STUDIES IN DAIRY BACTERIOLOGY

Major Thesis in Dairy Husbandry

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By

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Outline

The value of Dairy Bacteriology to the milk industry.

The cause of bitter cheese and bitter milk.

Yeast as an agent in producing bitter milk and abnormal fermentation in milk.

The search for yeast in local creamery cheese.

General procedure.

Preparation of media

Preparation of glass ware

Preliminary studies.

Bacterial counts from milk taken from herds I and II.

Bacterial counts as obtained from sterile water passed over cooler.

Summary of all counts made from above mentioned sources.

The life history of herd II.

General discussion of mastitis.

The prevalence of streptococci in the milk of cows.

Study of udder flora of individual cows with special attention given to streptococci.

Results from platings of samples from individual cows.

Conclusion.

Studies in Dairy Bacteriology.

The types of bacteria, most commonly found in milk, may be conveniently grouped as to their action on the milk and their effect upon the consumers of same. Quite often certain types of organisms which are very troublesome to the milk dealer or producer never affect the consumer. And yet, on the other hand, there are still other types that do not materially concern the producer or dealer but are of the very greatest significance when viewed from the stand point of the consumer, due, however, to the presence of Pathogenic organisms which may be very easily and oftentimes carried by milk. Therefore, it is plainly brought out that there are organisms found in milk which may cause little or no trouble to either the producer or dealer and still others whose presence would cause trouble anywhere.

The development of our knowledge of the relation of bacteria to the wholesomeness of foods has led to a study of the bacterial content of milk as a means of determining its purity. The methods used for this purpose have followed very closely those of the water bacteriologist, viz; the plating and the direct microscopic methods.

For many years dairy bacteriologists have endeavored to determine the number of organisms in milk by plating it into nutrient agar or gelatin. By this method the number of colonies developing in the plates is assumed to represent the germ content of the milk. But even when the best methods are employed, the plate count represents only the approximate and not the

exact number of bacteria in any batch of milk. It should also be borne in mind that such counts are always underestimates, due to the fact that all species will not develop in any given medium or incubation temperature. Different kinds of media are used to assist in the differentiation of types and species.

The plating method is expensive because of the large amount of time and materials needed. It is not possible for one person to handle large number of samples at one time. In order to decrease the labor and give greater possibilities to the work a method by which the bacterial condition of milk can be studied by direct microscopic examination was devised. Therefore, at present the microscopic method for determining the approximate numbers as well as the general species present in a given sample of milk has the advantage over the plating method, in that it is a labor and time saver.

Regarding the value of bacteriological standards for milk there is still some difference of opinion among bacteriologist. The germ content of any lot of milk is largely dependent upon three factors; the number of organisms getting into the fresh milk; the temperature at which it is kept; the age of the milk when analysis is made.

The high bacterial count in any lot of milk may be the result of any one of these conditions or a combination of them. A high count means that there has been carelessness either in the Broduction, resulting in high initial contamination; in the subsequent handling permitting a rapid multiplication of the organisms; in being unprotected from diseased udders or if the milk is old.

On the other hand, milk with a low germ content can be obtained only from healthy cows where the original contamination is small and the milk has been held at low temperature. A low count, therefore, means care both from the standpoint of production and later handling of the milk.

While the germ content may be regarded as a general index to the care the milk has received, it may not at all indicate its wholesomeness. A high count may be the result of the rapid growth of the lactic acid bacteria, in which case the milk may be perfectly safe and wholesome. On the other hand, the count may be quite small but contain Pathogenic species. The bacteria count is valuable as showing the sanitary conditions of production and handling, but much care should be used in the interpretation of such results. In some ways a direct microscopic examination of the milk sediment is much more satisfactory. The skilled analyst can recognize certain types which may indicate the sanitary quality of the milk. With sufficient experience one can recognize streptococci, certain other groups and leucocytes. The presence in large numbers of one or more of these may indicate the nature of the original contamination and the existence of diseases in the udders of cows. The presence of unusual numbers of streptococci and pus cells may indicate the existence of disease in the cows and when this condition is found in the milk it is often possible to trace it back to the farm and locate the diseased cow and prevent her milk being used for human consumption.

The Bacteriological Aspects of Cheese Ripening.

In making a study of cheese to determine the direct cause of bitterness, a condition which has given the cheese manufacturers of the State undue trouble, very little literature could be obtained. Due to this fact abstracts were taken from New York Experiment Bulletin Number 8 in which the opinion of the investigators mentioned in this paper are given. Having given the source of the literature on cheese ripening it now becomes necessary to enter into the discussion of the subject proper.

The ripening of cheese has long presented an interesting though complex problem. Chemists and bacteriologists alike have been unable to advance an acceptable explanation of the Phenomena involved. This fact has stimulated the interest of many investigators in the field of dairy bacteriology and dairy chemistry.

The changes produced during the ripening stages of cheese are neither wholly bacteriological nor wholly chemical. Acceptable conclusions have been reached only by correlating the results of both lines of investigation. This review of literature deals with the bacteriological aspects of the problem. The review of such literature, however, is not to signify that the biological changes are more important but expresses rather the feeling that chemical work should be covered by one better acquainted with the chemistry of casein compounds and their appearance during the ripening process.

The ripening of a cooked rennet cheese involves two distinct, yet closely related phenomena. The first of these consists

chemically of the gradual transformation or splitting of the insoluble casein of the milk until nearly all has been converted into simpler,soluble, and digestible products. The other factor- and this distinguishes a ripened from a green cheese- is the development of flavor. The flavor does not develop as rapidly as do the chemical changes in casein and its appearance seems to depend upon the presence of a certain amount of these soluble proteins. Though beginning later it progresses rapidly and the two processes develop simultaneously in the later stages of ripening.

At first, investigators in approaching the problem of cheese ripening gave undue weight to one or the other of these processes- Duclaux, Adametz, Weigmann, and their followers believed that if the cause of the development of flavor could be established, the general problem of cheese ripening would be solved.

Frendenreich, Jensen, and other investigators of their group regarded the changes in cheese ripening as centered around the breaking down of the insoluble proteins and the ability of the bacteria involved to attack these substances either in milk or in cheese.

Both groups of these investigators later appreciated the fact that normal cheese ripening involves both processes and that neither process alone could produce a normally cured cheese.

Prior to 1878, the changes involved and the cause of the ripening of cheese had not been studied as a distinct problem. Cohu, (1872) mentions the possibility that organisms which produce butyric acid play a part in cheese ripening, but offers no experimental data to support this conclusion. Duclaux, a student of Pasteur, in (1878-80) was the first investigator to make a

careful study of this particular problem and to attack it as an individual line of investigation. His purpose was not only to determine the factor which caused the ripening changes but to place cheese manufacture upon a scientific basis and so help the local cheese makers out of many difficultures.

