BACTERIA IN MILK

AND ITS

PRODUCTS

CONN

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DESIGNED FOR THE USE OF STUDENTS IN DAIRYING AND FOR ALL OTHERS CONCERNED IN THE HANDLING OF MILK, BUTTER OR CHEESE

BY

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43 ILLUSTRATIONS

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PREFACE.

Successful dairying at the present time depends, to a very large extent, upon skill in handling bacteria. It is impossible to meet the present conditions of the city milk supply, of butter-making and cheese-manufacture, without a knowledge of the relation of these microscopic organisms. Bacteriology has become, therefore, a necessary part of dairy courses. The subject is, however, equally important in other directions. The demonstrated connection between milk bacteria and the distribution of certain diseases has brought the subject of bacteria of milk products forcibly to the attention of boards of health and sanitarians. To meet the needs of such persons and all others interested in the handling of milk is the purpose of this work.

Most of the facts given in the following pages have been published in scientific papers which have appeared in the last ten years. Some of them, however, are the result of personal investigations not as yet published. A list of the more important recent references to literature upon milk bacteria is given at the close of the text. Wherever it seemed to be necessary references to this literature have been inserted in the body of the text.

In the last chapter are given the methods of bacteriological analysis in use at the present time, but, since some of these are admittedly unsatisfactory in certain respects, it is quite likely they may be replaced by better ones in the near future. The growing appreciation of the necessity of bacteriological study of market milk has made it desirable to insert careful directions for laboratory work, even though recognizing that these may not long remain the best methods of laboratory technique.

Middletown, January, 1903.

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BACTERIA IN MILK AND ITS PRODUCTS.

CHAPTER I.

THE NATURE OF BACTERIA.

THE last fifteen years have seen a very great change taking place in the methods of handling milk products. These changes have affected not only the handling of milk distributed for public consumption, but also the manufacture of butter, and they are rapidly producing improvements in the manufacture of cheese. The modifications of dairy methods have been more rapid in the last twenty years than ever before, and we may almost say that greater changes have taken place within this short time than in the previous two centuries. A variety of new conditions and new discoveries have contributed to this end, but nothing has had a greater influence than the knowledge which has developed concerning the nature of bacteria and their relation to milk. Dairy bacteriology has to a large extent revolutionized modern dairying. Sometimes the connection between modern methods and bacteriological discoveries is very apparent; sometimes it is less apparent or only incidental, but there is no one series of facts which has produced such a profound effect upon dairy processes as those associated with bacteriology.

WHAT ARE BACTERIA?

Bacteria are microscopic, colorless plants which are extremely abundant in earth, air and water. The term bacteria is a comparatively recent one. In the early work of the nineteenth century these plants were not recognized as forming a logical group, and not until about twenty-five years ago were they grouped together under the name *Bacteria.* To-day, while we regard them as a distinct group, we recognize that they are closely related to *yeasts* and only a little more distantly related to *molds.* In the following pages both yeasts and molds will be occasionally referred to, but the part they play in dairy products is far less important than that of bacteria.

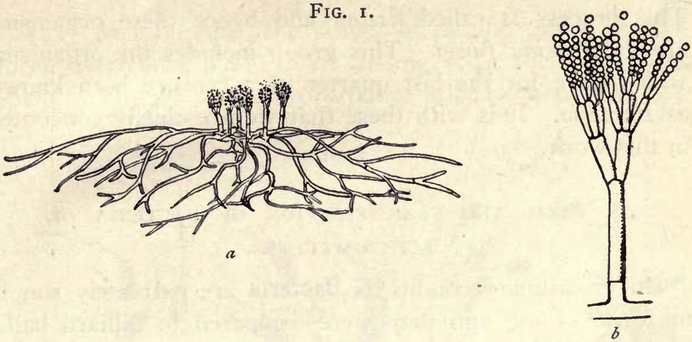
In order to understand bacteria and their allies a brief account of their structure, classification and general functions is necessary:

I. CLASSIFICATION OF FUNGI.

Bacteria belong to the group of colorless plants called **Fungi.** All of the group of **Fungi** are of great significance to agriculture, performing important functions based upon their power of decomposing organic substances and using them as food. Unlike the ordinary green plants, they are commonly unable to live upon mineral foods and are in consequence extremely important agents in disposing of the great quantities of dead organic matter in nature. These **Fungi** may be for our purpose conveniently divided into three divisions:

1. **Higher Fungi.**—Under this head we may group together a large variety of colorless plants comprising several large classes, including such forms as *molds, mushrooms,* etc. We are only incidentally concerned with them in this work, although many are of great importance in agriculture. They are generally characterized by the development of long slender threads (*mycelium*), which grow like delicate roots through the substance of the material upon which they are nourished (Fig. 1). These threads make it possible for them to force their way into hard substances, like wood, and to effect their decomposition.

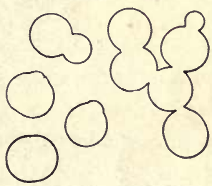
FIG. 1.



One of the higher Fungi, the common bread mould. *Penicillium glaucum.* *a*, the whole plant; *b*, one of the spore-bearing branches more highly magnified.

2. **Saccharomycetes.** (*Budding fungi. Yeasts.*)—These immensely important plants are wholly microscopic. They consist of simple oval or spherical cells, usually separate from each other but sometimes adhering in irregular masses (Fig. 2). Their distinctive character is in their method of reproduction, which is as follows: From the sides of the cells small *buds* arise which increase in size by growth until they are about as large as the original, when they may separate as distinct cells. This method of multiplication is called *budding* and the yeasts are consequently called the *Budding fungi.* The importance of yeasts in the great fermentative industries is well known.

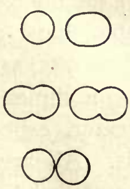
FIG. 2.



Yeast, showing method of budding.

3. **Schizomycetes.** (*Fission fungi. Bacteria.*)—These plants are also microscopic. They differ from yeasts in being smaller and somewhat different in shape, but chiefly in their method of reproduction. Instead of forming buds they multiply by lengthening somewhat and then dividing into two equal halves (Fig. 3).

FIG. 3.



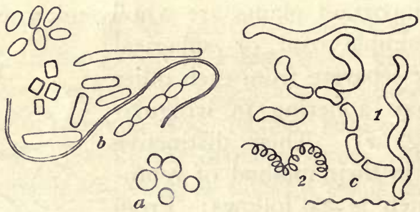
Bacteria, showing method of division by fission.

This process is called *fission* and hence these organisms are the *Fission fungi.* This group includes the organisms which have for the last quarter of a century been known as *Bacteria.* It is with these that we are chiefly concerned in this work.

II. FORM AND CLASSIFICATION OF BACTERIA OR SCHIZOMYCETES.

Under ordinary conditions **Bacteria** are extremely simple in form. Long ago they were compared to billiard balls, lead pencils and cork screws (Fig. 4), and even the most

FIG. 4.

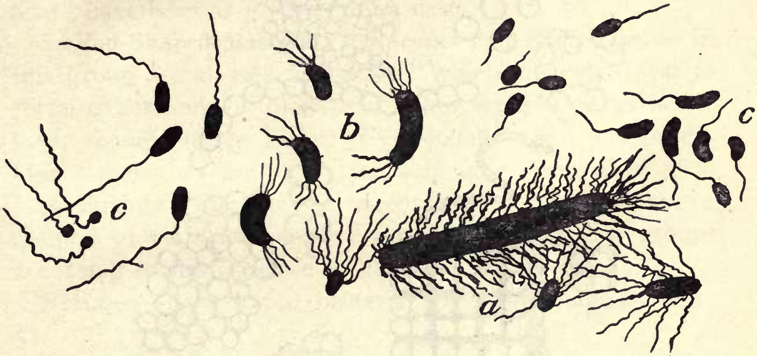


General shape of bacteria : *a*, spheres ; *b*, rods ; *c*, spirals.

careful work with the modern microscope has hardly improved upon this somewhat crude but striking comparison. Nearly all bacteria are either spheres, cylindrical rods of greater or less length, or spiral rods. In size they are inconceivably minute, being by far the smallest living organisms known, and demanding the highest powers of the microscope for their study. The spheres, for example, have a diameter varying from .25 μ to 1.5 μ (0.000012 to 0.00006 inch). The rods have a diameter of about the same dimensions, but their length may be considerably greater, especially when they grew into long slender threads. All are, however, far below the limits of human vision unaided by the microscope.

Many bacteria have the power of motion which is produced by slender motile hairs arising from their body and which by lashing to and fro produce a locomotion (Fig. 5).

FIG. 5.



Bacilli showing flagella. (*Migula.*)

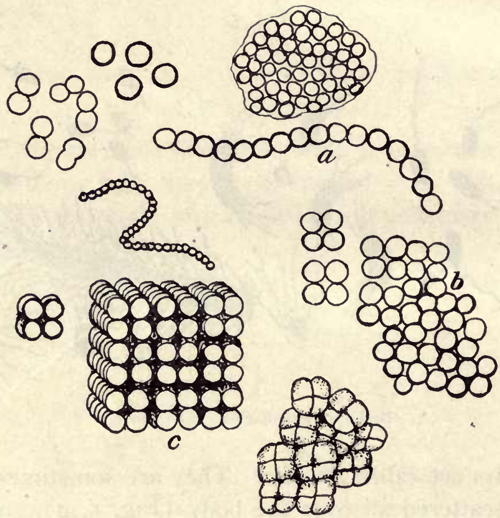
These hairs are called *flagella*. They are sometimes numerous and scattered all over the body (Fig. 5, *a*), sometimes few and grouped at one or both ends (Fig. 5, *b*), and in other cases only a single flagellum is found (Fig. 5, *c*). In many bacteria they are entirely wanting.

By the use of these characters bacteria are divided into three easily recognizable divisions:

1. **Spherical Bacteria.** *Coccus.*—This group includes all bacteria which are spherical. They are further subdivided in accordance with their method of division. In some of them the successive divisions follow one another in the same plane. If the spheres break away from each other after division, separate spheres, of course, arise, but it frequently happens that they remain clinging together for a time after division, so that long chains are produced (Fig. 6, *a*). In other cocci the second division plane is at right angles to the first (Fig. 6, *b*). In these cases, if the spheres remain

attached they form irregular masses (Fig. 6, *b*). In a third type the divisions are in the three planes of space at right

FIG. 6.



Cocci or spherical bacteria: *a*, Streptococcus; *b*, Micrococcus; *c*, Sarcina.

angles to each other (Fig. 6, *c*). In such cases there result solid masses of fours or some multiples of four. In accordance with these methods of division the cocci are divided as follows:

Streptococcus.—Division in one plane only, frequently forming chains (Fig. 6, *a*).

Micrococcus.—Divisions in two planes, the spheres either separate or forming irregular masses (Fig. 6, *b*). When forming masses they are sometimes called *Staphylococcus* and when in twos occasionally called *Diplococcus*. These terms are going out of use.

Sarcina.—Divisions in three planes, forming solid masses in groups of four or multiples of four (Fig. 6, *c*).

The cocci as a rule have no flagella and are consequently stationary.[1] A few forms have been found which possess flagella, and in recent classifications there have been introduced the terms *Planococcus* and *Planosarcina* to include the coccus and sarcina forms provided with flagella. These terms have been as yet very little used.

2. **Rod-Shaped Bacteria.** *Bacillus* and *Bacterium*. — In this group are classed all bacteria which are cylindrical in form, either long or short, and either straight or variously bent, though never spiral. Occasionally they are hardly longer than they are broad, while in other cases they form long threads (Fig. 4, *b*). According to the most recent method of classification they are divided into two groups according to the presence or absence of flagella.

Bacillus.—Rod-shaped bacteria possessing flagella (Fig. 5).

Bacterium.—Rod-shaped bacteria without flagella (Fig. 4, *b*). It must be noted that this method of separating the two genera, *Bacillus* and *Bacterium*, according to the presence of flagella, is a very recent one. Although these two terms have been used in all works on bacteria they have not had the significance above given in any except the most recent publications. In all books on bacteriology published before the last few years the term *Bacillus* was applied to almost any rod-shaped bacterium, while the term *Bacterium* had a very uncertain meaning, commonly referring to short rods without spores. The names of many of the best known bacteria which are in use do not agree with the classification above given. For example the organism which produces tuberculosis is called a *Bacillus* (*Bacillus tuberculosis*), although it has no flagella and, according to the classification given, should be a *Bacterium*. It is not likely that the name will ever be changed. The distinction of *Bacillus*

[1] Recent work has seemed to show that all cocci have flagella, which are very difficult to demonstrate.

and *Bacterium,* based upon the presence of flagella, is a convenient one, but its adoption would produce considerable confusion in the terms which have been commonly accepted in the last few years. At all events in most bacteriological literature the term *Bacillus* does not have the significance above mentioned and simply refers to any rod-shaped bacterium. It should be noted also that whereas the word *Bacteria* refers to the whole group of fission fungi which we are to study, the genus *Bacterium* has reference only to a small division of rod-shaped bacteria.

