OUTLINES OF DAIRY BACTERIOLOGY

RUSSELL

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OF

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BY

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PREFACE.

With the rapid advances made in modern dairying that are based directly upon the effect that bacteria have upon milk and its products, the need of an exposition of the principles that underlie bacteriology especially in reference to dairying is being more and more felt. No dairy course that purports keeping up to date can afford to omit some reference to this subject that is growing more and more important. Having felt the need of some text-book that could be placed in the hands of my own students, the following outlines have been prepared primarily for the use of those who are making a special study of dairying, although the attempt has been made to free the subject matter as far as possible from technical expressions, so that it may aid those practical students of dairying that are forced by circumstances to forego a course of dairy instruction. A brief glossary is appended of those technical terms that are necessarily employed that will be of aid to those that are not familiar with bacteriological expressions.

Numbers in italics at upper inner corner of page refer to paragraph number with which page begins.

Illustrations have been entirely omitted on account of the general unsatisfactory nature of the ordinary processes used in reproducing bacteria, and because these pages are intended to supplement the class lecture where much better facilities for illustration are at hand.

I take this opportunity of expressing my thanks to Dr. S. M. Babcock for valuable suggestions that he has kindly made in the preparation of the work.

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mainly out of experience. The fact has often been learned, but the *why* of that fact is frequently shrouded in mystery.

Modern dairying is attempting to build its more accurate knowledge upon a surer foundation and in doing this is seeking to ascertain the cause of well established processes. To assist in this, bacteriology comes as a potent ally and is striving, together with chemistry, to broaden and strengthen the underlying principles upon which advance in modern scientific dairying is rendered possible.

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PART I.

SYNOPSIS OF THE BACTERIA IN GENERAL.

CHAPTER I.

STRUCTURE AND FORM OF THE BACTERIA.

1. What are bacteria?

When Leeuwenhoek in 1675 first discovered these tiny, rapidly-moving organisms, that are now known as bacteria, he thought they were animals. Indeed, under a microscope, many of them bear a close resemblance to those minute worms found in vinegar that are known as "vinegar eels." The idea that they belonged to the animal kingdom continued to hold ground until after the middle of the present century. With the improvement in microscopes, a more thorough study of these tiny structures was rendered possible, and with this their vegetable nature was demonstrated. The bacteria are to be considered as a separate class from the fungi and other low forms of microscopical vegetable life. They differ essentially from the fungi and yeasts in their method of growth, and are as a class much smaller in size.

2. Structure of bacteria:

The bacteria as far as structure is concerned stand on the lowest plane of vegetable life, as they are composed of a single cell. The inner structure of the bacterial cell does not differ essentially from that of many other cell types of plant life. This tiny structure is composed of protoplasm, and is surrounded by a thin membrane that separates it from neighboring cells that are alike in form and size. In some cases there is a thick gelatinous capsule surrounding a single cell or a small group of cells.

3. **Form and size:**

Every species of plant and animal life has a form or shape more or less peculiarly its own. With the higher types of life, there is a great diversity of form, because each species is made up of many thousand cells. With these single-celled plants there can be but little difference in shape and size, as the individual cell is so small. Among the bacteria, there are three different types as to form:—

1. *Coccus*,—round cells of varying size, when isolated called *micrococcus* (little ball).

2. *Bacillus*,—straight, elongated and cylindrical rod-like cells. In the older literature, the term bacterium is used indiscriminately with bacillus in referring to the rod-like cells.

3. *Spirillum*,— curved, cylindrical cells of varying length and size.

In outline, a ball, a short rod and a corkscrew will represent the different types of bacteria.

Concerning size, it is difficult to gain any accurate conception, as they are so extremely small. If hundreds of germs could be placed side by side even then the aggregate length would not exceed the thickness of a single sheet of paper upon which this book is printed.

4. Arrangement of cell unions:

It is at once apparent, in a classification based upon no wider foundation than this that it will be extremely difficult to satisfactorily classify all species if the form of the individual cell is the only character considered. For this reason, there are numerous subdivisions based upon the way in which the different kinds of cells are attached to each other, as for instance:—

1. *Diplococcus*,—dumb-bell cocci where two germs are closely united to each other.

2. *Streptococcus*,—chain-cocci; a string or chain of coccus cells produced by continual growth in one (linear) direction.

3. *Staphylococcus*,—bunch cocci; an irregular grape-like cluster of cells produced by continued division in two directions.

Among the bacillus forms there is a marked difference in the groupings of the cells with reference to each other. Sometimes the cells adhere in long threads or filaments, but usually they are more or less completely divided so that they appear in an irregular heterogeneous mass.

In still another group, growth occurs in three directions making a cubical packet-shaped mass of cells (*sarcina*). By far the great majority of bacterial forms are included under the bacilli. There are other more minute subdivisions, but they have no special interest to us in connection with dairy work. The bacillus group is of most interest in dairy studies, although the coccus forms are not at all uncommon.

5. Manner of reproduction:

There are two ways in which the bacteria reproduce themselves.

1. The commonest, and at the same time the most important way, is by the direct division (*fission*) of the mother cell into two young individuals of smaller size. A single cell elongates in the direction of its long axis, and a new cell wall is formed across the middle of this growing cell making simultaneously, two smaller, although distinct cells.

This method of growth enables the bacteria to increase with astonishing rapidity. In a favorable food medium and with abundant moisture growth usually occurs in this way.

2. Under certain conditions, the bacteria reproduce in another way, viz., by the formation of *spores*. The spore-cell of the bacteria and some of the lower fungi are analogous to the seed of the higher plant; it enables the species to be perpetuated in unfavorable surroundings, and from one season to another. All bacteria do not form spores, but those species that do reproduce in this way possess an advantage, because, on account of the high resistance of these structures toward external conditions, (see 13) they are much more difficult to kill.

Two types of spores are known among the bacteria.

a. Endospore.

b. Arthrospore.

With the endosporous bacteria, the protoplasm of the cell undergoes a change, drawing itself together in a small round or oval mass within the cell and finally acquiring a bright glistening appearance. So far as is known at present, only one spore is to be found in each cell, although not every cell actually does form a spore in its interior.

Many forms, especially the micrococci, do not form internal spores but the whole cell itself changes into a spore (*arthrospore*) by the thickening of the outer membrane.

For the germination of spores certain conditions are required just as with seeds. Spores will not develop under conditions that sometimes allow the slow growth of cells already in the vegetative stage. This point is important, for if milk, for instance, contains bacteria in spore condition only, it is possible to keep these from germinating under conditions in which actual growth would occur, if the germs were in a developing stage.

6. Rapidity of multiplication:

The rate of growth of actively vegetating bacteria is in many instances perfectly astounding. With many species, under the best of conditions, a single cell will divide in twenty minutes. This rapid rate cannot be maintained indefinitely for the bacteria soon limit their own development by the production of bye-products that are unfavorable to their own multiplication. The sour milk bacillus thrives readily in milk until the lactic acid that is formed as a result of its own development exceeds a certain per cent. then growth ceases. If this acid is removed, the germs will start again to develop and produce acid to the point of excess. There is a marked difference with different forms in their rate of growth, and the conditions under which development best occurs.

7. Motility:

A great many different species of bacteria possess very evident powers of locomotion. If a bit of decaying matter is examined under a microscope, infinite numbers of tiny organisms will be seen darting across the field. These bacteria move by means of extremely delicate threads of protoplasm (*cilia*) that protrude from the wall of the cell. These cilia resemble tiny whip lashes and by alternate movements backward and forward, the cell is moved through the surrounding fluid. Sometimes these locomotor organs are attached to one end of the cell, then again the whole surface is girdled with numerous processes. So extremely delicate are these minute threads that their presence was merely conjectured until by a most careful process of staining, their true nature was brought out. Many bacteria, especially the coccus forms, are devoid of these appendages and therefore are immotile. These immotile forms often have an oscillatory motion that closely resembles a true vital movement, but this so-called Brownian movement is purely a physical phenomenon and may be noticed in any extremely fine particles of inert matter like dust, if examined in water under a microscope.

CHAPTER II.

THE PHYSIOLOGICAL FUNCTIONS OF THE BACTERIA.

8. Conditions essential for bacterial development: The growth of bacteria like all other living organisms bears a direct relation to their external surroundings. Certain conditions are absolutely essential before germ life can develop. Other conditions though often present are not of such vital importance.

1. *Adequate* and *proper food supply.* Concerning this there is a very great difference with different forms of germ life. Many of those that have developed the property of producing disease are very choice in the selection of their food. With those forms that are met with in milk, such delicacy of choice is not common. The *essential food elements* that must be present are nitrogen, carbon and oxygen together with minute quantities of mineral elements. The nitrogen and carbon are more available when in the form of organic compounds than as simple inorganic salts. Albuminous or proteid substances are the best adapted for the nitrogen supply, while sugars are available for the carbonaceous part of their food. The nitrogenous element is, however, the most indispensable.

The *concentration* of the food medium is important. If the fluid is too dense, bacteria cannot grow; on the other hand, it is impossible to so dilute a nutritive fluid as to completely stop all growth of germ life. Some of the water bacteria find even in redistilled water, a sufficient food supply so that they are able to increase.

The *chemical reaction* of the medium is another point of considerable importance. As a rule, bacteria can be said to prefer an alkaline to an acid substratum, but there are so many exceptions to this common rule, that the value of it as a general statement is now much modified. Those organisms that are normal inhabitants of milk are as a rule less susceptible to slight variations in the reaction of the food medium than many others.

2. *Temperature.* A certain degree of heat is absolutely necessary before the spores of bacteria will germinate, just as the seed of any grain will not sprout when the ground is too cold. As the temperature of a fluid increases, the rapidity with which the bacteria multiply also increases for a certain time. Beyond a certain point, however, a heat rigor sets in that destroys the activity of the protoplasm. There is, therefore, an *optimum* or heat temperature for growth and a *minimum* and *maximum* point as well, below and above which, development is impossible. These three cardinal growth points vary much with different germs.

The limits of growth of bacteria in general, i. e., the range between the maximum and minimum points of development, are much wider than with almost any other forms of living matter.

Several species have been found that thrive easily at the freezing point while a few have been described that have their optimum growth point in the neighborhood of 140° F. With the great majority of bacteria, especially those growing in milk, the range is not so great. Most of them fail to develop at a point below 45°–50° F. while the maximum growth point does not exceed 105°–110° F. With those species that have developed parasitic tendencies and have adapted themselves to the conditions furnished by the animal or human body, the optimum growth point is somewhat higher, usually approximating that of the body of their host (98°–100° F).

3. *Gaseous environment.* To most forms of life, atmospheric air is a necessity, in order to supply the oxygen used by the plant or animal in its growth. With the bacteria, the great majority require the free access of air and if denied this, they fail to grow. Such bacteria are called *aerobic.* There are, however, quite a number toward which oxygen acts as a direct poison. Only when they are surrounded with an atmosphere other than air, such as hydrogen or nitrogen gas, can they develop in any degree. These forms are called *anaerobic.* All bacteria are not divided sharply into these two classes. While some of them are subject strictly to either one condition or the other, hence are called *obligate* aerobes or anaerobes, there are other forms that are seemingly indifferent to their gaseous surroundings. To this class, the name of *facultative* or optional aerobe or anaerobe is given depending upon the dislike or the predilection of the germ with reference to oxygen.

Most milk bacteria as well as the great majority of germs in general belong to the aerobic class, but there are quite a number that are included among the facultative and obligate anaerobes.

4. *Moisture.* On a dry medium, bacteria can not grow, neither can the spores germinate. While they are unable to thrive in a dry atmosphere, many of them are able to withstand a certain amount of desiccation without being greatly injured. The cholera germ is one of the most susceptible to drying, an exposure of a few hours being sufficient to kill the germ.

9. Nutrition of bacteria:

Mention has already been made of the elements that are necessary for the development of bacterial life and the predilection that the bacteria have for organic foods. Those living upon organized material, assume a destructive role like the fungi and all animal life, in contradistinction to the green plants and the nitrifying bacteria which are constructive in their habits. The constructive organisms are those that take the inorganic constituents of the soil and air, and from these elaborate higher and more complex compounds. Until recently, this function has been considered as one of the distinguishing traits that separate the green plants from all other forms of life, animal or vegetable. Recent discoveries have shown, however, that the organisms concerned in the processes of nitrification are also constructive, inasmuch, as they take the simple inorganic salts and from these elaborate higher compounds that serve as food for plants.

The great majority of bacteria are destructive in their vital activities. Their energy is expended in tearing apart and breaking down organic matter into simpler compounds or elements. The value of this disintegrating process is evident at a glance when we consider what would be the result, if all decay, putrefaction and decomposition were at once arrested. Not only would the world's supply of carbonic acid gas be very soon absorbed and thus the development of chlorophyll-bearing plants stopped, but nature would be clogged and soon buried under its own debris. Dead and dying vegetable and animal matter, unable to rot and decay would soon bury all under the constantly accumulating mass. Owing to the energies of the lower types of plant and animal life, all of this dead effete matter is slowly consumed. The splitting of these materials into the simpler elements of their structure is largely due to the activities of the bacteria. Almost without exception, they prey upon organized matter, absorbing sufficient energy for their maintainance, and at the same time, giving off the unused elements to form new combinations which are absorbed again in the ceaseless cycle that matter undergoes in the change from the inorganic to the organic and *vice versa*. Bacteria affect organic matter under two conditions,—1, in a dead condition,—2, in a living state. Those germs that utilize dead organic matter as a nutritive base are called *saprophytes:* those subsisting on living tissues are called *parasites.*

10. Saprophytic bacteria:

Presumbly, this class is the more fundamental as it is the more common method of nutrition. Among the saprophytic organisms are to be included the great mass of the different forms of germ life, not only those resident in water, milk and various fluids, but the many species that are to be found on the surfaces of solids.

11. **Parasitic bacteria:**

More attention has been given to the parasitic class of bacteria because they are of special interest to mankind, inasmuch as many of them are able to set up diseased conditions in the human body, if they once gain access. These pathogenic or disease producing bacteria are germs that have acquired an especial ability to penetrate living animal or plant tissue. In acquiring this parasitic function, many of them have lost the power to thrive under ordinary conditions, especially in their relation to temperature changes. These parasitic bacteria possess more than an ordinary interest for us as they are the cause of many of the contagious diseases that afflict man and animals, such as typhoid fever, consumption, diphtheria and lockjaw. Not all of those bacteria that are disease-producing are exclusively parasitic. Some germs, like the bacillus of leprosy, can only grow with great difficulty outside of its host, while other bacteria like cholera or typhoid thrive quite as well in an artificial medium as within the human body. The first instance approaches the obligatory stage of parasitism, the other the facultative or optional parasitic state. There are numerous peculiarities among the saprophytic class that tend to separate these many forms into smaller groups. In most instances these groups are natural divisions in which certain physiological characters are strongly developed.

12. Bacteria associated with fermentation of organic substances:

Just as certain forms have acquired the ability to grow within living tissue, other forms have developed the peculiarity of transforming relatively complex substances into simpler compounds by regular steps. To this orderly pulling down process, the name of fermentation is given. In this class are to be included a large number of fermentative changes that occur in milk, such as the production of lactic acid in the souring of milk, the formation of butyric acid and others that will be mentioned later. In fermentation, there are often large quantities of the fermentable substances changed and while a certain amount of the energy released in the breaking-down of this material may be utilized by the bacterial cells, yet the disruptive changes set in motion by the living germs are out of all proportion to the results that are seen in the end.

Fermentation, although one of the best known processes that occurs in nature is yet a partial mystery. Our knowledge of the changes that fermentable solutions undergo during this process has been vastly augmented during the latter half of this century, but even yet, the problem has not been completely solved. The results of fermentation have been recognized and used from time immemorial, but no adequate conception of the changes involved was ever recognized until this century. The earlier theories of this action were mainly chemical and it was not until the important studies of Pasteur were made that the relation of these changes to the action of living organisms was fully proven. He showed that fermentation was closely connected with the growth and multiplication of minute forms of organic life and that the process was usually inaugurated by vital forces rather than purely chemical activities.

The action of rennet upon milk had been long known previous to this and the difference between this fermentation and other types had been recognized by Schroeder and Dusch. They showed that the rennet ferment was unaffected by alcohol and other chemicals, while the other fermentations were checked in these fluids. Pasteur emphasized the distinction between these two sets of fermentative action and soon was able to classify these series of changes into two distinct types.

1. *Organized ferments*—those in which the change takes place as a result of the presence of a living germ.

2. *Unorganized ferments* — those in which the change is caused by a chemical substance, devoid of vitality, that is not itself changed in the fermenting process. These unorganized, non-vital ferments are known as *enzymes*. Among the better known of these are rennet, which has the power of coagulating milk; diastase, the enzyme that converts starch into sugar; pepsin and trypsin, the digestive ferments of the animal body. Some of these unorganized ferments can withstand a high degree of heat as well as the influ-

ence of deleterious chemicals such as alcohol and various salts without injury.

Among the organized class of ferments are those that directly affect the character of sugar containing fluids, such as the yeasts and most of the bacteria. A great many of these organized ferments accomplish their effect indirectly through the agency of the enzymes that they themselves excrete.

With the exception of the rennet fermentation, the fermentations in milk that are of any importance are produced through the agency of the organized class.

13. Relation of bacteria to external conditions:

Bacteria, even in a vegetating stage possess a much greater resistance toward external forces than other forms of animal or vegetable life. When they are in a spore stage, this resistance is still further increased. A thorough knowledge of the effect of these external forces is valuable, for it is by the action of some of these that we are often able to destroy undesirable forms of germ life. In considering these forces in their relation to bacteria, the subject may be divided into two heads, the influence of physical and chemical forces on bacteria.

14. Influence of physical forces:

1. *Heat.* The bacteria possess a high power of resistance toward heat; but this relation varies, depending upon the condition in which it is applied, whether it is in a dry or moist state. Moist heat is far more effective in destroying germ life than dry. The temperature at which any form of bacteria is

killed is called its *thermal death point.* For the majority of germs in a spore-free, developing condition, a temperature from 130°–140° F. for ten minutes in a liquid medium is fatal.

A shorter exposure necessitates a somewhat higher temperature. When in the more resistant spore-stage many of them are able to withstand moist heat in the form of steam (212° F) from one to three hours. To destroy spores by dry heat, a temperature varying from 260°–300° F. is necessary from three-quarters to one and one-half hours. Germ life may be more rapidly destroyed in super-heated steam under pressure; a temperature of 230°–240° F. for fifteen to twenty minutes being sufficient to kill most species. Many of the milk bacteria, like the sour milk germ, are very easily affected by heat, as they do not form spores. Other forms, like the hay and potato bacilli, are often difficult to eradicate on account of the great resistance that their spores offer toward heat.

High temperatures are by far the most efficient means that can be used to render any substance germ-free and are most often employed for this purpose.

2. *Cold.* While the maximum temperature that the different spore-bearing species can stand has been accurately determined in many cases, the minimum temperature has in most instances never been reached. But little reliance is to be placed upon this method in attempting to free any substance from bacterial life. Even an artificial degree of cold as low as —220° F for a day has been found to be insufficient to destroy some forms.

3. *Desiccation.*—Bacteria behave very differently when subjected to drying. The cholera germ dies in three hours if it is dried, while anthrax retains its virulence unimpaired for decades. Tuberculous sputum withstands drying and is often found to be infectious after the lapse of eight or nine months. Those species that form spores resist desiccation much better than those that do not form these structures.

4. *Light.*—The influence of light on bacterial growth has not been generally appreciated until within a few years. Exposed to the rays of direct sunlight, many forms are killed in a few hours. Even diffused daylight often exerts a powerful inhibiting effect, if the exposure covers any considerable length of time. The value of sunlight as a disinfectant has undoubtedly been much underrated, for recent experiments show that the natural death of bacteria as they occur in nature is largely due to the influence of this agent.

5. *Pressure.*—The influence of pressure does not seem to have any direct effect upon development. Freudenreich and Schaffer found that under a pressure of ninety atmospheres, the development of certain forms took place at an ordinary rate. In studying the deep sea flora of the Mediterranean, the writer found the same species of bacteria at the surface that were present on the sea bottom at a depth of 3600 feet, or under a difference in atmospheric pressure of over one hundred atmospheres.

6. *Electricity.*—Exact knowledge of the effect that this agent has upon bacterial development is as yet

quite meager. Spilker and Gottstein succeeded in killing *Bacillus prodigiosus* in a few hours when subjected to a strong current.

15. Influence of chemical forces:

A great many chemical substances exert a powerful effect upon bacterial protoplasm, although on the whole, bacteria resist the action of chemical poisons more successfully than other forms of vegetable life. Among the more prominent chemical substances that are destructive to bacteria are mercury in the form of corrosive sublimate, carbolic acid, the mineral acids like nitric, sulfuric and hydrochloric, and alkalies especially the potash and soda series. The value of the above chemicals in killing bacteria, either in the vegetative or spore stage, is so great that these substances are extensively used for disinfecting purposes where it is possible.

16. Antiseptics and disinfectants:

An antiseptic substance is one that inhibits or restrains bacterial growth. A disinfectant is one that kills bacterial life with which it comes in contact. These two terms are often confused but it will aid in a clearer comprehension of their exact meaning if the above distinction is made. All substances possessing disinfecting power must of necessity be antiseptic in their action, but all antiseptics are not disinfectants.

Sugar or salt has a retarding effect on bacterial growth, yet these substances do not kill out the bacteria. The same is true with boracic acid and sundry prepared fluids that are used to increase the keeping quality of milk. In some instances the difference in effect is accomplished by varying the degree of concentration, as for example, carbolic acid in the proportion of 1 : 400 is antiseptic toward the typhoid germ, while 1 : 200 is a disinfectant. While the value of these chemical disinfectants for hygienic and sanitary purposes is very great, they are of but little worth in the dairy, except in occasional instances, (see 63). Their use in milk should be excluded because in almost all instances they render it unfit for human food, (see 71).

CHAPTER III.

METHODS OF STUDYING BACTERIA.

17. Necessity of bacterial masses for study:

Bacteria are so infinitesimally small that it is impossible to study individual germs separately without the aid of first-class microscopes. For this reason, but little advance was made in the knowledge of these lower forms of plant life, until the introduction of culture methods whereby a single germ could be cultivated and the progeny of this cell increased to such an extent in thirty-six to forty hours that they would be easily visible to the unaided eye.

18. Culture methods:

This pure culture system, as it is called, is based upon the supposition that the food medium in which the germ is grown is first freed completely from all pre-existing forms of life, or in other words is perfectly sterile. The pure culture processes of the bacteriologist may be said to be in a sense refined methods of seeding, such as the agriculturist employs. Just as the seed grain will in due season bring forth a harvest after its kind, so any kind of bacteria planted in a favorable culture medium will produce a crop of its own. If the farmer's seed is foul, it shows in his crop, and the same is true with bacterial farming. Bacteria are so universally distributed that it becomes an impossibility to grow any special form, unless the soil is first freed from all existing forms of germ life. To accomplish this, it is necessary to subject the nutrient medium used for a culture to some method of sterilization, such as heat or filtration whereby all forms of organic life are thoroughly eliminated. Germ free culture material is kept in sterilized glass tubes and flasks and is protected from outside infection by plugs of sterile cotton. Material thus prepared, if protected from evaporation, will keep indefinitely, the cotton allowing the air to filter through but restraining the passage of any particles of matter.

19. Media used for cultures:

For culture media, many different substances are employed. In fact, bacteria will grow on almost any organic substance whether it is in a solid or a fluid state, provided the essential conditions of growth are furnished it. The food substances that are used as culture media are divided into two classes; solids and liquids.

Solid media may be either permanently solid like potatoes or they may retain their solid properties only at certain temperatures like gelatin or agar. These last are of utmost importance in bacteriological research, for their use which was introduced by Koch permits the separation of the different forms that may happen to be in any mixture. Gelatin is used advantageously because the majority of bacteria present wider differences in their appearance upon this medium than upon any other. It remains solid at ordinary temperatures, becoming liquid in the neighborhood of 75° F. Agar, a gelatinous product derived from a

Japanese seaweed, has a much higher melting point and can be successfully used especially with those organisms whose optimum growth point is above the melting point of gelatin. Besides these solid media of various kinds, there are different liquid substances that are extensively used, such as beef broth and milk. Skim milk is of especial importance in studying the bacteria in milk and may be used in its natural condition, or a few drops of litmus solution may be added in order to detect the presence of any acid in the fluid.

These various kinds of culture media are of value for purposes of study, only when the germ is seeded in them in a pure state. To secure any special form of bacteria free from all contamination, it is necessary to use some solid isolating medium like gelatin or agar.