The technic which he developed included dilutions of an emulsion of cheese in milk until he obtained a practically pure culture of the predominating organisms; this in turn was inoculated into sterile milk and incubated. The inoculated milk cultures when examined microscopically, in a majority of cases, showed heavy rods of various sizes to which he gave the name "Tyrothrix". These organisms when grown in milk cultures had a decided cheese odor and had the power of attacking casein, both points tending to substantiate his claims that these liquifying organisms played an important role in the ripening process. With the production of this cheese odor it was thought that cheese ripening was identical with the development of flavor and aroma. Although, these conclusions were disapproved many times, it should not be forgotten that Duclaux was the first investigator to connect the ripening of cheese with micro organisms. The result of which opened this interesting field of bacteriological research.

Milk alnne is a perishable product. In cheese the nutrients of milk may be held in a condition for consumption a much longer period. Therefore in giving a definition for cheese, one might justly say that it is a solid protein food product manufactured form milk. Its solidity of course depending on the curdling of coagulating of part or all of the protein and the expulsion of the water or whey present.

No sooner than the milk is drawn does the process of cheese-making begin. The care and treatment and its subsequent handling has all to do with the qualities of cheese made. There are really five factors that influence the qualities of milk for cheese making. viz; (1) its chemical composition; (2) Flavor of feed eaten by cow; (3) Absorption of flavors and odors from the atmosphere; (4) The health of the cow; (5) The bacteria present. Of these five number 5 is of the most importaance, and is the more often neglected. However, from here on it is my intention to point out some of the beneficial as well as harmful effects brought about by bacteria in cheese making.

Bacteria might be defined as microscopic unicellular plants without chlorophyl. There are other forms of lower plants found in milk, such as mold and yeasts - while bacteria are of more importance yet mold and yeast may produce changes in milk and other dairy products. Bacteria are widely distributed through nature. They are so small that there are many of them clinging to the particles of dust afloat in the atmosphere, still others are found in the surface water, soil and organic materials. Many of them are of a very resistant kind. viz. spore forming thereby withstanding

an immense amount of exposure before injury might result. While in this state if favorable conditions for growth are brought about, the bacteria recuperate and become active again. We have a great many groups of bacteria with which to deal. Some are beneficial while others are harmful. Bacteria are so small that it is hard to differentiate between the harmful and beneficial only by the results produced. Bacteria reproduce very rapidly by means of fission, or in other words by cell division. Like other plants bacteria are very sensitive to food supply, temperature and moisture for optimum growth. In so much as bacteria are plant cells they must absorb their food from materials in solution. They may live on solid materials, but the food elements must be rendered soluble before the bacteria can use them. Most bacteria prefer a neutral or slightly acid medium for growth. Therefore, ordinary milk makes a very desirable medium for the growth of bacteria, because the food supply is readily available.

In milk, certain groups of bacteria are usually present, however, many others that find their way into it, live and multiply rapidly. In order for bacteria to multiply with rapidity, a favorable temperature is essential. By experiments it has been found that there is a temperature for optimum results and then a retrograding temperature either above or below the optimum. Under normal conditions milk soon begins to undergo changes due to bacteria. Changes produced in this way are called "Fermentations"; the agents causing the them, "Ferments". Normally the acid fermentation takes place first; with other fermentations or changes

later which after a time render the milk unfit for cheese making or human consumption.

The grouping of organisms found in milk based on their effects are as follows:

I Acid-producing types.

II Peptonizing types

III Inert types

IV Alkali-producing types.

V Butyric fermenting types.

Each type of bacteria produces more or less some specific change in milk. The reaction due to certain of these bacteria can be utilized by the dairyman in the manufacture of dairy products while others have deleterious effects.

One of the most important fermentations taking place in milk is brought about by the action of lactic acid forming bacteria on the milk-sugar. The bacteria that bring about this fermentation may be divided into several groups. However, in as much as lactic acid is the principal substance produced, they are called lactic acid organisms. In this group may be found. B.lactis acidi, B. colon-aerogenes acid peptonizing group, B Bulgaricus and the acid cocci group.

In bringing out some of the effects whether of a harmful or beneficial nature, it might be said that B. lactis-acidi is the most desirable group of bacteria with which we have to deal in the handling of milk whether it be for the making of cheese or other purposes. These bacteria grow and multiply very rapidly under normal conditions and it is through the work of these bacteria that a smooth and consistant curd is produced. These bacteria are

very essential to the production of the initial acidity necessary in most types of cheese. So important are these bacteria that in the manufacture of cheese a starter is used which has a predominance of lactic acid-forming microorganisms in an active state. If, however, all milk could be clean and sweet and only fermentation were from the clean acid type there would be no need for a starter. However, under adverse conditions a starter is necessary in overcoming gas forming bacteria, yeasts and bad flavors or taints.

In the colon-aerogenes group is found the B-coli species, which is a normal inhabitant of the intestines of man and animals. The presence of these bacteria in milk is indicative of fecal pollution or unclean methods of production. These organisms grow and develop very rapidly in milk at high temperature, therefore, the necessity of cooling the milk,no sooner than drawn from the cow, can be clearly seen. The acidity produced by this group is not the undersiable factor, but,due to the formation of CO_2 and H gases and the coagulation of a lumpy and ragged curd, is very objectionable. Following this group very closely is that of the acid peptonizing group, which is often associated with the colon organisms. This group contains those bacteria which coagulate milk with an acid curd and subsequently digest it. These bacteria grow and multiply rapidly between 65° and 98° F. Every precaution should be taken to prevent the growth of such bacteria, due to the fact that the presence of these bacteria necessarily impart undesirable flavors and odors to milk whether it be for cheese-making or other purposes.

There is no harmful effect brought about in milk by the presence of B Bulgaricus. They produce lactic acid and impart no

undesirable flavors or gas in milk. The acid cocci is not a very well defined group. The presence of bacteria from this group usually show that contamination has been brought about by the udder. As yet, there have been no deleterious effects brought about by these bacteria. Freshly drawn milk at times will have a bitter taste. Again milk acquires such a taste on standing a few hours. The former is usually doe to some feeds fed the cow, such as lupine, turnips, cabbage, etc. It was formerly thought that bitter substances would pass from such feeds as mentioned above, directly through the mammary glands into the milk. It is now thought that food merely acts as a carrier of micro-organisms which afterwards gain access to the milk.