3. **Spiral Bacteria.** *Spirillum.*—In this group the rods are spirally coiled to form either long or short spirals (Fig. 4, *c*). They are not so abundant as the cocci and rod forms, although some of them are of importance, inasmuch as they play an active part in the decay of organic tissues. They are sometimes motile and sometimes stationary. The only two divisions of the group that we need to notice are as follows:

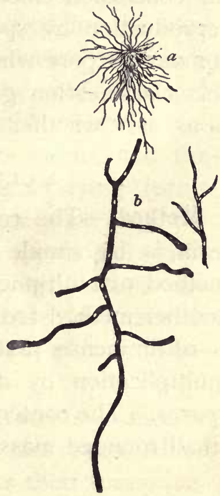
Spirillum.—Ordinary spiral rods, stiff and inflexible (Fig. 4, *c*, 1).

Spirochæta.—Spiral rods which are flexible like a spiral spring (Fig. 4, *c*, 2).

4. **Higher Bacteria.** *Cladothrix, Leptothrix, Streptothrix, Actinomyces.*—(Fig. 7.) Under this head are included a few forms of fungi which resemble other bacteria in some respects, but differ in others. They are composed of threads which are commonly larger than the threads of bacteria and which may show frequent *branching,* a characteristic not usual in bacteria. They also have a peculiar method of forming reproducing bodies. The group is not one of very great importance.

This classification gives only what are usually recognized as the *genera* of bacteria. A further classification of the group into *species* is at the present time in a condition of the greatest confusion. Many hundreds of species have been described by different bacteriologists, but there is great difficulty in giving any distinctive description of such minute organisms, which have so few characters, and it is quite uncertain whether these many hundreds of described species represent entirely distinct forms or whether they should be reduced to a much smaller number of species. It is frequently uncertain whether a species described by one bacteriologist is the same as that described by another under the same name. The difficulties which have been found in the way of a proper description and classification of the species of bacteria have been hitherto insurmountable, and at the present time the subject is in such extreme confusion that no one except an expert can understand it. Fortunately, for our purpose this confusion of species is of no importance. We are at present concerned in the results of the *action* of microörganisms, and only slightly concerned with the problem of the specific characters of bacteria. All that is necessary for us to know in connection with our subject will be referred to in the separate sections in the following pages, and the subject of the classification of bacteria may be left without further consideration.

FIG. 7.



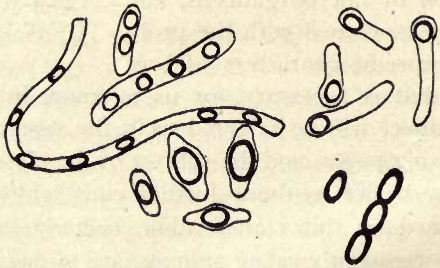
Actinomyces: *a*, a small colony; *b*, single rods. (*Boström.*)

As indicated by this classification, bacteria, although in earlier years frequently called animals, are to-day universally regarded as plants. The reason for calling them plants is not at first sight evident. They are colorless, unlike most plants. They are frequently endowed with a power of independent motion, a character which would naturally lead to their being called animals. Biologists find it very difficult to separate low plants from animals, all of the characters which distinguish the two groups among higher types disappearing when we come to the microscopic forms. The microscopist has, however, after long study, reached the conclusion that bacteria are to be regarded as plants, basing his conclusion chiefly upon their form and their method of reproduction by spores. It is a matter of no significance for our purpose whether we call them animals or plants, for this classification gives us no suggestion as to their functions, nor whether they are beneficial or detrimental.

MULTIPLICATION OF BACTERIA.

Method.—The common method of reproduction of bacteria is by simple division (Fig. 3). But although this method of multiplication is common to all bacteria, there is another method frequently found which, for certain reasons, is of immense practical importance. In addition to their multiplication by division some species of bacteria form *spores*. The contents of the organism collect in one or more small rounded masses (Fig. 8), after which the body of the

FIG. 8.



Showing the formation of spores.

bacterium commonly breaks up and the oval or spherical bodies are set free. These are *spores*. They are resting forms and their function seems to be to enable the bacteria to exist through conditions of adversity. They have very great power of resisting adverse conditions. They may be dried for months or even years without losing their power of growth. They may be heated to a temperature even above that of boiling water without injury, for, if they are later brought into proper conditions for growth, the spores germinate and develop new organisms like the original that produced them. They are evidently developed for the purpose of enabling the species to resist the adverse conditions of drying, to which it must occasionally be subjected, and to preserve it under conditions which would otherwise destroy the bacteria. Not all bacteria form spores, and the question whether a species which we are studying forms spores or not is one of great practical significance in teaching us how to handle it, since, while the spores can withstand heat and drying, the active growing bacteria are commonly killed by a moderate heat. These facts are of especial importance in all matters connected with disinfection.

Rapidity.—One of the most important factors connected with the life of bacteria, and the chief fact upon which their significance in nature is dependent, is their exceptionally rapid power of multiplication. The elongation of a rod and its division into two, followed by a repetition of the process, may be extremely rapid. Frequently it takes not more than half an hour for the whole phenomenon to occur, and sometimes even less time is required. Such division in geometrical ratio results in an increase in numbers which is almost inconceivably rapid. If this rate of multiplication could be maintained for twenty-four hours there would be produced, as the offspring of a single bacterium, some seventeen million descendants, and in five days a mass sufficient to fill the oceans. This rate of multiplication is, of course, not continued for any great length of time, for various checking influences are at work to stop the growth. But this possibility of reproduction represents an almost unlimited power which is constantly curbed by the lack of proper conditions. Bacteria may thus be looked upon as possessing this wonderful possibility of reproduction, a force of inconceivable magnitude, held more or less in check by adverse conditions, but ever ready to exert its influence when the conditions are favorable. It is this reserve force, possessed in greater or less degree by all bacteria, which makes them such wonderful and powerful agents in producing the great changes in nature which we are now forced to attribute to them.

GENERAL PROPERTIES OF BACTERIA.

Relation to Food.—A few general facts concerning the conditions of life of bacteria must be mentioned as necessary to an understanding of their activities. They are colorless organisms and consequently are commonly unable to make use of the energy of sunlight, but must, like animals, depend upon the decomposition of organic foods as a source of life energy. Hence organic material is commonly needed for food. Some of them obtain this organic food from the dead bodies of animals and plants existing in abundance throughout nature. Such bacteria are called *saprophytes,* and they find their sustinence in the lifeless organic matter in water and soil. Other species have the habit of feeding upon living organic matter, a habit which makes them *parasites.* These live upon the living tissues of either animals or plants. It must be noted here that a few bacteria are exceptions to this need for organic food and can subsist upon mineral food.

Relation to Oxygen.—Most bacteria demand oxygen to enable them to carry on their life processes, thus agreeing with animals and plants in general. But there are some species, quite numerous and abundant as we now know, that can live without a supply of oxygen, and some indeed, that can grow and multiply only when in an atmosphere devoid of oxygen. This property of living *without oxygen* makes them quite unique organisms, for no higher animals or plants have this power. This unusual power is one of considerable significance in explaining the conditions of bacterial life and action. The bacteria which demand oxygen, the great majority of known species, are called *aërobic.* Those that can only live in the absence of oxygen are called *anaërobic.* Some species that can live either in the presence or absence of oxygen are called *facultative anaërobic.* Between the two extremes of aërobic and anaërobic bacteria are numerous intermediate grades.

Relation to Temperature.—Bacteria have the same relation to temperature as do ordinary living organisms. At freezing or below it they cease to grow. As the temperature rises above freezing they begin their life activities and, up to a certain limit, grow more rapidly as the temperature rises. Above this upper limit they stop growing, and are eventually killed by higher temperatures. The temperature at which the maximum growth occurs is quite variable. Some grow best at 70° F., many at about 95° F. and others at still higher temperatures. The most remarkable bacteria, in this respect, are certain species, recently discovered, which are unable to grow at ordinary temperatures, but require a temperature above 125° F., and some even demanding a temperature as high as 140° F. before they can grow most vigorously. At temperatures as low as 100° F. they will not grow at all. These have been called *thermophilous bacteria.* What may be the significance of these bacteria in the processes of nature we cannot say at present. If the temperature is raised above 160° F. most bacteria are quickly killed, although bacteria *spores* can stand a much greater heat.

Pure Cultures.—In nature the different species of bacteria are found associated in all sorts of indefinite mixtures. Earth, air or water, or any other medium which supports bacterial life, will be found in most cases to contain numerous species side by side. It is only under some very exceptional conditions that large numbers of a single species are found together, wholly unmixed with other species. Such a condition, where great quantities of a single species of bacterium are associated, uncontaminated with any individuals of another species, is called by the bacteriologist a *pure culture*. While such pure cultures are very unusual or almost never found in nature, they are easily enough produced in the bacteriologist's laboratory by artificial methods. Pure cultures have been coming into prominence in recent years, and are to-day prepared by bacteriologists for various purposes. They always represent artificial preparations and, therefore, are usually unlike any natural conditions of bacterial life.

BACTERIA AS AGENTS IN PRODUCING CHEMICAL CHANGE.

When we consider the extremely minute size of bacteria it seems strange that they can be important agents in nature. No single one can accomplish much, but their rapid multiplication gives them almost unlimited power. A possibility of reproduction which will produce seventeen millions of offspring in twenty-four hours is never actually realized, but bacteria do multiply with inconceivable rapidity, and it is this immense possibility of multiplication which makes them agents of importance in nature. While growing and multiplying so rapidly they are sure to produce profound chemical changes in the food in which they live, and it is these chemical changes produced in the medium surrounding them that makes them agents of such significance.

The chemical changes which are produced by bacteria may be looked upon as of two general types:

1. **Synthetic Processes.**—These consist of chemical changes by which complex bodies are built out of simpler ones. Bacteria have a greater or less power of producing such changes. For example, when the bacteria multiply

and produce new individuals the increase in the total amount of living material implies the production of a considerable quantity of new organic material. The living bodies of the new bacteria are made out of the food upon which the bacteria live, and this involves a chemical **synthesis** of complex out of simpler material.

2. **Analytical Processes.**—These are processes of decomposition by which complex bodies are reduced to simpler ones. The great majority of bacteria live upon complex chemical foods, and these are broken to pieces to serve them as food and are reduced to simpler compounds. Bacteria are, indeed, one of nature's chief agents for breaking complex compounds into simpler ones. Throughout nature bacteria are constantly engaged in this process; in the water, in the soil, in every bit of decayed material, the world over, we find myriads of bacteria actively engaged in producing such chemical destruction. The extreme significance of these processes for the general life of the world need not be considered here, having been treated of in another work. In considering the relation of bacteria to dairy problems we are concerned with both the synthetic and the analytical processes. In general, however, it is those processes by which complex bodies are reduced to simpler ones that form the larger part of the changes which bacteria produce in milk products, and the synthetic processes concern us only slightly. But even of these **analytical** processes we know very little, and our study of bacteria in milk products tells us as yet little of the actual chemical changes. But the exact chemical nature of these products is a matter of little practical significance to the dairyman. He is interested in the general effect, rather than in the chemical nature of the new products formed by bacteria. We shall not attempt, therefore, to follow out in any detail the chemical results of bacteria growth in milk, and we may leave this general subject with this brief outline.

CHAPTER II.

SOURCE OF BACTERIA IN MILK.

MILK FROM HEALTHY UDDERS.

At the moment it is secreted by the healthy udder milk is absolutely free from bacteria. This, though certainly true, has been difficult to prove. Thirty years ago Lister and Hall, pioneers in dairy bacteriology, endeavored to determine whether milk could not be drawn from the cow in such a way that it would contain no bacteria. Their experiments, consisting of drawing milk into sterilized vessels under special precautions, were successful in a sufficient number of cases to demonstrate to their satisfaction that the pure, freshly secreted milk contains none of these organisms. In more recent years, however, such experiments are rarely successful in spite of the very greatest precautions that are taken. With all of our improvements in aseptic methods it is a practical impossibility to draw any considerable quantity of milk from a cow in such a way that it contains no bacteria. It is, however, occasionally done, provided care is taken to collect milk at the end of the milking only, and to collect small samples. But though thus occasionally possible no methods can be adopted which result in uniformly obtaining such milk, and it is quite impossible for the dairyman to draw milk from the cow in such a manner that it is free from bacteria. That the milk while in the glands is free from bacteria is indicated by other methods of work The udder has been examined by bacteriological methods directly after the slaughter of the animal, and while in some cases bacteria are found in various parts of the gland, in other cases, if the precautions taken are sufficient and the gland is examined without the possibility of contamination, the inner part of the gland is free from bacteria (34).[1] This fact and the possibility of occasionally drawing sterile milk forces upon us the conclusion, which is not disputed at the present time, that the healthy milk gland does not secrete bacteria, and that the bacteria which commonly get into the milk, even before milking, come from the exterior, making their way into the milk from the exterior through the milk ducts. In short, the milk of a cow with healthy udders is never contaminated by microörganisms at the moment that it is secreted.