20. **Methods of isolation:**

Suppose for instance one wishes to isolate the different varieties of bacteria found in milk. The method of procedure is as follows: sterile gelatin in glass tubes is melted and cooled down so as to be barely warm. To this gelatin which is germ free a drop of milk is added. The gelatin is gently shaken so as to distribute the milk thoroughly, and then the whole is poured out in a sterile flat glass dish and quickly covered. This is allowed to stand on a cool surface until the gelatin hardens. After the gelatin plate has been left for twenty-four to thirty-six hours, if germs are present, they will begin to appear in tiny spots on the surface or in the depth of the culture medium. These patches are called colonies and are composed of infinite numbers of individual germs, the result of the continued growth of a single organism that was in the drop of milk which was firmly held in place when the gelatin solidified. The number of these colonies represent in general the number of germs that were present in the milk drop. If the plate is not too thickly sown with these germs, the colonies will continue to grow and increase in size, and as they do, minute differences will begin to appear. These differences may be in the color, the contour and the texture of the colony, or the manner in which it acts toward gelatin. In this way, the different forms are separated from each other. To further study their peculiarities, the separate colonies are transferred to other sterile tubes of culture material and thus pure cultures of the various germs are secured. These cultures then serve as a basis for continued study and must be planted and grown upon all the different kinds of media that are obtainable. In this way the slight variations between different forms are detected and the peculiar characteristics are determined, so that the student is able to recognize this form when he meets it again.

These culture methods are of essential importance in bacteriology, as it is the only way in which we are able to secure a quantity of germs of the same kind.

21. Use of the microscope in bacterial investigation:

The microscope is in constant demand throughout all of the different stages of the isolating process in order to verify the purity of the cultures. For this purpose, it is essential that the instrument used shall be one of strong magnifying powers, combined with sharp definition, so that these tiny organisms shall stand out clear and distinct.

The microscopical examination of any germ is quite as essential as the cultural characteristics; in fact, the two must go hand in hand. An examination of this kind reveals not only the form and size of the individual germ but the manner in which they are united with each other, and any peculiarities of movement that it may possess.

In carrying out the microscopical part of the work, not only is the organism examined in a living condition but preparations are made by using anilin stains as a dye. These are of the greatest service in bringing out almost imperceptible differences. The art of staining has been carried to the highest degree of perfection in bacteriology, especially in the detection of germs that are found in diseased tissues in the animal or human body.

In studying the peculiarities of any special organism, not only is it necessary that these cultural and microscopical characters should be closely observed, but special experiments must be carried out along different lines, in order to determine any special properties that the germ may possess. Thus, the ability of any form to act as a fermentative organism can be tested by fermentation experiments; the property of causing disease, studied by the inoculation of pure cultures into animals; and, in this way the physiological peculiarities of any form are determined.

CHAPTER IV

GENERAL DISTRIBUTION OF BACTERIA.

22. Specialization of bacteria:

No class of living organisms is more widely distributed than the bacteria. Wherever organic matter is found, there bacteria will be present. This universal and ubiquitous distribution throughout the earth is due to their great resisting powers. No form of life can endure such wide ranges in physical environment, so that bacteria are thus able to live where other organisms are destroyed. Decomposition and putrefactive forms abound wherever dead organic matter exists and myriads of species are inhabitants of the soil and water masses. In the distribution of germ life throughout the world there are certain well defined facts that are to be borne in mind. Some species have become habituated to certain substrata so that they are always to be found in these localities. Thus, for instance, there are a large number of forms that are especially common in the soil layers. These organisms include the nitrifying bacteria, also those that are concerned with the manifold processes of decay and decomposition of organic matter; then again, certain forms are natural inhabitants of the intestinal tract of various animals or of the mouth cavity or on the surface of the skin. In water, there is a multitude of species that are so well adapted to this medium that they grow better, even in distilled water, than they do under any other condition. This adaptation to their environment is a prominent feature of bacterial life and forms as interesting a chapter in their distribution as it does with higher forms of life. We may speak of these forms that are habituated to certain conditions as *indigenous*. This does not preclude the presence of other types that we may call *adventive* or introduced, because they have gained access from some other source Thus the tubercle bacillus is adventive in milk even though it may be derived from the udder direct, while the sour milk bacillus is a natural and therefore indigenous inhabitant of milk.

23. Bacteria in the soil:

In the earth, the bacteria are most widely disseminated. The superficial soil layers teem with myriads of individuals and a multitude of forms. Only the upper layers are, however, rich in germ life. The bacteria suddenly diminish in numbers at a depth of two or three feet, and at a depth of six or seven feet, they have almost entirely disappeared. Cultivated soil contains naturally many more than virgin soil, but even the undisturbed woodland or prairie teems with millions in its upper layers. Numerous disease germs are to be found in the soil, that come from the bodies of decaying animals. They often retain their virulence unimpaired, and, when opportunity offers, gain an entrance into susceptible animals, causing a recurrence of an epidemic in the same locality from time to time.

24. Bacteria in the air:

The numerous germs in the air are largely derived from those that are resident in the soil. Bacteria are blown about in the dust, thus getting into the air.

Those that are found in the air are usually forms that are able to survive drying to a considerable extent. Germ life cannot in any way derive nutriment from this medium, therefore growth is impossible. They simply exist in a latent, resting condition. The number of bacteria in the air varies directly with the condition of the soil beneath and the conditions favoring the movement of dust particles. For this reason, the air of large cities is peopled with larger numbers than in country districts. Likewise, they are more numerous in summer than in winter when the ground is covered with a mantle of snow. They decrease in number as the distance from the earth's surface increases, and in samples taken from air over snow-covered mountains or oceanic bodies, their number is insignificant, the air being sometimes perfectly sterile.

In illy ventilated houses and outbuildings, they are numerous, and particularly is this true with reference to barns and stables where dust from the hay and manure particles fill the air.

25. Bacteria in water:

Water always contains enough organic matter so that many species find in it an ample food supply. A great many of the bacteria in water, especially in the smaller water masses like streams and lakes, come from the surrounding land, but there are numerous forms whose natural home is in water. It almost invariably contains some bacterial life; an exception to this is found in the condition of ground water that is free from germs, owing to the poor conditions to growth here present, and the fact that the bacteria are filtered out in passing through the upper soil layers. Stagnant pools rich in organic matter possess of course the maximum number, but even running water is often richly peopled with them. The oceanic masses from surface to abysmal depths contain a varying population of these germs. Spring water as it emerges from the soil is usually the freest unless it is contaminated by its surroundings. Not only is water the natural home of a number of saprophytic forms, but even several of the disease producing organisms are able at least to live, and in some cases to multiply in water rich with organic matter. Among the more important of the infectious diseases that are transmitted by water are cholera, typhoid fever and diphtheria.

26. Bacteria resident on and in living organisms: Naturally many bacteria are to be found on plants and animals that are normal either to soil, water or air. Nevertheless there are certain forms that have chosen special places under special conditions and have become habituated to these so that they may be called normal inhabitants. Thus the surface of certain plants have a specialized group like the hay and potato bacilli. The skin of man is notoriously contaminated with certain pus cocci; the mouth, the stomach and the intestines (which are in a sense external surfaces) are also peopled by regular habitues. The same is true with the inner tissues of the body.

A whole host of parasites feast on the blood of the living body, and while abnormal to the host, yet they have adapted themselves especially to the conditions that are here found.

Reference Works on the General Subject of Bacteriology.

This very brief résumé of the general biology of bacteria and the methods used in cultivating and studying these organisms is intended to bring out only the most salient points in this science. Students wishing to learn the details of the various processes employed in the laboratory, will find the subject treated at length in the many text books on the general subject of bacteriology. Among the most important of these in English are the following:

Frænkel's Bacteriology, translated by Linsley. 1891.
Sternberg's Manual of Bacteriology. 1892.
Woodhead's Bacteria and Their Products. 1892.
Abbott's Principles of Bacteriology. 1892.
Migula's Practical Bacteriology. 1893.
Hueppe's Methods of Bacterial Study, translated by Biggs. 1890.
Salomonsen's Bacteriological Technique, translated by Trelease. 1889.
Ball's Essentials of Bacteriology. 1894.
Frankland's Micro-Organisms in Water. 1894.

PART II.

RELATION OF BACTERIA TO MILK.

CHAPTER I.

COMPOSITION OF MILK AND CONDITION OF SAME IN UDDER.

27. Chemical composition of milk:

The chemical character of milk is such that it is especially adapted for the development of bacterial life. The essential nutritive elements are all present in proportions that enable many pathogenic species as well as a whole host of earth, air and water forms to find in it suitable conditions of growth.

The composition of milk varies not only with reference to the kind of animal but even in the milk of the same species. As our attention is to be directed mainly to cows' milk in its relation to bacterial growth, only this kind of milk will be considered to any extent.

Milk is an emulsion of butter fat in a serum containing sugar, casein and certain ash ingredients. The chemical constituents of this fluid may be classified as follows:

Water (87.5%)				87.5%
Solids, (12.5%)	organic, (11.8%)	nitrogenous, (3.9%)	casein	3.2%
			albumin	0.6%
			protein	0.1%
		carbonaceous, (7.9%)	butter fat	3.4%
			milk sugar	4.5%
	inorganic			0.7%

This may be taken as an average composition of cows' milk. The butter fat and the casein are liable to the greatest fluctuation in different breeds of cattle and under different conditions.

28. Value of milk as a bacterial food medium:

The proteid substances are perhaps the most important as they furnish the indispensable nitrogen for germ development.

Casein is by far the most important of the nitrogenous compounds. Its exact condition in milk is not satisfactorily known even by chemists. It is known that part of it at least is insoluble, because it cannot pass through a filter. It is easily precipitated by the addition of dilute acids either mineral or organic; also coagulated under the influence of rennet and by high heat. Under the influence of many bacteria it is rendered soluble, i. e., peptonized, so that the milky whiteness of the fluid quite disappears.

The other nitrogenous constituents, such as lactalbumin and lactoprotein, exist in only minute quantities. They approach in composition analogous compounds in the serum of animals and are of only general interest in bacteriology, as their presence is not known to exert any special influence on germ development.

From an economic standpoint, the *butter fat* in milk is perhaps the most important element, but from a bacteriological point of view, it has but little value. Fat exists in milk in the form of extremely fine globules and is a permanent emulsion in the fluid serum. It is a substance rich in carbon, but the element is in such combination that it cannot be utilized by germ life as food. While it may be possible that some of the volatile fatty acids are formed from fat through the action of bacteria, yet our present knowledge does not warrant the assertion that the fat is affected in any known way by them.

The *sugar* of *milk* is another ingredient that is utilized by bacteria, especially those forms that possess fermentative properties. Those species forming lactic acid, of which there is a large number, decompose this carbon-containing food product very easily, giving off a large series of waste products.

The inorganic elements, such as the mineral salts of potassium, sodium, calcium and magnesium that are combined with phosphoric and sulfuric acids, make only a minute fraction of the solid matter, yet these elements are essential to the growth of any kind of protoplasm and are used by the bacteria in the formation of new cell material.

29. Secretion of milk :

The milk of animals is secreted in certain glandular organs (milk glands). During the activity of these structures the milk flows from the separate glands and is collected in small channels which ultimately unite into large reservoirs (milk cisterns). The udder of the cow has four of these reservoirs and each of these is connected by a narrow milk duct (teat) with the surface of the animal.

30. Milk in udder is sterile :

Milk as it is formed in the milk glands of a healthy cow is germ-free. If it were possible to secure the fluid directly from the gland itself, it would remain sweet indefinitely, but the opening from the milk cistern to the outside is of such a nature that bacterial germs adhering to the mouth of the milk duct can make their way up into the teat for a short distance. Here they find rich food in the few drops of milk that nearly always remain in the teat, and warmth from the heat of the animal; consequently, the small number that gain an entrance increase so rapidly that when the animal is milked the next time, these bacteria are washed out into the milk, thus contaminating the whole milking. Owing to the lack of free oxygen in the upper portion of the teat and in the milk cistern, the great majority of germs (aerobes) are unable to develop except at the lower end. It is for this reason that the bacterial content of the first or fore milk is so much higher than that which is secured later. In one instance in which the surface of the teat was thoroughly disinfected with a dilute corrosive sublimate solution and the milk received into a sterile flask directly, so there was no chance of air infection, there were 2800 germs per cc. found in the first milk as against 330 per cc. found in mixed milk afterward. Perfectly sterile milk may sometimes be secured by the use of a sterile milking tube, but even with this precaution, it is difficult to obtain a sample that is absolutely free from germ life. These conditions refer only to healthy cows. Animals suffering with tuberculosis of the udder, for instance, might of course have germs in the milk that were derived from the affected glandular tissue.

CHAPTER II.

SOURCES OF BACTERIAL INFECTION IN MILK AND METHODS OF PREVENTION.

31. The contamination of milk:

Although the milk from healthy cows is sterile when it is in the glandular tissue, yet it is rarely free from germs as it comes from the cow. After it has undergone the usual treatment in the stable, this fluid will almost always be found infected more or less badly with different forms of germ life. The bacteria seeded in milk come from widely different sources and while experience has taught many a dearly bought lesson, a study of the sources of contamination will immediately suggest a rational system of treatment that will go far toward diminishing the possibility of infection in these multifarious ways.

The main sources from which milk obtains the great majority of the germs that it contains may be considered as follows:

32. Contamination arising from the use of unclean dairy utensils:

This applies not only to the vessels used during the milking, but to all dairy utensils that are brought in contact with it at any time after it has been drawn. Not only must the milk pail be cleaned with especial care but even more stress should be laid upon the condition of such articles as strainers and the cans in which the milk is set or transported. An ordinary cleansing of these objects is not sufficient, but they must be as near sterile as possible, for, if germs are present on the inner surface or in the joints of these vessels they easily contaminate the whole milk.

Storage cans when in use are rapidly fouled with bacteria, so that unless they are entirely freed from the innumerable germs that may be present in them, they remain a source of positive danger. A momentary application of hot water, or even boiling water or steam is ineffectual. On account of the resistant spores that are often present, many germs are able to withstand such a treatment for a considerable length of time. This time limit varies much with different forms, but it is necessary to subject all vessels of this sort to the maximum length in order that all may be killed. Where it is possible, steam should be used on all occasions as a final cleansing agent. Pails and other utensils should first be washed in water that is not too hot, in order to free them from the milk particles. After this is thoroughly done, they should be rinsed in scalding water, and then if possible turned over a steam jet for several minutes. Cans and pails should be left to drain in an inverted position so as to exclude dust and dirt. Steam to be effective as a disinfecting agent, must be confined, so that the jet should be applied to the inner surface of the vessel with considerable force. In this way, it penetrates all the joints much more thoroughly, destroying the bacterial life that may be harbored in such places.

It is quite as essential that strainers and dippers should receive as careful a cleaning as any other vessel, for they are brought in contact with so much more milk than a can, that if they are unclean, they may be the cause of spoiling a large quantity of milk. Cloth strainers are objectionable, for the fine mesh of cotton or linen retains so much moisture that they become a veritable hot bed of bacterial life, unless they are daily boiled or steamed.

Wooden milk pails should not be used, as they are much harder to keep clean and the chance for infection is much greater. Where possible, milk vessels should be made of pressed tin. If this is not practicable and it is necessary to have joints, they should be extra well soldered. Joints in the bottom of pails and corners of vats should always be filled flush with solder, so as to present a curved surface that may be more perfectly cleaned.

In order to show the effect of a sterilized milking vessel upon the changes that take place in the milk, the following experiment will be of value.

Two covered cans were taken, one of which had been cleaned in the ordinary way, and the other sterilized by steam for half an hour. Before the milking, the udder of the cow chosen for the experiment was thoroughly washed, and special precautions were taken to avoid the raising of dust; the fore milk was also rejected. These conditions were taken so as to eliminate as far as possible all factors of contamination except those due to the pail. Immediately after the milking, gelatin cultures were prepared with the following results.

In the milk in the sterile pail there were 165 germs per cc. while that milked in the ordinary pail contained 4,265 in the same volume. The milk taken under ordinary conditions soured in twenty-three hours while that received in the sterile pail remained sweet five and one half hours longer. This experiment was conducted in September, the thermometer registering from 70° to 85° F A repetition of the experiment in November showed nine hours' difference between the times in which the two milks soured.

32a. Use of milk cans as transport vessels for waste factory products back to farm:

In this connection, attention should be called to the methods in vogue in many factories of returning the bye-products of the factory, such as skim milk or whey, to the patrons in the same set of cans that are used for the transportation of the milk. Ordinarily, this is to be deprecated, unless the greatest of care is taken in the cleaning of the cans afterward, for the reason that these refuse products are very much richer in germ life, especially in those forms that are capable of causing offensive putrefactions. Both skim milk and whey are rich in bacteria on account of the high temperature, at which these products are separated. Too often, they are allowed to remain in the waste vats until they are completely sour and give off putrefactive odors. In this way not only is much of the nutritive value of the fluid lost for feeding purposes, owing to the decomposition of the milk sugar, but the growth of germ life is highly favored, so that the carrying cans are much more contaminated, rendering them harder to thoroughly cleanse.

A large proportion of the taints and troubles that bother creameries during the summer season are due to this cause. Where patrons insist upon such a method as good enough for *their* purpose, they are not apt to be overzealous in the matter of cleanliness on their own farms. A leading creameryman in this state informed me lately that during a certain season *all* of the trouble that he had experienced from tainted milk came from a few patrons who insisted in using their milk cans to carry back the bye-products of the factory to the farm. It was plainly evident that the trouble arose from the fact that the cans were not rigorously cleansed after they reached the farm.

Not only is it due the factory authorities that such a course should be carried out, but for each patron's own safety this method is advisable; for, in this way, abnormal ferments that may prove difficult to eradicate are often transferred from one dairy to another. Suppose there appears in the dairy of A an infectious milk trouble, such as bitter milk. This milk is taken to the factory and passes unnoticed into the general milk supply. The skim milk from the separator is of course infected with the germ and if conditions favor its growth, the whole lot soon becomes tainted. If this waste product is returned to the different patrons in the same cans that are used for the fresh milk, the probabilities are strongly in favor of some of the cans being contaminated and thus infecting the milk supply of other patrons. If the organism is endowed with spores so that it can withstand unfavorable treatment, this disease may spread from patron to patron simply through the infection of the vessels that are used for the transportation of the bye-products.

The danger from these bye-products can be largely obviated at the factory by proper treatment. Whey and skim milk reservoirs should be constructed out of some material and also arranged in such a manner as to admit of thorough cleansing; then, these products should never be allowed to accumulate and remain in the waste vats from one day to another (see 92).

33. Contamination by means of the fore milk: Another of the chief factors in the contamination of milk, the importance of which is rarely recognized, comes from the bacteria that gain access to the milk, by the mixing of the first milkings or fore milk with the whole of the milk. Even when the milking is most thoroughly done, there remains in the milk cisterns of the udder and in the teat, a few drops of milk that afford ample nutriment for the development of any germs that may gain access to these localities through the opening of the teat. Particles of filth and manure, if moist, easily adhere to the udder and belly of the animal as it reclines on the ground or in the stable. If these filth particles come in contact with lower open end of teat, bacteria in this way gain access, and when once they have established themselves, they easily pass up into the milk duct and possibly into the udder proper. Under these surroundings, they find the most favorable conditions of development that are possible with perhaps the single exception, that atmospheric air is wanting in some degree. Here they have a high temperature, approximating the body heat of the animal, so that the teat, if once infected, becomes an ideal incubator.

Under these conditions, a few germs will multiply enormously, so that if the fore milk is examined with reference to its bacterial contents many more germs will be found in this than is received in the later milk or in the strippings.

Schulz*[1] determined in fresh milk received under careful conditions the following numbers of bacteria.

Number of germs per cubic centimeter:

Fore milk.	*Strippings.*	*Average of total yield.*
55,500	00	2070
50,800	00	...
97,200	500	2590
78,700	665	....

The bacteria that are present in the fore milk will be largely expelled in the first part of the milking, but we have never been able to secure any samples of the strippings that were perfectly free from germs. One marked characteristic of the bacterial flora of the fore milk is that the number of species is usually small, one or two kinds usually predominating in a large degree. This is due to the fact that these special forms are rapidly multiplying in this warm chamber. For this reason, their growth in the mixed milk is more rapid than those forms that are in a spore or dried state that come from the dust and air. Generally speaking, it may be said where a reasonable degree of cleanliness is found that those species that predominate in large numbers in any sample of milk are derived from the fore milk of the cow. Those

*For citations see end of Part II.

that are present in relatively small numbers are due to the contaminations from the exterior.

Under ordinary conditions, the germs that are commonly found in the teat are those that produce lactic acid as these microbes find in milk their best medium for growth. It is, however, not impossible that noxious forms capable of producing serious changes may likewise obtain access, as is often experienced in an inflammatory condition of the udder. How far these different forms of germ life are able to penetrate into the healthy udder is as yet unknown. In all probability, the glandular tissue of the udder is not affected, although it is possible that microbes might work their way up the open channel of the teat into the udder proper.

34. Contamination from animal and milker:

Another most fruitful source of milk contamination comes from the dirt and filth that is derived directly from the animal and also from the person of the milker. This danger is considerable by reason of the fact that the germs on the surface of the animal are in a dried condition. They are, therefore, easily dislodged by any movement of the milker or the cow, and so gain access to the milk. The hairy coat of the animal offers exceptional facilities for the harboring of dust and dirt. It is, therefore, extremely rich in various forms of bacterial life that are derived from the particles of excreta that stick to the flanks and under parts of the animal when they lie down. These foreign particles are continually falling into the milk, and so adding bacterial life to this liquid. They may often be so numerous as to be easily recognized by the unaided eye. The amount of actual impurities that are to be found in milk, even after it is strained, will surprise the casual observer. Renk[2] has made a large number of determinations of the solid impurities found in the market milk of several European cities with the following results. In the Leipsic milk supply he found, by weight, 13 grains of impurities; in Munich, 31; in Berlin, 35; and in Halle, 51 grs. per 100 lbs. of milk. From his experiments, Renk deduced the following rule: If a sample of milk showed any evidence of impurity settling on a transparent bottom within two hours, it was to be regarded as containing too much solid impurities. These solid particles, most of which are bits of manure, are always teeming with bacteria, especially with those forms that belong to the putrefactive and decomposition classes. Improper stable conditions greatly favor the amount of filth that may adhere to the animal. The high feeding that is practiced at present produces a softer and more liquid manure, and one in which putrefactive organisms are found in greater numbers owing to the higher nitrogenous contents of the excreta. Then, too, the animal serves to transmit to the milk organisms found in stagnant waters, as the cow is often covered with slime and filth that adhere to its legs and udder as it wades through stagnant mud holes that may happen to be in the pasture. The filth with the myriads of germs that it contains, dries on the coat of the cow and is easily displaced by any movements of the animal or milker. In these ways, myriads of germs are always present on the animal's coat, and are constantly being brushed into the milk as it is milked.

What is true of the animal is likewise true concerning the person of the milker. Clothed in the dust-laden garments that he has in daily use, he himself is covered with innumerable bacteria in a dried condition, and by means of the movements that are made during the milking, where his person comes in contact with the cow, numerous dust particles are dislodged that bear their quota of germs into the rich nutrient fluids below. A large amount of this filth and dirt can be easily avoided, but even the best cleaning in a dry manner of the particles of excreta and loose hairs from the animals' coat only partly diminish the number of germs. Bacteria are dislodged from a dry surface with great ease, but a thoroughly moistened surface, however rich in germs, affords very little chance for them to be shaken off. Applying this course of treatment to the cow, a very marked diminution in the amount of bacterial life that finds its way into milk will be noted. The flanks and under parts of the animal, especially the udder, should be thoroughly moistened with water and then wiped, so that it will not drip from the surface. The hairs of the coat must be thoroughly wet, and, if this is done, the myriads of germs that are invariably present, even under the most favorable conditions, will be quite effectually held in place.

The hands of the milker should also receive a similar treatment. Just previous to the milking, they should be thoroughly cleaned with soap and hot water and then dried. The habit of moistening the hands with a few drops of milk just before milking is to be deprecated from every standpoint, but especially so when considered from our present point of view. It is worth while to have the milker clothed in a suit kept for this purpose, especially the upper portion of the body. An outer garment could easily be slipped over the regular working clothes. This garment should be laundried at frequent intervals. In order to show what effect the animal has upon the possible contamination of milk, the experiment described below teaches a valuable lesson.

One of the cows that had been running in a lowland pasture was taken for the experiment and the milking done out of doors, so as to eliminate as much as possible, the influence of the germs in the barn air. Without any special precautions being taken, the cow was partially milked, and during this operation a covered glass dish containing a thin layer of sterile gelatin was exposed underneath the belly of the cow in close proximity to the milk pail, for sixty seconds. The udder, flanks and legs of the cow were then thoroughly moistened, and all of the precautions referred to before were carried out and the milking then resumed. A second plate was then exposed for the same number of seconds, and in the same place as the first; a control also being exposed at the same time at a distance of ten feet from the animal and six feet from the ground to ascertain the germ content of the surrounding air. These cultures were kept intact for several days until the germs that had fallen on the surface had developed into visible patches or colonies. By counting these and determining the ratio between the exposed culture plate and the open surface of the milk pail, some idea may be gained of the extent of the contamination derived from the animal.