Bitter milk may more commonly be ascribed to the presence of specific bacteria. In some cases bitterness is thought to develope with advanced lactation. Bitterness produced in this way was studied by Eckles and Shaw. They used several cows of which the conditions of feeding, care and handling of the milk were uniform. As described by these investigators, the milk within twenty-four hours after drawn from the cow, developed a strong rancid odor, suggestive of butyric acid. This odor was accompanied by a very bitter flavor. These conditions were also developed in the cream separated from the milk. In neither case did the addition of formalin prevent the abnormal odor or flavor. Churning of cream under these conditions is accomplished only through extreme difficulty.

That the conditions already described are not uncommon in the

commercial production of milk is indicated by a statement from Eckles and Shaw, that, "numerous inquiries are received each year regarding abnormal flavor in milk from cows near the end of lactation period".

It is generally believed that sufficient data have been obtained to warrant the conclusion that the abnormal flavor and odor of certain cow's milk whose lactation period is more or less advanced is due to the secretion of an abnormal quantity of lipase in the milk. In as much as lipase is apparently absent under normal conditions, the Physiological problem to be solved in the case of the bitter milk of advanced lactation is the factor underlying its appearance at this particular time. It is clearly seen that the conditions causing its appearance do not occur for all cows at the close of lactation. Advanced lactation is, therefore, merely a secondary cause. Obviously the whole matter is much more deep seated, and statements which may be advanced to explain the phenomenon can only be speculative.

In many sections cream and milk are shipped considerable distances, either from receiving stations or individual farmers. Much of this milk and cream is sent without refrigeration and as a result the organisms present soon become very active. Through the warm months the fermentation of most importance is that producing the "yeasty" or "Foamy" fermentation.

*A typical "yeasty" or "foamy" milk or cream has a definite yeasty odor and shows a formation of much gas. Very often cans in transit have part of the milk or cream forced out by the development of gas. Beside the mechanical loss there is a deterioration in the quality of the milk or cream as a result of the development of the organism at issue. There seems to be no restraining influence on the development of the typical "foamy" condition due to the presence of acid.

Lactose-fermenting yeasts have been known to be the cause of an abnormal condition in cheese quite often. Bochicchio, in his studies on Italian cheese, found a yeast capable of fermenting lactose with the production of CO_2, responsible for the undesirable swelling. Hard cheese made from milk inoculated with a culture containing this yeast soon developed a swollen appearance with large holes in the surface layers. In whey the yeast produced a foaming beverage with a pleasant taste.

Harrison** described under the name of Torula Amara a lactose-fermenting yeast causing a bitter taste in milk and cheese. The bitter taste was detected in milk incubated at a period of five or six hours at 37^0 C., and after fourteen hours the taste and smell were disagreeable and strong. In about ten days the milk was slightly acid and slightly coagulated.

*Iowa Experiment Station Bulletin Number 361.

**Harrison,F.C. Torula Amara, Bulletin Ontario Agr.College,120.

In addition to the lactose fermenting yeasts already mentioned many others responsible for rather extensive abnormal conditions in dairy products have been isolated from various sources and described.

From the standpoint of elimination of such organisms, the logical thing seems to be the prevention of the growth of the yeasts. Their frequent presence in milk suggests a wide distribution of the organisms and under these conditions it is inadvisable to attempt to exclude them from milk. The prevention of their growth is the next possibility. According to the present knowledge, this can best be done by the use of a low temperature in case of a small shipper. If large shipments are made or the volume of milk sufficient to justify the expense for effective pasteurization, the loss of milk or a deterioration in quality is practically eliminated. Furthermore yeasts are comparatively non-resistant to heat and pasteurization affords a satisfactory method of destroying them.

Abnormal flavor and bitterness in cheese has been frequently met with in the State. Often abnormal development of curd in the cheese vat causes annoyance in cheese manufacturing. Studies reported here were entered into with the hope of throwing some light on the problem and suggesting a possible solution. It is not assumed that both disorders are caused by the same agency but rather the reverse.

The cheese used for this experiment looked to be normal in every respect from a general appearance. But on close examination the texture was found to be poor, and the flavor off. The bitterness, already mentioned, was very pronounced shortly after

eating a small piece of the cheese. This bitterness never left the cheese.

Experimental Data.

Description of cheese on hand.

The samples of cheese examined for the different types of bacteria present were obtained by means of a trier and from the middle portion of the plug one gram was taken for a sample. The sample thus obtained was placed in a motor containing sterilized sand and then it was finely ground by the use of a pestle. The mixture was placed in a 99 cc sterile water blank and vigorously shaken, after which the desired dilutions were made.

Platings on beef peptone agar, litmus lactose agar, lactose bile agar, acid milk agar, purple lactose agar, and acid or yellow lactose agar, were made. The different media were used for the purpose of giving an estimate as to the number of bacteria present, the gas producers, the acid producers, and non-acid formers. In the table to follow, results obtained from these media will be given.

Table I

Date	Media	Incubation temperature	Average Count per gram
Dec. 5, 1921	Beef peptone agar	37° C.	134,700,000
Dec.5. 1921	Lit. Lact. agar	37° C.	83,375,000
Dec.5, 1921	Whey Agar (acidified)	37° C.	150,375,000
Dec. 5, 1921	Lact.Bile Agar	37° C.	28,925,000
Jan.28, 1922	Lact. Purple Agar	37° C.	81,375,000
Jan. 28 1922	Lact. Agar (Acidified)	37° C.	48,125,000

From the platings in table I, three types of organisms were noticed. These were acid, non-acid and gas producing species. The most outstanding of these was that of the acid producing type, closely followed by the gas producing organism. There were only a few colonies of the non-acid type to be noticed.

On December 8, 1921, from the platings of table I isolations were made. The media used were litmus milk agar, lactose bile agar, litmus lactose agar. These were used so that if there were non acid, acid and gas producing organisms present, they would be revealed. The colonies from which the inoculations were made were given the following symbols as a means of distinction, ch_1, ch_2, ch_3, ch_4, ch_5, ch_6, ch_7. These were incubated at 37° C. for 48 hours after which they following results were noted:

ch_1 gave acid reaction in litmus milk agar.

ch_2 showed digestion of the milk in litmus milk agar.

ch_3 was a colorless, round colony, showing a good zone of digestion about 6 mm in diameter. The internal structure showed zonations. This inoculation was made on lactose bile agar.

ch_4 this colony in general appearance resembled very much that of ch_3, but it was very much smaller having a diameter of not more than 1 mm. Lactose bile agar was used as a medium for the growing of the organisms.

ch_5- the colony from which this inoculation was made showed signs of the presence of gas producing organisms. The colony itself, was very indistinct. The medium used for ch_5 was lactose bile agar.

ch_6- the colony used for this inoculation was of a different type as compared to those already mentioned. This was a whittish cone shaped and rather deeply set colony having a diameter of about 2.5 mm. Instead of showing the presence of gas or acid an alkaline condition existed. Here litmus lactose agar was used as a medium.

ch_7- like that of ch_6 gave an alkaline reaction but instead of having a whittish color a dull brown color was noticeable. This inoculation was made on litmus lactose agar.