MILK FROM DISEASED UDDERS.

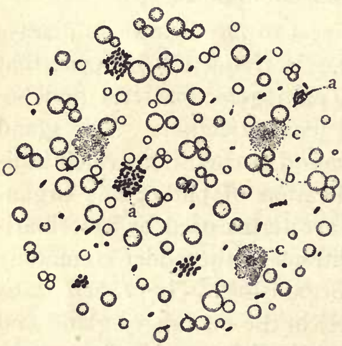
If, however, the udder is the seat of any infectious disease the case may be quite different. It frequently happens that the milk gland is attacked by pathogenic bacteria and becomes the seat of a variety of local infections. The gland may contain centers of inflammation, a condition which indicates the presence and multiplication of pathogenic organisms. *Mammitis* and *mastitis* are terms used by veterinarians as applying to certain conditions of the udder commonly due to the growth of microörganisms. The *tuberculosis bacillus* occasionally locates itself in the mammary gland and produces local infection. The infection produced may, in such a case, be so slight that no indication of it can be seen externally and yet the mammary gland may have one or more minute tubercle centers of activity. When the udder is attacked by any such trouble the milk which is drawn from the cow can no longer be relied upon as free from bacteria. Even in these cases it is incorrect to say that the cow secretes contaminated milk, but it is certainly a fact that the milk at the moment of its secretion is contaminated with bacteria associated with these udder diseases, so that even in

[1] Numbers inserted in the text refer to numbers in the list of references that follows Chapter IX.

the very depths of the milk ducts the milk may become crowded with microörganisms.

The kind of microörganisms that find their entrance into milk from such sources is somewhat varied. The tubercle bacillus thus undoubtedly sometimes finds entrance into the milk from diseased udders. Indeed this is practically the only source from which this organism invades milk. Other types of udder troubles contaminate the milk with bacteria, of which there are several species. The more common among them appear to be certain forms of *streptococci* (35). The inflammatory troubles of the udders are usually associated with the growth of streptococcus forms, and milk drawn from such udders is quite likely to be filled with certain types of streptococci. The question whether these streptococci render the milk unwholesome has not yet been positively answered, but experiment has indicated that, at least in some cases, milk with streptococci from such diseased udders is capable of producing intestinal disturbances in experimental animals, and the conclusion is a strong one that such milk is unwholesome for man (100). The frequency of milk contamination by such diseased udders is seemingly greater than would be imagined. Milk contains quite frequently a greater or less abundance of pus. The presence of a considerable quantity of pus must be looked upon, in all cases, as indicating some inflammatory condition of the udder that secreted the milk, and the presence of pus is also undoubtedly associated with the presence in the milk

Fig. 9.



Milk as seen under the microscope: *a*, bacteria; *b*, fat globules; *c*, pus cells or leucocytes.

of the bacteria which were the original cause of the inflammation in the mammary gland. The simple presence of streptococci, however, does not necessarily indicate any diseased condition of the udder, for streptococci are very commonly found in milk drawn from perfectly healthy udders.

MILK FROM DISEASED COWS.

The question has frequently been raised whether a cow suffering from a bacterial disease that is not located in the udder may transmit the bacteria through the milk glands into her milk. Probably no definite answer can be given to this question which would be true for all conditions. Certain forms of bacterial disease are located in somewhat narrow centers; for example, in the case of tubercular glands in the neck. In these circumstances, provided there are no tubercles in the udder, it is quite certain that the milk will not contain tuberculosis bacilli. In the case of other infectious diseases, like anthrax, the microörganisms enter the circulatory system and pass all over the body in the blood, and may be found even in the lymph. Under these conditions the probability of the bacteria being secreted by the milk is greater. The experiment has been occasionally tried of injecting certain bacteria into the blood of animals to determine whether they would be secreted through the mammary glands (18). Although no universal answer can yet be given to the question, the results appear to indicate that the secretion of bacteria into the milk under these circumstances is extremely unusual and perhaps does not occur at all. The milk gland is not an excretory organ designed for the purpose of getting rid of waste products, and there is no reason for believing that bacteria circulating in the blood will be secreted through the milk gland, provided the milk gland is itself in a normal, healthy condition. Bacteria pass through the gland only when it is itself the seat of inflammatory trouble. It is probable that in all cases where the microörganisms are present in the milk at the moment of secretion, the mammary gland itself will be found to be infected with local centers of disease. It is really a matter of little practical importance how this case is settled. If an animal is attacked by pathogenic disease there is quite likely to be local udder infection, which may frequently be so slight as to be incapable of detection, and even if the udder is not infected there is always a chance for secondary contamination of the milk. If an animal is attacked by a contagious disease her milk is thus quite likely to contain the bacteria, even though the scientific evidence seems to indicate that the bacteria are not secreted through the gland.

BACTERIAL CONTAMINATION OF NORMAL MILK.

Whatever the condition of the milk the moment it is secreted it is always contaminated with bacteria by the time it reaches the milk vessel, or even before, for there are a variety of sources from which bacteria are sure to find an entrance to the milk during or immediately subsequent to its passage through the milk ducts. The sources from which these bacteria are derived are several.

1. **The Milk Ducts.**—If the mammary gland of the cow is examined it will be found to have a structure shown in Fig. 10. In this figure the upper part of the gland is seen to be moderately solid. The lower part of the gland is filled with irregular ducts and spaces, and at the upper portion of the teat there is rather of a large space called the milk *cistern*. It has been demonstrated in a variety of ways that these ducts and spaces in the milk gland are more or less infected with bacteria (26). The milk first drawn at each milking, called " fore milk," is quite certain to be filled with bacteria in considerable quantity even at the moment of leaving the milk teat (20), whereas at the close of the milking, after the various ducts have had an opportunity of being washed out by the stream of milk, the number of bacteria

in the milk is very much reduced, and frequently the milk at the end of the milking is nearly or quite sterile. There is

FIG. 10.

A cow's udder cut across and showing the milk ducts.

a constant, though not a regular, reduction in the number of bacteria from the beginning of the milking to the end. The presence of the bacteria in these milk ducts is also demonstrated by a direct examination of the gland itself. If the glands of slaughtered animals are carefully cut open and the contents of their milk ducts tested directly with all proper bacteriological precautions, it is easy to demonstrate satisfactorily the presence of bacteria in the ducts.

Although bacteria are certainly present in the milk ducts it is not at the present time positively known how far within the gland they extend. That the bacteria come from an external source, passing in through the milk teat and living within the gland, is perfectly evident, but experiments hitherto reported are not in agreement as to the depth to which the bacteria can penetrate into the glands. In some tests it has appeared that bacteria may be found in all parts of the gland, even in the small ducts in its upper portions. Other experimenters have found that the milk in the upper ducts of the milk gland is sterile, the ducts here containing no bacteria, whereas the lower ducts near the cistern, and particularly below it, are always filled with bacteria (34). The only conclusion we can reach at present is, therefore, that bacteria are present in the milk cistern and extend for a considerable distance above, and that they are present in sufficient quantities to contaminate the milk during the milking, especially the first portion drawn at each milking.

The bacteria which are present in these ducts find here their normal habitat, and live, grow and multiply here in a perfectly normal fashion. This follows from the fact that, although the ducts are quite thoroughly washed out at each milking, so that the milk at the close is nearly sterile, bacteria are always present at the beginning of the next milking in considerable abundance, a fact which proves that they must have multiplied during the interval between the milkings. It is a curious fact, however, that it makes comparatively little difference whether the intervals between the milkings are moderately short or very long (17). If the interval between the milkings is as long as six hours the number of bacteria which are found in the fore milk is practically the same as it is if the milking occurs at intervals as long as two days, a fact which seems to indicate that the bacteria develop to a certain extent and then are checked in their growth by some unknown cause.

It is also an interesting fact that not all bacteria—not even normal milk bacteria—are capable of finding a habitat and growing within the milk ducts. Experiments have been made of injecting bacteria into the milk ducts and then testing the milk drawn upon the following days (31). The results show that the bacteria that have been used are not able to colonize in the milk ducts. For a few days after injection they are present in some abundance, but they soon become reduced in numbers and finally disappear. In these experiments the bacteria that have been used for inoculation are not those which are commonly found in the milk ducts but are in some cases unusual forms like *B. prodigiosus*. In one case, however, the ordinary lactic bacteria found in milk have been used, but even these did not succeed in living long, a result which is not surprising in view of the fact that these bacteria are not commonly found in the milk ducts.

The species of bacteria which are found living in the ducts are somewhat variable. There do not appear to be a very large number of species normally inhabitating the duct, and the variety which is found, even in fore milk, is small compared to the variety of species found in milk shortly after the milking. The species which are found in the milk ducts are different with different cows, but apparently, in most cases, there may be found within these ducts certain species of *streptococci*. If the milk gland is the seat of an inflammatory process due to any of the pus-forming cocci the bacteria present in the milk at the beginning of milking will be the pus-forming streptococci. But the normal milk from the healthy gland is almost sure to show the presence of other species of streptococci. Streptococci, indeed, are found so frequently that they may probably be regarded as normal inhabitants of the milk ducts (35). These milk streptococci, however, are not all alike, and a variety of types have been described. Further, it has appeared that the species of bacteria found in the milk gland of any cow are tolerably constant. Examination of the fore milk of a cow extending over a considerable interval of time shows that certain species of these bacteria are quite persistent, being found in the milk over a long period of time. The milk from the four teats of the same cow may show a slightly different bacteriological content, and the bacteria remain tolerably persistent in each of the four quarters.

The relation of these streptococci to the wholesomeness of milk is as yet not determined. Some of the common streptococci found in fore milk have no visible effect upon the milk, neither souring it nor curdling it, nor having any other visible influence upon it. Others, however, appear to render it slightly alkaline, a fact which indicates that they do produce certain changes in the milk. But the changes are at all events very slight. It is quite certain, however, that occasionally some of the dairy troubles which are attributable to milk bacteria come from the bacteria which live in the udders. These problems have not as yet been satisfactorily worked out in many cases, but at least in one instance a bitter milk infection has been traced to bacteria that come directly from the milk ducts (80), and were, therefore, in all probability inhabitants of the milk duct; a single infection of blue milk has been similarly located. It is quite probable that some other milk infections are traceable to bacteria from a like source, but very little is as yet known in regard to this matter.

It is important to notice that the lactic bacteria, which have, from the beginning of the study of dairy bacteriology, been looked upon as the dairy bacteria *par excellence,* do not apparently inhabit the milk ducts. Indeed, in the majority of cases where the subject has been carefully tested there appear to be no typical lactic organisms in the milk while in the duct. Milk drawn directly from the duct contains bacteria, but in most cases contains bacteria incapable of souring and curdling milk. In no experiment thus far recorded have the common lactic bacteria, *Bacterium lactis acidi* (see p. 66), been found under conditions which indicate that they come directly from the milk duct. It is true that milk drawn immediately from the milk duct sometimes contains lactic organisms and will subsequently sour, but, so far as experiments have thus far indicated, the bacteria found in these cases, although they produce lactic acid, are not the usual lactic bacteria which, in the majority of cases, cause the souring of milk.

From the facts given it will be evident that a contamination of milk by bacteria during the milking is quite unavoidable. The bacteria that are in the milk ducts will inevitably be washed out into the milk vessel, and no means that can be devised can protect the milk from them. The contamination from these sources may be in a measure reduced by allowing the first few jets of milk drawn to run to waste, inasmuch as the first few jets will contain a larger number of bacteria than any subsequent portion of the milk. This practice of rejecting the first few jets is quite common and is of use in reducing the number of bacteria, but it never gets rid of them all. In other words, the bacteria contamination that comes from the milk ducts is absolutely unavoidable in the common method of milking. The only possibility of obtaining sterile milk from the cow would be to collect small quantities of the milk drawn at the close of a milking. By this means, if sufficient care is taken, sterile milk can actually be obtained. The contamination by these bacteria has also, in some cases, been avoided by passing a small sterile tube into the duct and forcing it up into the udder for some distance. But such a method of milking has proved impractical.