From this experiment the following instructive data were gathered. Where the animal was milked without any special precautions being taken, there were 3,250 germs *per minute* deposited in a ten inch milking pail. Where the cow received the precautionary treatment as suggested above, there were 115 germs per minute deposited on the same area, besides 40–50 spores of moulds. In the control plate that was exposed to the surrounding air at some distance from the cow, there were sixty-five bacteria and over one hundred moulds. From this it will be seen that the shaking loose of a large number of organisms from the dry under parts of the animal can be almost entirely done away with if some such simple precautions as these are carried out.

35. Contamination from the air:

Besides the dust and dirt that are directly derived from the animal and the milker, a considerable number of germs always find their way into the milk from dust in the barn air. Barn air owes its germ content to the influence of several factors. The use of dry fodders, like hay, straw and corn and the custom of bedding the animals with coarse refuse and litter adds greatly to the number of germs to be found in barn air. Unless manure is removed at frequent intervals, the coarser particles become dried and these contribute their quota of organisms to the air. The presence of these dust particles is always accompanied by large numbers of microbes, especially those forms that can withstand drying, so that the air of the barn is invariably richer in germ life than the open air. Several epidemics of milk troubles have been traced to this cause. Weigmann mentions a case that came to his attention in which the milk had a soapy flavor (see **55**). He isolated the organism from the milk that was able to cause this change and also found it in one instance to come from the straw that was used as litter for bedding. In another case, it was present in the hay and doubtless gained access to the milk by the dust that was raised during feeding.

It is of course, impracticable to attempt to free the barn entirely from dust, but much could be done toward this condition with a little forethought. The common practice of feeding while milking, on the ground that the animal is more contented and will give her milk more freely, aids materially in increasing the germ life in the air of the barn, if the fodder is of a dry character. Moistened fodder is not so objectionable on this account, and if it is deemed necessary to feed in this way, this moist feed can be substituted for the dry coarse fodder and so lessen materially the germ content of the air.

If it is necessary to use dry fodder, the danger can be minimized by feeding it an hour or more before milking. If this is done, most of the dust that is raised during the feeding will have settled by milking time. The majority of bacterial germs present in the air are attached to dust particles so that they quickly settle by gravity if the air is not disturbed. Even a short space of time suffices to clear the air of the greater part of these microbic impurities.

The experiment of exposing sterile gelatin plates at the same point in the barn during the time in which the herd was being fed with hay and also fifteen minutes thereafter, showed that three-fourths of the germs present in the air during the dry feeding were deposited even in this length of time.

36. Removal of milk from barn and straining:

The common custom of allowing the milk to remain in the barn until the milking is completely finished and also the straining of the milk in the stable are entirely contrary to the teachings of the preceding pages. If the milk is removed to a separate room, and for this purpose in a well organized dairy a separate room should be arranged, then the constant deposition of germ life from the infected barn air will be largely excluded. Still more important is it to strain the milk in a separate room arranged for this purpose. The process of straining exposes so much surface to the air that it should be done under conditions that do not favor the easy access of organisms as is the case when this operation takes place in the stable.

37. The influence of temperature on freshly drawn milk:

In considering the influence of these various factors that assist in peopling milk with bacterial life, another most important element must be included—that of the temperature at which the milk is kept. The actual number of germs present in any milk is dependent more on this factor than upon any other single one, for if only a very few germs gain access to the milk, these are able to multiply in a short time to an enormous extent, if the temperature of the fluid is at the optimum growing point. The rate of development of different species of bacteria varies somewhat but in general the warmer temperatures in the neighborhood of 90° F favor the more rapid growth of most forms. Von Freudenreich found that the bacteria in a sample of milk increased in the following ratios when kept at varying temperatures :

	3 hrs.	*6 hrs.*	*9 hrs.*	*24 hrs.*
When kept at ....59° F.	1.+	2.5.	5.	163.
" .... 77° F.	2.	18.5.	107.	62100.
" .... 95° F.	4.	1290.	3800.	5370.

It will be noted that an increase in temperature usually produces an acceleration of the rate of development although this does not uniformly take place. Many species develop better at comparatively low temperatures than at a high temperature, like that of the blood heat. For this reason, it is indispensably necessary that milk should be cooled with the greatest possible alacrity as soon as it comes from the cow. The temperature at which the milk leaves the animal greatly favors the growth of the germs that will gain access even under the most favorable conditions. If this milk is allowed to cool naturally, it will take several hours before it reaches the temperature of the surrounding air. During this time, the organisms that are derived from the udder of the animal are continuing their rapid growth, while those forms that come from dust and dried surfaces, and are presumably in a latent state on account of their condition awake from their lethargy under the influence of these favorable surroundings. If organisms once gain an entrance and begin to germinate, it requires a considerably lower temperature to successfully check development than it does to hold latent organisms, like spores, in a condition where germination will not take place.

To hasten this lowering of temperature, the use of aerators has been recommended. Coolers using running water or ice water are also very efficient in reducing the animal heat to a point where development is much checked; but in using apparatus for this purpose it is well to take into consideration, the amount of exposure of the milk to the air, also the ease with which these devices can be thoroughly cleaned.

38. Results of experiments:

These foregoing factors account for the presence of the different bacterial germs that are to be found in milk. They are, however, subject to such great variations, that only general deductions can be drawn from them. A systematic adherence to the suggestions indicated above will materially decrease the number of microbes present, especially the more noxious ones that are derived from filth masses, and at the same time increase the keeping quality of the milk to a marked degree. This is a condition to be desired whether the milk is used in a natural state or whether it is worked up into other dairy products. In a series made to determine how much the bacterial flora of ordinary milk could be diminished, by the simple observance of the above principles, the following data were collected. In the month of October, the mixed milk of the University herd taken under ordinary conditions had 15,500 germs per cc., while that of a cow that had been carefully cleaned and the milking done in the manner already suggested had only 330 bacteria for the same volume. A repetition of this same experiment in February under winter conditions revealed 7,680 germs per cc., in the milk secured without special precautions, while the number in the milk that had been taken with care was reduced to 210 germs per cc. These results were obtained under conditions that can be put into actual practice, and in this way, the germ content of the milk can often be reduced eighty or ninety per cent. and sometimes even more.

39. Extent to which factors of milk contamination can be controlled:

The influence of these various contaminating factors can be largely minimized and in some instances quite eliminated. A goodly number of the bacteria present in the fore milk can be thrown out by milking the first few streams on the ground or barn floor, although this factor can be controlled to a less degree than any of the others. Dust in the air of the barn is a minor factor, yet the influence of this can be materially reduced by exercising care relative to feeding dry fodder or bedding the animals just previous to or during the milking. The number of germs derived from the animal itself and the milker can largely be decreased by thoroughly moistening the udder and flanks of the animal and having the milker milk with clean hands. The effect of contamination arising from imperfectly cleaned milk vessels can be entirely excluded by sterilizing all such utensils in steam or even in scalding water for a short time.

40. Precautions easy to observe:

These precautionary measures were carried out by an ordinary workman who had charge of the milking and in no sense are to be regarded as being made with more care than should always be given to such work as this. It must be borne in mind that all of the fermentative changes that occur in milk are due to the presence of these living germs that largely gain access to it subsequent to its withdrawal from the cow. If these are excluded, milk will remain normal. To completely exclude all these forms is of course impossible and impracticable but by constant watchfulness a great deal can be done in this direction. This subject is deemed of such importance that a summary of the above suggestions is here appended.

41. Résumé of precautions to be noted in securing milk as free as possible from bacteria:

Precautions before milking.

1. Remove fouled straw from stall that has been used for bedding.

2. Do not feed dusty fodder like hay or cornstalks just before milking.

3. Ensilage, turnips, etc., should not be fed *immediately* before milking.

4. See that all vessels that come in contact with the milk are thoroughly cleansed with steam or scalding water.

5. Brush with a stiff brush all loose hairs and filth from the udder, belly, flanks and tail of the cow.

6. Thoroughly moisten these under parts with pure clean water.

7. Remove excess of water so that no drip occurs.

8. Milker's hands should be carefully cleaned with soap and water, and then dried immediately preceding the milking. A clean outer garment (kept for the purpose) should be slipped over working clothes during the milking.

Precautions during and subsequent to milking.

1. Reject the first few streams from each teat.

2. Hold milk pail so as to diminish the opportunity of hairs falling into milk as much as possible.

3. Remove milk from barn to a clean, dry room, as soon as pail is filled.

4. Strain, aerate the milk and cool as rapidly as possible by immersing in ice water or cold running water.

5. See that cans used for setting are protected from the dust.

CHAPTER III.

SOURCES OF MILK INFECTION IN FACTORIES.

42. **Treatment of milk in factories and creameries:** The suggestions in the previous chapter apply primarily to the treatment of milk while it is being handled on the farm. Where it is taken to a creamery or cheese factory, other factors come in to increase the amount of infection and thereby lessen its keeping properties.

Butter and cheese making both require the presence of bacteria, but the germ life must be of the right kind, otherwise they are undesirable. For this reason, the maker wishes the milk to be as free as possible from all contaminating influences. If the previous suggestions (see **41**) concerning the handling of milk are intelligently followed, the raw material will be turned over to the factory in prime condition; but the rational treatment must be continued in the creamery if the best of products is to be expected. Numerous influences are at work in the factory that add their mite to the milk in different stages of its manufacture and ultimately affect in a serious way the final product. Of course, very much depends upon the proper observance of physical conditions, but a brief outline of some of the possible dangers will also be pertinent.

The cardinal precept in the factory as well as the farm dairy should be cleanliness. Cleanliness here must must not be taken to mean a mere absence of dirt and filth, but all utensils that come in actual contact with the milk should be rendered as germ-free as possible.

From the time that the milk enters the weigh can until the butter is in the tub, and the cheese on the curing shelf, it should be remembered that many opportunities for infection are always present.

43. Infection from unclean utensils:

In the creamery there are nearly the same set of factors of infection at work as are to be found in the farm dairy. The milk receives continually a large quota of organisms from cans, vats, churns and other utensils with which it comes in contact. In the factory where steam is at all times accessible, there is no excuse for uncleanliness of any sort. Most of the tin ware can easily be arranged so as to be steamed daily. Open vats can be successfully sterilized by covering them with a heavy canvas vat cloth that is used in cheesemaking. The separator bowls, churns, cans and dippers should all receive a daily steaming. The rational nature of this treatment is seen in the case of cans and dippers that are used indiscriminately in handling the fresh milk or the more highly contaminated waste products as buttermilk and whey.

44. Infection from air:

The air of a good creamery should be relatively free from dust, because as a rule the floors are damp most of the day and consequently but little dust can arise from them. The majority of the germs found in the air of a well regulated creamery come from the person of the attendants. Germ life from this factor can be largely minimized by having the attendants properly attired in caps and suits provided for this purpose. These should be of white material so that any dirt may be quickly recognized and should be laundried at necessary intervals. If this precaution is observed, the value of it is largely minimized, if the separator and ripening rooms are accessible to the general public, especially to those persons such as farm hands that come continually in contact with dirt of all descriptions. Ripening vats should at all times be covered with a tight canvas frame so as to exclude dust and dirt of all kinds.

45. Infection from water and ice:

The water and ice supply of a creamery should also be carefully inspected. Water, and to a less extent ice, always contain bacteria in varying numbers so that it is possible that a serious fault may be introduced from this source. Most creameries derive their water supply from private wells and in using these, care should be taken that they are arranged so as not to receive any surface drainage. A deep well from which the water is used in large quantities, if properly arranged, will contain the minimum number of germs as the bacteria in the water of the soil are filtered out in passing through thick soil layers. The majority of the organisms in the ice are destroyed by freezing (60 to 90 per cent.) so that water in this form is relatively freer from microbes than that in a fluid condition. Often, however, ice is secured from shallow, still ponds where the germ content is extremely high, in which case, water even when it is frozen, may contain a large number of organisms.

CHAPTER IV

DISTRIBUTION OF BACTERIA IN MILK.

46. Numbers of bacteria in milk:

The germ content of milk varies so greatly that unless the conditions are all known, it is impossible to foretell what may be found therein. An examination of milk will often reveal a difference in numbers ranging from a few score of germs to hundreds of millions per cc. The presence of such an enormous multitude in any milk is entirely dependent upon certain factors, such as the age of the milk, the care taken during the milking, and also the way in which it has been handled since that time. Disregarding milk of different ages, the number of germs present in any sample is often dependent upon the amount of dirt and filth with which it has come in contact since it was drawn from the cow. Bacteria and filth of all kinds are so intimately associated with each other that the presence of one rightly presupposes that of the other.

As to the numerical content of any milk, there is such a wide variation that figures are of little worth. In fact, the determination of the number of germs that any sample possesses has only a relative value because of this extremely wide variation.

The studies by different observers have been carried out under such diverse conditions that no comparison of the results can well be made. Here in this country but little work has been done in this direction, but in my experience, milk as it is sold here to the consumer seems to contain fewer bacteria than that retailed in European cities. A few determinations of the bacterial contents of European milks that have been analysed biologically will illustrate this point.

Renk[2] found in Halle milk supply 6–30,000,000 germs per cc.; Cnopf[3] in Munich milk supply 200,000–6,000,000 per cc.; Uhl[4] in Giessen milk 83,000–170,000,000 per cc.; Clauss[5] in Wurzburg 222,000–23,000,000 per cc.; Bujwid in Warsaw an average of four million per cc. and Knochensteirn[6] in Dorpat 25,000,000 per cc. Sedgwick and Batchelder[7] report fifty-seven samples of Boston milk as containing from 30,000–4,220,000 per cc. In the country, they found in the milk fresh from the cow 30,000 and in the milk as used on the table, about 70,000 organisms per cc. In my experience, the mixed milk of a herd that is kept with any reasonable degree of cleanliness if examined immediately after it is milked usually will not exceed from 5–20,000 germs per cc. The number present in any milk is due to the influence of so many factors that it is practically impossible to establish any number as a *normal*, although Bitter[8] sets 50,000 germs per cc. as a maximum limit for a milk intended for human food. The milk as delivered by the milkmen to their private customers in the city of Madison ranges from 15,000–2,000,000 organisms per cc. varying mainly with the season of the year.

The presence of such an enormous multitude of germs in a food product need not necessarily occasion alarm from a hygienic standpoint, although it is quite probable that the presence of large numbers of decomposition bacteria may have an irritating effect upon a deranged digestive tract, and thus produce intestinal disturbances, especially with infants during the summer months.

If we compare the bacterial flora of milk with that of sewage, a fluid that is always rich in germ life, it will almost always be observed that milk when it is consumed, is richer in bacteria by far than the sewage of our large cities. Sedgwick[9] found that the sewage of the city of Lawrence, Mass., from January, 1888, to October, 1889 contained at the lowest, 100,000 germs, while the maximum number was less than 4,000,000 per cc.

47. Kinds of bacteria in milk:

In connection with this, it is only necessary to observe that the numerical contents of a fluid are not as important as the kind of bacteria that are present. While milk may contain forms that are known to be injurious to man, yet the great majority of them have no apparent effect on human health. In their effect on milk itself, it is however much different. Most bacteria that gain access to milk, influence the keeping quality of the fluid to a marked degree. They set up a series of manifold changes of a fermentative character, whereby the value of milk as a food is greatly diminished. In some of the dairy industries, the presence of certain kinds of bacteria in milk are of undoubted advantage, but where they are introduced through careless handling, it is impossible to assume that favorable species alone are introduced. Left to this haphazard method, many forms gain access that are more or less harmful, owing to the undesirable changes that they bring about in milk.

It is therefore, highly important not only for the milk-handler but for the butter and cheese-maker, that the germ contents of milk should be reduced to the lowest possible numbers. It will be impossible, owing to the universal distribution of bacteria and to the methods by which milk is secured, to eliminate entirely all germ life from milk, but this can be accomplished to such a degree as to materially lengthen the period during which milk will remain in a marketable condition.

The kinds of bacteria that are found in milk depend upon the treatment that the animal receives. The milk from cows kept on pasture land, especially if they are milked in the open air, contains less bacteria than that secured from herds that are continuously stabled. Not only is there a marked difference in numbers but also in kinds. Those species that are found commonly associated with animal excreta and which as a rule are the most detrimental to milk, abound in larger numbers in the confined stable than in the open. Undoubtedly there is also a difference in regard to milk from cows that are kept on different kinds of pasture, although this point needs further study.

CHAPTER V

FERMENTATIONS IN MILK AND SUGGESTIONS AS TO TREATMENT.

48. Introduction:

In one sense, the action of almost any species of bacteria in milk may be regarded as some sort of a fermentative change, for almost all forms of germ life that gain access to this medium are able to grow therein and modify to a certain extent the chemical constitution of the milk.

In discussing the fermentations that occur in milk, it is impossible in the present state of our knowledge to classify them in any other than a most provisional way. While our knowledge of some of them from a bacteriological standpoint is fairly complete, so many of them, as yet, have been studied only superficially that we are not in a position to classify them as we would other chemical changes.

The various fermentations are sometimes classed according to the substances in the milk upon which they chiefly feed, such as those affecting the milk sugar and the casein. This division is more or less confusing because so many organisms draw from the different food elements, the materials for their growth. A more satisfactory division would be one based upon the products manufactured during the fermentation, but even here confusion is liable to enter to some extent, because in many cases there are several distinct substances formed.

Milk is such a complex substance that the changes produced by a single germ are often so numerous that the processes cannot be separated in their reactions. We must then, in speaking of these fermentations remember that we are considering under one name, perhaps, a single fermentation, but it is more than probable that there are a number of different fermentations, in which the most marked and pronounced change is alone considered. For example, there is a fermentation classed under the head of the butyric changes, a decomposition process in which butyric acid is the chief product formed, but this may be associated with an alkaline condition of the milk and the production of a bitter taste in the same. Thus, the subdivision followed here will of necessity be imperfect and occasional instances will be noted where some changes in milk might well be described under several heads.

Some of these fermentation changes occur so constantly that they are to be regarded as normal in their character. Normal, however, only in the sense that the bacteria that cause them are so widely distributed that the change is inevitable and not that the milk itself would undergo any of these changes if it were not for the presence of the different germs that bring them about. Of such a nature is the lactic acid fermentation, by far the most common change that occurs in milk.

49. Lactic acid fermentations:

Milk naturally undergoes a spontaneous change in its composition and appearance known as souring if left undisturbed for several days at ordinary temperatures. Milk so affected assumes a semi-solid condition and is sharply acid to the taste. This change is so universal that it may in a certain way be called normal, although it is now well known that the change does not take place in milk, in and of itself, but requires the presence of certain forms of organic life.

In this fermentation, the milk sugar is the element that is directly affected. This breaks up under the action of the various acid-forming bacteria. The casein is present in sweet milk, partly in solution, partly in a suspended condition. This nitrogenous compound will remain in this state so long as the milk does not possess more than a small amount of acid, but when the acidity exceeds a certain percentage, then the casein is precipitated as a white semi-solid mass and the milk is said to curdle or coagulate.

Earlier studies on lactic fermentation. Although this lactic fermentation has been studied longer than any other of the fermentations in milk, yet our knowledge of it is by no means complete.

The fact that sour milk contained living organisms had been known since early in the eighteenth century, but no comprehensive study of the subject was made previous to the work of Pasteur,[10] which was done between 1850 and 1860. He saw and described an organism that he considered the cause of the souring process. Of course his descriptions are imperfect in the light of our present knowledge, because he knew nothing at that time about purifying his cultures. Pasteur's work was perfected by Lister,[11] the great English surgeon, who succeeded in separating the lactic acid germ from other bacterial forms. The organism which he called *Bacterium lactis* was found plentifully distributed in the neighborhood of dairies, but the air of houses, hay barns or the open air was almost always free from it.

More recent investigations. Lister's work was still further advanced by Hueppe[12] to whom we owe the most complete study of this question that has ever been made. He worked with the more modern and accurate bacteriological methods and was able to isolate a number of different organisms from souring milk that were concerned in this process. The principal germ in this connection he called *Bacillus acidi lactici* which is probably the same germ that Pasteur studied. This short and plump bacillus is usually found either singly or in pairs. In gelatin, it forms colonies that are merely tiny white points. When growing on the surface of the medium, they spread somewhat, developing an irregular edge that is translucent. Often the colonies have an appearance in reflected light resembling porcelain. In gelatin tube cultures, growth occurs all along the track of the inoculation needle and later spreads out on the surface to a limited distance from the needle point, but does not liquefy the gelatin.

According to Hueppe, it begins to grow in the neighborhood of 50° F., but forms no acid until the temperature reaches 59° F At 140° F. its development is stopped. Although it is found in different localities in Europe, it is by no means the only organism that is concerned in the souring of milk. Grotenfelt[13] and Marpmann[14] have described a large series of other organisms possessing the similar function of changing milk sugar into lactic acid and other products. Conn[15] claims that he has never yet been able to isolate Hueppe's bacillus from any sample of milk that he has examined in this country, although he has found a large number that affect milk in this way.

This lactic acid fermentation is then not to be ascribed entirely to the action of any single, specific germ, but to a series of organisms more or less closely related, whose most prominent physiological function is expressed in this change.

Chemical changes involved in process. From a chemical standpoint, this conversion can take place very easily, but it is well known that it does not always occur in such a direct manner. Thus, a molecule of glucose can break up into two molecules of lactic acid directly, as is shown in the following equation.

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} = \underset{\text{(lactic acid)}}{C_3H_6O_3} + \underset{\text{(lactic acid)}}{C_3H_6O_3}.$$

In the souring of milk, there are other compounds, such as carbonic acid gas (CO_2), acetic ($C_2H_4O_2$) and formic (CH_2O_2) acids, hydrogen (H), nitrogen (N), and marsh gas (CH_4) that are evolved so that the process as it occurs in nature, cannot always be represented as taking place in the direct manner mentioned above.

A peculiar feature concerning the sour milk fermentation is that the activity of the germs concerned is checked and finally stopped by the accumulation of its own waste products. After the acid produced by the growth of the sour milk organism exceeds a certain percentage, (0.8% in the case of Hueppe's germ), the continued formation of lactic acid ceases altogether, although the amount of milk sugar that is in the milk is much greater than that which has been consumed. If the acid present is neutralized, so that the reaction of the liquid becomes once more favorable to the growth of these organisms, the continued growth of the germs will be resumed.

Lactic acid bacteria are so universally present in milk that their presence exerts a rapid and powerful effect on this food product, soon rendering it worthless for consumption. In the manufacture of milk into dairy products, this fermentation is not entirely obnoxious. In both cheese and butter making, the production of a certain amount of acid is deemed necessary. However, the presence of some of the acid forming bacteria is not especially desirable, as for instance, those that form gases like CO_2 and H, in the continued decomposition of lactic acid into simpler substances.

In cheese making these fermentations of the milk sugar are often very troublesome, as they cause the cheese to swell and crack, owing to the gaseous fermentation that is carried on in the curd.

50. Rennet and peptonizing fermentations:

Often in milk that has stood for some time, a change is to be noted that is entirely different from the ordinary souring. The milk loses its pure white appearance and becomes watery. The casein usually is more or less coagulated although the appearance is by no means constant. The curd formed by this coagulation is markedly different from that which is formed by the lactic acid organisms. Sometimes the soft curd masses undergo only a partial digestion; sometimes they are completely dissolved, so that nothing remains but a watery fluid of varying consistency and color, and a small mass of flocculent material on the bottom of the vessel.

These changes that often occur in milk are brought about by the growth of a large class of germs of both aerobic and anaerobic character, and the series of chemical changes that occur vary widely although the result may be ascribed to two different types of fermentative action. The curd formed by these changes is almost always characterized by a soft gelatinous condition. The reaction of the fluid is often alkaline but with some germs it is somewhat acid (mainly butyric acid) although the amount of acid is rarely ever as much as it is in the pure lactic acid fermentations.

Sweet curdling of milk. The formation of this curd in an alkaline condition of the milk is produced by the action of a ferment allied to animal rennet, that is excreted from a series of different organisms that have this characteristic in common. Our knowledge concerning the chemical changes in these fermentations is as yet very meager, but the labors of Duclaux,[16] Warington[17] and Conn[18] have already thrown much light on these imperfectly known problems.

