisms from the outside.

The Cow's Udder.

The particular glands in which the milk originates,- the milk glands - form the most important portion of the milk secreting udder. The cow's udder is divided by a strong fibrous partition, running longitudinally. Each of the halves contains a large milk gland of a reddish-gray color, or more correctly speaking, an accumulation of glandular structures called the gland basket. The milk glands vary greatly in size in different individuals and contain embedded in them a white connective tissue, the delicate gland-lobules in which occur numerous round cavities, the microscopic gland-lobules, or alveoli, which are terminal or lateral dilatations of numerous and extremely fine canals in which the material for the formation of milk circulates through numerous lymph tracts, uniting into ever widening ducts - milk ducts - and eventually into large, hollow cavities - the so-called milk cisterns or milk reservoirs. Four of these are present in each udder and corresponding to these are four teats.

The teat is a canal surrounded by a muscular wall and closed at the extremity by an involuntary sphincter muscle which varies much in rigidity in different animals. Often, the

pressure of a small amount of milk in the canal is sufficient to open it and permit the milk to escape. In other individuals it requires strong effort of the hand of the milker to draw the milk. This canal with a temperature of the animal body and containing always, even after the most complete milking, a small amount of milk offers ideal conditions for bacterial growth. When the animal lies down, be it in the stable or elsewhere, the udder and teats come in contact with dust and dirt teeming with bacteria. The pressure exerted by the animal upon the udder, added to the pressure of the milk in the udder, opens the canal of the teat and thus provides an avenue for the entrance of microorganisms into the udder.

By studying the anatomical structure of the udder it is observed that its different parts are not closely connected. This fact has been confirmed by observing certain infections to which the udder of the cow is often subject; for example, mastitis, which has been proved to be a local infection which does not travel from one quarter to another.

We are in a position now to return to the theories of our discussion. Do the organisms gain access to the udder through the blood stream?

The shifting of the balance of opinion on this question may possibly be traced to the statement in regard to Bacterium abortus in the udder, given by Cotton (10). He says: "The bacillus of infectious abortion may and in some cases does persist in the udders of cows that have aborted for years and possibly for the balance of their lives and, during this time, is eliminated more or less continually with their milk. It

makes its appearance in the milk months before abortion occurs, even before a conception that is terminated by an abortion. It may be eliminated for years from the udders of cows that never aborted."

This periodical appearance of these particular kinds of organisms in the udder has to have some source of supply. That the genital tract is not a source of supply is proved by the conclusions of the same writer that Bacterium abortus persists in the genital tract for only 46 days (or even a shorter time). That it does not come from the outside is shown by the fact that Bacterium abortus persists, when exposed to weather conditions, for a very short time, even as short as ten days.

Then it is left for us to assume that it gains access to the udder through the blood stream. The assumption that bacteria gain access to the udder through the blood stream is improbably for various reasons.

First; If the bacteria of infectious abortion gain access to the udder through the blood stream, we would expect to find the abortion germ equally distributed in each of the quarters of the udder which is far from being the case; this is also true of other bacteria appearing in the udder.

Second; Cooledge, by means of the agglutination tests, found that antibodies for this organism could be demonstrated to exist in the milk coming from some quarters of the udder, but they were not found to be present in the milk coming from all of the quarters. And further in the discussion of this point, Huddleson (22) joins Cooledge when he says: "the antibody content of the milk and that of the blood in the same

cows is not in a direct proportion." This shows again that the infection does not come through the blood stream.

It is a well known fact that some drugs, especially bitters and aromatics, are very largely excreted in the milk and guided by this fact there is reason to believe that bacterial toxic products may become excreted through this channel, but yet this does not appear to be the case with microorganisms.

As a conclusion to this side of the question, Williams (41) writes: "We are beginning to believe that the tubercle bacillus does not pass from the mammary capillaries into the milk acini while the secretory epithelium is intact, and that the danger of tubercular infection from the milk is chiefly or wholly dependent upon tubercular disease of the gland or to contamination of the milk from bacilli elsewhere eliminated, especially to bacilli voided with the feces." Again he says: "The diseases of the genital organs of cows may be related to dairy inspection from three different viewpoints:

First; Certain types of genital diseases may induce sapraemia, pyaemia or septicaemia, and through such avenues imperil the wholesomeness of the milk by the products of disease being excreted in the milk.