From this group of platings, ch_3 seemed to be suspected as showing a type of bacteria noted for causing trouble. Therefore, additional efforts were expended in order to arrive at some conclusion as to the ability of these organisms to produce gas as well as their effect upon normal milk.

Fermentation tubes were prepared in the usual laboratory way, using lactose broth as a medium. These tubes were inoculated by means of a sterile inoculating needle from ch_3 and incubated for 48 hours at a temperature of 37° C. At the end of the incubation period there was present 55 percent of gas. A concentrated solution of NaOH was made, and a few centimeters added to the tubes containing the gas approximately seven-tenths of the gas was absorbed by the NaOH. The three tenths of unabsorbed gas when ignited gave evidence of the presence of hydrogen.

Having found this particular organism to be a great gas producer inoculations of ch_3 were made direct into flasks containing about 100 cc of unpasteurized milk. A check sample for each inoculated flask was also kept. These samples were tested daily for at least five days for acid, presence of gas, and flavor. In table II is given the incubation temperature, the percent of acid each day, the general odor of samples, and whether they be gas or non-gas producing.

Table II

Sample	Inoculation temperature	Percent Acid. 2/7/22	2/8	2/9	2/10	2/11	
I -check	37° C	.91	.261	1.203	1.291	1.220	Clean odor & no gas produced.
I-ch$_3$ inoculation	37° C	.191	.418	.767	1.308	1.133	Cowy odor with gas produced
II-Check	37° C	.191	.209	1.151	1.308	1.377	clean lactic acid odor no evidence of gas
II-ch$_3$ inoculation	37° C	.191	.418	.802	1.220	1.220	Cowy & disagreeable odor, production of much gas evident.
III-check	37° C	.191	.470	1.220	1.308	1.220	Clean lactic acid flavor no gas pre sent
III-ch$_3$ inoculation	37° C	.191	.418	1.220	1.273	1.220	Cowy & undesirable flavor with the production of gas.

Samples		2/20	2/21	2/22	2/23	2/24	2/25	
I-check	37° C	.157	.348	1.046	.994	1.255	1.657	clean flavor no gas.
II- ch$_3$	37° C	.157	.540	.959	.994	1.308	1.657	Cowy flavor, gas produced.
II Lactose Bacilli	37° C	.157	1.133	1.133	1.081	1.046	1.116	Clean flavor, some gas present.

In noting the development of acid from day to day in the samples given in table II with the exception of seven readings the acid in the inoculated samples developed more rapidly than that of the check samples. It is of further interest to note that all check samples gave a clean lactic acid flavor with no signes of gas being produced, whereas on the other hand, all inoculated samples gave a cowy and very disagreeable flavor. These samples produced enough gas to fill the curd full of large sized holes, something that is very undesirable in any dairy products. With the presence of gas producing bacteria the dairyman is suspected of not handling his milk under the proper sanitary conditions. Furthermore, milk containing such organisms will invariably cause trouble that is oftentimes not suspected.

Considering the nature of ch_3 it might not be erroneous to suspect its being at least partially responsible for the abnormal condition in the cheese vat mentioned above.

The cheese that was used for obtaining the data already given was made from, as thought, a comparatively clean milk, but after finding the types of organisms already mentioned in the cheese, attention was given to the milk cooler, cans, and buckets and finally each milking individual of that particular herd in an effort to locate the exact cause if possible.

At first, in order to get an idea as to the average bacterial count from herds I and II, samples were only taken from the milk cans on the dates mentioned in the table. With a fair estimate as to the average count, a more thorough and detailed search was made to locate the exact cause of high bacterial

count. At this point the cans, cooler and milk buckets were used as the suspected sources of contamination. The sample of milk was taken from the cans in the usual way. Sterile water was run over the cooler and samples of the first and last water passing over was obtained. Milk from the first cow milked into each bucket was taken as a sample from each of the buckets.

Why agar, lactose bile agar, beef peptone, agar, litmus lactose agar, and lactose purple agar were used as the media on which the various dilutions were planted. These media were used in order to bring out yeasts, non-acid, acid and gas producing colonies as well as giving a fair estimate of the bacterial content of the milk.

The media for this work was prepared in the usual laboratory manner.

For beef peptone broth or bouillon the following ingredients were used:

Beef-extract (Liebig)	2. grams
Dried peptone (Powdered)	10. "
Sodium chloride (table salt)	5. "
Distilled water	1000 cc

These were mixed and thoroughly dissolved. Boil for fifteen or thirty minutes, filter, place in small flasks or test tubes and sterilize for 15 minutes in an autoclave at 15 pounds pressure. For nutrient agar the same ingredients as used for buoillon plus 10 grams of agar-agar are used. The same process of sterilization is used.

The same ingredients as used in nutrient agar with the use of whey instead of distilled water + Beef extract are found in whey agar.

In preparing litmus lactose agar, from 1 - 5 percent of lactose is added to the agar solution and enough litmus solution to give a dark blue color.

Preparation of veal broth.

Place 500 grams chopped lean veal in a liter of distilled water and allow it to stand in a refrigerator or cool place over night. The juice is then pressed out with a convenient press, boiled for half an hour, the coagulated albumins filtered out, the liquid made up to a liter with H_2O. 10 grams of peptone added, and heated sufficiently to dissolve. The reaction is adjusted to the proper point in this case to PH 7.2 by titration, or the medium is simply neutralized by addition of normal NaOH, using Phenolphthalein paper as an indicator if a high degree of accuracy is not required. The broth is then autoclaved at 15 lbs. pressure or boiled for fifteen minutes, allowed to cool and then filtered. The cooling throws down a preparation of magnesium ammonium phosphate, which may be removed. The finished bouillon or broth is placed in test-tubes and flasks, and sterilized in the autoclave under a pressure of 15 pounds for 15 minutes.

To prepare veal broth agar add to the broth already prepared 5 gm. NaCl and 18 gm. of agar-agar per liter, dissolve, filter and sterilize.

Cows blood was used for this work. It was obtained in the following manner: under aseptic conditions

Sterile flasks containing a few glass beads were used to catch the blood. The trochanter was sterilized. Alcohol was

used as a disinfectant. The juglar vein of the neck was tapped. The blood was collected in the flasks mentioned and shaken vigorously for ten to fifteen minutes in order to defibrinate the blood. ~~Do not sterilize blood~~.