Digestion of casein. In almost all cases, the presence of a rennet ferment capable of coagulating milk is associated with another that is able to digest the casein. Conn and Adametz have shown in a few instances that the digestive property may be present in a species that has no curdling power although this is rare. This digestion of the curd formed by the milk makes a profound chemical change in the constitution of this fluid. The casein is the main substance that is affected and this is rendered soluble, so that peptones and soluble albuminoids are always present in this digested milk. This action is quite comparable with the tryptic change that occurs in the digestion of proteid materials by animals, where the trypsin, the active principle of the pancreatic fluid, changes the insoluble proteids into a soluble form. Duclaux,[19] who has studied these milk changes very thoroughly, has given the name of *casease* to this enzyme. The casein is changed under the influence of this ferment into *caseone.* Weigmann has also succeeded in isolating this unorganized ferment casease, from cultures having this digestive function. It is worth noting in this connection, that this digestive action in milk is almost always associated with the power of liquefying gelatin which is another expression of the peptonizing function.

Although the rennet and peptonizing functions of various species are closely associated, yet they should be regarded as separate and distinct chemical changes.

The earlier workers in this line failed to isolate the two ferments from each other, but this has recently been done by Conn who succeeded in 1892, in separating the rennet enzyme from bacterial cultures in a tolerably pure condition. The appearance that milk presents after being acted upon by these digesting enzymes varies greatly. Sometimes the casein is entirely consumed leaving either a clear transparent fluid or a cloudy turbid liquid that may be almost any tint or color. Notwithstanding this great variety as to color, consistency and general appearance, these changes are in the main to be classified for the present under the rennet and peptone forming ferments.

Their importance in milk is considerable. In ordinary milk, they often appear as a subsequent fermentation to the regular lactic change, and in the ripening processes in cream and cheese-making, their effect is more marked. In all probability, they are intimately concerned in the curing of cheese in which the casein is broken down into soluble compounds.

51. Butyric acid fermentations:

Another fermentation that may be considered as a class fermentation is the one in which butyric acid is formed as one of the chief products of decomposition. This property is now known to be present in quite a number of the bacteria of both aerobic and anaerobic classes, and the number of species is constantly increasing as the subject receives more careful study.

Pasteur was the first investigator to separate a butyric ferment from the numerous other changes that occur in milk. Although he did not succeed in obtaining his cultures in a perfectly pure condition, he undoubtedly studied an organism that had the property of forming butyric acid in milk. He separated his germ from boiled milk, and it possessed the peculiar property of being able to thrive without access of atmospheric air. These anaerobic germs in milk are now known to be largely butyric acid forms, although the production of this acid is by no means confined to this class of organisms, for Loeffler and Hueppe have both found organisms growing in milk under ordinary conditions with free access of air that were able to produce butyric acid. Recently several other anaerobic forms have been isolated by Kedroski[20] and Botkin[21] from milk. Botkin's germ in milk under anaerobic conditions undergoes a violent fermentation giving off large quantities of gas. The casein of the milk is rapidly dissolved and the milk soon changes into a transparent yellow fluid. Often this butyric fermentation is associated with an alkaline condition of the milk, but this is not always the case. Several butyric organisms from milk have been studied that rendered it acid in its reaction so that the alkaline reaction is not necessarily associated with the production of butyric acid.

Most all of the butyric acid organisms are spore-bearing so they are frequently to be found in samples of sterilized or boiled milk. Germs of this type are almost always present in ordinary milk, but owing to their predilection for anaerobic conditions, and the fact that the acid usually present inhibits their growth, they rarely appear in raw milk. Our knowledge concerning this fermentation is far from being complete, but if it is regarded as a chemical change induced by a certain class of organisms instead of a specific germ, then much of the confusion that exists can be understood.

The formation of butyric acid cannot be regarded as a distinct process, for it is often a bye-product in other proteid decompositions. In some instances, the butyric acid is the chief end-product of the breaking down process, then again, it is associated with the production of leucin, ammonia, butyl alcohol, tyrosin and other compounds.

52. Bitter fermentations:

Another abnormal condition in milk is where it acquires a bitter taste that is often so pronounced that the milk is rendered unfit for food purposes. Bitter milk is due to several causes, some of which are due to improper feeding, while with other cases, the trouble is directly caused by the presence of various forms of bacteria that produce as a result of their growth in milk, these extremely bitter soluble substances. With cows that are far advanced in lactation, a bitter salty taste is occasionally to be noted. This is an individual peculiarity of the animal and is in no sense a bacterial trouble. Cows often eat herbs such as lupines that contain a bitter principle and the milk is affected in this way.

All of these non-infectious troubles, however, can be easily separated from those of an infectious nature. Troubles due to bacterial origin are always more prominent as the age of the milk increases. In regard to positive knowledge concerning the production of those substances that cause a bitter taste in milk, nothing definite was known prior to 1890. Earlier observers noting this condition, had associated it with the formation of butyric acid, but no thorough study of the subject was made until Weigmann[22] published his researches in this year. He showed that the bitter taste might arise without any butyric acid being present; the bacillus that he isolated from milk producing the bitter condition as a result of the decomposition of the casein in the milk.

In this same year, Conn[23] isolated from a sample of bitter cream, another organism belonging to the coccus type that rendered milk strongly acid and extremely bitter. In this case, the bitter taste was associated with the production of butyric acid. Hueppe has suggested that as the bitter taste is sometimes caused by proteid decomposition, it may be due to the formation of peptones that often have a bitter taste.

Most cases of bitter milk due to bacteria are found in milk that has been cooked. After standing for a time, the bitter taste develops. The explanation of this is due to the fact that the bacteria producing the bitter substances are usually those that possess endospores and that while the boiling or sterilizing of milk easily kills the lactic acid germs, these forms on account of their greater resisting powers are not destroyed by the heat.

53. Slimy fermentations:

One of the most troublesome of the abnormal fermentations is that where the viscosity of the milk is greatly increased, so that it may be drawn out in long threads. Milk so affected is called slimy, stringy, ropy or thready, to indicate the peculiar character of the fluid. The viscosity of milk varies much with different slime producing organisms; some making it merely thick-flowing, while with others, the milk adheres to anything that touches it, drawing out into threads from several inches to several feet in length.

This ropy condition in milk often occurs during the warm season and is a source of considerable trouble in some creameries and factories. Its presence prevents perfect creaming, as the globules of butter fat are unable to rise, owing to the viscous nature of the fluid. It has naturally an injurious effect also upon the quality of the cream and renders milk useless for drinking purposes.

Desirable cases of slimy fermentation. While in general, slimy milk is undesirable, yet there are some cases where it has been used to advantage. In Holland, in the manufacture of Edam cheese, a slimy fermentation of the milk is sought for, and in Norway, a popular drink (Tättemjolk) is made from milk that has been infected with the leaves of the common butterwort, *Pinguicula vulgaris*, that seems to have the property of setting up a slimy change in milk. Weigmann has isolated a bacterial form from the leaves of this plant that is able to cause this slimy change in milk so that the change is due to the germs on the surface rather than to the plant.

This ropiness in milk has been ascribed to various causes, such as an inflammatory condition of the udder, errors in feeding, etc., but in the main these are not the real causes. In almost every case, the viscous condition is brought about through the influence of some form of germ life. All of the different phases of this slimy change in milk are not due to the action of a single form, but under this head are grouped a series of fermentations, inaugurated by various organisms, but possessing the common peculiarity that the milk is changed into a much more viscous fluid.

Various species of slime-producing organisms. Nearly a score of different species of bacteria have been found that have this property of turning milk into a more or less slimy fluid, although all of these are not of equal importance in connection with milk as it occurs under natural conditions. Among the earlier studies on the cause of slimy milk were those of Schmidt in 1882.[24] He separated from a milk that had undergone a slimy degeneration, a motile micrococcus that was the true cause of the change, although he failed to study the organism carefully by culture methods. This germ grew best between 86°–104° F., and developed mainly at the expense of the milk sugar. In cultures grown in milk for ten days, there was only 2.37 % of milk sugar remaining, while the fresh milk contained 4.77 %. When grown in nutrient peptone solution to which pure milk sugar had been added, the slimy change was also to be noted. The same was true with other sugars like glucose, cane sugar and mannite. This germ is easily destroyed at 140° F., but frost seems to have no effect upon it.

Adametz[25] studied very carefully a germ that he found in milk and also in fresh streams near Vienna. This germ which he called *Bacillus lactis viscosus* made milk very stringy so that it could be drawn out into long threads. The change at the room temperature, however, took place so slowly that in ordinary cases, this germ could hardly have any effect.

The chief cause of ropy milk that has been found to be present in Switzerland is *Micrococcus Freudenreichii*. This germ has been isolated by several observers in different places, so that it is not a local trouble. The organism is a large immotile coccus that is able to liquefy gelatin. So marked an effect has this germ that in five hours, a change is to be noted in the milk. M. Freudenreichii is easily killed by heat, two minutes at 212° F., being sufficient. When dried, it possesses great resisting powers so that if it once gains a foothold in a dairy, it is difficult to get rid of it unless very thorough measures are resorted to.

Weigmann[26] has isolated from the viscous whey that occurs in the manufacture of Edam cheese, a micrococcus form that grows in long chains and is capable of producing ropy milk in twelve hours.

The ordinary potato bacillus, *B. mesentericus vulgatus*, is able to produce a viscous condition in milk although it does not render it ropy or stringy. This germ is so widely distributed that it is a common inhabitant of milk and on account of the very resistant spores that it forms, is difficult to eradicate it if it once gains the supremacy in the milk.

Several other slime producing organisms have been isolated from different sources and probably further study will reveal a still larger number that are concerned in this change in milk. A careful study of almost any sample of milk examined during the hot weather will show the presence of a few organisms of this class, and it only needs favorable conditions of growth for them to obtain the ascendency from time to time.

In some cases, the slimy material is merely the swollen outer cell membrane of the bacteria themselves; in others it is due to different substances formed from the proteids in the milk, and occasionally the milk sugar.

54. Alcoholic fermentations:

Most solutions containing sugar are very apt to undergo an alcoholic fermentation if they are allowed free access to the air. This change is usually induced by the presence of yeasts of different kinds that have the power of breaking up the majority of sugars and producing alcohol and carbonic acid gas. These yeast organisms are not to be confounded with bacteria. They are much larger than the bacteria and grow in a different manner.

Lactose or milk sugar, however, is not readily acted upon by the ordinary organisms of alcoholic fermentation, so that the production of alcohol in milk in any considerable quantities is not a common occurrence. Alcohol is often present in small amounts as a result of some of the lactic acid fermentations, especially with those in which CO_2 is disengaged.

Duclaux[27] mentions an instance in which an alcoholic fermentation of milk in a dairy proved to be a serious trouble. The milk would not keep and it was impossible to make butter from it. A change of pasture and stables also failed to improve the quality of it in the least. He was able from the affected milk to isolate a peculiar form of yeast that brought about the change; and after this was excluded by sterilization of vessels, etc., the trouble disappeared.

Although milk does not often undergo alcoholic fermentation naturally, there are two artificial preparations of an alcoholic nature that are made from this fluid.

One of these is *kumiss* (also koumiss, kumys) an alcoholic beverage that has been used for centuries by the wandering Tartar tribes of the Orient. This beverage is prepared from mares' milk. A similar product is now prepared from cows' milk by the addition of a small amount of cane sugar and the subsequent infection of it with yeast. This beverage possesses certain advantageous properties for invalids, inasmuch as the casein is precipitated in a finely divided state and consequently is more easily digested than ordinary milk, which curdles in large masses under the influence of the gastric juice. Our knowledge concerning the exact changes that occur in natural kumiss and the causes of the same, is extremely vague. Several different organisms, yeasts as well as bacteria have been isolated, that probably take part in the fermentative process. The different elements in the milk are decomposed under the action of this mixed series of organisms. A portion of the milk sugar changes into lactic acid; also a part into alcohol. The casein is likewise profoundly affected, a large part of it, according to Dochmann, being changed into soluble substances.

Another alcoholic beverage that is in common use among the people of Caucasus is *kephir* (also kefyr, kéfir). This is a sour, effervescent alcoholic fluid prepared from the milk of goats, cows or sheep. The direct cause of the fermentation is the so-called kefir grain, a yellowish, irregular mass about as large as a walnut that is added to the milk. These grains are left in the milk for about a day; the milk is then poured off and the grains dried and preserved for future use. This milk is then mixed with fresh milk and kept in leather flasks and soon a mixed fermentation sets in.

This alcoholic change has been studied considerably from a biological point of view, but as the ordinary methods of preparation are so crude, it is difficult to tell just what germs enter into the process. It is evidently a mixed fermentation, and one in which no single organism can produce all of the essential ingredients. The sugar of milk is partially converted into alcohol but at the same time the casein is coagulated and digested to a certain extent so that the process is exceedingly complicated. Quite a large number of different organisms have been isolated from this material. Some of them have been found to be able to produce a change allied to that which is seen under natural conditions. A yeast form is probably the main cause of the alcoholic fermentation while different forms of bacteria enter in to change the other constituents of the milk.

55. Soapy Milk:

Weigmann and Zirn[28] have recently detected the cause of a new milk trouble. The milk which they examined had a sharp pungent odor and foamed when shaken. It did not sour properly and in the process of butter making, the butter came only after a long delay. The milk when made into cheese acted quite normal but later developed a bad taste. The milk was also characterized by a strong soapy flavor. These investigators traced this soapy appearance and flavor to the action of a germ that they isolated from the milk. They also succeeded in finding out the source from which it was derived. In one case, it was found in large numbers on the straw that was used for bedding; in another, it was present in the hay that served as fodder. During the milking, the milk became infected from these sources and the peculiar fermentation noted was traced directly to this source.

56. Fermentations producing color changes in milk:

Besides these fermentations that affect mainly the taste of the milk, there are quite a series of changes that occur rather sporadically, whose main character seems to be expressed in the production of certain pigments. The color changes in milk are very noticeable and are perhaps better known than almost any other class. These chromogenic or color troubles in milk often do not impair the nutritive value of the milk, although their presence is so noticeable as to deter consumers from using the infected fluid.

57. **Red milk:**

Red or bloody milk is due to different causes and is not always to be ascribed to bacterial action. It may arise from the presence of blood in the milk due to some wound in the udder. It is also said to be caused by animals eating sedges, scouring rush and plants that contain a large amount of silica in their tissues. Cows' milk has been found to be reddened where animals have eaten plants like the madders. These instances can always be separated from bacterial troubles, because if due to actual blood, the color will be noted at the milking. There are several chromogenic bacteria that have been isolated from milk that have the power of turning milk red. The most widely known form able to bring about this change is *Bacillus prodigiosus*, the so-called "bleeding bread" bacillus. This species has not been found spontaneously in this country, but in Europe it has been known for centuries. This bacillus is interesting from an historical standpoint, inasmuch, as it has been found to be the cause of the bloody bread that has been the source of much superstitious fear. On bread and solid substances, it forms bright red patches which grow deeper in tint with age until they present a dark bloody red color. Troubles in milk have been traced to this bacillus where it has gained access to this fluid. Its growth in milk is marked by the production of a coloring matter that is diffused throughout the milk, especially near the upper surface. This germ requires free contact with oxygen in order to produce its characteristic pigment.

Another interesting form that produces a red coloration in milk has been described by Hueppe[29] and also by Grotenfelt.[30] *Bacillus lactis erythrogenes* or the red milk bacillus is a germ that grows easily in milk and other nutrient substances, but it possesses a curious property of being able to produce the red color only in the dark and in milk that is not strongly acid in its reaction. When grown in the light, this germ forms a yellow pigment of varying tints. In milk, the casein is slowly precipitated and gradually dissolved. This germ thrives best between the temperatures of 86° and 95° F

Several other cases of red milk have been reported that have been traced to other germs. Menge[31] found in the milk supply of Rendsburg, Germany, a red sarcina that was the cause of trouble in the milk supply of that city. Here in this country, it is not at all uncommon to find on old milk, red patches that are caused by the growth of a yeast-like germ, *Saccharomyces glutinis*, that is often found in the air.

58. Blue milk:

Blue milk is historically a better known disease than almost any other. As long ago as 1838, Steinhoff showed that the trouble was communicable from one lot of milk to another. It manifests itself in the course of one to three days by the appearance of isolated flecks of bluish or grayish color on the surface of the milk. In fresh milk that is only slightly acid, the gray tints prevail but as the amount of lactic acid increases in the milk, the blue coloration becomes more marked.

The blue patches on the surface may remain isolated or by continued growth, the whole surface may be covered with a blue coating. This appearance is due to the growth of a certain bacillus in the milk, now known as *Bacillus cyanogenus*, a form that has been carefully studied by Heim and others. It does not materially affect the milk, but the butter made from infected cream has very poor keeping qualities. Heim[32] found that this bacillus was especially resistant toward drying, or the influence of chemical agents like soda and potash, but a moment's heating at 176° F sufficed to kill it.

59. Other kinds of colored milk:

Two or three chromogenic forms producing still other colors have been found in milk. Schroeter discovered in a sample of cooked milk, a peculiar form that produced a citron yellow appearance and which precipitated and finally dissolved the casein. Adametz, Conn, and List have described other species that confer tints of yellow on milk. Some of these are bright lemon, others orange, and some amber in color.

Still other color-producing bacteria, such as those that produce violet or green changes in the milk, have been observed. In fact, almost any of the chromogenic bacteria are able to produce their color changes in milk as it is such an excellent food medium. Under ordinary conditions, these do not gain access to milk in sufficient numbers so that they modify the appearance of it except in occasional instances, although a biological analysis of almost any sample of milk will show the presence of several chromogenic forms that are for the most part derived from the air.

60. Suggestions as to the treatment of various milk troubles:

The foregoing pages have outlined in a brief manner some of the most marked fermentations that are known to occur in milk. This list does not by any means include all of the changes that take place in this fluid as a result of bacterial action, for our knowledge is far from being complete on this subject. As yet, we may say that we have just begun to recognize the very great importance of this study of milk fermentations and that the subject is one, in which future research will give us much more light than we now possess. In treating of this subject as a whole, attention should be drawn to the differences that mark the bacterial changes in milk as contrasted with those due to other causes and also to present ways and means by which these troubles can be successfully combated. It should be borne in mind, that not all troubles and disorders that occur in milk are due to the action of bacteria. A great many difficulties that the practical milk handler has to contend with are produced by a number of causes that have no relation to the germ content of the milk. Errors in feeding, physiological causes sometimes of an unknown origin, actual impurities, such as dirt, blood, etc., produce changes that render milk less palatable and wholesome than normal.

Recognizing the workings of these causes then, we must see that tainted milk of any sort brings us face to face with a complex problem. The general comparison can well be made between those troubles that are present in milk and the diseases that afflict the human being. A large number of the ills that flesh is heir to are self-engendered, i. e., they are produced by some error in nutrition due to improper nourishment, lack of exercise or some such condition affecting the body. Among such diseases as these may be mentioned as examples, jaundice, diabetes and dyspepsia.

Then there is a large class of diseases that originate from some cause entirely extraneous to the body and only produce their effect if they once gain an entrance, by breaking down the natural barriers. In this class are to be found such maladies as diphtheria, consumption, typhoid fever and the grip.

The same distinction holds with the troubles present in milk. A great many taints are due to the absorption of impure and noxious vapors arising from decomposing and putrefying masses, general debilitated condition of the cow, bad food and such causes. These are in a sense self-engendered in the cow, although feeders are primarily to blame owing to their ignorance of the physiological processes that go on in the animal.

Many other taints are due to an entirely different set of causes. They are analogous to the contagious class of diseases that are to be observed in man. Fortunately, we have an almost infallible rule that enables us to separate those troubles that we may designate as infectious milk troubles from those that are produced by other causes.

61. Distinction between infectious and non-infectious troubles:

Bacterial troubles in milk can nearly always be distinguished from taints due to physiological defects if the milk is fresh.

If the taint grows more pronounced as the age of the milk increases, it is probably due to living organisms, as the taint-producing bacteria must have gained an entrance after the milk was drawn from the cow. Taints due to errors in feeding will be strongest when the milk is first drawn, and will gradually be dissipated as it increases in age.

Milk itself absorbs or gives off gaseous impurities and odors with great ease. As long as it remains warmer than the surrounding atmosphere, it will continue to give off any flavor with which it is impregnated. This teaches us the important lesson of aeration, as a means of eliminating the gases and odors that may be present in the fluid. When milk becomes cooler than the surrounding air, it acts as an active absorbent and will often acquire any flavor or odor that is in close contact to it. Cold milk placed near fruit will acquire the fruity aroma, and the same is true if it is in close proximity to any offensive or decomposing material. This absorption of flavors by old milk is sometimes puzzling to distinguish from a bacterial trouble in the milk, but if the surroundings are closely noted, the cause can usually be detected if it is due to filthy store rooms.

In detecting a tainted condition in milk, it is much easier to recognize it if the milk is heated slightly, for then the fluid exhales the impurities that are present and unnoticed in the milk when it is cold.

In attempting to combat these abnormal fermentations that occur in milk, the first necessary step is to locate the source of the trouble. In most instances where the difficulty is due to bacteria, it is caused by filth and dirt in some of its many phases gaining access to the milk. Scrupulous cleanliness will therefore in most cases eliminate the trouble. So efficacious is this course that cleanliness in every detail in dairy pursuits is almost a panacea for troubles and taints of all sorts that occur in milk.

However, in order to apply a remedy efficiently, the source of the offending cause must be exactly determined.

If the taint is recognized in the mixed milk of the herd, it is necessary to ascertain, first, whether it is a general disease or whether it is restricted to a few cows or to a single cow. Often the whole milking may be infected from the milk of a single animal that has been fouled in some way by contact with germ life derived from decomposing material of some sort.

To prove whether the trouble is a general or an incidental one can easily be done by separating the milk of the different cows, or if that is not feasible by massing that of a few together and so narrowing the number down by degrees.

Where the trouble is a general one and is not due to the spreading of the infection from a single source, the fault is usually to be traced to some error in handling the whole mass of milk. Imperfectly cleansed cans that are used to set the milk in often contaminate the entire lot (see 32); then too, noxious germs derived from the cow often gain access to the milk. The herd, especially in late summer, when the upland pastures are dry and the grass is short, are often turned on to marsh or lowland fields. The stock seek the low places, frequently slime-covered mud holes, and in passing through these their lower parts are fouled with this scum and slime that is filled with putrefactive forms (see 34).

Sometimes the source of the filth may be in the barn itself. Unclean stalls filled with moist and decaying matter may be the source from which the milk is often seeded (see 35).

With the great majority of these troubles, cleanliness is a sufficient cure, but sometimes a hardy, noxious form becomes so well established that it is a serious matter to eradicate it.

62. Antagonizing abnormal changes with normal fermentations:

Sometimes it is possible to oppose one kind of fermentation with another, if the two are in any way antagonistic to each other. An illustration of this principle is seen in the following example. A dairyman applied for assistance in determining why his milk did not naturally undergo a lactic acid change. The milk lobbered in an alkaline condition, and the curd was always soft and slimy. The trouble proved to be caused by one of the rennet producing bacteria (see 50). These curdlers of milk in an alkaline condition are much restricted in their ability to grow, if the milk is acid to any considerable degree, but if they once gain the ascendency they possess considerable powers of resistance on account of the spores that they form. The trouble had appeared with considerable regularity and the owner was unable to obtain the proper degree of acidity for his cream, but he succeeded in restoring normal conditions by taking a lot of good sour milk from a neighboring dairy and infecting thoroughly all cans and pails that were used. In this way, he gave the lactic acid germs the ascendency, and they produced the requisite acid to check the growth of the alkaline curdlers.

63. Use of chemicals as disinfectants:

In exceptional instances only, will it be necessary to employ chemical disinfectants to restore the conditions to the normal. Of course with such pathogenic diseases as tuberculosis, very stringent measures are required as they are such a direct menace to human life.

In case it becomes necessary to employ chemical substances as disinfecting agents, their use should always be preceded by a thorough cleansing with hot water so that the germicide may come in direct contact with the surface to be disinfected.

Sulfur is often recommended as a disinfecting agent but its use should be carefully controlled, otherwise the vapors have no germ killing power. The common practice of burning a small quantity in a room or any closed space for a few moments, has little or no effect upon germ life. The effect of sulfur vapor (SO_2) alone, upon germ life is relatively slight, but if this gas is produced in the presence of moisture, sulfurous acid ($H_2 SO_3$) is formed which is very much more effective. To use this agent effectively, it must be burned in large quantities in a moist atmosphere (three lbs. to every 1,000 cubic feet of space) for at least twelve hours. After this operation, the space should be thoroughly aired.

Bleaching powder or *chloride of lime* ($Ca Cl_2$) is to be recommended where a chemical can be used to an advantage. This substance is a good disinfectant as well as a deodorant and if applied as a wash, in the proportion of four to six ounces of the powder to one gallon of water, it will destroy most forms of organic life. In many cases this agent is inapplicable on account of its odor.

Corrosive sublimate for most purposes is the best disinfectant known but it is such an intense poison that it is dangerous to use in places that are at all accessible to stock.