The Cow.—From the moment the milk leaves the milk ducts it is subject to a number of external sources of bacterial contamination. The most important of these is probably the cow herself. At the present time much greater attention is given on the part of the dairyman to cleanliness of his cows than a few years ago, and hence the contamination of milk from this source is probably less to-day than it was ten years ago. But in the best dairies the skin of the cow is likely to be more or less covered with dirt unless the cow is carefully groomed daily, and even daily grooming does not keep her clean. In the less carefully kept dairies, and particularly in poorly kept dairies, which two classes represent the majority, the condition of the cow in matters of cleanliness is surprisingly bad. In many barns the animal is rarely groomed and becomes covered with excrement and all sorts of miscellaneous filth, especially on her flanks. This filth dries upon her skin and, being completely loaded with bacteria, the result is that such dried filth on the flanks of the cow becomes a constant source of bacteria in the milk. Every motion that the cow makes, and every time she switches her tail, showers of filth, loaded with bacteria, drop from her body, filling the air in her immediate vicinity. Indeed, even when she is perfectly quiet there is a considerable quantity of bacteria-laden filth and dust which falls from her flanks. During the milking, when her body is more or less rubbed by the milker and when she is herself undergoing various motions, there is a constant shower of bacteria from the animal into the milk pail. This is not a matter of simple theory, but one which has been demonstrated by bacteriological tests. The dust and dirt that falls into the milk pail during the milking has frequently been collected, analyzed, and found to contain bacteria in very large numbers (38).

The kinds of bacteria which come from this source are naturally more varied than those from the milk ducts, since the filth is of a varied character. In general, the bacteria from this source are commonly *cocci* (17). By this it is not meant that the rod-shaped bacteria are not found in such filth, but the predominant type of bacteria are cocci. The species found, however, will vary widely with conditions and will depend upon the particular kind of filth that happens to be attached to the animal. The filth from the cow is probably the largest source of bacterial contamination.

The Air.—The air has always been regarded as one of the important sources of bacterial contamination, but this statement requires a slight explanation. The ordinary out-of-door air on a farm does not contain bacteria in large numbers, and if the milking were done in the open, the air would not be a very important source of contamination. The case is quite different in the somewhat small barn or milking room where milk is commonly drawn. The presence in such a room of many cows, which are constantly giving off particles of dust and dirt from their skin, renders the air much more liable to be filled with bacteria-laden dust than is the air in the open (24). Moreover, in many barns it is customary to throw down hay or other dry food in the front of the cattle and allow them to eat it in the same room where the milking is simultaneously or subsequently carried on. The hay itself is crowded with bacteria, and the dry dust from the hay scatters itself readily and abundantly through the air of the room. If the milking is done while the air is thus filled with the dust from the hay, large numbers of bacteria may settle into the milk pail. Here, again, we have a conclusion that is not dependent upon theory but has been readily demonstrated. It has been found by bacteriological tests that the kinds and number of bacteria in the milk are generally modified by the kind of food and the condition of feeding. In a single experiment, made at Storrs, it was found that, upon one day, when the feeding was allowed to take place shortly before the milking, not only did the number of bacteria in the milk rise markedly, but the milk was filled with a species quite different from those which were found in the same barn under ordinary conditions. The use of the particular food in question just before milking had totally modified the character of the bacteria found in the milk.

The Milk Vessels.—A further source of contamination is the vessel into which the milk is drawn. Under the ordinary conditions this is a large source of contamination, for upon the ordinary farm it is practically impossible to wash the milk vessels in such a way that they will be *bacteriologically* clean. The most thorough washing that may be given them in hot water, such as is commonly available on the farm, is insufficient to wash out the bacteria. After such a washing many bacteria will remain in the vessel, attached to its sides and walls, and, although drying between the successive milkings, they are not at all injured and are ready to begin to feed, to grow and multiply, as soon as the pail is again filled with milk. The more thorough the washing, the less the chance of contamination from this source, but except by the use of live steam, which is rarely available on the farm, the milk vessels can hardly be washed absolutely clean. This source of contamination is, therefore, one which the dairyman must always expect and for which he must make allowance.

The Milker.—A source of contamination that is quite liable to be one of more serious importance is the milker. The pathogenic bacteria which are capable of producing diseases in man are more liable to be found associated with the milker than they are with the cow, and contamination of the milk, during the milking, by bacteria from the milker's hands or clothing is, therefore, more likely to affect the wholesomeness of the milk than the bacteria from other sources. The hands and clothing of the milker are the two chief sources of bacterial contamination. The clothing that an ordinary farmer wears becomes very soon loaded with bacteria and these are quite sure to be detached by his motions during the milking and to find their way into the milk pail. If his hands are not clean it is inevitable that the friction of the teats in the milking will rub from the hands the bacteria which may be attached to them, and these will inevitably fall into the milk. While this source of contamination is not so great as some of the others, it is one which is frequently of more importance than the others and one that should be especially guarded against. It should always be remembered that *bacteria from man are more dangerous to human health than those from the cow,* and this applies not only to the milker, but to all who subsequently handle the milk.

EXTENT OF CONTAMINATION.

The amount of contamination which will be found in any sample of milk varies widely with conditions. From a dirty stable and poorly kept cows milk will be produced that is much more highly contaminated with bacteria than that from a cleanly kept dairy. Actual experiment has shown that variations in extent of the contamination are very wide indeed. By very strict precautions, such as can be adopted by an extremely careful expert, the number of bacteria which find their way into milk during the milking may be reduced to a score or two in each cubic centimeter. Sometimes the number may be even less than this, although more commonly even the greatest precautions will give a number somewhat larger. From this small number the contamination is greater with the decrease in the precautions that are taken. In a common cleanly kept dairy the number may be 1,000–10,000 per cubic centimeter. In a dairy not so carefully kept the number will rise to 30,000–50,000 per c.c., and under very poor conditions the number may rise up to 300,000–400,000 per c.c. in the milk as the result of the original contamination. The number of bacteria found in milk at the close of the milking is directly dependent upon the conditions of cleanliness, and one may, within narrow limits, determine the extent of precautions that are taken by the dairyman in his dairy by a simple quantitative examination of the number of bacteria contained *in the fresh milk*. If the number is not more than two to three thousand it may be assumed that the condition of the dairy, of the milking vessels, and of the cows is very good. If the number is less than this it indicates an exceptionally good condition; if the number is twenty thousand, or more, the indications are that the conditions are rather uncleanly.

It is, however, necessary to remember that the contamination of milk during the milking is subject to great irregularities which are quite beyond explanation at the present time. It occasionally happens that a dairy that is kept in excellent condition and produces milk containing a comparatively small number of bacteria, will suddenly, for some unexplained reason, produce milk of a very different character. In a single experiment, for example, a careful record of the bacteria in the milk of a well-kept dairy was made for some months. During that period the number of bacteria in the fresh milk ranged from 1,000–10,000 per c.c., and month after month this number was tolerably constant. Suddenly, upon a single day, there was obtained a sample of milk drawn under identical conditions, so far as could be determined, which showed at the outset 400,000 bacteria per cubic centimeter. This milk soured and curdled within eighteen hours, whereas the samples of previous weeks had been easily kept two days without difficulty. What sudden incident produced this extraordinary change it was quite impossible to determine, for, so far as the dairyman who was carrying on the experiments could determine, there was no difference of conditions in the milk drawn upon this day and previous days. This high number of 400,000 per c.c. was found, however, for only a single day. In the next sample that was taken the number had dropped down again to the normal average. Of course, there must have been some extraordinary source of contamination in this case, and if a careful investigation could have been made it would probably have been determined. The example is mentioned, however, as indicating that, even under the best and most careful conditions, there will be occasionally unexplained irregularities. This phenomenon has also frequently been noticed by dairymen; for, in special dairies, where extreme care is taken and where the milk is in general of a high character, there are occasionally incidents such as that just mentioned. Upon one or two days the milk sours very rapidly, due doubtless to the presence of a much larger number of bacteria than usual, although the conditions under which the milk has been obtained are, so far as can be determined, identical with those which were found in the dairy upon other days. To what such great irregularities are due we can at present give no explanation.

HOW TO REDUCE BACTERIAL CONTAMINATION.

There are no means of avoiding the bacteria from the milk duct; but these form really a small part of the bacteria in ordinary milk, most of which come from external sources. Though the latter cannot be wholly avoided, they may be very greatly reduced in numbers by simple precautions that may be adopted in any dairy (19, 29). In general it may be stated that an increase in cleanliness will result in a reduction of the amount of bacterial contamination; but in order to understand this statement and its applications it is necessary to consider the various points to which attention should be especially directed. They are as follows:

The Cow.—Since the dirt which is allowed to accumulate upon the skin of the cow is one of the largest sources of bacterial contamination it naturally follows that an efficient means of reducing the number of bacteria in milk is to clean the cow. Filth should be prevented from accumulating on her skin. She should be groomed as frequently as possible; indeed, if groomed two or three times a day the result upon the bacterial contamination of the milk is very noticeable. All particles of dirt, which by any means may become attached to the hairs of her flanks should be carefully removed. The long hairs on the end of the tail are particularly liable to become contaminated with urine and feces, and these should be especially guarded. It is sometimes a custom to cut off the long hairs upon the tail or around the flanks of the animal, thus reducing greatly the chance of filth collecting on her skin. The greater the attention paid to cleanliness in this respect the more satisfactory the results.

The Stable.—The stable in which the cows live, and particularly that in which the milking is done, should be kept in as clean a condition as possible. In the stalls where the cows stand care should be taken to have only clean bedding. The manure should be removed frequently, preferably two or three times a day. The walls of the rooms should be kept clean and free from cobwebs and dirt, so far as possible. The practice of whitewashing the walls is an excellent one, not so much because the whitewash in itself does much toward reducing the amount of dirt, but because the whitewash increases the light in the stable, shows quickly the presence of dirt and draws the attention of the person who looks after the stalls to the impurities in the stable. Whitewashing should be done twice a year. Floors, walls and windows in the stables where the cows are kept should all be as clean as possible, and this is especially true if the milking is carried on in the same room. The habit of removing the cows to a separate milking room is one which is undoubtedly very useful in reducing the chance of bacterial contamination. At best the stable in which the cows are living cannot be kept as clean as a smaller room where the cows are taken only for a few moments for the purpose of milking. A special milking room is, therefore, advantageous from the standpoint of cleanliness, and it is probably also useful in its influences upon the cow. Cows are creatures of habit, and soon learn that they go into the milking room to be milked and respond accordingly.

The Barnyard.—It is desirable that the place where the manure is stored should be removed at some distance from the milking stall, several hundred feet if possible, and no manure or stagnant water should be allowed in the vicinity of the barn. Not only will the manure be a source of bacteria which will inevitably be carried back and forth from the manure heaps to the milking room, but it will also be a breeding place of flies which are themselves a great nuisance in the dairy. One fly falling into the pail has been shown to be capable of introducing 250,000 bacteria into the milk.

Employees.—Especial care should be exercised in regard to the condition of those who do the milking or have anything to do with the handling of the milk. It is very desirable that especial clothing should be used during the milking, and many of the high class dairies furnish their milkmen with white clothing which is carefully washed and steamed, sometimes each day, and must be worn by each milker when he enters the milking stall. That the milker should carefully wash his hands, arms, face and beard before entering the milking stall should be a matter of course, although this is a habit which only recent years has emphasized, and is rarely followed in ordinary dairies. Special care should be taken to exclude from the milking and from the handling of the milk any employee that has any form of contagious disease. As will be seen in a later chapter, there have been numerous instances where typhoid fever, diphtheria, scarlet fever, and possibly other contagious diseases have been transmitted through the milk to the consumer from some person suffering or recovering from such diseases. The better dairies to-day allow no one who has contact with any contagious disease to have anything to do with the handling of the milk. Dairymen should be on the watch for such contagious disease and *no one who is recovering from any contagious disease, or who has anything to do with the nursing of persons suffering from them should be allowed to have anything to do with the milk supply.*

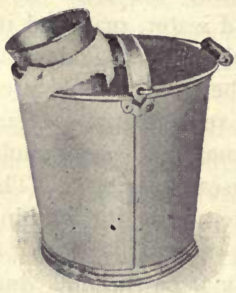
Milk Vessels.—That milk vessels should be washed as cleanly as possibly is a fact that every dairyman understands. A perfectly satisfactory method of washing is hardly available on an ordinary farm. As already noticed, the bacteria which are in the milk pail can only be thoroughly removed by live steam, and this is rarely found on the farm. Where such steaming is not possible the dairyman must do as well as he can to wash the vessels clean without it. They should be very thoroughly scrubbed with hot water containing some alkali, like sal soda, should be scalded in boiling water and then, without rinsing in cold water, they should be turned upside down or inclined at about 45° and allowed to stand in the air until needed for use. They should not be rinsed in cold water unless the source of the water is absolutely reliable, nor should they be wiped with a rag after scalding. Such a washing will not sterilize them, and they will always be a source of bacterial contamination; but if the water is very hot and the washing thorough the bacteria in the vessels will be greatly reduced. If it is possible to send them to a creamery occasionally for treatment with live steam this should be done, especially in hot weather, for it will be an efficient aid in preventing the souring of milk which is so common at these seasons. The milk vessels should not be allowed to stand in rooms where any patients are suffering from any form of contagious disease. The air of such rooms is liable to contain pathogenic bacteria which may find their way into the milk and become a subsequent source of the disease to consumers of the milk. Such instances have been recorded, and it is therefore necessary to emphasize the precaution of keeping all milk vessels away from any possible contact with contagious diseases. The vessels should not be allowed to stand open near barnyards or piggeries, under trees, nor beside roadways where dust can blow into them.