In order to get the veal blood agar, about 2 cc of blood was added for every 10 cc of veal agar, just before pouring the plates, and thoroughly mixed.

For lactose purple agar, 18 grams of a specially prepared media for regular laboratory work were used.

In preparing lactose bile agar a specially prepared media for laboratory work was used.

The method of sterilization for all media is the same as previously described.

The glass ware was sterilized in the usual manner for bacteriological work. The pipettes and petri dishes were sterilized in the hot air oven for a period of 3-4 hours. The sample bottles, water blanks, etc were sterilized in the autoclave at 15 pounds steam pressure for 15 minutes.

Table III.

Source of Sample	Date	Media	Incubation temperature	Average counts.
Herd I from can	Nov.3,1921	Whey agar	37° C	numerous
		Lact.B agar	37° C	numerous
		B.pept.agar	37° C	numerous
		Lit.lact.agar	37° C	numerous
Herd II from can	Nov.3,1921	Whey agar	37° C	numerous
		Lact.B agar	37° C	130,000
		B.pept.agar	37° C	very numerous
		Lit.lat.agar	37° C	800,000
Herd I from cans	11/13/21	Whey agar	37° C	174,000
		lact.B.agar	37° C	4,036,000
		B.pept.agar	37° C	2,500,000
		Lit.Lact.agar	37° C	900,000
Herd II from cans	11/13/21	Whey agar	37° C	470,000
		Lact.B.agar	37° C	400,000
		B.pept.agar	37° C	4,180,000
		Lit.Lact.agar	37° C	3,200,000
Herd I from cans	11/18/21	B.pept.agar	37° C	1,700,000

Table IV

Source of Sample	Date	Media	Incubation temperature	Average counts.
Bucket 1	11/18/21	B.pept.agar	37° C	2,990,000
" 2	"	"	37° C	320,000
" 3	"	"	37° C	1,280,000
" 4	"	"	37° C	2,700,000
" 5	"	"	37° C	625,000
Cooler	"	"		very numerous
Cooler	11/21/21	"	37° C	10,600,000
First portion of sterile H_2O passed over cooler	12/9/21	B.pept.agar	37° C	numerous
		Lact.B.agar	37° C	numerous
		Lit.lact.agar	37° C	580,000
Last portion of sterile H_2O passed over cooler	12/9/21	B.pept.agar	37° C	numerous
		Lact.B.agar	37° C	numerous
		Lit.lact.agar	37° C	600,000
Milk taken from cans	12/9/21	B.pept.agar	37° C	1,740,000
		Lact.B.agar	37° C	numerous
		Lit.lact.agar	37° C	1,400,000
First portion of sterile H_2O passed over cooler	3/16/22	B.pept.agar	37° C	3,550,000
		Lact.purple agar	37° C	1,230,000
		Whey agar	37° C	No results.

Table IV continued.

Source of Sample	Date	Media	Incubation temperature	Average counts.
Last portion of sterile H_2O passed over cooler	3/16/22	B.pept.agar	37° C	2,370,000
		Lact.purple agar	37° C	1,100,000
		Whey agar	37° C	no results
Milk from cans	3/16/22	B.pept.agar	37° C	1,400,000
		Lact.purple agar	37° C	1,500,000
		Whey agar	37° C	no results
Sterile H_2O from cooler	3/29/22	B.pept.agar	37° C	3,650,000
		Lact.P. agar	37° C	720,000
Milk from cans	3/29/22	B.P.agar	37° C	520,000
		Lact.P.agar	37° C	240,000

Table V.

Herd I	1,800,000
Herd II	1,530,000
Buckets	1,580,000
Cooler	2,500,000

In these platings there were found gas, acid and non-acid producing colonies. Inoculations offermentation tubes with the gas forming organisms showed that a very uniform amount of gas was produced. In the addition of concentrated NAOH to the fermentation tubes all but a slight trace of the gas was absorbed.

In summarizing the data given in table IV with reference to the average bacterial count of herds I and II, the buckets, and the cooler, it is a noticeable fact that in regard to the highest count, the cooler takes first place; Herd I, second; the buckets, third; and Herd II fourth. As indicated in table V the cooler showed an increase of about one million bacteria per cc over any of the other sources mentioned. This being true, special attention was given to the cleaning and care of the cooler. Later other counts were made, which revealed the fact that the average bacterial content was still too high. Having now aroused a suspicion of contamination from other sources, it was deemed advisable to make an attempt at locating theexact cause. Thus it is that a general study of Herd II was undertaken.

From the above results it seemed advisable to make a close study of the herd examining the health record of each cow and a study of their udder flora in as much as streptococcus infection of the udder would cause high bacteria count and, from work of others, may be the cause of bitter taste in cheese made from such milk.

For convenience the cows will be given symbols of this nature C-1, C-2, etc and then the information already designated.

C-1, This cow has dropped five calves and thus far her health in general has been exceptionally good.

C-2, The general health of this cow has not been quite so good. She has had udder trouble, off feed at times and rather irregular in milk flow at times. She has freshened five times.

C-3. This cow has had six lactation periods, all of which seemed normal up to fourth calf. On March 14, 1920, She went off feed, developed a severe case of garget. Milk from the affected quarter was stringy, clotty, bloody and had a very disagreeable odor. The quarter was not entirely lost. Later little pustules came out on the udder. These would go away and appear again at frequent intervals. Every since the first attack, this cow has had occasional udder trouble as well as being off feed at times.

C-4. The general health of this cow as gathered from her record was very good. She has had five lactation periods and all seem to have been normal.

C-5. With four lactation periods to her credit, there was no indication of any kind of trouble until June 30, 1920. At the date mentioned there was a considerable drop in milk flow which was never regained during that lactation period. Slight indications of the presence of garget have been noticed since, but in a very mild form. The milk at times was slightly stringy, lumpy, the odor was of a disagreeable nature. This condition would last for only a few days.

C-6. This cow was a low milk producer. She never had any

udder trouble. Placenta was retained on two or three occasions after freshening. Health had been good throughout. She has had four lactation periods.

C-7. This particular cow has had some trouble ever since her second calf. She has had five lactation periods. At times she is off feed, milk flow decreases, some evidence of a mild case of garget which at times were brought out by small pustules on the udder. The right front quarter is almost entirely lost, only a few streams of milk being obtained at any milking. The placenta was retained after freshening the fourth time.

C-8. The general health of this cow has been exceptionally good. She has dropped four claves and during no lactation period has she showed any signs of garget or any kind of udder trouble. She has been a very good and consistant milker. Had slight hardness and soreness of the udder with an increase in temperature after last calf. Milk flow and condition of milk seemed to be apparently normal.