For the disinfection of walls in stables and barns, common thin *white wash*, milk of lime, is admirably adapted if made from freshly burned quick lime. It possesses strong germicidal powers, increases the light in the barn and is of course exceedingly cheap.

Carbolic acid, creosote and such products while having a good germ killing power cannot well be used because of their odor, especially in factories.

For gutters, drains and waste pipes in factories, *vitriol salts* (sulfates of copper, iron and zinc) are sometimes used. These are deodorants as well as disinfectants and are not objectionable to use on account of their odor.

These suggestions as to the use of chemicals, however, only apply to extreme cases and should not be brought into requisition until a thorough application of hot water, soap and the scrubbing brush have failed to do their work. The strong disinfecting power of sunlight should also be borne in mind in this connection, as this is often a very efficient means of disinfection.

CHAPTER VI.

RELATION OF DISEASE-PRODUCING BACTERIA TO MILK.

64. Manner of infection:

The bacteria that are of the most importance as affecting the keeping quality of milk are those that are purely saprophytic in their habits of growth, but as milk is such an excellent food medium for parasitic forms as well, and in numerous instances a direct relation has been traced to this group, a general survey of the subject is advisable.

The bacteria that are able to produce a diseased condition in the human subject, and bear any relation to milk may be considered under three heads.

1. Those forms that are known to be pathogenic, and are able to thrive in milk, if they gain access to it after it comes from the cow.

2. Those that are derived from a diseased animal, and at the same time are dangerous to man, by being transmitted from the animal directly to man by means of the milk.

3. Those that are saprophytic but which are able to form poisonous substances either in the milk directly, or after it is taken into the alimentary canal.

In the first catagory, will be included all of the disease-producing organisms able to grow in milk that may have accidentally gained an entrance to this fluid. This will include quite a list because milk is so admirably adapted to the growth of most germs that are able to thrive outside of the body at all.

Milk destined for public use may be handled by persons that are convalescing from some such disease as diphtheria, or the contagious virus of the disease may be directly implanted in the milk, or, it may have been handled simply by an exposed party and even in this way, sufficient germs have gained access to produce the disease. The saprophytic germs that are always present in milk have no doubt a restraining influence upon those disease organisms that are introduced accidentally; but they do not always succeed in choking them out for epidemics of these diseases have often been traced directly to the milk and the actual presence of the specific microbe demonstrated upon examination.

65. Tuberculosis:

Of those diseases that are communicable from the animal to man by means of the milk, tuberculosis is by far the most common. The importance of this disease with reference to public health and successful animal industry is so great that as full a consideration of the subject should be given as is possible. Tuberculosis or consumption is the term now used to indicate a number of apparently different maladies that affect warm blooded animals and is caused by the growth of the tubercle bacillus. In this connection, reference can only be made to the bovine type of the disease and the relation that this bears in milk and dairy products to the human race. It is now quite generally accepted that the disease is caused by the same germ whether it be present in the human being or the lower animals and the danger of infection exists in the direct transmission of the virus from one to the other. The tubercle germ, *Bacillus tuberculosis*, was discovered by Robert Koch in 1882. Many years previous to this the infectious nature of consumption had been recognized. Indeed, experiments were made in 1868 which showed that tuberculous material was capable of reproducing the disease in a healthy animal if it was properly transplanted.

Conditions of growth. Koch isolated the specific germ able to cause this disease, and finally succeeded after many failures in cultivating it in artificial media. In this way the peculiarities of the species could be ascertained. This organism is remarkable for the narrow temperature limits within which growth will take place; the minimum being 86° F., while the maximum is 104° F.

This is important because there is then, no danger of multiplication if a few germs should accidentally gain access to a milk supply. This organism can withstand drying easily, in fact, by virtue of this property it is most widely distributed. In the later stages of consumption, tubercular material is thrown out of the living body. This dries quickly and is mixed with the dust; in this way the germs are easily blown about and gain an entrance into a new host. Dried material of this sort will often retain its infectious properties for several months. Putrefaction and decomposition even, will not quickly destroy its vitality. Sunlight is, however, an efficacious agent in killing the germs that are exposed to the direct rays; and, even when subjected to a diffused light, the bacilli are destroyed in a few days.

Bovine tuberculosis. The main danger to mankind from bovine tuberculosis is due to the possibility of the transmission of bacteria in quantities from a diseased animal by means of the milk and meat.

Tuberculosis is a common disease in almost all of our domesticated animals, particularly with cattle. Unfortunately this disease is more prevalent with dairy stock than any other. This is largely due to the unhygienic conditions under which this class of animals is often kept. Close confinement in badly ventilated stables, immature and continual breeding, prolonged periods of lactation do not cause the disease, but each of these factors exerts a depressing influence upon the animal that hastens the progress of the malady if the disease once gains a foothold.

It is practically impossible to estimate at present, the prevalence of this disease in dairy herds in America. The recent introduction of the tuberculin of Koch as an aid in the diagnosis of the disease in cattle has shown the trouble to be more widely spread than was at first believed, but sufficient data have not yet been collated to enable an accurate estimate to be made.

Tuberculin test. The tuberculin test[33] while not always an infallible guide to a correct diagnosis, has proven itself so far superior to the ordinary physical methods of examination, that the results obtained by its use are held to be a more correct condition of the actual state of the disease than has heretofore been known. Its use in some of the finer bred herds that have been subject to much change by continual sale and purchase, has often revealed an alarming prevalence of the disease, but the supposition that the great bulk of dairy cattle are as badly affected as these herds is entirely unwarranted.

Location of the disease. In a great many cases in cattle, the disease assumes a chronic form that is not recognizable by a purely physical examination. This chronic type may sometimes pass into a more acute phase, if the vitality of the animal body be severely taxed, as in calving.

While the lungs are the organs that are most frequently attacked, the other organs of the body are by no means exempt. Often the disease develops in the udder, causing an enlarged, indurated condition of that gland. When this organ is affected the milk is unfit for food purposes, as the bacilli of this disease are usually present. Woodhead[34] found in fourteen out of nineteen cases that the milk or the sediment from it contained bacilli numerous enough to produce the disease in guinea pigs inoculated with small quantities of it.

The writer found one case in which one cubic centimeter of milk from a diseased animal sufficed to kill a rabbit inoculated with it. In this same case, the bacilli were also demonstrated microscopically in the milk.

Often the udder may contain tubercle bacilli and still not show any external symptoms of the disease. Bang,[35] Ernst[36] and others have shown that in quite a percentage of animals with apparently healthy udders the milk possesses infectious properties. The disease may sometimes be more or less generalized in the system and the disease germs be found in the milk, while it is apparently normal to the eye. For this reason, the greatest of caution should be exercised in using milk from cows that may have the disease even in the most incipient stages.

Methods of treating milk. The possible danger from tubercle bacilli in milk may be greatly diminished, if not entirely eliminated, in two ways, by dilution and by the use of heat (Pasteurization, see 75).

The milk of a cow that is so infectious that a few cc. of it will kill a rabbit, will be rendered comparatively harmless, if it is diluted with several times its volume of healthy milk. This dilution reduces the number of germs absorbed in equal volumes so much that they fail to establish themselves in the intestinal tract and consequently the disease does not gain a foothold.

66. Typhoid fever:

This disease stands next to tuberculosis in importance in its relation to milk. The germ producing this fever does not develop in the animal itself, consequently, no danger need be apprehended from milk, if it is properly cared for after it comes from the cow. The typhoid fever bacillus, however, finds in raw milk such favorable conditions for development, that if it is once introduced, it is often able to thrive for a considerable length of time. The disease usually spreads by means of the water supply, yet quite a number of epidemics have been traced directly to milk as the original and only source of infection. Von Mering gives the history of an epidemic that occurred in one of the Strasburg prisons, in which seventeen cases were traced directly to the milk supplied by a milkman that had had but a single case of the fever in his family.

The contamination of milk by this disease has been found to have occurred in the following ways:

1. Infection by the milker who had been near a person sick with the fever, and whose clothes had become infected.

2. Infection of the milk by allowing it to stand in a room that was next to that occupied by a typhoid patient.

3. Direct infection of the milk vessels by infected water used for cleansing purposes.

All of these means of access can be readily governed with care and caution. If typhoid fever is present in the family of a milk-handler, especial care should be taken that no one that has access to the patient has anything to do with the handling of the milk. Water used in cleansing vessels should either be boiled or taken from a source that has no possible chance of contamination. Wells in the vicinity of dwellings are sometimes infected by the disease germs passing from excreta deposited in vaults along rifts and cracks in the soil, and so gaining access to the well that may be used for household purposes.

67. Cholera:

Milk also functions as a medium for the transmission of cholera, although the danger from this source is somewhat minimized on account of the inability of this germ to thrive in acid fluids. Heim found that the cholera germ would live in raw milk from one to four days depending upon the temperature. This variation is based rather upon the amount of acid that is formed in the milk by the lactic acid bacteria; acid in any degree being fatal to the development of this germ.

Cholera epidemics in India have more than once been traced directly to contaminated milk. Simpson[37] records a good example of infection in this way, in the case of the brig Ardenclutha at Bombay. Ten sailors out of twenty-four partook of some milk that had been purchased from a milk-man, in whose district cholera had recently broken out. Of these ten, four died, five were severely sick and one that used the milk sparingly was slightly ill. Careful investigation proved that the milk had been diluted by adding to it some water that had been taken from a drainage pool in this infected district.

68. Diphtheria:

Diphtheria is another parasitic disease whose specific bacterial germ is able to thrive in milk. Several epidemics are reported from England that claim to be traced to milk as the source of infection. Klein[38] claims to have found diphtheritic bacilli in the milk of cows inoculated with this germ although Abbott[39] failed to confirm these observations in a recent experiment.

69. Scarlet fever:

Although the specific organism of scarlet fever has not, as yet, been isolated, yet in a number of cases of this disease the claim has been made that the trouble originated with the milk. According to Hart[40] fourteen epidemics of scarlet fever in England have been traced to milk that had been infected in some way.

Besides these diseases there are still others, in which milk has been proven to have been the medium of transmission. Among these are anthrax and hydrophobia, while among those that are distinctively animal diseases are the foot and mouth disease, and swine plague.

70. Cases of poison traced directly to milk:

Besides these germs that are recognized as parasitic organisms capable of producing a diseased condition in man or in animals, there are other forms, probably quite numerous, that are saprophytic in their tendencies, yet, they are able to form poisonous or toxic compounds if they once gain access to the milk.

Among this class of germs are included those microbes that are concerned in the production of bacterial poisons that come to our notice in cases of milk and ice cream poisoning, as well as those found in cheese. Vaughan[41] has isolated the poisonous principle from some of these substances, and has called it *tyrotoxicon* (cheese poison). Our knowledge of the organisms producing these poisons is, as yet, quite indefinite, but it is more than probable, from Vaughan's researches, that the germs causing these changes are putrefactive organisms that gain access to the milk where it is kept under filthy conditions.

Severe intestinal disturbances, especially those observed with children, are also doubtless due to organisms of this class that produce their constitutional effect more by the action of the poisonous alkaloids and bases that they form in the intestines than by the material ingested.

CHAPTER VII.

ON THE PRESERVATION OF MILK.

71. Keeping quality dependent upon germ life: Ordinarily, milk inevitably undergoes a change in a few days that renders it worthless not only for human food, but at the same time destroys very largely its value as an animal food. To prevent this loss, which is considerable from an economic standpoint, it is desirable that some method should be introduced whereby these fermentative changes can be checked or if possible inhibited entirely.

As the changes in question are brought about, almost, if not entirely, by the presence of the various forms of bacteria, methods of milk preservation, to be efficacious, must either prevent the entrance of these organisms into the milk, or destroy them after they have once gained access to this fluid. Bacteria are so widely distributed, and milk is such a nutritious medium for their development, that to entirely prevent their entrance into this fluid is not feasible. Scrupulous care in the dairy both before and after the milking will do much to reduce the germ content of milk (see 39 and 40), but even the best of care will not completely exclude these organisms.

There remains then, only a single alternative, that of destroying the bacterial life with which milk is so abundantly seeded. In speaking of the preservation of milk, reference will only be made to milk intended for economic purposes. Milk intended for analytical use would be treated in entirely a different way, since for this purpose, it is not necessary to preserve its food properties. In the preservation of milk by eliminating or checking germ development, two different courses may be applied.

The results desired are attained by the use of

1. Chemical agents.
2. Physical agents.

72. Preservation of milk by chemical agents:

Under the head of antiseptics and disinfectants (see 16), the action of different chemicals on bacterial life has been discussed.[42] Those substances that are inimical to the development of these organisms are usually too strong for use as preservatives, because the protoplasm of bacteria as a rule is more resistant than other forms of organic life.

For this reason, the application of strong disinfectants like carbolic acid, mercury salts, strong acids and alkalies is excluded. The chemical agents that can be used in the preservation of milk fall into two classes.

1. Those that unite chemically with certain products of germ growth to form more or less inert substances in the milk.

2. Those that restrain or inhibit the development of fermentative organisms in the milk.

To this first class, belong those alkaline salts such as bicarbonate of soda, etc., that combine with the acids that are formed in the milk. While salts of this sort neutralize the acidity produced by the lactic acid bacteria, they do not kill these organisms. By this neutralization of the milk, the bacteria that develop best in an alkaline medium (rennet and peptonizing forms) are furnished with much more favorable conditions of growth so that this method is of little value in diminishing germ life.

As representatives of the second class, salicylic acid, boracic acid and their derivatives may be mentioned. These substances can be added to milk in quantities not recognizable to the taste (salicylic acid 0.75 gr., boracic acid 0.5 gr. per liter) and they will materially increase the time that milk will remain sweet, but their use, like that of all other chemicals, is strongly to be deprecated. In many of the European countries they are prohibited on account of the harmful effect that the continued use of the ingredients in their manufacture is known to exert upon living tissues. A large number of preserving agents of this sort, sold under various proprietary names, are on the market, but all of them depend for their efficiency upon the antiseptic properties of some chemicals that are used in their manufacture (see 16).

Recently hydrogen peroxide (H_2O_2) has been suggested as a chemical disinfectant. If applied to milk in the proportion of 1:10, it will keep raw milk according to Heidenhain[43] sweet from three to eight days. The use of these materials in milk is quite unnecessary as other methods of preservation are applicable to which objection cannot be made.

73. **Preservation of milk by physical agents:**

1. *Filtration* through clay cylinders is a valuable means of eliminating germ life from some fluids, but

it is inapplicable to milk because the fat and casein that are held in suspension cannot pass the filter. Successful attempts have been made to purify milk by filtration through sand.[44] This principle is somewhat the same as that which is applied to sewage and consists in forcing the milk upward through fine gravel and sand. In this way a large number of organisms can doubtless be removed, but as the fat globules in milk are larger than ordinary bacteria, its use is confined to the purification of milk from solid impurities rather than rendering it germ free. The claim is made that there is but very little loss in fat in using this method. The inconvenience of sterilizing such a filter as this militates much against its general use.

2. Successful attempts in preserving milk have been reported where a current of *electricity* has been employed, but the method has not as yet passed the experimental stage.

3. *Temperature variations* are best adapted to this purpose because within certain limits they do not injuriously affect the physical characteristics of the milk. Bacterial life possesses a wide range of temperature in which growth is possible, but both heat and cold have an inhibitory effect on the development of these organisms as well as all other forms of living matter.

a. Low temperatures.—While low temperatures (see **14**, 2) do not easily destroy all germ life, yet they affect strongly the keeping quality of the milk. If the temperature of milk is reduced to within four to six degrees of the freezing point, the growth of bacteria will be practically stopped and while kept under these conditions the milk will retain its freshness for a very long time. Artificial methods of refrigeration are used for this purpose.

Attempts have been made to preserve milk by freezing and Guerin[45] introduced this method into Paris on a successful scale. Milk in this condition can be readily transported but certain technical difficulties concerning the separation of the fat militate against the general use of the method.

b. High temperatures.—The oldest and best known method of preserving milk is by the use of heat. Cooked milk has long been known to have better keeping qualities than raw milk, and even boiled milk is often recommended for invalids' and children's use on account of its freedom from toxic germs that set up gastric and intestinal disturbances.

Cooked or boiled milk, however, has one disadvantage, i. e., the peculiar flavor it acquires when heated above the coagulation point of the proteids in it.

Two methods* are in use for the preservation of milk by means of heat, the technique of which is dependent upon bacteriological principles.

One is the application of a comparatively low degree of heat for a short period of time; the other, the use of a high heat for a longer time. For these two methods the terms *pasteurization* and *sterilization* are employed.[46]

Neither process as carried on in actual practice will always render milk absolutely germ free. The first condition requisite for the successful use of either, is

*No reference is here made to condensed or evaporated milks.

to have the milk to begin with just as free from organisms as possible. The forms that are most resistant in milk are the spore-bearing bacteria, such as those found in dry feed or dried manure. These often find their way into the milk in a spore condition mainly by means of the dirt and dust that fall into the milk during the milking.

The attempt has been made to send milk by rail for considerable distances, keeping it heated above the growing point of bacteria while it is in transit, but the prolonged heating even at a comparatively low temperature, changes it is said, the physical characters of the fluid.

74. Pasteurization of milk:

Pasteurization is the name given to that process of rendering fluids as germ free as possible by means of heat where the temperature employed ranges considerably below the boiling point. This process was first used extensively by Pasteur in 1868 (from whom it derives its name), in combating the various maladies of beer and wine. Its importance as a means of increasing the keeping quality of milk was not generally recognized until a few years ago, but the method is now growing rapidly in favor as a means of purifying milk for commercial purposes from germ life of all sorts. Pasteurization does not entirely eliminate all forms of bacterial life, indeed, it is difficult to do so at a single operation even where sterilization at 212° F is resorted to. By taking advantage of the principle used by Tyndall, that is known as the discontinuous or intermittent method, it is possible to render milk absolutely germ-free by employing no higher heat than that ordinarily used in pasteurization, if the operation is repeated for several times on successive days. This method of procedure, however, increases the expense of the process very materially and would not be applicable for practical purposes.

75. Advantages of pasteurized products:

The advantages that pasteurized products enjoy in comparison with raw uncooked milk are two-fold:

1. The keeping qualities of the fluid are much increased, so that from an economic standpoint alone the process is a valuable one in preserving so important a food as milk and cream and some of their products.

2. The pasteurizing process, properly carried out, eliminates not only the great majority of saprophytic bacteria but destroys the seeds of contagious diseases that may be transmitted by means of the milk.

Pasteurized milk has not the keeping qualities of sterilized milk, because it is not entirely freed from all forms of germ life, but for milk that is intended for consumption in the near future it fulfils all necessary conditions.

It possesses a marked advantage over a sterilized product, inasmuch as its physical characteristics are not perceptibly changed. Boiled, steamed or sterilized milk acquires a peculiar cooked odor and taste that to many is disagreeable. The question as to the digestibility of boiled milk is yet an unsettled one, but recent experiments show that the casein and other proteid elements are rendered more resistant toward the digestive fluids by the use of high heat.

76. Conditions as to the pasteurizing temperature: The necessary conditions as to temperature in pasteurizing are that the degree of heat used shall exceed the thermal death point of all the vegetating bacteria in the fluid, and yet not be high enough to change the proteids of the milk.

Most species of bacteria are killed when in an actively growing condition in the neighborhood of 133°–140° F.

Two conditions enter in to determine the thermal death point of the various forms of germ life.

1. The temperature of heat used.
2. The length of exposure to the heat.

With either condition fixed, the same result is accomplished more rapidly by an increase either in temperature or duration of exposure, so that an exposure for a longer time at a lower temperature is quite as effective as a shorter exposure at a relatively higher temperature. The importance of this fact is recognized in pasteurization, where the degree of heat used is limited to a certain point—that at which the milk acquires the cooked taste. This upper limit is fixed by the coagulation point of the proteids in the milk, yet, under practical conditions, it is subject to certain variations. Milk that is heated to about 158°–168° F will evolve a cooked odor and will have a peculiar taste at the time of heating, but this same milk, if cooled, loses this flavor, and, when thoroughly chilled, no change can be detected. If this temperature is exceeded the objectionable taste and odor does not disappear, so that a higher temperature than this can not be used when milk or cream is being handled.

With the great majority of bacterial species that have been individually tested as to their thermal death point, 140° F. for ten minutes has been found fatal. This degree of heat suffices to kill all of the disease-producing bacteria with the exception of the tubercle bacillus. As this is the organism that is most dreaded in milk, on account of the prevalence of this disease among dairy cattle, it is necessary that any process employed should be able to annihilate all danger from this source. The exact relation of the tubercle bacillus to heat has been studied by numerous investigators and while there are some slight differences in the results reached, the following conclusions seem to be warranted by experimental evidence.

Thirty minutes heating at 149° F., 15 minutes at 155° F. and 10 minutes at 167° F. suffice to destroy the tubercle germ. As this germ is the most resistant one found in milk, its death point will serve as a maximum temperature necessary in pasteurization. This temperature should always be maintained so as to eliminate any possibility of danger from this source.

77. Apparatus for pasteurization:

There are many pieces of apparatus that have been designed for the purpose of pasteurization. The great majority of them have originated in Germany where this method is largely used for the preservation not only of milk but all inferior dairy products like skim milk and whey.

The first devices for this purpose to be used with milk were those of Thiel and Fesca that were introduced early in the '80's. In these the milk was warmed for only a short time as it flowed over a heated surface and then cooled by passing it over a cold corrugated surface. These machines were introduced primarily to increase the keeping quality of the milk without any reference to its contents from a hygienic point of view.

At present there are over a dozen different machines intended for the pasteurization of milk and other liquids that have been put upon the European market. In many cases, there is a marked similarity, so that it will be quite unnecessary to describe in detail the different designs. The greater part of them fall into two general classes.

1. Those in which a thin sheet of milk is allowed to flow over one side of a corrugated surface that is heated on the other side either by hot water or by steam directly. This method gives a continuous delivery of the milk. The milk is heated only while it is in contact with the radiating surface. After it is thus treated, it is conducted over a cold surface in the same way, thus materially reducing the temperature. Among the more prominent machines of this class are those of Thiel, Kuhne and Hochmuth.

2. Those in which a reservoir is used to hold the milk. This reservoir is surrounded by an exterior shell that contains the heating agent either steam, or water heated by steam. The more efficient machines of this class are provided with an agitator in the milk

reservoir so as to hasten the equalization of temperature in the inner chamber and at the same time to keep the milk in motion in order to prevent the coagulation and the adherence of the proteids on the wall of the vessel.

The larger number of these pieces of apparatus are arranged for a continuous delivery, the milk flowing in at the lower end and displacing that already pasteurized which flows out above into a cooler. In some of them the agitator even mixes the fresh supply with that which has already been heated, so that the efficacy of the process is much lessened. Among the more prominent of these machines belonging to this class are those of Ahlborn and Fjord which are composed of two cylinders, the milk reservoir being the inner one. The milk is kept in constant motion by means of a rotating vane in the milk chamber. The apparatus of Dierks and Möllman has a series of closely set rotating brushes in the milk that act upon the heating surface so as to prevent the collection of proteids.

The majority of these machines are subject to the very serious objection that the product they deliver is not always uniformly heated.

Where it is constructed for continuous delivery, the length of exposure must necessarily be quite limited and as the temperature cannot exceed that of 167° F. without affecting the physical characteristics of the fluid, it very often happens that the pasteurizing process is not efficient. In the case of the harmless souring organisms from the hygienic standpoint, it makes but little difference, but to insure absolute freedom from disease germs, the temperature and the time of exposure must be thoroughly under control. Very few of the machines intended for this purpose have been subjected to a rigid bacteriological test and the lack of this has allowed the introduction of many designs that may be adapted for the pasteurization of bye products intended for animal food, but certainly do not deliver a product that can be relied upon for human food.

78. Essential conditions in pasteurizing apparatus: A pasteurizing apparatus needs to fulfil two conditions. The cream or milk must be heated to a certain temperature; then, after a certain lapse of time during which the temperature remains constant, the product must be thoroughly and quickly cooled. The factor of cooling is quite as essential to the success of the operation as that of heating, for if the temperature of the heated milk is allowed to fall naturally, this takes place so slowly, at the room temperature, that the few spore-bearing bacteria that are always present in the pasteurized milk, have the best of conditions for their development and the keeping quality of the milk will only be slightly increased. It is therefore necessary to hasten the chilling process rapidly through that period during which the bacteria find a favorable growing temperature. The extremes of this period vary much with different forms, but in general the danger points are included between the limits of 104° F., and 55°–60°F. This operation should be done as rapidly as possible, for if the spores are once allowed to germinate, they are able to grow at a temperature considerably lower than one in which germination would be possible. Therefore, if the germinating stage can be held in abeyance until they are once chilled, the most of them will be unable to develop.