The Water Supply.—The water supply of the dairy is one of the most important factors in relation to the healthfulness of milk. Instances are now quite numerous of typhoid fever being distributed through the milk, the source of which is sometimes traced directly to the water supply. So distinctly is this true that some large dairy companies refuse to accept milk from a farm unless they have had an opportunity of making a chemical and bacteriological analysis of the water used in the dairy. This does not apply to the water which the cows drink, but to the water which is used in the dairy for washing the milk vessels. The water drunken by the cows rarely, if ever, has an opportunity of contaminating the milk, for, as we have seen, bacteria do not pass through the milk glands; but the water used in washing the milk cans, particularly if they are rinsed with cold water, may find its way into the milk and produce trouble. Every dairyman should, therefore, be particular as to the water supply used in the dairy, and it should be always remembered that no well which is located near a house, or near any privy vault, is safe from contamination with disease bacteria. The farmer's well, if near his house, is quite unsafe for washing the milk vessels.

Milking.—If milk could pass directly from the milk duct to a sterilized milk vessel the number of bacteria it would contain would be small. These would frequently include no acid bacteria and those that were present would usually be of comparatively little importance. Such a method of milking, however, is practically impossible. Several attempts have been made to devise milking machines which shall carry the milk directly from the milk glands to the milk vessel without contact with the air. Although these machines have been ingenious and have apparently prevented the ordinary contamination with dust and dirt of the stable, they have hitherto not been practical. They are usually somewhat complicated, and the difficulty in keeping them clean is very great. Some experiments made with such milking machines have shown that the milk is, on the whole, rather more contaminated with bacteria than when milked in the ordinary way, and none of the results obtained up to the present time promise much that is useful along this line. The milking machines hitherto devised, besides being expensive and complicated, are really of no great value. Until some radical improvement in them has been made milk will be drawn from the cow in the old-fashioned way.

But a few simple devices can reduce the amount of external contamination that may get into the milk during an ordinary milking. It is possible to use a specially devised milk pail which does not expose such a large area for collecting dirt as the ordinary pail. Sometimes this is accomplished by having a milk vessel with a narrow mouth; sometimes by having a milk pail with a cover in which there is a small opening only, near one side, through which the milk is drawn. In some dairies the entrance of dirt into the milk is still further prevented by a simple device shown in Fig. 11. Here a bit of sterilized cheese cloth can be stretched over the small opening in the milk pail cover, thus keeping out a considerable quantity of the dirt which would otherwise enter the milk. Sometimes the milk pail is provided with a special cover of such shape that the whole sur-

FIG. 11.



A milk pail with a special cover designed to keep out the dust which falls into the pail during milking.

face of the milk is protected from the entrance of dirt, the milk being drawn in at the side into a vertically open hood. Other devices have been used for this purpose which it is not necessary to consider. In general it may be recognized that any plan of milk vessel which exposes a small amount of surface to the air will result in reducing considerably the amount of dirt that may enter the milk during the milking, and hence the bacterial contamination will be less. Actual experiments with such milk pails have demonstrated that they do keep out a considerable quantity of dirt, and as a result, a considerable proportion of the bacteria. The pail shown in Fig. 11 keeps out about 66 per cent. of the dirt. Some such specially devised pails are coming into use in dairies where special care is taken for producing a good quality of milk. They are not much more inconvenient to handle than an ordinary milk pail, and their use is certainly to be recommended in any dairy where it is desired to produce a pure grade of milk.

A useful practice is to moisten the under side of the cow's body, particularly the udder, but not the teats, just before milking. This largely prevents the dirt from falling from her body during the milking, and thus improves the quality of milk. In cases where exceptional care is desired the practice of washing the teats and udder with warm water or even a two per cent. solution of boracic acid, is sometimes adopted. The value of the use of such disinfectants is probably hardly sufficient to compensate for the trouble and slight expense, except in cases where, for special reasons, it is desired to obtain a milk as free from bacteria as possible, or in instances where it is desired to get rid of some dairy trouble, like bitter milk, etc. The cow's tail may be fastened during the period of milking in such a way as to prevent its being swung to and fro.

The use of dry feed for the cow should never be allowed in the milking stall immediately before or during the milking. The throwing of the dry hay from the mow is sure to fill the air with dust, and if the cows are eating the hay during the milking they are scattering in the air millions of bacteria. The dairyman should remember that the use of dry hay in the milking room is, under all conditions, a source of troublesome bacterial contamination, for the bacteria are so light that they will float in the air many hours after they have been disturbed by the throwing of the hay from the mow and, even if the feeding occurs after milking, the air does not become wholly free from bacteria before the next milking.

The so-called "wet milking" should never be allowed. Wet milking only results in washing the hands of the farmer in the milk, for the moisture with which the hands are wet will cause the bacteria which chance to be on the hands of the milker, or the outside of the teats, to run down and mingle with the milk.

Treatment of Milk after Milking.—The longer milk is *exposed to the air,* other things being equal, the greater the amount of contamination. This, of course, represents a general truth of which there can be no doubt. But it must be remembered that it is the dusty, dirty air of the barn and milking stall that furnishes the greater quantity of bacterial contamination, while the pure out-of-door air does not contain enough bacteria to produce much trouble. If the milk can be immediately removed from the barn and carried to a clean milk room the subsequent contamination from exposure to the air is not very great. But even though this be true, it is always wise to cover the milk as quickly as possible after it has been drawn. The use of *sterilized bottles* for milk distribution has produced an improvement in the quality of the milk so far as concerns external impurities. It is quite a general practice to run the milk over a milk aërator, and then to bottle at once. The milk cooler and aërator, of which there are several kinds in use, always exposes a considerable surface of milk to the air for a few moments, and in this respect is, therefore, a source of bacterial contamination. If, however, this cooling is done, not in the barn but in a clean dairy or a room that is kept clean, the amount of contamination that comes from the air during cooling is not very great. That the milk should be cooled immediately is beyond question, but it can be cooled just as efficiently, and perhaps more satisfactorily, by being placed at once in the sterilized bottles, immersed immediately in cold water and kept there until it has had an opportunity to cool. Those who have carefully used this method are convinced that the results are fully as satisfactory as those obtained by passing the milk over a cooler, which at the same time aërates the milk.

Many dairymen think it necessary to aërate the milk for the purpose of getting rid of the so-called animal odors. The milk, as drawn in an ordinary dairy, does have a certain odor which is removed by the aëration. But these odors are due in large degree, if not wholly, to the filth contamination of the milk. If the milk could be drawn directly from the cow without becoming contaminated with the various forms of filth already referred to, the animal odors would, under ordinary circumstances, be slight if at all appreciable. Such milk would not require any aëration. Dairymen who are particularly careful in their dairies and give the greatest attention to cleanliness are not troubled with animal odors, and find that the immediate bottling of milk, without aëration, and its rapid cooling in cold water gives the very best results. In other words, the necessity of aëration will be proportional to the amount of filth contamination of the milk during the milking. The best kept dairy will feel the need of aëration the least, while the poorly kept dairy feels this need the most. But under any circumstances, whether the milk is aërated or not, *immediate cooling* is an important factor in the keeping power of the milk.

Value of such Regulations.—The general suggestions for the dairy above given have been the result of experience and not of theory, and when they are adopted the result in protecting the milk from bacterial contamination is quite appreciable. Exactly how efficient these general methods are in reducing bacteria cannot be stated, but experiment has shown that where they are regularly performed the number of bacteria that find entrance in the milk may be reduced to a few score per cubic centimeter, and sometimes even less. Even moderate precautions will produce a considerable reduction, and simple rules that may be adopted without very great trouble by any dairyman may reduce the number of bacteria to 1,000–5,000 per cubic centimeter.

The question of reduction of bacteria is not one of possibility but of practicability. The dairy methods adopted will always be a compromise, and the particular regulations adopted by any individual dairyman will depend upon their expense compared with the price he receives for his milk. It is perfectly possible for any dairyman so to protect his milk by the precautions pointed out that the number of bacteria will be very small indeed, but whether he will do this will depend upon the price that he can obtain for such milk. The price that most milkmen receive for milk is not sufficient to warrant them in adopting all the precautions and suggestions that have been here pointed out. These regulations require care and expense, and it is quite impossible to expect that the dairyman will adopt them unless those to whom he furnishes the milk are willing to pay him for his trouble. With the price of milk which the ordinary dairyman receives it is quite impossible to adopt proper methods. It is only in special dairies, where a particular price can be obtained for the milk, that the adoption of such sanitary regulations is possible. In other dairies, where milk is supplied for the general market at ordinary market prices, the rules that are adopted will depend upon the price which the dairyman receives. It can never be hoped that there will be a complete modification of dairy methods until there can be such a change in the milk industry as will give to the milk producer a price for his milk sufficient to warrant him in carrying out such regulations.

Nevertheless, there are some of these regulations which are simple and can be adopted without very great expense. Probably the first point where care should be given, and one which can be adopted with no expense at all, is to prevent the cows from feeding, especially upon dry food, before or during the milking. The second point, in view of practical results, is the condition of the cow. If the cow can be kept groomed and tolerably clean there will be a marked improvement in the character of the milk. The practice of moistening the udder of the cow with a sponge before the milking is a simple one, and, since it involves no expense, it may be adopted by any dairyman, and will be extremely useful. The other suggestions given above are applicable only in dairies where exceptional care can be taken.

DISINFECTION OF DAIRY PREMISES.

It sometimes happens that a dairy experiences trouble due to certain microörganisms, as shown in the next chapter. If it is possible to determine exactly where the mischievous bacteria come from it is not difficult to apply proper remedies, for a disinfectant applied at the source of the trouble will remove its cause. In many cases, however, the exact location of the source of trouble is unknown, and it becomes necessary to institute a general cleaning and disinfection of the dairy premises. Such a general cleaning is also useful occasionally for the purpose of improving the general condition of the milk. In such cases the disinfection should be applied to the milk vessels, the barn, the dairy and the cow.

Milk Vessels.—The disinfection of the milk vessels has already been referred to and we need only repeat that the best method of disinfection is by live steam, which should be resorted to if possible.

The Barn.—The disinfection of the barn is a matter of considerable difficulty on account of the roughness of the lumber of which the stables are usually made. The first thing to be done is to remove all dirt from the surfaces of the stable, and this must be done *thoroughly*, or a disinfection of the premises is impossible. Water should be used freely to moisten up the dry filth and facilitate its removal, and, by scraping and washing and brushing, every bit of accumulated filth must be removed. After such cleaning the whole interior surface of the stable should be washed with some disinfectant. Probably the best solution is corrosive sublimate dissolved in water one part to a thousand (one ounce of the sublimate to eight gallons of water). This may be applied to the inner surface of the barn, either by washing with a broom, or better, by spraying, if a proper spraying apparatus can be obtained. It must be remembered, however, that an ordinary spraying apparatus made of metal cannot be used, inasmuch as the corrosive sublimate corrodes metals badly. The farmer must, therefore, usually apply the material with a broom. After having thoroughly covered all of the surfaces of the stable with the disinfectant, the whole should be again washed in water so as to rinse away the disinfectant, which is itself an intense poison. After this, a whitewashing of the walls is very desirable to complete the process. Instead of corrosive sublimate a solution of chloride of lime (one pound to six gallons of water) may be used for washing the floors and walls. This has the advantage of not being so poisonous as the corrosive sublimate.

The Dairy.—In disinfecting the dairy essentially the same methods should be used. The dairy, however, being ordinarily in a more cleanly condition, can be more easily disinfected. Every part of the dairy, including the woodwork, should be washed with a disinfectant solution, either corrosive sublimate or chloride of lime. It is perhaps better to use the latter solution in the dairy than the corrosive sublimate, because of the intensely poisonous nature of the former. None of these solutions should be used by washing the inside of any of the vessels or vats which are to contain the milk. These vats should be washed with nothing but boiling hot water. After the disinfection, every part should be thoroughly washed with clean water for removal of the disinfecting material, so far as possible.

The Cow.—The fact that some of the dairy troubles are due to microörganisms which become attached to the cows, makes it necessary occasionally to apply the disinfection to these animals. All of the long hairs around the cow's flanks and udder, and especially those on the tip of the tail, should be cut off with shears. Then the cow should be washed, first with water and later with a three to five per cent. solution of boracic acid. The teats should also be carefully washed and, if one wishes to take special pains in the disinfection, the interior of the milk ducts may be rinsed with a three per cent. solution of boracic acid, which may be injected into them through a small tube, like an ordinary milking tube, with a rubber bulb on its end. Such a disinfection applied to the animals is usually sufficient to remove from the animals the microörganisms which are a source of trouble.