C-9. So far as recorded, the health of this cow has been normal. Her milk flow normal but after her last calf a slight case of garget developed and for some time thereafter little pustules were noticed on the udder. With the exception of these pustules the cow seemed normal in every respect. She has passed through three lactation periods.

C-10. There has been no indication of any kind of trouble either from the standpoint of health or that of milk production. She has had three lactation periods.

C-11. This cow was a very consistant milker. She has passed

two lactation periods. A short time after the last calf was dropped, the udder became caked, feverish and rather sore to touch, this condition existed for only a few days. Through the entire time the milk seemed to be normal. After the hardness disappeared all quarters were left normal so far as the quantity of milk produced is concerned.

C-12. This cow freshened twice. The first lactation was normal in every respect. After the second calf, udder trouble was contracted in a very severe form and three quarters were completely destroyed. All four quarters were affected. The cow became very poor, off feed and for two or three months she showed a very dejected condition. She was dried off and never milked again.

C-13. The health of this cow was not very good after freshening. Her appetite was poor, and had an amatiated appearance for more than a month, no indication of udder trouble was shown. She has had only one lactation period.

C-14. First calf. Health normal, no indications of udder trouble.

C-15. First calf. Slightly off feed, had rather dejected appearance but no signs of udder trouble. This condition existed for at least one month.

C-16. First calf. Healthy and no signs of udder trouble.

C-17. Cow healthy, normal milk flow through entire period. First calf.

C-18. First calf. Parturition difficult, off feed for several days. No evidence of udder trouble.

C-19. First calf. Placenta was retained. Cow became infect-

ed and interitis developed giving a virulent discharge with a disagreeable odor. She went off feed, got sick, became very poor, and in general had a very dejected appearance. She was treated for more than six weeks in which time no signs of recovery could be noticed. She was killed about May 1st, 1922. A postmortem examination revealed the fact that she was very badly infected and would have never recovered.

C-20. This cow had four lactations to her credit. The first two periods were recorded as being normal in every respect. In March 1920, about three weeks after freshening for the third time a severe case of mastitis developed. All four quarters were involved. The milk was lumpy, stringy, bloody, yellowish and had a disagreeable odor. The cow went off feed, got poor, looked amatiated and with little or no prospects of recovery. Potassium iodide was given in large dosess She never fully recovered from this attack. In September of 1921, after the fourth calf, the same trouble developed. This time to make matters more complex, she had digestive troubles along with bloating. If fed more than an ordinary amount of silage she would bloat and later her health got to the point of going down and down which finally terminated in her being killed on December 15, 1921.

At times mastitis was thought to have been caused by exposure or bruising the udder but now it is thought that the most common cause of mastitis is attributed to streptococci, and yet by no means are these bacteria the sole cause. Other organisms such as Bacillus Coli and Staphylococci and occasionally members of the Paratyphoid group of bacilli are held responsible.

Streptococcus mastitis is extremely common. Once it gains a foothold in a herd it is difficult to eradicate, especially if the habit of spilling the first few streams of milk on the floor is practiced. By such means mastitis streptococci are disseminated through the air and by direct contact. Dissemination becomes less general with more care and cleanliness.

Mastitis, or garget, is a disease of the udder which is very wide spread, even more so than is generally assumed. It has been stated by some authorities that there is probably no herd entirely free from mastitis. The disease assumes either a chronic or an acute form. The symptoms in the chronic form may be so insignificant as to escape attention for some time. It is found that between the two forms, there are intermediate stages, so it is not surprising to actually find cases of mastitis in herds which have been subjected to a painstaking examination and thought to be entirely free from the disease.

When cows suffer from mastitis the milk undergoes material changes in appearance, taste, and composition. As the disease becomes more acute, these changes become more pronounced. The changes are slight and oftentimes difficult to detect at the beginning of chronic attacks or quite often at the commencement of the acute stage of the disease. In well-developed, acute cases parts of the blood pass into the milk also and enormous increase in the number of body cells is noticeable. However, there is no definite relation between the number of body cells and pathologic conditions. If the disease is acute the udder enlarges and usually cakes, temperature rises, and the milk becomes stringy,

bloody or yellow. During chronic attacks the changes come about slowly. At first the milk seems to be normal, but later becomes thick, slimy and may contain red blood-cells. In standing a yellowish sediment appears. In some cases, instead of becoming thick and slimy the milk may appear thin, as though watered. The taste turns bitter.

Some of the changes most commonly brought about which may arouse suspicion are: The fat content becomes abnormally high or low; the sugar may diminish; the total solids and plasma solids may decrease; casein may decrease while heat coagulable albumin increases. Coagulation due to acid or rennet action is usually delayed.

The intensity of the disease depends upon several factors; the most important of which is perhaps the relative virulence of the organism, which may vary within wide limits. The affected part or parts of the udder, as a rule, degenerate as a result of the disease. In many cases the animal never recovers. Accidental lesions of the udder may afford an opportunity for the streptococci to gain entrance, so a very important factor would be that of individual susceptibility. Therefore, it is of great importance to recognize the presence of mastitis at an early period in order to possibly prevent the disease from running its course.

With the general health of the cows studied most for this particular piece of work before us, it now becomes necessary to arrive at the cause for an abnormally high, bacterial count of the milk obtained from these cows. At first, platings were made to get the average bacterial flora of the milk from this particular

herd after reaching the creamery. The samples were always taken from the cans containing mornings milk. This was done because of the fact that if taken otherwise the bacteria would have had from twelve to fourteen hours time for development which would necessarily have meant an abnormal count.

The average bacterial content of the milk from Herd II is given in the following table.

Table VI

Source of Milk	Date	Media	Incubation Temperatures		Average count.
Herd II	12/12/22	Agar	27° C.	37° C.	200,000
Herd II	12/14/22	Agar	27° C.	37° C.	500,000
Herd II	1/5/23	Agar	27° C.	37° C.	400,000
Herd II	1/6/23	Agar	27° C.	37° C.	400,000
Herd II	1/12/23	Agar	27° C.	37° C.	300,000
Herd II	1/15/23	Agar	27° C.	37° C.	450,000
Herd II	1/18/23	Agar		37° C.	2,800,000
Herd II	1/21/23	Agar		37° C.	70,000

Platings of milk from dilutions of 1:100 and 1:10,000 were made on endo-agar in order to bring out a specific type of bacteria. Eight different counts were made on agar mainly to get the bacterial flora of the milk from the entire herd. The platings were incubated 48 hours at 27° C and 37° C. Those incubated at 37° C showed the quickest growth. The average number of colonies were approximately the same for both temperatures. In 1:10,000 dilutions the colonies were numerous and similar throughout. In 1:100,000 dilutions the number of

colonies varied anywhere from 1 - 47. On January 18, the cows were not washed nor was the milk cooled, which in all probability caused such an abnormal count on the date mentioned. The general average,not including the count of January 18th, is around 7 colonies or 700,000 per cc.