Second; Diseases of the genital organs tend to cause a discharge through the vulva which, soiling the tail, thighs, and udder, threatens finally to drop in part into the milk while being drawn.

Third; Genital discharges passing downward from the vulva along the tail, thighs, and udder, come finally in contact with the teat orifice and the infection may pass along the teat canal, gain the secretory surfaces of the

gland, multiply there, establishing a new disease focus, and contaminate the milk directly before it is drawn."

But if it is true that this occurs in a diseased condition it is within the same ground of possibility that it should happen during the normal condition of the animal, although to a lesser degree.

Finally, in support of this latter view it has been pointed out by some investigators that the fact that organisms are generally found in larger numbers in the rear quarters than in the front quarters of the udder may explain the relation that these organisms may have with the genital discharges. Although the writer's personal observations do not warrant him in making a definite statement at this time, however, he is inclined to believe that the fact that microorganisms are found in larger numbers in the rear quarters than in the front ones lies in the assumption that when the cow lies down the rear teats are more exposed to the contamination from the floor than are the front ones. The writer is thus encouraged to believe that neither of the theories here stated is supported by their corresponding evidences, and the attention of the observer needs to be directed to other outlooks in order to elicit proofs that will be sufficient to settle this question of conveyance of the contaminating organisms to the inside of the udder.

The organisms from the genital organs of the cow were isolated and studied by Mr. Keck* and Mr. Bolton* and the following is taken from their observations:

* Mr. W. C. Keck and Mr. R. B. Bolton of this laboratory were working on the flora of the genital organs.

Cows from which studies were made:

Number of cows from which swabs were made	30
Number of swabs made from the vaginas	30
Number of swabs made from the uteri	23
Number of cows from which swabs were made of both vaginas and uteri at the same time	4

Organisms isolated:

Number of organisms isolated from the vaginas	34
Number of organisms isolated from the uteri	24
Total number of different organisms isolated	52
Number of organisms occurring in both uterus and vagina	5

In going over their results they found a very wide range of organisms. There were no two cows which showed an identical flora in either the uterus or the vagina, and, besides, they found very little similarity between the organisms isolated from any two cows. In accord with their morphology, they divided the organisms isolated as follows:

Number of Bacilli isolated	22
" " Micrococci "	11
" " Streptococci "	4

Due to the fact that the complete study of the organisms isolated by them has not terminated, the descriptions of their morphology, cultural and physiological characteristics, hemolytic power, etc., are here omitted.

General Discussion and Conclusions.

The results set forth in this paper have been based upon the study of a number of organisms isolated from 55 samples of milk drawn from the normal udders of 25 apparently healthy cows and compared with those similarly isolated from the normal genital organs of 30 different cows.

The samples of milk were taken from cows which did not show any sign of genital discharges. In the majority of the cases, both samples of milk for study were drawn and swabs from the genital organs were made from the same cows, although in a good many instances only one of these was taken from each individual.

In comparing the organisms isolated from these two sources, the writer has found that a wide range of various organisms prevailed in the genital organs of the cow. A large number were bacilli while none were isolated from the normal udder. Streptococci were found in the genital organs, but none were isolated from the samples of milk taken from the normal udders. The majority if not the entire number of organisms found in the udders were Micrococci, and in the genital organs only 11 Micrococci were isolated and were found in less numbers than the bacilli of which 22 representatives were studied. Furthermore, the Micrococci isolated from the genital organs did not seem to possess a similarity to those isolated from the udders.

The result of the writer's present observations, while not determinative in character, lead him to assume the following brief conclusions:

First; That the normal flora of the udder comprises largely of microorganisms which belong to two types, the Micrococci and the Staphylococci, varying in pigment production, fermentation reactions, hemolytic power, and the other tests to which they have been subjected.

Second: That although in the few samples of milk here studied these two types have been considered as representing the bacteria of the normal udder, this does not preclude the possibility that had a large number of samples been studied and more colonies subjected to examination, other types might have been encountered.

Third; That a comparison between the organisms isolated from the udder and those isolated from the genital organs shows that there is not a close similarity between the bacterial flora of these two sources.

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