The bacterial contamination thus far considered relates only to the bacteria that find entrance to the milk during the milking. The numbers given above, ranging from a few score to several thousand, are the numbers that are found in fresh milk immediately after the milking process is over. But the number of bacteria that are found in any sample of milk is dependent upon other factors beside the original contamination. These factors are chiefly the age and the temperature at which the milk is kept. Before we can understand satisfactorily the effect of temperature and age upon the bacteria of milk it will be necessary for us to consider the kinds of bacteria that may be found in the milk. We, therefore, turn now to the consideration of the types of milk bacteria.

CHAPTER III.

TYPES OF MILK BACTERIA.

In classifying the bacteria of milk for our purpose it is best to divide them into groups according to their action upon milk. Although this is not the best scientific method of classifying bacteria, it is the most useful for the purpose of understanding their relations to milk.

Practically all of the common changes which occur in milk, subsequent to the milking, are due to the action of micro-organisms. Milk, kept free from bacteria, may be preserved for an almost indefinite period with very little change. A few years ago it was supposed that this was absolutely true and that milk, if protected from bacterial contamination, would remain indefinitely without any chemical change. Within the last few years it has been found that, even in fresh milk, there is present a chemical ferment or enzyme which has been called *galactase*. This is a normal ingredient in milk, derived from the cow, and it is capable of producing a slow chemical change. Hence, even if uncontaminated by bacteria, milk will in time change its chemical nature quite materially. This galactase probably plays a part in the ripening of cheese, but its action is comparatively slow and it has very little to do with those changes in milk which take place rapidly.

Odors and Flavors of Fresh Milk.—Pure milk has very little flavor or smell, but it frequently happens that our market milk has strong **tastes** and **odors.** In some cases these flavors are strongly developed in the milk at the moment it is drawn. If, for example, a cow is fed upon *garlic* or *turnips,* their odor shows itself in the milk, and in such cases the odor comes directly from the cow, the milk being at the moment of secretion filled with volatile products which give rise to these flavors. Such tastes and flavors in milk are, however, not very common and associated with only a few strongly flavored foods. The *animal* or the *cowy* odor, already referred to, is one which has been in general regarded as due to volatile products directly transferred from the animal to the milk at the moment of secretion. It appears, however, that this animal odor is chiefly due to contaminations and is primarily caused by manure. The soiled boots and clothing of the farmer have the same odor. Milk which is produced in a clean dairy, under the exceptional precaution already mentioned, does not show these animal odors. They may be largely, if not wholly, prevented by sufficient care in the dairy.

Flavors due to Microörganisms.—Aside from these tastes and odors there are others which are due to the development of bacteria in the milk. There is little difficulty in determining whether a particular taste in milk is due to the growth of bacteria in the milk or to products derived directly from the cow and probably due to her food. Odors which are due to volatile products transmitted from the animal to the milk, like the garlic flavor, are strongest in the fresh milk. The milk when drawn shows these flavors developed at their height and, if the milk is kept for a few hours, the tendency will be toward a reduction of the flavors rather than their increase. On the other hand, the odors and flavors, or any chemical change which occurs in milk as the result of a growth of bacteria, will never be found in the freshly drawn milk, but will appear later, being slight at first and continuing to increase hour by hour as the bacteria have a chance to multiply and produce their action.

In giving an account of the common milk bacteria it must always be recognized that no statements will apply to all localities. Certain species are common in some places which are rare or uncommon in others. A few species, however, appear to be very widely distributed and these we shall notice particularly. The effects produced upon milk by different bacteria are very numerous, but they may be grouped into a comparatively few types as follows:

THE LACTIC FERMENTATION.

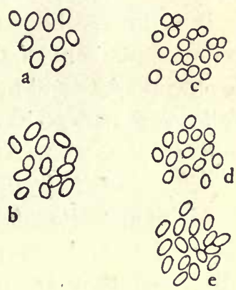
By **lactic bacteria** are meant species which give rise to a considerable amount of *lactic acid*, the production of this acid being, therefore, a primary characteristic of their growth so far as concerns their relation to milk. Along with the lactic acid, however, they all produce other products and they have numerous secondary characters. For example, there is frequently produced *carbon dioxide, hydrogen,* traces of *alcohol, acetic acid* and *salicylic acid,* and other more obscure chemical products. The development of these secondary products is somewhat variable, and is in a measure dependent upon temperature. At moderately high temperatures the secondary products are developed in larger amount than they are at low temperatures. But the chief product of this group of bacteria is lactic acid, which is produced from the milk sugar, and since this is a prominent characteristic of a considerable group of bacteria we recognize them as lactic bacteria. The lactic acid produced is not always the same. At 70° F. it is the type of lactic acid that turns the plane of polarized light to the right. At 98° it is likely to be both the right-handed and the inactive type (50). In old cultures at high temperatures the left-handed variety is more apt to be present (50).

The Souring of Milk.—The souring of milk is a well-nigh universal phenomenon. It is true that occasionally normal milk does not sour, due to factors to be noticed later, but these cases are exceptional and, in the vast majority of cases, milk becomes acid after a few hours and eventually the acid precipitates the casein and the milk curdles. Curdling may also be produced by *rennet,* and souring and curdling may be produced by certain *yeasts* or certain species of *molds* (39). But in the great majority of cases it is caused by bacteria. This change in the milk is the one which produces the greatest amount of trouble to the dairyman, and milkmen in general look upon it as the one troublesome fermentation in milk which they desire to avoid. Hence the lactic bacteria must be regarded as the dairy bacteria *par excellence,* and ever since the beginning of the study of bacteria of milk they have been looked upon as the chief dairy organisms. In a sense this is true, but in another sense it is quite false. Although the lactic bacteria are the predominant dairy organisms, this does not mean that they are more abundant in the dairy than any other kind of bacteria, nor does it mean that, of the bacteria that get into the milk by the ordinary contamination in the milking, the majority are lactic bacteria. Indeed, as we shall see presently, the reverse is the case, and the larger proportion of the bacteria in fresh milk belong to other types than the lactic bacteria. In saying that they are primary dairy bacteria we mean simply that this group of organisms finds milk more favorable to its rapid growth than any of the other miscellaneous bacteria which contaminate the milk and that it causes an almost universal souring.

The Lactic Bacteria.—These include a large number of varieties, over one hundred different types of bacteria having been described as producing lactic acid. Although many of these have nothing to do with dairy problems, a large number have been found in milk capable of producing lactic acid, and these may, in general, be regarded as normal milk bacteria. This large number of species would doubtless be very much reduced if the organisms could be compared together, for the same species has been described several times under different names by different investigators. This confusion has been increased by the fact that the same lactic species shows variations under different conditions, and the descriptions of the same organism do not always agree because of different conditions and different methods of study. As the result of these facts it has been quite impossible until recently to state what were and what were not the primary lactic bacteria. At the present time, however, enough work has been done to enable us to see some order in this chaos and to reduce the facts to an intelligible system.

Nearly all of the lactic bacteria described are short, oval rods or cocci, resembling each other closely in size and shape. Practically all of them agree in failing to develop spores and in being consequently killed *by moderate heat.* Among the large list of lactic species described are many that are purely incidental, found only occasionally in milk and in small numbers, but present so rarely and in such small numbers that they have little or nothing to do with ordinary dairy problems. If we omit these incidental species from our list the rest may be readily grouped into a few simple types.

FIG. 12.



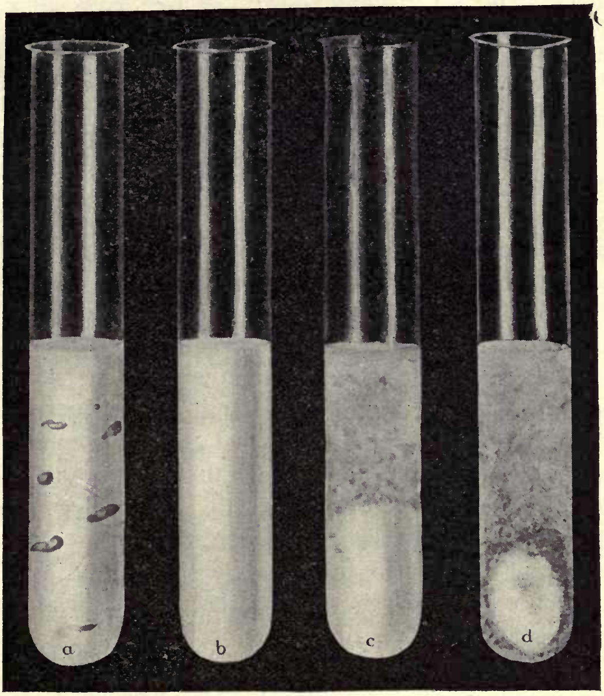
Lactic Bacteria of the common type showing variations in shape. *a* and *b* are the same species. *c*, *d*, and *e* are three forms of a second species.

1. **A Facultative Anaërobic Type.**—The first type consists of bacteria which do not flourish in the presence of oxygen, growing better where the amount of oxygen is slight. They are what is known as facultative anaërobes. The bacteria included under this head have the following chief characters (43): They are short rods, showing considerable variation in length from forms so short as to be practically almost spherical to others that are twice as long as broad. The same species show these variations and have been sometimes described as *micrococci* and sometimes as *bacilli* or

bacteria (Fig. 12). The types described as cocci, bacilli and bacteria are, therefore, probably the same. Since none of them grows readily in the presence of oxygen they grow better under the surface than on the surface of culture media. They sour milk more readily in deep than in shallow dishes, the former condition furnishing them with less oxygen than the latter. They also fail to grow readily in most artificial culture media used for cultivating bacteria in the laboratory. None of them produces gas and they all curdle the milk into a solid, hard, acid curd, without bubbles and with very little, if any, odor (Fig. 13, *b*). The acid they produce is primary right-handed lactic acid, and the amount which they develop varies with the temperature. Most of them grow best at a temperature between 70° and 98° F. The amount of acid which they may develop at 98° F. is represented at about .88 per cent. to .90 per cent. (see p. for explanation of these numbers). At a temperature of 83° they produce 1.01 per cent. in about 48 hours; at a temperature of 65° they produce 1.10 per cent., requiring six days for the purpose (56, 57).

It is impossible to say how many species belong to this type. Members of the group have been described by many bacteriologists and have been given a variety of names. The following names have been used: *Bacillus acidi lactici I. and II.* (Esten), *Streptococcus acidi lactici* (Grotenfelt), *Bacillus acidi lactici* (Gunther), *Bacterium lactis acidi* (Leichman), *Bacillus lacteri* (Dinwidie), *Bacillus a* (Freudenreich), and several others. All of these are so closely alike that they are probably to be regarded as identical, so that perhaps all these names apply to the same species. It is true that there are some variations in the descriptions given by the different authors, but it is also true that there are variations in the characters of the same species as given by the same author. For example, some cultures of the same species are found to produce acid in such small quantities that they do not curdle milk, while others curdle it in a few hours. Other variations are also found within the limits of the same type, and the probability is that we have in this list of species one organism, showing slight variations

FIG. 13.



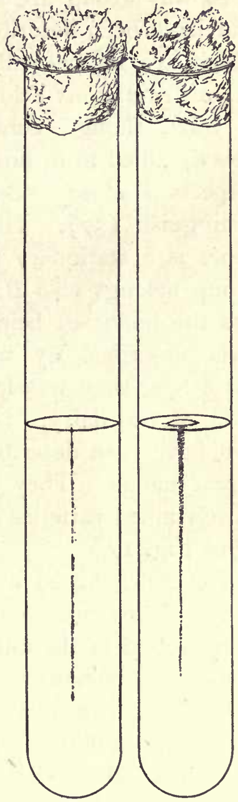
Showing the action of different types of bacteria upon milk. *a*, the aërobic type (*B. aërogenes*); *b*, the anaërobic type (*Bact. acidi lactici*); *c* and *d*, the peptonizing type, with the curd in different stages of digestion.

as studied under different conditions in different localities by different bacteriologists. Probably the best name to be reserved for this species is *Bacterium lactis acidi.*

Source of Bacterium lactis acidi.—This microörganism does not live in the milk ducts of the cow (40). It was long ago found that milk would not sour in the milk gland, even if allowed to remain there for a long time, and if this bacterium were present it would be expected that the milk would sour because the conditions in the duct are favorable for its growth. Moreover, careful study of the bacteria in the milk ducts, and of milk drawn with aseptic precautions have demonstrated that this bacterium is not commonly a normal inhabitant of the milk duct. A study of the external sources of contamination has shown that this organism may probably always be found upon the hair of the cow, upon the hay, in dust on the floor, in the air of the stall; but that it is not found in the ordinary dirt of the stall, in water, or in the excretions from the animal (44). Its primary source in ordinary milk appears to be the skin of the cow, from which it drops during the milking, and perhaps it comes also from hay dust. It is extremely probable that the organism must have some other normal habitat. It cannot grow upon the skin of the cow, which is hardly moist enough for proper bacteria growth. Moreover, the bacteria that get into the milk show, as we shall notice later, indications that they are not at first in a condition for vigorous growth. Probably, therefore, there is some other source in nature where these organisms live naturally and where they grow and multiply. What that source is has not as yet, however, been determined, though it is possibly the leaves of grass.