Stains were made from a few of the different shaped colonies found on the endo-agar plates. On examination the presence of streptococci were revealed. According to Dr. J. Howard Brown*, we find that streptococci have been recognized in milk for many years. They have also been known to cause disease for many years. The fact has also been revealed that not all streptococci are dangerous or equally dangerous. It may be further stated that a great deal of work has been done in trying to differentiate the harmless from the pathogenic streptococci. Streptococci are found on practically all portions of the animal body both external and internal.

A great deal of work has been done with the streptococci of bovine mastitis or garget. It is stated that streptococci are usually the cause of mastitis in cows.

There are at least two kinds of streptococci,hemolytic and non-hemalytic, that may be linked with mastitis. The hemalytic type is recognized by their ability to destroy or break down red blood cells; the non-hemalytic do not affect the red blood cells. Streptococcus mastitis may take on various degrees of

* Twelfth annual report of the Rockefeller Inst. for Med. Research.

severity. Of course milk from diseased udders is not knowingly allowed to enter the certified or any other milk supply. Yet, a case of mastitis may be so mild as to escape detection by the herdsman or veterinarian, and it may be produced by the same streptococcus which has produced a severe case of mastitis in another cow. Again, streptococci in the early stages of infection, are present, even before the milk or udder show any signs of the disease. Furthermore, after the disease has subsided, the cow is more than likely to become a carrier and in that way pass large numbers of mastitis streptococci into her milk long after the disappearance of the disease. These incipient and recovered cases of mastitis constitute the high counters in the herd. Such a cow giving apparantly normal milk gave counts of over 180,000 mastitis streptococci per cubic centimeter of milk drawn directly from the udder. It can, now, be well appreciated that even the milk of this particular cow if mixed with that of 17 others, whose milk was practically sterile, the count for the mixed milk would run 10,000 streptococci per cc regardless of how well the milk has been handled about the dairy. Thus it can very readily be understood how even"certified milk is likely to contain a relatively larger number of mastitis streptococci than ordinary milk" In certified milk the contamination from filth is reduced to the minimum, which leads one to naturally conclude that almost all of the streptococci found are those that have come directly from infected udders. In making such a statement, does not mean that the streptococci are more numerous in certified than in ordinary milk, but relatively speaking they may be.

Not only are the cows harboring mastitis streptococci a menace to the other cows of the herd, but they also increase very materially the bacterial content of the milk. Experiments seem to indicate that the disease is transmitted from one cow to another by the hands of the milker and that infection is by way of the milk ducts.

Having thus obtained a high average count for the herd and the presence of streptococci brought out, it was deemed advisable to take up and study the udder flora of each milking individual of herd II.

For this work milk was drawn directly into a sterile bottle. The cows were washed and dried before the sample was drawn. The first few streams were discarded from each quarter. A sample from each quarter was milked into the same bottle. The milk was iced at once and usually plated within three to four hours thereafter. 1 cc of milk was added to a 99 cc sterile water blank and shaken vigorously. 1 cc was added to petri dishes in which about 12 cc veal infusion agar plus 2 cc of defibriuated cow blood were added and thoroughly mixed. After 48 hours incubation at a temperature of 37° C. the final counts were made which will be given in the tables to follow. Some of the most suspicious colonies were fished and inoculated into beef peptone broth tubes and these cultures were examined microscopically after 24 - 48 hours incubation at 37° C, cultures when showing a number of chains containing four or more cells were considered streptococci.

Hemolysis: This was determined by plating on the veal infusion blood agar as previously described. The plates were incubated at 37° C for 48 hours after which the final counts were made.

The beta type of reaction on the blood plates as described by Smith and Brown* consists of a sharply defined clear zone about the colonies. Under the microscope no blood corpuscles are noticed near the colony. Furthermore, after refrigeration for 12-24 hours, the hemolyzed zones show up well. This type was very common among the platings made.

Still another type existed on the platings. This type known as the non-hemolytic type, produced no clear zone around the colony but instead, they were rather deep set and resembled the more common types in general.appearance.

In the following table a symbol to represent each individual cow, Date, Media, temperature, average counts, average number of hemolytic and non-hemolytic-types will be given.

* Jour. Med. Research, 1915, 31,p. 455.

Table VII

Number of cow	Date	Media	Incubation temperature	Average counts	Hemolytic	Non-hemolytic
C-4	2/9/23	Agar	37° C	1100		
	2/9/23	Veal blood agar	37° C	2000	825	1175
C-8	2/9/23	Agar	37° C	10200		
		Veal blood agar	37° C	12,800	4,100	8,700
C-17	2/9/23	Agar	37°C	1,800		
		Veal Blood agar	37°C	1,500	750	750
C-17	3/22/23	Agar	37°C	1,500		
		Veal Blood agar	37°C	5,300	1,500	3,800
C-10	2/9/23	Agar	37°C	3,900		
		Veal Blood agar	37°C	4,800	1,200	3,600
C-10	3/22/23	Agar	37°C	1,900		
		veal Blood agar	37°C	6,700	2,400	4,300
C-11	2/9/23	Agar	37°C	8,400		
		veal Blood agar	37°C	8,600	2,600	6,000
C-18	2/9/23	Agar	37°C	4,800		
		Veal Blood agar	37° C	4,200	2,500	1,700
C-18	3/22/23	Agar	37°C	2,500		
		Veal Blood agar	37°C	6,700	1,500	5,200
C-3	2/9/23	Agar	37°C	2,700		
		veal blood agar	37° C	6,200	1,800	4,400

Table VII Cont'd.

Number of cow	Date	Media	Incubation temperature	Average counts	Hemolytic	Non-hemolytic
C-3	3/22/23	Agar	37°C	1,500		
		Veal blood agar	37°C	4,200	1,400	2,800
C-16	2/14/23	Agar	37°C	5,900		
		Beal blood agar	37°C	4,000	600	3,400
C-16	3/22/23	Agar	37°C			
		Veal blood agar	37°C	2,300	900	1,400
C-9	2/14/23	Agar	37°C	9,500		
		Veal blood agar	37°C	51,200	2,600	48,600
C-9	3/22/23	Agar	37°C	4,200		
		Veal blood agar	37°C	8,800	2,200	6,600
C-15	2/14/22	Agar	37°C	1,800		
		Veal blood agar	37°C	2,100	50	2,050
C-15	3/22/23	Agar	37°C	2,600		
		Veal blood agar	37°C	2,000	800	1,200
C-14	2/14/22	Agar	37°C	3,800		
		Veal blood agar	37°C	13,400	3,700	9,700
C-14	3/22/23	Agar	37°C	1,900		
		Veal blood agar	37°C	3,800	1,900	1,900
C-7	2/14/22	Agar	37°C	5,000		
		Veal blood agar	37°C	43,600	2,800	40,800
C-7	3/22/23	Agar	37°C	3,400		
		Veal blood agar	37°C	7,200	2,200	5,000

Table VII Cont'd.