Another important point is the deleterious effect on growth where the transition from one temperature to another is rapid. Germ life heated to the highest possible point that it is able to stand, is much weakened. Pasteur uses this method in attenuating the virulence of his germs that are used for vaccine purposes. When, however, to this method of attenuation by heat, a sudden change of environment is made, such as a drop of 100° in less than two hours, the paralyzing effect is still further increased. Spore bearing germs subjected to this treatment are still further retarded in their ability to develop, so that cultures made from heated samples need to incubate two or three days longer than unheated material, before growth is apparent.

While it is possible to incorporate in a single piece of apparatus both the heating and the cooling features and thereby lessen the expense of the outfit, most of the machines already designed are merely for heating purposes. A single piece of apparatus serving both purposes is probably applicable for establishments on a small scale, but for a pasteurizing plant of any magnitude, a separate pasteurizer and cooler are desirable, as there is considerable loss of available cooling power utilized in the cooling of the apparatus itself, after it has once been heated.

There are several conditions that must be fulfilled by a pasteurizing apparatus before it may be regarded as thoroughly efficient. The following apply to both heater and cooler:

1. Simplicity of construction so that it can be managed by ordinary help.

2. All surfaces in contact with milk must be easily and thoroughly sterilized.

3. Adequate capacity so that a large amount can be handled with the greatest possible economy in time.

4. Ability to control temperature exactly at any degree, for any length of time.

5. The radiating or absorbing surface should be so arranged that the whole mass of milk will have an equable temperature.

The heater should be arranged so as to conform to the following conditions:

1. The milk should be rapidly heated so as to economize in time.

2. The source of heat should be so arranged that the proteids in the milk will not be scorched.

3. The temperature of the milk must be uniform during the process.

4. The milk must be protected from all danger of contamination in the heater and while being transported to the cooler.

79. Dr. Bitter's apparatus for pasteurization: Bitter[47] of Breslau, has constructed an apparatus that is designed especially with reference to the fulfilment of the hygienic conditions that should always be observed in this process. His machine holds a definite quantity of milk. The heating is done by direct steam that is conducted through a series of removable coils that are immersed in the milk. The milk is kept in motion by means of a number of small rotating vanes that are geared either to run by hand or by steam power. The apparatus allows an intimate mixture of the milk so that it is evenly heated. It can be held at any temperature and can be withdrawn with no danger of infection.

Bitter found that an exposure at 155° F for fifteen minutes sufficed to destroy the various pathogenic germs, even the tubercle bacillus, as well as numerous varieties of saprophytic organisms. At this temperature, an exposure of fifteen minutes destroyed the vegetative bacteria quite as thoroughly as when they were heated for thirty-five minutes.

The keeping qualities of the milk that had been pasteurized, he found to be as follows: Kept at the temperature of 86° F., it remained sweet from six to eight hours longer than raw milk. At 77° F., ten hours; at 73° F., twenty hours; at 58° F., from fifty to seventy hours. In taste and appearance no change was noted.

A bacteriological analysis of the raw milk showed from 37,000–250,000 germs per cc., while the pasteurized had from 0–40 germs per cc.

He also tested skim milk in the same way. Skim milk being much richer in germ life, he used a higher temperature, that of 167° F. Biological tests made during the heating at five minute intervals showed that skim milk containing 2,000,000 organisms per cc., had only 250 germs after being heated for five minutes; 100 germs after ten minutes heating; 90 after fifteen minutes, and 100 after twenty minutes, so that the efficiency of a ten minute pasteurization at 167° F equals that of a longer exposure.

80. Apparatus and methods used at Wisconsin Dairy School:

Experiments in this line have been made at the Dairy School, and the details of apparatus and methods are here presented:

The vat used is a long, narrow, deep, tin reservoir surrounded by a wood covering making a six-inch water chamber on all sides, except on top which is covered by a tin cover overlapping on the edges of the milk reservoir. The milk or cream is heated by the surrounding water jacket that encloses the inner reservoir; the outer chamber being connected with steam and cold water pipes. The milk chamber is provided with a stirrer that may be shoved back and forth either by hand or may be geared to a crank power.

After the milk has been pasteurized (usually at 155° F. for twenty to thirty minutes) it may be cooled in the same chamber or withdrawn into a separate cooler. Our practice is usually to draw off the cream or milk into cream cans after it has been lowered to 50°–55° F. These cans are then kept in the refrigerator until thoroughly chilled. If cream is to be bottled, it is drawn from these into sterilized glass bottles by means of a sterile siphon and capped with paper covers that have been sterilized in paraffin for several minutes.

In all of these transfers, the greatest care is taken to prevent dust and dirt from gaining access to the pasteurized fluid. A separate room for this work is advisable. The pasteurized product must be stored in a refrigerator for several hours, preferably fifteen to twenty, before it is taken out for distribution. In this way it is thoroughly chilled, and the full benefit of the process gained in the sudden cooling to a point below the germinating temperature of the spore-bearing bacteria that remain in the milk.

All of the bottles, cans, dippers, cloths, etc., that are used in the process are thoroughly sterilized in a steam sterilizer before using. We have for this purpose a galvanized iron box into which steam is introduced and the large cream cans are inverted over a row of small steam jets, thus gaining the full benefit of the steam when it is of the most value as a sterilizing agent.

Ice should be used in the latter part of the cooling process in order to hasten the fall of the temperature of the milk when it approaches that of cold water. The length of time that the milk remains between the upper and lower germinating limits of the bacteria in the milk should be diminished with all possible speed.

Where cream or milk is sold directly to the consumer, it is necessary to use special precautions in the delivery of the same. Our plan is to take the bottled cream from the refrigerator and pack it in a tin-lined box, adding a small amount of chipped ice. Cream in this way is delivered to the consumer at about three or four degrees above the freezing point, and if kept under proper conditions will retain its sweetness from three to six days. We have had milk that had received but a single pasteurization that remained sweet for over a month when it was kept in a good refrigerator.

This apparatus that has been described, was intended primarily for the pasteurization of milk and cream that is supplied to consumers in this condition. It can, however, be used for the heating of cream, where it is to be made into butter, a system that is being advanced considerably at present and which is used quite extensively in Denmark and northern Germany (see 95, 3).

The apparatus considered above, is, of course, intended primarily for factories and creameries, but the principle of pasteurization is so simple that any person can employ it in private families, with very little trouble.

81. Freeman pasteurizer for family use;

An ingenious method has been suggested by Dr. R. G. Freeman,[48] of New York, that is applicable particularly to family use. The principle upon which it is constructed is as follows: In a definite amount of boiling water (212° F.), the source of heat having been removed, a certain amount of cold milk in bottles can be immersed, under such conditions that the temperature of the milk will be raised to the proper pasteurizing point and held there sufficiently long to thoroughly kill all vegetating germs.

The success of this method depends upon the temperature of the milk, but if this is first chilled in a refrigerator, this factor will not vary much. The milk in this way is pasteurized in sterile bottles and is intended mainly for family use, although the system has been introduced into New York city on a large scale.

82. Sterilization of milk:

To absolutely sterilize milk requires the application of either a very high heat (215°–240° F.), for several hours, or the repeated heating of the milk at a lower temperature (175°–212° F.), for several consecutive times. Neither of these methods is practical in its application; the first changing the character of the fluid and the second consuming too much time and labor. For this reason absolute sterilization is not used in milk preservation, but a comparative sterilization can be secured in a single application by the heating of milk at or near the boiling point of water. Milk so treated will usually keep longer than pasteurized, and like pasteurized, it is free from all disease organisms and all but the most resistant saprophytes. All sterilized milks, however, have one disadvantage, and that is the cooked taste that is always present when it is heated above the coagulation point of the proteids.

Most of the earlier methods of sterilization did not overcome this defect in any satisfactory degree, but the more modern improved sterilizing methods claim to have reduced this objection to a minimum.

Neuhauss, Groenwald and Oehlmann have recently introduced an apparatus that conveniently and quickly handles large quantities of milk. The milk is first heated in steam at 190° F., then allowed to cool for a time, after which it is sterilized at about 215° F for thirty minutes under a slight pressure. By an ingenious contrivance, the bottles are opened and closed during the heating process, the steam penetrating the milk and driving out all of the air, so that when the milk is cooled, the space in the bottle above the milk is a complete vacuum. The exclusion of the oxygen deprives the spores of the aerobic germs of the necessary conditions for growth, and consequently they are unable to germinate. This, however, furnishes a favorable environment for any anaerobes that may happen to be in the milk, and as these forms are frequently spore-bearing, and consequently able to resist high heat, they are quite apt to be present. Where milk is intended for export purposes, or it is desired to keep it unchanged for several months, sterilized milk possesses an undoubted advantage over the pasteurized product, but for all ordinary purposes for which it is used, pasteurized milk meets the conditions.

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PART III.

RELATION OF BACTERIA TO MILK PRODUCTS.

CHAPTER I.

ROLE OF BACTERIA IN LIQUID PRODUCTS OF MILK.

83. Necessity of studying different products separately:

No sharp and distinct line can be drawn in studying the relation of the bacteria that are found in milk from those that are present in various dairy products, as in most cases, the germs in milk are transferred directly to the various manufactured products.

Their action, however, often differs in the various substances made from milk on account of the different surroundings under which they are kept; for this reason, they should be studied separately according to the product that is made from the milk.

84. Cream:

In an economic way, cream and milk are so closely associated with each other, that it is easier to consider them together, in so far as they are sold in their original condition.

The presence and effect of bacteria in cream, differs in some ways so much from that of milk that a brief consideration will not be amiss. Cream viewed from the standpoint of a food medium for germ life, can not be said to be as good as milk, for the fat content in cream is much increased at the expense of the other more suitable food elements, milk sugar and casein. For this reason, one might expect to find less bacteria in cream than milk of the same age, but the reverse is true.

Such wide variations in the actual numbers of germs present are to be noted that quantitative analyses have but little value, nevertheless a rough comparison of the bacterial flora of milk and cream can be made with safety.

85. Bacteria in cream due mainly to mechanical causes:

Cream whether raised by the gravity process or separated from the milk by means of a centrifugal machine is invariably richer in bacteria than the skim milk of the same age. A sample of the milk taken from the bottom of a deep vessel may contain less than 100,000 germs per cc., while the bacterial contents of the cream layer in the same, would be several millions for the same unit of volume. This is largely due to the filtering out of the microbes during the process of creaming. It is a well-known fact in sewage filtration that the addition of some chemical that will cause the precipitation of certain organic elements always present in the sewage, will eliminate, by far, the majority of the bacteria in the settling of this precipitate. The same principle seems to be operative in the process of creaming, except that the fat globules instead of sinking to the bottom rise to the surface. The richness of this substance in bacterial life is due, then, largely to mechanical causes, although in gravity raised cream, the freer access of air at the surface, no doubt hastens somewhat the development of the aerobic organisms.

The quantitative distribution of bacteria in cream obtained by the separator process shows conclusively that the richness of this fluid is due to this mechanical cause. There are in full milk separated by the centrifugal method, three well marked products, the skim milk, the cream, and the slime that adheres to the separator bowl. If milk is thoroughly mixed just previous to separating and examined with reference to its bacterial life just before, and then immediately after the separation, these three materials are re-examined in a similar way, the following results will be obtained: The slime which is composed of particles having a greater specific gravity than the milk is thrown out on the edge of the revolving fluid by virtue of the centrifugal movement. This material, if examined microscopically will be found to be very rich in germ life.

The cream will almost always contain larger numbers of bacteria than the skim milk. Popp and Becker found in a sample of whole milk, containing 73,000 germs per cc., the following germ contents after it had been separated: Cream, 58,275 germs; skim milk, 21,700 germs, and the separator slime 43,900 per cc. This centripetal movement of a large number of germs with the cream would seem to indicate that they adhered to the tiny fat globules, for this peculiarity in distribution can hardly be explained on the ground of their specific gravity, because in separating milk they remain in the skim milk in considerable numbers in spite of the great centrifugal pressure.

86. Relation of tubercle bacillus to separator slime: According to Scheurlen[49] and Bang,[50] tubercle bacilli, if present in a milk, are largely thrown out with the slime in the separating process. This separation is, however, not so complete that tuberculous milk can be purified for use in this way. Coupled with this peculiar relation of the tubercle germ to centrifuge slime, is the fact that tuberculosis among swine is much more prevalent in Denmark and North Germany where the centrifuge process of creaming is extensively used, and where the swine are fed on uncooked separator slime. Ostertag[51] has pointed out this condition, and has drawn attention to the numerous cases of intestinal tuberculosis in hogs. These facts may be correlated with each other, or merely coincidences, but they are striking enough to be made the subject of further investigation.

Gravity-raised cream is usually richer in bacteria than separator cream, but this is mainly because it is older.

As germ life is so abundant in cream, it is to be expected that this product will be the seat of manifold changes of a fermentative character. These changes are of course undesirable where cream is intended for consumption in its natural form, because they sooner or later result in the formation of various products that render it worthless for table use.

Cream, however, is not subject to such an extensive series of fermentations as milk, because the conditions favoring germ development are not present to the same extent. Although it is numerically, much richer in bacteria, yet the rate of growth of the germ life present is slower than in milk, so that the latter product often spoils sooner than cream. For this reason, cream will sour sooner when it remains on the milk than it will if it is separated as soon as possible. This fact indicates the necessity of early creaming, so as to increase the keeping quality of the product, and is another argument in favor of the separator process.

87. Variation in bacteria found in different creaming methods:

The method used in creaming has an important bearing on the kind as well as the number of the bacteria that are to be found in the cream. The difference in species is largely determined by the difference of temperatures used in the various processes.

Old gravity methods. In the old-fashioned, shallow setting process, the temperature of the milk, and consequently the cream, is relatively high, as the milk is allowed to cool naturally. This comparatively high temperature favors especially the development of those forms whose optimum growing point is near the air temperature. By this method the cream layer is exposed to the air for a longer time than any other, and consequently the contamination from this source is the greatest. Usually, cream obtained by the shallow setting process will contain a larger number of species, which fact is accounted for mainly by this exposure; and also, because the temperature undergoes a slower change that affords a better opportunity for the growth of a larger number of varieties. Cream secured by this process contains more acid than that which is obtained in any other way.

Modern gravity method. In the Cooley process, or any of the modern gravity methods where cold water or ice is used to lower the temperature, the conditions do not favor the growth of a large variety of species. The variation in the cream will depend largely upon the way in which the milk is handled previous to setting. If milked with care, and if handled so as to exclude outside contamination, the cream will be relatively poor in bacteria. Only those forms will develop in abundance that are able to grow at the low temperature at which the milk is set. Cream raised by this method is less frequently infected with the noxious forms than that which is creamed at a higher temperature.

Centrifugal method. Cream obtained by the separator process should be freer from germ life than that which is secured in any other way. It should contain only those forms that have found their way into the milk during the milking and immediately after, for the cream is ordinarily separated so soon that there is but little opportunity of infection, if care is taken in the handling. As a large part of the infection of fresh milk is due to the contamination from the fore milk which usually has but a few species, separated milk, if handled with caution, will contain for the most part those species that are to be found in abundance in the milk while it is still fresh.

The high temperature at which milk must be creamed if the separator process is used makes it necessary that some precaution be taken with the cream after it comes from the separator or it will rapidly change. To accomplish this purpose, coolers are used in some creameries. In this way the growth of bacterial life can be checked so that the cream will not undergo those undesirable changes, that are consequent upon a high temperature. The pasteurization of cream to accomplish the same end has been suggested, and is used to some extent in Europe, but this process necessitates the addition of another factor—that of a starter for ripening purposes—a subject that will be more fully considered under a subsequent head (see **95**).

88. Factory bye products:

While the bye products in the manufacture of butter and cheese are in a certain sense waste products, yet all of them possess enough nutrient value to make it worth while to utilize them either in the preparation of human food or in animal feeding. As ordinarily handled, they very soon become unfit for use, even for feeding purposes, as the nutritive value is much lessened by the continued fermentations that occur in them. All of these products are rich in bacteria owing either to their age or the treatment they have undergone in the manufacture of butter or cheese. It is, therefore, all the more essential that they should be handled in such a manner as to check the continued development of germ life within them.

89. Skim milk:

Skimmed milk varies much in its bacterial content, depending upon the way in which the full milk has been treated. Milk from which the cream has been removed by the shallow setting process is usually very rich in germs, and often has so much acid that it is easily recognized by the taste. Where the cream is raised by the aid of ice water, the temperature is reduced to such an extent that the skimmed part is relatively poor in bacteria. Separator skim milk is treated in such a radically different way that it is quite a different product. The skimmed part is separated from the fat when the milk is only a few hours old so that the opportunity for germ growth has been relatively slight. The conditions under which it is separated are such that unless certain precautions are taken, the waste product will be badly contaminated in a very short time. To efficiently separate milk by the centrifugal process, the milk must be warmed to about 95° F., and if the skimmed part is not cooled at once artificially, it radiates its heat so slowly that the organisms present have the best facilities offered them for growth. It is for this reason that separator skim milk sours so rapidly after it has been through the separator. This difficulty can be obviated in two ways:

1. Rapid and efficient cooling below the growing point of the contained bacteria.

2. Pasteurization and subsequent cooling.

Where skim milk is used immediately for feeding purposes, it is perhaps hardly worth while to pasteurize, for, if it is properly cooled it will remain sweet for several hours.

90. Buttermilk:

Buttermilk contains a large amount of casein and sugar and possesses considerable value although not as much as sweet skim milk for feeding purposes. Buttermilk is usually very rich in bacteria, sometimes containing even more than ripened cream. Pammel[52] found 1,700,000 germs per cc. in buttermilk while the organisms in butter were less than half a million. This highly infected condition is due to its age, for while it is in the ripening cream, bacterial growth is taking place rapidly and when the buttermilk is strained from the fresh butter, the majority of the organisms are washed out.

91. Whey:

This is another bye product of cheese making that has for feeding purposes some value, if it can be used in a sweet condition. Like separator skim milk, it is drawn at a temperature that greatly favors bacterial development. Compared with the germ content of the raw material, whey possesses fewer organisms than skim milk or buttermilk. The greater number of the germs present in the milk are caught in the curd as it is coagulated by the rennet. An analysis of the full milk just before the rennet was added gave 12,500,000 germs per cc. while the whey tested immediately after it was drawn showed 1,500,000 for the same volume. The presence of this number in whey if left to cool naturally soon sours the product, as the milk sugar is further converted into various acids. Both whey and skim milk for feeding purposes should be carefully handled in order to get the most out of them. If the acid producing ferments are allowed to develop, the sugar is changed into various acids and much of the feeding value is lost.

92. Cleanliness of whey vats:

The above condition is that which is found in most factories and creameries. The vats for waste products are usually made of wood, consequently they are difficult to clean thoroughly, even if that procedure was carried out daily. If not carefully cleansed and sterilized by steam each day, the particles of milk or whey that adhere to the walls, quickly sour, and so infect the next lot of waste that is stored in the vat on the succeeding day. In this way the whey vat becomes a center of infection. Often, too, this already contaminated waste product is allowed to stand in the cans after it is taken back to the farms until it is thoroughly soured. Such fermented food has only a minimum value, as much of its nutritive worth is gone. Vats for these bye products should be constructed from galvanized iron and arranged so that the fluids may be drawn into the cans by gravity instead of pumping the material into the same. The vats and waste pipes should be carefully scalded each day as much as any other part of the factory.

The trouble arising from sour whey and sour milk is made still worse by the practice often seen of returning these highly contaminated fluids to the farm in the same set of cans that are used for the transportation of milk. The danger from this source has already been pointed out (see **32a**). Filthy whey vats and cans are responsible for much of the tainted milk that is seen in factories. It is necessary that the cheese-maker should have his waste vat free from sour and half fermented whey before he can charge *all* of the blame upon the patron who brings bad milk.

CHAPTER II.

ROLE OF BACTERIA IN BUTTER AND BUTTER MAKING.

93. Sweet and sour cream butter:

The good qualities of butter are so dependent upon the relation of bacteria to cream that in order to understand the subject aright, it is necessary to consider, first, their effect in cream. Butter can be made from fresh cream, or what is more usually the case from cream that has undergone a certain amount of fermentation. Sweet cream butter is that which is made from fresh cream in which there is no development of acid. Sour or acid cream butter is made from cream that is allowed to stand for a certain time, during which it undergoes a fermentative change called "ripening." This change is entirely conditioned upon the presence of living organisms that are in the milk and are allowed to develop where the cream is held for some hours. Sweet cream butter is obtainable only from cream secured either from newly separated fresh milk, or from milk that has been set at a temperature below the developing point of bacteria.

Only a small percentage of the butter that is manufactured in this country is made after the sweet cream process. The market has become accustomed to the sour cream product, and as yet there is but little demand for the other brand. Fresh sweet cream in butter making produces an excellent butter, but it lacks that pleasant acid flavor that is so characteristic of the ripened cream. Butter made from fresh cream has a very mild aroma and flavor, one that is very evanescent; consequently, its keeping qualities are poor when compared with butter made from ripened cream.

94. Ripening of cream for acid butter:

If a mass of fresh cream is allowed to stand for a day or two at ordinary temperatures, a marked change will be noted. As the cream increases in age, there is a noticeable development of acid; and with this acid, a peculiar aromatic odor and flavor of a mild, pleasant type is produced that is so greatly to be desired. Butter possessing this delicate flavor commands a higher price in the market, so that this condition is eagerly sought after by all first class butter makers. The source from which this delicate aroma is derived is not definitely known. However, this much is certain, that the production of the flavor depends upon the activity of the living organisms that are in the cream, and, that if these are excluded, the flavor is lacking.

The view has been advanced that this aromatic odor is due to the decomposition of butter fat into fatty acids, but this hypothesis does not seem tenable in the light of our present knowledge. Storch[53] holds that the flavors are produced from the decomposition of the milk sugar and the absorption of the volatile flavors by the butter fat. Conn[54] believes that the casein in the cream functions as a food material from which are formed various decomposition products, among which is the desired aromatic substance. The change is unquestionably a complex one and cannot be explained by a single fermentation. If the steps of the ripening process, as it is ordinarily carried on, are closely observed, it will be seen that the conditions under which the cream is kept during this change are those that favor the development of germ life. This fermentation as at present conducted is purely a chance matter. Experience has found that cream ripened under certain conditions produces first class butter, but the cause of the change is left to chance. Ordinarily the product is good, as scrupulous care and attention to details usually succeeds in producing a satisfactory product. Very often, however, the butter is of an inferior quality, and the reason of this deterioration, a mystery. In the light of our present studies of the fermentations that occur in milk and subsequently are transferred to cream, the causes that produce this inferior grade ought to be apparent. Where no control is exercised over the kind of fermentation that goes on in the raw material, it is a wonder that the product made under such conditions is as good as it is. As no especial attention is paid to the manner in which milk is secured and kept, innumerable numbers of many species of bacteria inevitably gain access to the milk and set up their growth changes. In this way a manifold series of fermentations are going on simultaneously, and it is mere chance whether those producing favorable products are in the ascendency or not. Fortunately, the good-flavored bacteria usually predominate, and if a reasonable degree of care and cleanliness is exercised, the ripening processes are on the average favorable for the production of good butter; but, even in the best of creameries and dairies, the product is far from being uniform, and much loss is occasioned by this variability of the output in value.

95. Methods of ripening cream:

Several methods of ripening cream are in vogue that are used in different sections of the country.

1. *Natural souring.* The simplest and oldest method is one that may be called the natural souring of the cream. In this old fashioned process, the cream is left to ripen naturally, the only artificial aid being a regulation in a crude way of the temperature of the cream. The whole process is a haphazard one and the ripened product varies in degree of ripeness; also, in kind of fermentation, depending upon the various species of bacteria that have happened to gain access to the milk. By this method, the rate of ripening is often irregular, and to overcome this, dairymen have introduced the addition of various kinds of starters as a means of hastening and rendering more uniform the ripening process.