Distribution.—This species is very widely distributed, although it does not appear to be anywhere in very great abundance. It has been found in milk from nearly every state in the United States, and it is equally common in the milk in the dairies of Europe. When milk drawn directly from the cow is examined at once the numbers of this species are very few. Indeed, if exceptional care is taken to prevent external contamination during the milking they are so few in the fresh milk that they can rarely be found by ordinary bacteriological methods of study and are frequently totally absent (100). Hence milk uncontaminated by external material contains them in very small numbers or not at all (38). If the milk, however, has been subject to much contamination by filth the number, even at the start, may be considerably greater.

FIG. 14.



Showing the method of growth in gelatin of the two types of lactic bacteria. *A* is the facultative anaërobic type and grows only below the surface. *B* is the aërobic type growing well on the surface.

Although these bacteria are not particularly abundant in milk they are so widely and universally distributed that in the ordinary process of milking they are almost sure to find entrance into the milk at least in small numbers. In milk which has stood for several hours, as we shall notice in the next chapter, they become very abundant, and in old milk they are by far the most abundant bacteria.

II. **An Aërobic Type of Lactic Bacteria. Gas Producers.**—The distinction between this type and the first is that the bacteria grouped here grow best in contact with air. They grow luxuriantly upon the surface of culture media and better in shallow than in deep dishes (Fig. 14, *B*). Their morphology is, however, almost identical with that of the first type. They commonly, though not always, possess the power of producing gas in considerable quantity, and when they curdle milk the curd, instead of being smooth and solid, is apt to be broken and more or less filled with gas bubbles (Fig. 13, *a*). They play a part in the ripening of cheese which is probably in most cases an unfortunate one, since they are the organisms that produce the cheese fault known as *swelling* of cheese. They give rise to prominent odors, such as commonly found in sour milk.

In this group are also found quite a number of forms that have been described under distinct names and some of which are undoubtedly distinct species. The well known *B. coli communis* belongs here, a species frequently present in milk, though commonly not in very great quantity. Closely allied to it, however, is *B. aërogenes,* which in many respects so closely resembles the *B. coli* as to be difficult to distinguish (37). The two species differ in that *B. aërogenes* is a stationary rod, while *B. coli* is motile. To this group belongs also *B. acidi lactici* of Hueppe. This form has the honor of being the first of the various lactic bacteria described by modern bacteriological methods, and for a long time was looked upon as the primary lactic bacteria of the dairy. Here belong also several other forms that have been described by other bacteriologists under different names. They are all probably either the same or closely allied varieties and may all be grouped together (58). (See Fig. 17.)

These organisms also play a very important part in the dairy. They are commonly secondary contaminations, not being found in the milk ducts, except perhaps under exceptional circumstances. They find their way into the milk from some of the contaminating material that enters the milk during the milking. They are not so frequently concerned in the normal souring of milk as are the bacteria of the first type. Some samples of sour milk have been studied in which the souring has been produced by this type of bacteria, and some bacteriologists have, therefore, regarded this as the primary cause of milk souring. This may be doubtless true in some individual instances, but in the majority of cases, as has been determined by the study of the last few years, the organisms of the first group are the chief milk-souring organisms, while those belonging to this group are of secondary importance. In many samples of sour milk these aërobic bacteria are present in small numbers, perhaps 2% to 5% of the total, whereas the organisms of the first group will comprise 90% of the total. Moreover, the species of this group do not multiply very readily in the milk in the presence of those of the first group. All these facts together indicate that they are secondary factors in ordinary milk souring, although occasionally they alone are concerned.

The fact, however, that they produce gas in abundance makes the type of much importance in certain dairy problems. Sour milk very frequently contains bubbles of gas, and when this occurs it must probably be attributed to the fact that some species of this type of lactic organisms are present. The production of a large amount of gas is the cause of one of the most troublesome "faults" which appear in the ripening of cheese, and it is this group of organisms primarily that are concerned in the process. While these gas-producing, aërobic bacteria, therefore, are commonly not the chief organisms concerned in souring of milk, they do have considerable importance in other dairy phenomena.

Other Lactic Types.—In addition to these two primary forms of lactic bacteria quite a number of others have been found in greater or less abundance. These may also be divided into a few types, but their importance in dairy problems is probably insignificant compared to that of those already referred to. Only brief mention is, therefore, needed.

A Liquefying Type.—This type of lactic organism has the power of producing an enzyme ferment, as will be explained in the next group of bacteria, and, as a result, after curdling the milk the curd is subsequently slowly dissolved. The organisms here classed belong, therefore, to the next type of bacteria as well as to the lactic type. They are rather common in milk and sometimes appear to be cocci and sometimes bacilli, which are probably different species. Whether they are of much importance in dairy processes is not yet known.

A fourth type consists of four or five species of bacteria which agree in producing a kind of lactic acid different from that produced by the common lactic organisms. Whereas the common bacteria produce lactic acid which has commonly no rotary effect upon polarized light or rotates it to the right, there are several species of lactic bacteria known which rotate the plane of polarization to the left. These four or five species do not resemble each other very closely, and are only grouped together because of the character of lactic acid they produce. Several species have been described as having this character: *B. acidi lævolactici* (Schardinger), *M. lævolactici* (Leichmann), *B. lævolacticus* (Leichmann), *B. acidi lævolactici* (Kozai) and others. Whether they are of much importance in dairy processes cannot be stated, although one of them has been found to be quite abundant, especially in milk that has been kept at a moderately warm temperature; so much so that Kozai believes that at warm temperatures this organism is the chief agent in the souring of milk (50).

In addition to these there are some other lactic bacteria which have characters different from any of these types. But all of these other forms are so unusual that they cannot be regarded as typical dairy organisms, and while they are found in milk incidentally, and sometimes in considerable quantities, they must be put down as occasional visitors and not as distinctively characteristic of the dairy.

In general, then, it will be seen that, of the numerous milk bacteria which have the power of producing lactic acid and souring the milk, the first type here mentioned is almost universal and, although not extremely abundant in any locality, is so widely distributed that milk will, in the vast majority of cases, become contaminated with this type of bacillus. At moderate temperatures the souring is ordinarily produced by this species. The second type is also almost as widely distributed as the first, but it does not grow so profusely in the milk, especially at moderate temperatures, and is, therefore, not so universally the cause of milk souring. The other types are more or less unusual and play a part in dairy processes which may be important, but is certainly secondary to the first two, although at higher temperatures some of the other species may be the significant types. Each of the first two types is represented by a number of varieties, but they are closely allied to each other and perhaps should all be regarded as belonging to the same species.

The amount of acid produced by different species of lactic bacteria is variable, the same species appearing to differ noticeably in this respect. The *Bact. lactis acidi* commonly produces acid abundantly and causes a speedy souring and curdling of the milk, but some varieties of this organism, agreeing in all other respects, produce acid in smaller amount and never in sufficient quantity to curdle the milk, although it becomes sour to taste and will curdle when heated. Other species produce only a slight amount of acid. The variations occur in different cultures of the same species of bacterium, and may be produced by modifying conditions.

The power of producing acid seems to grow stronger as the bacteria develop in the milk. If bacteria are taken from fresh milk their power of forming acid is rather feeble, but if bacteria are taken from the same milk after a two days' growth, their power of producing acid is noticeably greater. This seems to indicate that they get into the milk from some source where they exist in a dormant condition, and only develop their vigorous powers after growing in milk for some hours. The conditions in the milk stimulate their power of producing acid in marked degree.

Method of Producing Lactic Acid.—From the very outset the question of the exact method by which bacteria produce lactic acid from milk has been much discussed. That it is produced from the milk sugar seems certain, but a primary question arises, whether the lactic acid is produced directly by the growth of the microörganisms, or indirectly by the production of a chemical ferment or enzyme. These two views as to the possible action of the lactic organisms have been held from the very beginning of their study. In recent years there has been a tendency on the part of biologists to refer a large number of the bacterial fermentations to the direct action of enzymes. Bacteria certainly do secrete enzymes as the results of their life processes. This has been abundantly proved in regard to some bacteria, and a similar fact has been shown to explain the alcoholic fermentation by yeast. Whether the lactic bacteria produce a similar enzyme is not yet known, for no lactic enzyme has been yet separated from them.

There are, however, some reasons for believing that the production of an enzyme by lactic bacteria is the simplest explanation of the lactic fermentation. If the lactic acid was produced by the bacteria seizing certain ingredients in the milk sugar for their nutritive processes and thus producing a destruction of the milk sugar molecule and its conversion into lactic acid, it would naturally follow that the amount of lactic acid produced would be directly proportional to the growth of the bacteria. Now, while there is a general parallel between the growth of these microörganisms and the production of lactic acid, the parallel is by no means a close one. For a considerable time after milk is inoculated with lactic bacteria there is no increase in the amount of lactic acid, although the bacteria are themselves multiplying rapidly and becoming very numerous. Then there is a rapid increase in the development of lactic acid, accompanying a still further growth in the bacteria. Finally, the growth of bacteria to a considerable degree ceases, but the production of lactic acid goes on for some time. It is also a fact that the disappearance of milk sugar, out of which the lactic acid is doubtless formed, does not proceed parallel with the production of lactic acid nor with the growth of bacteria (47).

Now, while it is not an absolutely necessary conclusion that the production of lactic acid should be parallel with the growth of bacteria if the phenomenon is the result of the vital processes, still such would be the natural inference. The fact that the two curves are not parallel is certainly more in accordance with the belief that the lactic acid is the result of an enzyme produced by the bacteria. If an enzyme is thus produced it is easy to understand that for the first few hours of bacteria growth not enough of the enzyme is secreted to produce lactic acid, but that after the lactic bacteria have grown several hours they develop an enzyme which begins to work at once upon the milk sugar, causing a rapid conversion into lactic acid. Later the presence of lactic acid thus formed checks the growth of bacteria, as will be seen in the next chapter, but the enzyme which has been developed is not so rapidly checked in its action, and continues to develop lactic acid for some time even after the growth of the bacteria has stopped. These facts together, then, suggest that the production of lactic acid by these microörganisms is not the direct result of growth, but rather due to the action of some enzyme that is secreted by the bacteria.

ENZYME-FORMING BACTERIA.

We have just noticed that the lactic bacteria may possibly produce **enzymes,** although this has not as yet been demonstrated. The group of bacteria that we are now to consider certainly does produce enzymes. They are commonly not capable of producing acid, although a few, as mentioned on page 71, do produce a small amount. Many of them produce a slight alkaline reaction in the milk while some do not change the reaction at all. Their distinctive characteristic, however, is the production of enzymes. Two quite distinct types of action on milk are recognized, a *curdling* action and a *digestive* action. Not only do we see two types of action, but two distinct enzymes or chemical ferments have been separated from the bacteria cultures by chemical means, and we have, therefore, demonstration that the action of these organisms is through the production of certain chemical ferments. The enzymes produced by these bacteria are as follows:

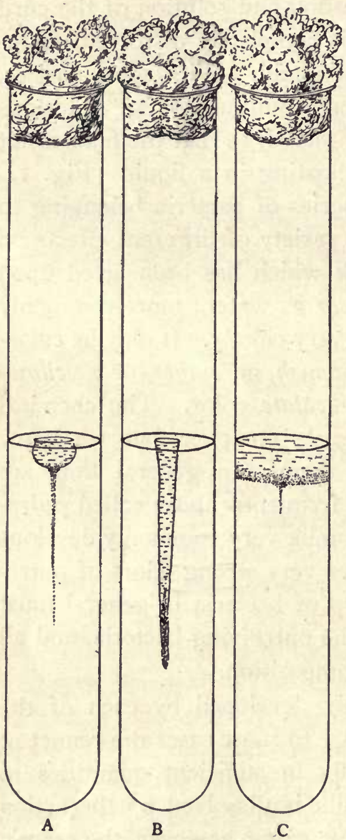
Rennet.—Most of the organisms in this group, perhaps all of them, produce a certain quantity of an enzyme that has the essential characteristic of **rennet** (62). Like the ordinary rennet, it is capable of curdling milk rapidly and producing a soft curd, which is commonly either alkaline in reaction or amphoteric. The curd thus produced is sweet rather than sour, and the action of these bacteria is, therefore, the common cause of a phenomenon appearing in the dairy known as the **"sweet curdling"** of milk. The bacteria commonly require two days' growth in the milk before they curdle it, although some of them act more rapidly and some more slowly. The curd may usually be distinguished from the acid curd by being soft, while the latter is hard.

Tryptic Ferment.—The second enzyme produced by these bacteria is akin to the **tryptic** ferment, called **casease** by Duclaux (3). It has characteristics very similar to the trypsin produced by the digestive glands, though it has not been studied in sufficient detail to determine whether it is strictly identical with trypsin. Its most noticeable characteristic is its power of **digesting** proteid material, which it converts more or less completely into soluble forms, like peptone, etc.