C-5	2/14/23	Agar	37°C	36,000		
		Veal blood agar	37°C	19,000	2,900	16,100
C-13	3/22/23	Agar	37°C	2,600		
		Veal blood agar	37°C	7,200	2,600	4,600

From table VII it is found that the bacterial flora of the individuals under consideration varied anywhere from 1000 - 36,000 per cc as shown on nutrient agar plates and from 1500 - 51,200 on the veal blood agar plates. The counts made from nutrient agar platings did not reveal anything but an approximate number of bacteria per cc of milk. The object of the platings made on veal blood agar was to bring out and show the approximate number of hemolytic as compared to non-hemolytic types of bacteria. As already noticed, the counts obtained from veal blood agar, are slightly higher than those taken from agar. This might be attributed to the presence of the fresh defibrinated blood which would materially aid in bringing about a condition, when properly incubated, under which the maximum growth may be obtained. The number of hemolytic bacteria varied anywhere from 50- 4,100 while the non-hemolytic type showed a variation from 750 - 48,600.

Because of the large numbers of hemolytic bacteria found in the milk of the individuals studied do not signify that the milk is unfit for human consumption nor does it signify that the hemolytic forms found are strictly pathogenic in their nature. But irrespective of all that has been mentioned to pay no attention to the udder flora of individuals, when the average count for the herd is unusually high, is a poor policy to follow.

In table VII it is a noticeable fact that the hemolytic type of bacteria is found regardless of the health of the individual. But on making a comparison of the health and high counts it is found that in practically all cases, those showing a large number of hemolytic bacteria have had udder trouble some time or other.

From the platings of table VII a number of cultures were made into beef peptone broth. In making these cultures different typed colonies were intended for each inoculation. However, upon microscopic examination there seemed to be a more or less general type present in all.

Before inoculation a description of the colonies used were recorded.

C-I. Diameter of colony is about 2 1/2 millimeters. Diameter of colony and haylo is 3 millimeters. The colony is tough and its surface convex.

C-II. Diameter of colony is 1 mm. Diameter of colony and haylo is 2 mm. The surface of the colony is slightly convex.

C-III. Colony is irregular; diameter 1 mm; has crosslike interior structure; and set deep in media.

C-IV. Colony is less than 1 mm in diameter, haylo is 2 mm in diameter. The colony is hard and raised.

C-V. Diameter of colony is 1 mm; of haylo 1 1/2 mm. This is very likely to be same type of colony as that found in C-I and C-II, only it is smaller. In 1:100 plate about 5000 per cc of this particular type is shown.

C-VI. Diameter of colony 2 mm. no haylo, colony is embedded. In 1:100 plate there are found about 2000 per cc of this type of organism.

C-VII. Large colony and appears to be a surface colony of the form as described in C-VI.

D-1. Colony very small, deep set, slight hemolysis, hard and white.

D-2. Colony about 1 mm in diameter; haylo 2 mm in diameter; soft and yellowish with complete hemolysis.

D-3. Colony deep set, tough, white, slight hemolysis, ovoid in shape and about 1 mm in diameter.

D-4. A surface colony, soft and white, diameter about 1/2 mm, and a haylo with a diameter of 1 1/2 mm.

B-1 Very small colony; small hemolytic zone, deep set, and very numerous.

B-2. Colony is small, hard, white, convex, with a haylo of about 1 mm in diameter.

B-3. Colony is very small, ovoid, hard, deep set, yellowish, with complete hemolysis.

B-4. Colony has flat surface, soft, pinkish in color, slight hemolysis, and about 2 mm in diameter.

T-1. Colony is deep set, round, very small, yellowish color and hemolytic.

T-2. Colony is small, convex, tough, white and hemolytic.

T-3. Colony is deep set, very small and shows complete hemolysis.

T-4. Colony is tough, pink in color, deep set with complete hemolysis.

S-1. Ahard and white colony with a double haylo measuring about 5 mm in diameter.

S-2. A very small colony, soft, avoid, with double haylo, but the first not quite so prominent as second and about 4 mm in diameter.

S-3. Colony is about 1 mm in diameter, white, tough, deep set, with a haylo of about 4 mm in diameter.

V.D. I. The colony is flat, white, about 2 mm in diameter and a small haylor.

V.D. 2. A very small colony with a pronounced ring out from haylo.

The effect of each inoculation on beef peptone broth is shown in the following table.

Table VIII

Culture Number	Media	Incubation Temperature	Growth
C-I	Beef peptone broth	37° C	Clear
C-II	"	"	Turbid
C-III	"	"	Turbid
C-IV	"	"	Turbid
C-V	"	"	Clear
C-VI	"	"	Clear
C-VII	"	"	Turbid
D-I	"	"	Turbid
D-2	"	"	Turbid
D-3	"	"	Turbid
D-4	"	"	Turbid

Table VIII Cont'd.

B-1	Beef Peptone broth	37° C	Turbid
B-2	"	"	Turbid
B-3	"	"	Turbid
B-4	"	"	Turbid
T-1	"	"	Turbid
T-2	"	"	Turbid
T-3	"	"	Turbid
T-4	"	"	Turbid
S-1	"	"	Clear
S-2	"	"	Clear
S-3	"	"	Turbid
U.D.-1	"	"	Turbid
U.D.-2	"	"	Clear

Of the twenty-four inoculations made in beef peptone broth only seven remained clear with seventeen of them turbid. Stains were made from all and the presence of streptococci was revealed in every case. In some the chains seemed to be longer and more abundant than in others.

In conclusion, it might be of interest to note that bitterness of milk may come from one or more of several factors, the most common of which are those caused by bacteria, kind of feed consumed, and advanced lactation.

In the study of Herd II the life history of each individual was obtained, average counts from milk taken collectively were made and then counts from each individual. Here it is noticed that

the average counts were abnormally high, and a number of individual counts were very high. Cows that had had udder trouble gave the highest counts. The presence of streptococci was noticed in all stains made from twenty-four different inoculations taken from platings of individual cows.