2. *Use of naturally prepared starters.* By the use of these starters, dairymen have practically attempted in a crude way to select mixed bacterial growths to assist in the ripening of cream. In doing so they have advanced materially from the standpoint of the previous method and are also able to exclude in this way undesirable fermentative changes. For starters of this sort, several different materials have been employed. All of them, however, are liquids rich in

bacteria, and they are chosen because they abound in forms that are usually associated with a good product.

a. Among the most common starters of this class is *buttermilk from the previous churning.* A small portion of this liquid is removed after the butter comes and is added to the ripening cream of the next day. Usually the amount used as a starter does not exceed a small per cent. of the entire volume of cream to be ripened. In this way, the ripening cream is seeded with a large number of organisms that are presumably concerned in a favorable ripening process of an earlier date. The trouble with such a starter is that if an undesirable ferment gets established in any creamery, it is constantly perpetuated by such a process. When such a trouble is experienced, dairymen often resort to the importation of a fresh starter from some other factory or dairy that is known to be free from such contamination.

b. Sometimes instead of adding buttermilk, *sour cream* is used. This is less desirable because the fermentation in the old cream has usually passed beyond the proper degree of acidity and is liable to be "off" in flavor.

c. *Skim milk* is also often employed for this purpose. In using this, the milk is taken from the mixed skim milk of the factory, or what is much better, a single cow is chosen and the milk from this animal is handled with care; after the cream has been removed, the skimmed part is kept at a good ripening temperature so as to sour properly for use as a starter. In this way there is usually a less number of foreign bacteria in the milk than there is where the starter is derived from a mixed supply that is considerably older. It is very evident, that while the use of these bye products from ripened cream is a step in the right direction, it does not overcome all possible difficulties, for, after all, any starter of this nature is almost certain to be a mixed one and may possess many undesirable organisms that effect noxious changes in the ripening cream. The process is at all times an empirical one, and, while it is susceptible of certain modifications that will make it very much more efficient, yet it can be handled, and often is handled, in such a way that no benefit is derived from its use. With a bacteriological knowledge of these changes that go on in milk and cream under various conditions, it is possible to use a starter of this sort so that it will be of value for its purpose. If sterilized vessels are used in which to develop the starter; if the greatest of care is taken in the selection of a pure fluid that is known to be associated with a first class product; if this is ripened at a uniform temperature; and, if it is at all times handled so as to exclude any contaminating factor from the outside, then a starter of this sort may be perpetuated by continued re-inoculations and will be tolerably uniform in its action.

3. *Use of pure cultures of bacteria as starters.* A much more scientific method of ripening cream has been introduced within the last few years and is now being extensively used in Scandinavian and German dairies. It consists in the use of pure cultures of certain bacteria that have the ability of producing a de-

sirable ripening change in cream. The use of a pure culture of known fermentative action was first introduced by Hansen[55] into the brewing industries. He separated and cultivated for this purpose, the different varieties of yeasts concerned in this process and introduced the use of an absolutely pure yeast from a biological point of view. In this way, he was able to obtain a uniform ferment and thus eliminate all of the "off flavors" and undesirable fermentations that occurred in the process. Storch, the Danish scientist in 1890, was the first to take up this subject with reference to dairying, and in studying the effect of the different organisms present in cream, he succeeded in obtaining a form that produced the proper ripening, and with it a delicate pleasant aroma. His investigations opened up a new field for research which has been diligently prosecuted by several Danish investigators, Weigmann in Germany, and Conn in this country.

96. Problems to be solved in using pure culture starters:

In studying the effect of these various pure cultures in cream ripening, it was soon found that two problems presented themselves for solution. Not only is it necessary that butter should have this delicate pleasant aroma, but it is quite as essential that the product should have a good keeping quality. If this is lacking, the value of the butter is much lessened, for it must be consumed immediately, and consequently only a local market can be supplied with such a product.

Weigmann[56] found that some species of bacteria produced the requisite aroma while others gave it a good keeping quality. It seems that these essential characteristics are not easily found in the same species; that where one is present, the other is usually lacking.

The essential requisites in a starter of this sort are:

1. Rapid growth at a comparatively low temperature (60°–75° F) so as to ripen the cream quickly.

2. The production of a high flavor in the butter.

3. The production of butter with good keeping qualities.

Quite a large number of organisms have been isolated that fulfil some of these conditions, but, as yet, only a few forms have been found that meet all of these requisites in the highest degree, the chief difficulty being that the flavor is not strong enough to meet the demands of the general market. The number of favorable forms in butter must be relatively large, because if the mechanical part of the butter making process is properly carried out, the product is usually a good one. More than a dozen species have been isolated already that are able to make a good product, but in the majority of instances the so-called "high" flavor,—the very delicate aroma desired—is to some extent lacking. In the manufacture of the best grades by the old way, several different species must take part in the process so that the flavor is a combination of the decomposition products of different bacteria.

Attempts have been made to imitate this condition by mixing several species together; some of these being able to produce the desired aroma, while others gave the necessary keeping quality to the butter. In

Denmark and Germany these pure cultures for use as starters are sold in the market, and are now quite extensively used. In most cases a single organism is not employed, but a mixture of several germs is made. These cultures can now be secured in a powdered form, and in this condition can be kept for some time. They should be treated with all the care that a pure culture in the laboratory would be handled so as to prevent any contamination. The details of the use of these pure cultures will not be entered into here as explicit instructions are given with each sample.

It is, of course, apparent that to gain the full benefit of a pure culture as a starter that the soil in which the starter is to be planted should first be freed as much as possible from all pre-existing bacteria. This implies that pasteurization of the fresh cream is necessary, and then by the subsequent addition of a starter of known value a definite sort of a ripening will occur. Ths use of a starter of this sort in unpasteurized cream will be helpful to some slight degree but not nearly to the extent that it would be if the field was clear for its development. The experiments of Lunde[57] and others have demonstrated repeatedly that when the starter is used with pasteurized cream the product is in every way higher in grade. In using a starter of any sort, it is necessary that the cream should first be warmed to the ripening temperature so that the development of the bacteria added in the starter may not be suddenly checked.

97. Advantages derived from the use of pure culture starters:

1. The main value of a pure culture starter is in *increasing the keeping quality* of the butter. Butter made from pasteurized cream to which a good pure starter has been added will keep very much better than the ordinary product, because if properly handled the butter will not contain those organisms so commonly present in milk that produce the "off flavors" as the butter increases in age.

2. Another decided advantage is the *elimination of obnoxious fermentations* that often gain a foothold in the dairy. Troubles of this sort are almost always overcome by the use of pure culture starters as the pasteurizing of the cream usually destroys the infectious agent in the cream.

3. Another advantage is the *uniformity* of product that is gained. Even the best butter-makers fail, now and then, to secure a product always up to the standard. This failure is largely conditioned upon the varying germ content in milk incident to changes in methods of feeding, ways of handling, etc. With the use of a pure starter, these difficulties ought to be largely overcome, as the fermentative process must of necessity be the same under all circumstances. Under ordinary conditions if the starter is used in connection with pasteurized cream, the buttermilk from the churning can be used from day to day as a new starter; the pure culture being reseeded after an interval of a week or two. The use of a pure starter is entirely dependent upon biological principles, and unless these

are explicitly carried out, it will be of little avail. It is no cure-all for dirty and filthy conditions in the manufacture of butter. While its use will not enable the high grade buttermaker to excel his present standard very much it will go far towards improving the quality of those that are troubled with an inferior product.

98. Bacteria in butter:

As ripened cream is necessarily rich in bacteria, it follows that the butter will also contain germ life in varying amounts. Butter being composed so largely of butter fat is not well adapted for a bacterial food medium; consequently, the number of germs found in butter are usually less than in ripened cream.

Just as the cream and the milk vary within wide limits as to their germ population, so butter has a variable number of bacteria. As yet, butter has been studied but little from a biological point of view, so that the numerical content and the general laws governing the distribution of organisms in this material are not thoroughly known.

Sweet cream butter is naturally much poorer in germ life than that made from ripened cream. In butter made from soured cream, the numbers of bacteria vary considerably. Grotenfelt[58] reports in his analyses from 2,000–55,000 germs per cc. Pammel[59] found from 125,000–730,000 per gram, while cultures that Lafar[60] made from butter sold in Munich had very much higher proportions, ranging from ten to twenty millions of organisms per gram.

The germ content of butter on the outside of a package is very much greater than it is in the middle of a mass; this being due to the freer access of air favoring the growth of aerobic forms.

99. Changes in germ content of butter:

The bacteria that are incorporated with the butter as it first comes, undergo a slight increase for the first few days. The duration of this period of increase is dependent largely upon the condition of the butter. If the buttermilk is well worked out of the butter, the increase is slight and lasts for a few days only, while the presence of so nutritious a medium as buttermilk affords conditions much more favorable for the continued growth of the organisms.

While there may be many varieties in butter when it is fresh they are very soon reduced in numbers as well as kinds. The lactic acid group of organisms disappear quite rapidly; the spore bearing species remaining for a somewhat longer time. Butter examined after it is several months old is often found to be almost free from germs. This fact is important, for in considering the after changes in old butter the relation of these changes to the germ life would naturally be considered (see **100**).

In the manufacture of butter there is much that is dependent upon the mechanical processes of churning, washing, salting and working the product. These processes do not involve any bacteriological principles other than those that are incident to cleanliness. The cream, if ripened properly, will contain such enormous numbers of favorable forms that the access of the few organisms that are derived from the churn, the air or the water in washing will have little effect, unless the conditions are abnormal.

Salting is thought by many to exert a strong influence on the keeping quality of butter. Although the addition of this substance may increase to some extent this quality, yet the belief that it is the chief factor does not seem to be justified. Salt is only a mild antiseptic when used in a state of considerable concentration and even in saturated solutions some germs retain their full fermentative properties, while others are quite sensitive toward this chemical agent.

CHAPTER III.

ABNORMAL CHANGES IN BUTTER.

100. Rancid change in butter:

Fresh butter has a peculiar aroma that is very desirable and one that enhances the market price, if it can be retained; but this delicate flavor is more or less evanescent and soon disappears even in the best makes. While a good butter loses with age some of the peculiar aroma that it possesses when first made, yet a gilt-edged product will retain its good keeping qualities for some length of time. All butters, however, sooner or later undergo a change that renders them worthless for table use. This change is usually a rancidity that is observed in all stale products of this class. The cause of this rancid condition in butter has been attributed to the action of living organisms, to the influence of light, of air, etc.

The labors of Duclaux[61] have given us data on this subject that have established some points beyond dispute. In rancid butter, butyric and allied acids are always found, and it was supposed for a long time that the change was a butyric fermentation inaugurated by some of the butyric organisms that are found so commonly in milk and cream.

Ritsert[62] found that sterile butter became rancid in three days if exposed to the action of the light, while unsterilized, normal butter exposed to the light did not change in five months, if the air was excluded from it. Duclaux has proven that the rancid change is largely a chemical action that takes place in butter fat where it is exposed to light and oxygen; that it is not necessarily inaugurated by the vital functions of any special kind of bacteria. While the change goes on largely in a purely chemical way in most cases, there are, however, certain organisms that are able to hasten this process if they are present in the butter. Among these are the alkaline ferments, especially those that are able to affect any of the casein that may be left in the butter. In this way, a soft butter containing considerable quantities of buttermilk, and therefore rich in nitrogenous material, undergoes a rapid change and quickly becomes rancid.

101. Defects due to abnormal bacterial fermentations:

Besides the rancid change that is so common in butter there are various other defects that occur from time to time. In the majority of instances, these faults are purely mechanical but in some cases the prime cause of the trouble can be traced either directly or indirectly to the action of some undesirable bacteria. Often, the "off" flavor in the butter is due to some abnormal fermentation that has been going on in the cream or even in the milk before the cream was separated. The conditions under which butter is made are subject to such fluctuations in ripening temperature, kind and degree of ripening that it is almost impossible to make the product uniform. These variations mean a marked difference in the organisms concerned in the process and when the ripening is left to chance, it is surprising that noxious flavors do not develop more frequently in the cream and be transmitted directly to the butter. The great majority of these "off" flavors are not sufficiently marked to have a very decided character, yet they are easily detected by the taste and at once diminish the market value of the product very materially. There are besides these indefinite troubles that may be perpetuated in the butter from the milk or cream, or may originally appear in the butter after it is made, several other serious defects that are sharply characterized by the production of some peculiar property. Some of these defects or diseases in butter have been traced to a biological cause.

102. Lack of flavor:

One of the most common defects that is to be noted in butter is where there is a lack of flavor in the product. Often this may be due to improper handling of the cream in not allowing it to ripen far enough, but sometimes it is impossible to produce the high flavor no matter how much acid may be developed. This lack of flavor in this case, is due to the absence of the proper flavor-producing organisms. This condition can usually be overcome by the addition of a proper starter. The relation between flavor and desirable bacteria is very intimate, and troubles of this kind usually arise because the proper forms commonly found in the cream have been supplanted by other species that do not possess the ability of forming these aromatic substances so desired in acid cream butter.

103. Foul-smelling butter:

The specific trouble referred to under this head is a disease that has been observed in Denmark and has been thoroughly studied by Jensen.[63] Butter affected in this way rapidly acquires a peculiar putrid odor that soon ruins it for domestic use. Sometimes even, this flavor may be developed in the cream previous to churning. Jensen found the trouble to be due to several putrefactive bacteria. One form in particular which he called *Bacillus fœtidus lactis*, a close ally of the common feces bacillus produced this rotten odor and taste in milk in a very short time. Fortunately, this organism was easily killed by a comparatively low heat, so that pasteurization quickly eliminated the trouble, where it was tried.

104. Turnip-flavored butter:

Butter sometimes acquires a peculiar flavor recalling the odor of turnips, rutabagas and other root crops. Often this trouble is due to feeding, there being in several of these crops, aromatic substances that pass directly into the milk, but in some instances the trouble arises from bacteria that are able to produce decomposition products, the odor and taste of which strongly recalling these vegetables.

105. "Cowy" odor in butter:

Often there is to be noted in milk a peculiar odor that resembles that of the cow stable and sometimes the odor of the animal itself. Usually, this defect in milk is ascribed to the absorption of this flavor by the milk as it cools. Occasionally it is transmitted to butter and recently Pammel[64] has isolated from a sample of butter, a bacillus that produced in milk the same peculiar odor so commonly present in stables.

106. Lardy and tallowy butter:

The presence of this unpleasant taste in butter may be due to a variety of causes. In some instances, improper food seems to be the source of the trouble; then again, butter exposed to direct sunlight bleaches in color and develops a lardy flavor. In addition to these, cases have been found in which the defect has been traced to the action of bacteria. Storch[65] found a lactic acid form in a sample of tallowy butter that was able to produce this disagreeable odor.

107. Oily butter:

Jensen has also isolated one of the causes of the dreaded oily butter that is reported now and then in Denmark. The specific organism that he found belongs to the lactic acid group of organisms. In twenty-four hours it curdles milk, the curd being hard and white, similar to that of ordinary milk souring. There is produced, however, in addition to this, an unpleasant odor and taste resembling that of machine oil. This peculiarity in the milk is transmitted directly to butter made from affected cream.

108. Bitter butter:

Now and then butter develops a bitter taste that may be due to a variety of different bacterial forms. In most cases, the bitter flavor in the butter is derived primarily from the bacteria present in the cream or milk. Several of the fermentations of this character in milk (see **52**) are able to transfer the bitter taste to the cream and so on to the butter.

109. Further research in this line necessary: The number of undesirable changes that are to be found in butter might be extended still further by adding those that have been more or less imperfectly described. The data already gathered on these defects, or diseases in butter, we owe mainly to the researches of Jensen, Storch and Weigmann. The subject has not, as yet, received general attention from the bacteriological standpoint, so that it is probable that the list of troubles induced by bacterial action will be increased materially as the subject is more thoroughly studied.

CHAPTER IV

ROLE OF BACTERIA IN CHEESE MAKING AND IN NORMAL CURING OF CHEESE.

110. Necessity of bacteria in cheese making:

If bacteria are desirable in butter making, they are an absolute necessity in the manufacture of cheese, for without the intervention of microbes to perform certain chemical changes, the casein in the cheese would remain an inert, indigestible mass and cheese would have but little nutritive value. Cheese making, like all other industries, especially those pertaining to the dairy, is a vocation based upon experience, and while a large number of methods for different products have been more or less perfected, all of them have been evolved in an empirical manner. The why and the wherefore of many of the operations that are traditional in cheese making are even now just beginning to be understood. The application of bacteriological ideas to this branch of dairy industry is timely and seems to promise more than ordinary results. As yet, the relation of bacteria to this subject is very imperfectly understood. A few investigators here and there in Europe have shown the bearing of this new biological science to this subject but the full details of this relation are by no means elucidated. The subject as applied to our American methods of manufacture has been investigated even less, so that as yet, it is only a field of promise.

Nevertheless in spite of the lack of exact knowledge, the application in some general way of bacteriological facts will be pertinent under this head.

Milk destined for the manufacture of cheese does not, as a rule, receive any special treatment to prepare it for this condition until it comes to the factory.

111. Different methods of cheese making:

From the same kind of milk, a great variety of different cheeses can be prepared, depending upon the treatment that the milk receives during the manufacture of the cheese and the way in which the cheese is handled after it is made.

Cheese is made by precipitating the casein of the milk in either of two ways:

1. By adding *rennet* to the milk, which has the power of coagulating the casein.

2. By allowing *acid* to develop in the milk until the casein is precipitated, as in sour milk cheese.

Cheese made by the addition of rennet is divided into two general classes, *soft* and *hard*, depending upon the treatment it receives during the manufacturing process. This difference in character is produced largely by the way in which the curd is handled. There is also a marked difference in the way in which the cheese is handled during the curing process.

112. Chemical changes in cheese ripening:

Green or fresh cheese is worthless as a food product. But if newly made cheese is kept under certain conditions for a time, a deep seated chemical and physical change occurs. The cheese acquires a certain flavor, and the insoluble casein breaks down into soluble digestible materials. The surroundings under which the various kinds of cheeses are ripened differ greatly and have much to do with the proper curing of the cheese. Concerning this factor the influence of temperature and moisture exerts without doubt a strong effect upon the character of the curing change. Much of this influence is purely physical, but these conditions also affect the rate and kind of bacterial growth to a certain extent.

From a chemical standpoint, the following changes are to be noted in cheese as taking place during the ripening process. Little or no change in the character or condition of the fat is to be observed, but the casein which is only very slightly soluble when fresh, is converted into a series of soluble compounds closely allied to the peptones. The sugar content in the milk usually disappears completely, different acids being formed, while the ash constituent practically remains the same. Much of the water also is lost by the gradual drying out of the cheese.

113. Relation of cheese ripening to living organisms: These chemical changes which are really fermentative processes, were for a long time supposed to be purely chemical in their nature, but when it was found that fermentation was often induced by the action of living organisms, the relation of the ripening processes in cheese to biological causes was suspected. No actual proof of this was shown, however, for many years, and it was not until the researches of Duclaux[66] that any definite knowledge on this point was gained.

His studies revealed the presence of numerous organisms, especially the bacteria, belonging to both aerobic and anaerobic classes, that are to be found in the cheese. The bacteria excrete the ferments that bring about these chemical changes. Duclaux has called the ferment having this power *casease*, and the material into which the casein is converted, *caseone*. These researches led the way to the view that the ripening of cheese is due to the action of minute living organisms. This conclusion was still further strengthened by the experiments of Adametz, who demonstrated that thymol, creolin and other disinfectants that have no effect on the casein, inhibited entirely the ripening process. While the curing of cheese is now quite generally ascribed to the influence that the different forms of organic life exert by their fermentative action upon the casein, many details of the process are as yet very imperfectly understood.

Cheese belonging to the soft type cure much more rapidly than the hard, firm cheese.

In the ripening changes that occur in soft cheese made with rennet, molds and other fungi, as well as bacteria play an important rôle, and the curing process extends slowly from the rind to the center. The changes in the firm cheese are much slower in their action, so that the hard type of cheese often require several months before they are fit for use. With this type of cheese, bacteria are intimately concerned in the curing process, which goes on throughout the whole cheese simultaneously.

When different varieties of cheese are made from milk in the same locality, the germ content of even the ripened product has a marked similarity as is illustrated by Adametz's work[67] on Emmenthaler, a hard cheese, and Schweitzer Hauskäse, a soft variety. Of the nine species of bacilli and cocci found in ripe Emmenthaler, eight of them were also present in matured Hauskäse.

In different kinds of cheese, or even in the same variety, there is a marked variation in the bacterial flora if made in different countries. In the nineteen species isolated by Adametz in Emmenthaler and cottage Hauskäse, not one was found that corresponded with the ten species that Duclaux[68] found in Cantal, a French cheese. So far as Pammel's descriptions[69] of forms prevalent in American cheddars have been published, and also in my own experience, no forms have as yet been isolated that correspond with those that are described as commonly present in Europe.

114. Bacteria in the cheddar process:

As most of the cheese manufactured in this country belong to the hard, firm type, the relation that bacteria bear to this branch of the dairy industry will be first considered.

Cheese as manufactured in this country is made almost entirely in large factories where the milk of many patrons is collected for this purpose. Under these conditions, it is always well seeded with all sorts of bacteria. Usually the lactic acid forms predominate, and here in cheddar cheese making their presence is not entirely undesirable. One of the conditions essential in this method of cheese making is the presence of a certain amount of lactic acid. This is developed in the milk by the fermentation of the sugar by the lactic acid organisms. The cheese maker hastens, if necessary, the development of this acid by ripening the milk still further before the rennet is added.

115. Bacteria in rennet:

The addition of rennet does not check the development of the bacteria in any way. The idea has been advanced that the germ life incorporated with the milk from the rennet exerts a considerable influence on the cheese; this view is however, hardly tenable, in spite of the fact that rennet always contains enormous numbers of organisms, for the amount of rennet that is added is so small that this does not appreciably increase the germ content of the milk. An analysis of the milk and rennet used in cheese making has shown that for every germ derived from the rennet, there are upwards of a thousand organisms that are naturally present in the milk. The growth of most bacteria is highly favored by the temperature used in the cooking of the curds. Although it is difficult to study the germ flora at just this stage, an analysis of the whey as it is freshly drawn indicates a very large number of organisms, so that when the curds are put in press, they are filled with bacteria.

116. Condition of green cheese:

If cheese is examined soon after it is pressed, a large number of germs will be found, included under a number of different species that are also demonstrable in the milk. In cheddar cheese as ordinarily cured, the conditions do not favor a very rapid development of bacteria as there is not an excess of moisture present. The organisms that may be present in the air or on the shelves of the curing room, exert no appreciable influence on the ripening of the hard firm cheese. In the maturation of some of the soft European cheeses, this factor is of more importance. As the curing process goes on in cheese, the bacteria increase in numbers, but at the same time a few forms thrive more rapidly than the others.

117. Bacteria concerned in ripening changes: It will be remembered that in milk there are several types of fermentative action due to different classes of organisms. One class of these, the ferments affecting the casein, so change this compound that it is easily dissolved. This dissolution is really a process of peptonization in which the proteid compounds of the milk are changed into soluble peptones. It is among this class of bacteria that we may expect to find those organisms that take part in the changing of the casein in cheese.

Whether this breaking down process is due to the influence of a single organism or not is not yet definitely settled. In the hard, firm cheese, the change goes on throughout the whole mass simultaneously, and has no reference to the free access of oxygen. Freudenreich[70] enveloped a cheese in sterile vaseline, another in quicksilver so as to exclude the air, and in both cases he found the cheese had ripened although it had a bitter flavor.

118. Normal ripening of Swiss cheese:

Baumann[71] has recently contributed a valuable addition to our knowledge of the normal changes, especially to those that occur in Swiss (Emmenthaler) cheese. In this kind of cheese the presence of the so-called Swiss holes are constant. The appearance of these holes in the cheese and their general nature indicates that they are due to the action of gas-forming organisms. The distribution of the holes more or less uniformly throughout the entire mass suggests that the organism is either a strict anaerobe or at least a facultative organism with anaerobic tendencies. In his study of milk, Baumann was soon able to isolate a common form from numerous samples of milk and also from cheese that had the property of forming compounds closely related to the peptones from the proteids in the milk, and at the same time of producing lactic acid at the expense of the milk sugar. This organism he holds to be causally connected with the normal ripening changes as they exist in Swiss cheese.

Von Freudenreich has also found a form that is concerned in the production of these large gas holes. There is now no question that the normal holes are the result of gaseous fermentations, and probably there are several species that are able to take part in the process.

As yet, no exhaustive study of the ripening of our cheddar cheese has been made, so that the specific nature of the curing process can not be considered in detail.

It is highly probable that in the maturing of cheese we have to deal with a more complex problem than that of butter making, as the nitrogenous elements in cheese offer a more favorable field for continued germ growth than the butter fat.

119. Influence of germ life on flavor:

The conversion of the casein into a soluble form is one type of the fermentative change. The production of the fine flavor is probably brought about by the action of another set of ferments. The two properties may be resident in the same organism, but as is the case in butter making, it is quite probable that they are due to the action of distinct organisms. This seems highly probable from the experiments of Freudenreich who found that the ripening went on normally even if the air was excluded, but that the taste of those ripened without air was bitter and abnormal. The suggestion has been made and it seems very probable indeed, that the good quality of cheese is often dependent more upon the character of germ life than it is upon the kind of feed to which the cows have access. Our domestic Swiss cheese, or even cheese of this class made in Germany rarely have the peculiar flavor that is found in the product imported from the Swiss valleys. For centuries this peculiar brand has been made in that country, until the factories and dairies have become stocked with the right breed of germs, capable of bringing forth the desired fermentation. These germs are so prevalent that the milk is infected with them under ordinary conditions; consequently the ripening changes bring about the production of the peculiar flavor that is so highly prized. Grotenfelt[72] states that in his studies on the Swiss type of cheese made in Finland, he found but very few of the species that Adametz described as occurring normally in the same kind of cheese made in Switzerland.

120. Normal ripening of foreign brands of cheese:

Although the bulk of cheese made in this country is prepared according to the cheddar process, the amount of foreign cheeses that are consumed is constantly increasing. In order to show the relation that exists between the preparation of these different brands and organisms like the fungi and bacteria, a very brief résumé of some of the fermentations that are connected with the ripening processes in some of these cheeses is here appended.

The manufacture of only a few of the many European cheeses has as yet been attempted in this country. The influx of foreigners has augmented the demand for these products materially, and at present there are made in various sections of the country considerable quantities of the Swiss (Emmenthaler), brick and limburger varieties of cheese.

121. Swiss or Emmenthaler cheese:

Emmenthaler is another representative of the firm type of rennet-made cheese, and differs mainly from the cheddar type in that it is made from a sweeter curd, i. e., the acid is not strongly developed. The green cheese always contain a certain amount of sugar and from the fermentation of this material, gas is formed which results in the production of the Swiss holes or "eyes" in the cheese. The formation of these eyes is a part of the normal ripening process. Von Freudenreich, Weigmann and Baumann have isolated organisms that are concerned in the formation of these gas holes. It has been shown that if the gas-producing germ is present in the milk in abnormal quantities, a too vigorous fermentation may take place, resulting in the production of a spongy structure.

Adametz, who has studied the Emmenthaler cheese very thoroughly, finds from six to eight different species always present. There is a marked numerical increase in bacteria (from 90,000 per gram in green cheese to 850,000 in ripe), as the cheese increases in age. This increase is mainly in solid forms, i. e., those bacteria that do not liquefy gelatine; the liquefiers being unable to hold their own in the conditions under which they are forced to live. As yet no single organism has been isolated that is able to produce all of the ripening changes that are to be noted in this cheese.

122. Relation of fungi to soft cheese ripening: While the majority of the firm cheese made with rennet are ripened almost solely by the action of bacteria, most of the soft varieties are dependent upon a fungus vegetation to assist in the ripening process. In some cases this fungous growth gains access to the cheese in purely a chance manner; then again, the cheese is seeded with the requisite organisms to produce the desired fermentation.

123. Roquefort cheese:

In Roquefort cheese, a French cheese made from goats' milk, the essential agent in the curing change is the ordinary blue-green bread mould, *Penicillium glaucum.* This organism is cultivated first in a most careful manner on specially prepared bread. It is then dried, pulverized, and when the curds are put in the moulds, it is sprinkled over the surface of them, thus mixing the mould organism thoroughly with the fresh curd.

At ordinary temperatures this organism develops too rapidly, so that the cheese to ripen properly must be kept at a low temperature. The town of Roquefort is situated in a limestone country, in a region full of caves, and it is in these natural caves that most of the ripening is done. These caverns are always very moist and have a temperature ranging from 35° to 44° F., so that the growth of the fungus is retarded considerably. The rapidity of the ripening varies somewhat. In summer it is more rapid than in winter, but the flavor is somewhat impaired by this rapid curing. The spread of the mould throughout the ripening mass is also assisted in a mechanical way. The partially matured cheese are run through a machine that pricks them full of small holes. These slender canals allow the mould organism to penetrate the whole mass more thoroughly; the straw matting upon which the ripening cheese are placed helping to furnish an abundant seeding of the desired germ.

124. Stilton cheese:

Stilton cheese, a very rich cream cheese made in

England, is another of these fancy cheeses that is ripened by the aid of a fungous growth. To distribute the desired mould in the fresh cheese, plugs are taken from ripened Stiltons, and the cores are interchanged so it is in a sense an inoculation process.

125. Brie cheese:

Brie cheese is one of the most important of the soft cheeses that are made in France. This cheese is made from full or half skimmed milk. The ripening process is a peculiar one and the cheese must be handled with great care, otherwise undesirable fermentations are sure to set in. There is no artificial seeding of the curd in the Brie cheese manufacture, the maker depending upon the germ content of the ripening cellar for the proper organism at the proper time. So necessary is it that the ripening room should be impregnated with these fungi and bacteria that when new curing cellars are constructed, the maker washes the walls and floor of the new room with scrapings from an old factory. In this way, he introduces the ferments necessary for the maturation of the green product. Brie cheese is ripened on mats and soon after it is placed in a relatively cool ripening cellar, the surface of the cheese becomes coated with a thick growth of the common mould, *Penicillium glaucum*. The cheese are turned often so as to render the growth of the mould as uniform as possible; the object being to cover the cheese with a thick felting of the mouldy growth, to diminish drying. The temperature must be kept at a low point, otherwise the mould will fruit in which condition the white fungus turns to the blue green stage.

This growth of the Penicillium merely prepares the soil for a more essential organism. The cheese as it goes to the curing room is very acid and consequently favors the growth of the fungi. The mould organisms derive their sustenance from this acid medium, slowly consuming it until the reaction of the cheese is made neutral. With this change in reaction, there appears in the course of a few weeks another coating beneath the blue felting. This is at first white but soon passes into a red color. This slimy coat below the mould layer is made up of diverse species of bacteria and fungi that are able to begin development after the acid is consumed by the blue mould. The red coating is a casein ferment producing an alkaline reaction that is unfavorable for the growth of the blue mould. The two sets of organisms are then essential; one preparing the soil for the ferment that later produces the requisite ripening changes. The process as carried on is purely empirical and if the red coat does not develop at the proper time, the maker resorts to all sorts of devices to bring out the desired ferment. The appearance of the right form is dependent entirely upon the proper reaction of the cheese, and if this is not suitable, the wished-for fungus will not appear.

126. Neufchatel cheese:

Neufchatel is another of the highly prized French soft cheese that is ripened under the influence of a mould. A white fungus covers these little cheese in the course of a few days after they are prepared. As this develops, they are stored in a ripening cellar for two or three days when they become covered with a blue growth that is merely the fruiting stage of the white felted coat.

In all of these soft cheeses where the ripening goes on under the influence of fungi, the change takes place from the outside toward the center. There is always an outer layer that is changed to such an extent as to be unfit for food. In the inner part, the casein is transformed by the slow diffusion of the ferment throughout the whole mass.

CHAPTER V

ABNORMAL FERMENTATIONS IN CHEESE.

127. Susceptibility of cheese to germ diseases:

Cheese more than butter is subject to undesirable fermentations incited by bacteria because it is so much better adapted for germ growth on account of its high nitrogen content. Then too, the method of curing is so often without control, especially with many of those methods that employ fungi as agents in the curing process. This inability to check the ripening at the proper moment often results in the decomposition processes being carried too far and in this way unpleasant flavors are developed.[73]

128. "Off" flavors in cheese:

Besides a number of more or less distinct fermentations of diverse characters there is to be seen a whole host of somewhat ill-defined defects in flavor and odor. Cheese possessing these may be thoroughly ripened and therefore digestible, but they lose much in market value by reason of these improper flavors. In many cases, troubles of this sort are undoubtedly traceable to faulty manufacture, but more often the defect arises from the presence of some fermenting organism that is the cause of the unpleasant flavor. Our knowledge of the influence that the different species have in the ripening changes of cheese is still too meager to enable us to trace in all cases, these undesirable conditions to their proper source. The great majority of the taints observed in the factory are due entirely to the abnormal development of some of these forms capable of evolving unpleasant or even putrid odors. The most of them are seeded in the milk before it comes to the factory and are due to careless manipulation of the milk while it is still on the farm. Others gain access to the milk in the factory, owing to the unclean conditions of one sort or another. Sometimes the cheesemaker is able to overcome these taints by vigorous treatment, but often they pass on into the cheese only to detract from the market value of the product.

In studying the effect of the different organisms found in milk in their relation to cheesemaking, I have isolated a number of different species that are concerned in the production of these "off" flavors in the curd. If these organisms are seeded in pasteurized milk and the infected milk made into cheese they develop in the curd odors that are often to be noted in factories troubled with taints. These indefinite "off" flavors and odors are often insufficiently pronounced to give them a name, consequently, it will be impossible to describe them under any well defined head.

129. **Fermentations producing gas in cheese:**

One of the worst and at the same time most common troubles in cheese-making is where the cheese undergoes a fermentation marked by the evolution of gas. The presence of gas is recognized by the appearance either of spherical or lens shaped holes of various sizes in the green cheese; often the gas holes appear in the curd even before it is put to press. Usually in this condition, the curds look as if they had been finely punctured with a pin, and are known as "pin-hole" curds. Often, however, the gas holes are larger, sometimes even approaching the large round so-called Swiss holes. When the gas bubbles are extremely numerous they are more apt to be restricted in size. The formation of gas may continue to such an extent that the curd even floats on the surface of the whey before it is removed. These "floating curds" are permeated through and through with gas bubbles, giving the curd an appearance somewhat resembling that of a coarse sponge. If "gassy" curds are put to press in this condition, an abnormal change is sure to occur within a few days. The fermentation goes on in the green cheese causing it to swell or "huff," until it may often be nearly spherical in shape and considerably enlarged. This bloating, which is due entirely to the gaseous fermentation products, diffuses with difficulty in the new cheese so that the mass rapidly swells. The fermentation may be so energetic as to actually cause the cheese to split, owing to the pressure of the contained gas. In the severer types of this gaseous fermentation, the product is rendered worthless, but even where the development is not so marked, the flavor of the cheese is impaired and the market value somewhat diminished. The difficulty may occur at almost any season of the year, but the trouble is most frequently observed in the late summer months. So common are these difficulties that they may be regarded as the worst cheese troubles with which our cheese-makers have to contend. Cheese-makers have by continual practice learned how to handle milks that develop these abnormal gaseous changes, and by the rapid development of acid in the whey, to hold in check this disease.

The cause of the difficulty has long been charged to various sources, such as lack of aeration, improper feeding, retention of gases, etc., but in all these cases, it was nothing more than a surmise. Very often the milk does not betray any visible symptom of fermentation when received, and the trouble is not to be recognized until the process of cheese-making is well advanced.

Recent studies from a biological standpoint have, however, thrown much light on this troublesome problem; and, with the *cause* of these fermentative changes more fully recognized, it is quite probable that improved methods of handling milk so as to exclude these troubles will be rapidly introduced.

130. Relation to living germs:

The formation of gas, either in the curd or after it has been put to press, is due entirely to the breaking down of certain elements, such as the sugar of milk, under the influence of various living germs. This trouble is then a class fermentation and is therefore much more widely distributed than it would be if it was caused by a single specific organism. There are present in all milks, numerous forms of organic life that are capable of producing a varied series of fermentations in which different gases may be given off, either as a chief product or as a bye product.

Among these organisms are a large number of the bacteria, although yeasts and allied germs are often present in milk and are likewise able in some cases to set up fermentative changes of this sort. There are a large number of the bacteria that possess the faculty of decomposing milk sugar in such a way as to evolve gas. Chief among these are some of the lactic acid organisms that instead of splitting milk sugar directly into lactic acid, decompose it in such a way as to give off CO_2 and H, and in some cases, alcohol. There is also a close relation existing between those germs that are able to produce an infectious inflammation in the udder of the cow and some forms capable of gas evolution. Several epidemics of "gassy" milk have been traced directly to animals suffering from an acute inflammatory condition of the udder in which it has been shown that the organisms producing this disease were the direct cause of the gas production in the milk. These germs are so numerous that they are almost always present in any sample of milk, especially if it is a little old. From a single sample of mixed milk examined in August, Bolley and the writer isolated six different species that formed gas in sugar solutions. Even in winter we have found milk to be constantly peopled with them in such numbers as to require special care in the manufacture of cheese. Under normal conditions, where care is taken in the handling of the milk, they are not usually found in large numbers, and when thus numerically restricted, are doubtless kept under subjection by the competition of numerous other bacteria in the milk. These fermentations are very often observed in foreign kinds of cheese, especially those that are made after the sweet-curd process. Adametz has collated data on the European forms that have been isolated and has found nineteen bacterial species (five cocci, fourteen bacilli) and eight varieties of yeast-like fungi that are gas-producing organisms.

If pasteurized milk is seeded with a pure culture of any of these gas-forming organisms and made into cheese, an intense fermentation is always to be noted. This oftens appears in the curd, sometimes even before the whey is drawn. The cheese when taken from the press develops gas rapidly, causing it to swell and a cross section of it at this stage will show a spongy structure. If the cheese is left intact, the fermentation may progress to such an extent that the pressure of the contained gas will cause the rind to split open. This fermentation does not last for more than a few days; then the swelled cheese sinks as the gas slowly diffuses throughout its mass. An analysis of the gas reveals the presence of CO and H.

131. Normal and abnormal gas fermentations in Swiss cheese:

It seems probable from the researches of v. Freudenreich,[74] Baumann[75] and Weigmann[76] that in Swiss cheese (Emmenthaler), certain gas-producing forms may take part in the normal ripening. Solid or "blind" cheese (*gläsler*), i. e., cheese devoid of these Swiss holes may ripen quite well but the market value is diminished on account of the absence of the round holes. The production of these large holes (eyes) in Swiss cheese is due to the gas formed by these fermentations. Where gas-producing organisms are sparingly present, they probably have in cheese cured under our American conditions but little effect, but if they once gain the ascendency in milk, trouble is sure to ensue. The original seeding of the mixed milk at the factory with quantities of these organisms is often due to infection arising from a single patron who brings tainted milk of this sort. The addition of a small batch of infected milk to the general milk supply is sufficient to propagate the disease to such an extent as to contaminate the whole mass used for cheese.

The size of the gas holes as they appear in abnormal cheese is by no means constant. Neither is the character of the hole dependent upon any specific germ. If the gas-forming bacteria are plenty and quite evenly distributed the holes will be small and numerous. This is the condition seen in "pin-hole" curds; Swiss cheese of this sort is called *Nisslerkäse.* Where the organisms are less frequent and develop in small groups, then the gas bubbles are much enlarged and the Swiss holes are present in the cheese. As the different organisms vary in their intensity of gas formation, this too, modifies the size of the gas holes to a marked degree.

132. Tests for the detection of "gassy" milk: In order to detect milk infected with gas-producing organisms, several different tests may be applied. Those that are effective are based upon the rapid incubation of the organisms in milk by subjecting them to a constant warm temperature, from about 87° to 95° Fahr. This hastens the growth and the consequent development of the fermentations to such an extent that fine gas bubbles will appear in the course of a few hours. The test may be made even more delicate by the addition of a small amount of rennet to a sample of suspected milk. In this way, as the gas is evolved, it is caught in the coagulated milk, until a bubble of sufficient size accumulates to be recognized easily. Often it gathers in sufficient quantity to force its way through the soft curd leaving rents and slits behind to betray its passage. In order to determine approximately the amount of gas evolved (as this is highly important in cheese-making), I have used with success the so-called fermentation tube that is employed in chemical work in the determination of gas given off in any fermentation. This tube, which is nothing more than a **U** tube closed at one end, is filled with some nutrient fluid like sterilized skim milk or whey that has been rendered neutral or nearly so by the addition of sodium hydroxide or lime water. After this is sterilized in steam and protected from air infection by a plug of cotton, a definite amount (1 to 3 cc.) of the milk is added. The tube, or tubes if the milk of different patrons is examined simultaneously, is kept at or near the blood heat (97° to 100° F). Treated in this way, a rapid growth of most germs takes place and if gas-producing organisms are present, fine bubbles will begin to collect in the closed arm of the tube in a few hours. The volume of gas can easily be measured in this way, so that the relative ability of different patrons' milk to form gas can be determined with ease. In using this test, its accuracy depends upon the way in which the fermentation tubes are handled. If they are first sterilized in steam and filled carefully with thoroughly boiled nutrient fluid, the only source of gas formation must come from the milk that is seeded in them. This method needs merely the observance of those rules that are the basis of cleanliness for its successful use.

133. Bitter cheese:

During the curing of cheese, an intensely bitter taste is sometimes noted, when the cheese is only partially ripened, but as the maturation of the raw material progresses, the bitter flavor is lost.

Guillebeau[77] found several of the organisms that he connected with udder inflammation, able to produce a bitter ferment when made into cheese. Von Freudenreich[78] has recently isolated a new form, *Micrococcus casei amari* (micrococcus of bitter cheese) from a sample of bitter cheese. This germ proved to be a new form although closely related to Conn's micrococcus of bitter milk. It develops lactic acid rapidly, coagulating the milk and producing an intensely bitter taste in the course of one to three days. When milk infected with this organism is made into cheese there is formed in a few days a decomposition product that confers a marked bitter flavor to the cheese. It is peculiar that some of the organisms that are able to produce bitter products in milk do not retain this property when the milk is worked up into cheese; in these cases it is probably the sugar that is affected, and as this material rapidly disappears in cheddar cheese, there is no nutritive substance well adapted for the growth of these organisms.

134. Putrid or rotten cheese:

Sometimes cheese undergoes a putrefactive decomposition in which the texture is profoundly modified and various foul smelling gases are evolved. These may begin from the exterior where they are first noticed as small circumscribed areas in which the rind is changed into a soft slimy mass. This area may slowly extend into the cheese. Then, again, the interior of the cheese is first to undergo this slimy decomposition. The soft varieties of cheese are more prone toward this rotting fermentation than the hard although the firm cheeses are by no means exempt from the trouble. The trouble with soft made cheese, especially Limburger that is known to the Germans as "Verlaufen" is similar to these. It is where the inside of the cheese breaks down into a soft semi-fluid mass. In severe cases, this interior mushy mass may even break through the rind, in which case, the whole interior of the cheese may flow out as a thick slimy mass having sometimes a putrid odor. The conditions favoring this putrid decomposition are usually associated with an excess of moisture, and a too low ripening temperature. As yet, this fermentation has not been studied closely, but it is undoubtedly connected with a series of putrefactive organisms that are incorporated in the milk from the beginning. It may be predicted that in most cases the organisms concerned will belong to those that are able to affect the casein. Cheese affected with this fermentation have usually a very sharp flavor, that soon becomes so pronounced as to render the product worthless for food purposes.

135. Pigment changes in cheese:

Occasionally with hard cheeses, but more often with the softer foreign varieties, abnormal conditions are noted that express themselves in the production of various pigments in, and on, different sorts of cheese. The production of these parti-colored pigments are due mainly to the action of bacteria, yeasts, and molds. More frequently they are merely superficial, and affect only the exterior layers of the cheese.

Adametz[73] has given a most excellent résumé of these troubles in his work on the "Abnormal Ripening Processes of Cheese."

1. *Red Cheese.* Adametz found in milk, two kinds of micrococci that are concerned in the production of small red flecks on the surface of Swiss cheese. Schaffer isolated in 1888 from a sample of cottage cheese, a yeast form capable of producing a red coloration that often extended into the middle of the mass.

Two or three different forms of filamentous fungi producing a red pigment have been isolated, that seem well adapted for growth on the surface of cheese.

In our American cheddar cheese, red spots are occasionally noted that may be merely on the surface, but sometimes extend throughout the cheese. In some instances, these places have been found to be swarming with bacteria but no specific organisms have been isolated that were proven to be the direct cause of the red coloration.

2. *Blue cheese.* In this connection, it will be proper to omit those cases where blue cheese have been found to be due to the action of copper and iron salts derived from the vessels used in the manufacture of the product. De Vries has described a contagious disease found in Edamer cheese in Holland. In some years the trouble is wide spread and is the cause of much loss. It makes its appearance as small blue points in the inside, increasing rapidly in size and number until the whole mass is badly affected. The organism producing the same is an anaerobic germ, for the color is lost and does not reappear when exposed to the air. Blue cheese have also been reported as having been caused by the action of blue milk bacillus, *B. cyanogenus*, (see 58).

3. *Black cheese.* Black cheese have now and then been noted, especially with Limburger products. This appearance is caused by the copious growth of different forms of low fungi, mainly those that spread out in tiny threads, like the molds. In one instance a yeast-like form has also been isolated. So far as known, troubles of this sort are not caused by bacteria.

136. Poisonous cheese:

The production of poisonous ptomaines in cheese is perhaps more common than with almost any other food product. Vaughan, to whom the most of the work on this subject is to be credited, reports over 300 cases of cheese-poisoning in two years. It seems to be very much more common here in America than it is in Europe. Vaughan has isolated from numerous samples of cheese, a highly poisonous alkaloid that he has called tyrotoxicon. This ptomaine he has also abundantly demonstrated in milk, cream and ice cream. The poisonous substances formed in the milk are probably produced through the agency of putrefactive organisms that gain access to the milk.

The poisons secreted in the milk are transferred to the cheese with their virulence often unimpaired, so that serious complications follow where infected material is used for human food (see 70). Danger from this source is to be noted particularly with the so-called sour milk cheese, as the methods by which these are made give the most favorable opportunity for the development of poisonous decomposition products.

137. Prevention of cheese diseases:

In attempting to treat the various defects or diseases found in cheese, no uniform method can be applied in all cases. Excluding faults due to manufacturing methods, the most of the troubles traceable to germ origin get their start in the milk before it comes to the factory. In some instances the maker can repress or subdue the influence of these undesirable ferments by changing his methods somewhat to suit the varying conditions, such as the rapid development of acid in gassy fermentations, but what he must insist upon under all circumstances is that the milk shall be handled by the patron in such a way as to exclude as far as possible all conditions favoring the access of any organisms into it. If this is insisted upon, and the way pointed out to each individual patron as to the manner in which his milk becomes infected, then the maker may expect to reap the reward for his teachings in the improved conditions of the milk.

138. Pure culture principle in cheese making: It is possible that future research may enable us to apply the pure culture principle to cheese making as it has to butter making, but the problem is far from being solved at the present moment. The normal ripening changes that take place in cheese made under natural conditions must first be thoroughly investigated before we can hope to apply any sweeping changes—such as these will necessarily involve—to our methods of cheese making. Until this ideal method is worked out and put upon a practical basis, the maker can avoid much of the trouble that he now encounters if he studies the subject in hand, from the biological point of view and utilizes the principles here expounded day by day in connection with his own experience from the maker's standpoint.

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GLOSSARY.

Adventive.—Species introduced from foreign sources.

Aerobes.—Bacterial organisms requiring free oxygen for growth.

Anaerobes.—Bacteria growing without free oxygen.

Albuminous.—Substances containing albumen (white of eggs).

Anthrax (splenic fever).—A contagious animal disease characterized by blood poisoning.

Antiseptic.—Any substance capable of restraining bacterial development.

Arthrospore.—Spore formed from the whole mother cell.

Bacillus (plural, bacilli).—Straight, rod-like bacteria of varying length.

Casease.—The enzyme capable of converting casein into soluble compounds.

Caseone.—Casein that has been rendered soluble by enzymes.

Chromogenic.—Bacteria capable of forming colored products.

Cilia, (singular, cilium).—Tiny, whip-like protoplasmic appendages of cells, serving as locomotor organs.

Coccus (plural, cocci).—Bacteria having a spherical shape.

Colony.—The progeny of a single germ growing in an isolated mass.

Constructive.—Organisms able to build up organic material from simpler compounds or elements.

Destructive.—Organisms whose function is to break down organic tissue.

Disinfectant.—Any substance able to destroy germ life.

Endospores.—Spore formed in a mother cell from a part of its protoplasm.

Enzymes.—Unorganized chemical ferments not endowed with life.

Facultative.—Forms that possess the faculty of growing under varied conditions.

Fission.—Division of a cell by direct partition.

Germicide.—Any substance capable of destroying germ life.

Indigenous.—Bacteria normally found in any given place or habitat.

Mother cell.—A typical vegetative cell capable of reproduction.

Obligate.—Bacteria that are obliged to grow under certain conditions.

Optimum growth temperature.—The best temperature for the growth of any form.

Parasites.—Living organisms subsisting on living matter.

Pasteurization.—The use of heat from 140° to 165° F. as a germ destroyer.

Pathogenic.—Bacteria able to produce disease in living tissue.

Pepsin.—Digestive enzymes present in the stomach of animals.

Peptonizing.—The conversion of insoluble proteids into a soluble form (peptones).

Proteids.—Complex nitrogenous substances usually of an insoluble nature.

Protoplasm.—The living substance of organic tissues.

Ptomaine.—A poisonous chemical product of bacterial growth.

Pure culture.—A bacterial growth of a single species in a sterile medium.

Saprophytes.—Living organisms subsisting on dead organic matter.

Silex.—The quartz element in rock (pure sand).

Spirillum (plural, spirilla).—Curved, or bent cylindrical bacteria.

Sterilization.—The use of heat in the neighborhood of 212° F. as a germ destroyer.

Thermal death point.—That degree of heat fatal to any species.

Toxicogenic.—Bacteria producing toxic or poisonous effects.

Trypsin.—The active principle of the pancreatic secretion.

Tyrotoxicon.—A poisonous ptomaine isolated from milk and cheese.

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