The action of these bacteria upon milk is striking. The casein in the milk is in the form of a solid or semi-solid material held in practical solution. The bacteria in question, by means of the rennet which they produce, first commonly curdle the milk into a soft curd; then the curd begins slowly to be digested by the tryptic ferment, and, as it is digested, it is also dissolved. This digestion and solution of the curd may continue until the whole is completely dissolved, forming a more or less transparent liquid. With some species, however, the digestion does not continue so far and stops after the curd has been half dissolved, so that the final result is a small mass of soft curd floating in a liquid (Fig. 14, *c* and *d*). There are many species of bacteria belonging to this group and they produce a variety of different effects on the milk. Sometimes the milk which has been acted upon by them becomes almost as *clear* as water; more commonly it is slightly *cloudy,* sometimes very cloudy. It may be *colorless,* or it may become of a *brownish,* an *amber,* or a *yellowish,* or even a *greenish* or *chocolate* color. The chemical changes that occur are extremely complex, but we know almost nothing in regard to them. In general they are changes of decomposition and frequently those called *putrefactive*. The resulting digested milk very commonly develops prominent odors, and sometimes very strong odors of putrefaction and decay. This group of bacteria in general must be looked upon as containing the putrefying bacteria, and all of them produce chemical decomposition.

Both enzymes are commonly developed by each of the species of bacteria in this class. In most cases the rennet at the outset is developed rapidly in sufficient quantities to curdle the milk. Later the milk is dissolved by the action of the tryptic ferment. In some cases, however, the tryptic ferment is developed so rapidly at first that the digestion and solution of the casein occurs before the rennet ferment has developed sufficiently to curdle the milk. In this case the milk shows no signs of curdling, but slowly becomes transparent or semi-transparent. Experiment has shown, however, that these bacteria do produce the rennet ferment, although the tryptic ferment is developed too rapidly to make it possible for the milk to curdle before it is digested. In all cases the milk is finally converted into a more or less perfect liquid, semi-transparent, having a complex composition of unknown chemical compounds, and showing a variety of tastes and odors which frequently indicate a close alliance to putrefactive products.

FIG. 15.



Showing growth of liquefier in gelatin. The lower half of the tube is filled with gelatin which is liquefied by the bacteria in the shaded portion.

This class of bacteria can be easily distinguished by bacteriologists. One of the culture media in common use is made of gelatin, and the enzyme which digests the casein also *liquefies* gelatin. The result is that this class of bacteria may all be distinguished by their power of liquefying gelatin when growing in ordinary culture media, and they are therefore perhaps the most easily recognized by bacteriological methods of all of the types of bacteria. The *liquefiers,* as they are called, are, therefore, in general the bacteria which produce enzymes and digest the casein in milk, and this term we will hereafter use. (Fig. 15.)

The liquefying bacteria include bacilli, bacteria and cocci (Fig. 16). They are abundant in fresh milk, apparently

FIG. 16.

A variety of bacteria liable to be found in milk. 1 and 2, typhoid bacillus (*Pfeiffer*); 3, pus and pus cocci; 4, *B. dysenteriæ* (*Shigar*); 5, *Proteus vulgaris*; 6, *Clostridium butyricus*; 7, 9, 10, 11, types of common lactic bacteria (*Conn*); 8, a coccus without influence on milk (*Conn*); 12, 13, 14, three bacilli producing slimy milk (Fig. 12, *Marshall*; Figs. 13 and 14, *Conn*); 15, 16, 17, 18, 19, types of liquefying and putrefying bacteria, which digest the casein (*Conn*).

coming from sources external to the cow, since the bacteria that are in the milk ducts are not liquefiers, as a rule. They are found in abundance in the feces of the cow and in various forms of filth, and the presence of large numbers of liquefiers in a sample of *fresh* milk is an indication that it is badly contaminated by bacteria. Indeed, the quality of milk, from a bacteriological standpoint, may be with tolerable accuracy estimated by the number and proportion of liquefiers which it contains. If the number of bacteria is large but they belong to the lactic type, the milk may still be looked upon as not fresh, but not badly contaminated (see Chap. IX.). If the number, however, is large and the majority of them belong to this liquefying type, the indications are that the milk has been subjected to some excessive source of filth contamination and is more suspicious.

This type of bacteria has very little to do with the changes which ordinarily occur in milk. It is true that they are able to produce profound changes in the chemical nature of the milk, even more profound than those of the first type, but their development is commonly prevented by the lactic bacteria, and in ordinary milk they get no opportunity to grow. It is rare that any changes occur in the ordinary milk supply which are to be attributed to this type of organisms, although it occasionally happens. Once in a while a dairyman finds what is called sweet curdling, due to some of these organisms. Occasionally also *putrefactive tastes* may develop in the milk, and these again are due in all probability to this type of microörganisms. But in the majority of cases the rapid development of lactic bacteria checks the growth of this type of organisms, so that they do not produce any noticeable effect upon the milk. That they probably have some influence in the changes that occur in ripening cream we shall see in a later chapter.

MISCELLANEOUS.

The lactic- and enzyme-forming bacteria may be regarded as normal to milk, inasmuch as they are expected in nearly every sample of milk. In addition to these, there is a large variety of occasional visitants which may produce more or less change in the character of the milk. Inasmuch, however, as they are found only occasionally they are properly to be regarded as *infections* of the dairy. When abundant they may produce trouble for the dairyman which is sometimes very serious. In their relation to dairying they differ from the first groups in one highly important respect. There are no practical means, so far as we know, by which the dairyman can avoid the presence of the lactic and enzyme bacteria, although care and cleanliness may greatly reduce their numbers. The types of bacteria now to be mentioned may be avoided, and if they produce trouble in a dairy the application of simple remedies will enable the dairyman to get rid of them. The following are the most important of these dairy troubles due to bacteria.

Slimy Milk.—The most troublesome infection in a dairy, outside of the milk souring, is the development of a **sliminess.** A sliminess in milk is not always produced by bacteria. It is a characteristic of the milk from animals suffering from certain diseases, particularly *garget.* But in the majority of cases the trouble is due to the development of bacteria in the milk subsequent to the milking. In some countries slimy milk is regarded as a delicacy and is produced by artificial means. In Norway the people have learned to produce slimy milk, which they call *taettamoelk,* by placing in the milk the leaves of a certain plant (*Pinguicula*) which causes the development of a sliminess in the course of a few hours. It has been believed that the plant in question has nothing directly to do with the sliminess, but that its leaves are the home of a microörganism which develops in the milk with the result noted, and a slime-producing bacterium has actually been found upon this plant. Within very recent times it has been insisted that the sliminess is actually due to certain secretions from the plant rather than to bacteria, possibly to *enzymes.* Whether this is true, or whether the more common explanation, that it is due to bacteria, is the correct one, is at present unknown. Another artificial preparation of slimy milk is used in the manufacture of Edam cheese, which is mentioned on page 246.

Except in a few such instances slimy milk is an undesirable fermentation, and sometimes produces great trouble in the dairy. It is one of the most common of the types of unusual fermentations of milk, is widely distributed and occasionally is the cause of much bother to dairymen. Its general characteristics are unfortunately too well known. The milk, though apparently treated as usual, fails to sour and curdle normally, but after a few hours begins to be somewhat slimy. The trouble increases until, at the time when the milk should normally sour, it has developed such an amount of slime that it can be drawn out into long threads. At the same time it has a sweetish taste. Such milk is practically worthless. It cannot be used for butter-making, for the cream will not separate. It will not be used for drinking or cooking purposes, although there seems to be no reason for believing that it is not perfectly wholesome. Most people, however, do not wish to drink slime and will throw it away. Sometimes such an infection may spread through a whole farming district, affecting many dairies and continuing for a long time. Although not always easily explained, when such infections have been studied, the trouble has generally been traced to some common source of distribution. For example, a central creamery receiving such milk from a single patron, may distribute it over the whole patronizing district by returning to the farmers milk vessels not properly sterilized by live steam.

Wherever such slimy milk appears it may always be traced to the action of bacteria. As in the case of other fermentations it is not a single species of bacterium which has the power of producing slimy milk, but rather a somewhat large class of organisms. More than a score of bacteria have been described as possessing the power of producing a slime in greater or less amount (see Fig. 16). Moreover it appears, according to recent work, that some of the common dairy bacteria (*B. aërogenes lactis*) are capable, under certain conditions, of producing slime. But of this large number of species probably only a few are concerned in the actual dairy infections, since most of them produce the effect very slowly and are not vigorous enough to render milk slimy when they have to contend with the ordinary lactic bacteria.

Slimy milk appears to be produced in dairies by one or two widely distributed bacteria. The most common one seems to be a short rod (Fig. 17) which does not produce spores and is, therefore, readily killed by heat. This bacterium has been found a common cause of this trouble in the United States (77, 73). Further, it so closely resembles a slimy milk bacterium described in Europe by Adametz that it is probably to be regarded as the same, and is consequently known by the name given it by Adametz, *B. lactis viscosus*. It happens to be a very vigorous organism and, when once present in milk, will grow so rapidly as to make it slimy in spite of the action of the ordinary lactic bacteria which are present.

FIG. 17.

B. lactis viscosus; the common cause of slimy milk. (*Ward.*)

The source from which this troublesome organism is derived has been, at least in part, determined. The bacillus is an inhabitant of water, and the origin of the trouble may be originally some water supply infected with the bacillus. Its continuance in the dairy has been in one case certainly traced to the dairy water supply. It has been found living and growing in the water used for washing the milk vessels, or for submerging them to cool the milk; and through such a source of contamination the bacillus gets into the milk. The trouble is found to disappear if the milk vessels are once thoroughly sterilized by live steam and then placed in fresh, pure water. Disinfecting the water with permanganate of potash has also been found sufficient to allay the trouble. These facts teach that upon the occasion of such an infection the water supply and the water tanks must be the first place of suspicion. The bacilli are not common enough in nature to be a very frequent source of trouble, and if the dairy is infested with the bacteria it generally means that the water tanks and cans have become infected with the organisms. Thorough sterilization of the milk vessels and of the tanks in which the water stands for cooling the milk, will be the most efficient means of removing the trouble. A second fact of importance to be remembered is that the water tank of a central creamery may be a source of distribution to a large dairy community. If, as occurs in some places, the milk cans, after standing in the water tank at the creamery for a day, are then given directly back to the farmers without sterilization, a slimy milk infection, starting in a single farm in the district, is pretty sure to be distributed presently, through the creamery, to the whole territory furnishing that creamery with milk.

It is not likely that this particular organism is always the cause of slimy milk, for, among the many species having this power, some occur under conditions which render it probable that they may produce a dairy infection. In Switzerland it is pretty certain that a micrococcus (*Micrococcus freudenreichii*) is the common cause of the trouble. This bacterium is commonly found, it grows rapidly, producing a sliminess in five hours, and is regarded as the agent for producing the common dairy infection. Possibly other species may be concerned elsewhere. The slimy milk used in making Edam cheese is produced by a *Streptococcus*, while a different species has been described as growing on the *Pinguicula* leaves and is said to be the cause of the artificially produced slimy milk of Norway. But whatever be the particular variety which produces the trouble it is certain that in all cases the bacterium is an unusual one. It does not belong to the dairy and is not common around barns. Except under peculiar conditions, therefore, the slimy milk bacteria are not likely to get into the milk, and when they do get into the dairy, the trouble may be quickly checked if the dairyman can only find the particular source of trouble in his dairy and apply some disinfecting agents at the right place. Cleaning of water tanks and milk vessels will be the efficient remedy in most cases. Slimy milk is a preventable infection and its remedy is simply cleanliness, with, perhaps, disinfection.

Bitter Milk.—There are quite a number of causes which may give a **bitter taste** to milk. Cows produce bitter milk after eating certain foods, like *ragweed* or *lupine*. A bitter taste is found in the milk of late periods of lactation. Certain udder diseases (*mastitis*) render the milk bitter. These phenomena are none of them very common and are rarely of importance in ordinary dairying. They may in general be distinguished from a bitterness due to bacteria by the fact that they appear at full strength in the milk when freshly drawn.

A true bitter fermentation of milk is known to be produced by the growth of bacteria. Quite a large number of kinds of bacteria may cause a bitter taste in the milk. Most of them, however, are capable of producing this flavor only after they have grown for a considerable time in the milk, and the examples that have been studied by bacteriologists are commonly milk that has been imperfectly sterilized, and has subsequently become bitter to taste instead of souring like ordinary milk (81). Such a bitter taste does not interest the ordinary dairyman. Occasionally, however, milk develops a bitter taste within a few hours from the time of milking, and in such cases the trouble becomes one of significance to the ordinary dairy (79). The phenomenon is quite rare, but there has been at least one instance of such bitter milk dairy infection studied, and one or two others recorded. A more common occurrence is a bitter taste in cheese and this has been proved also to be due to microörganisms. We have, therefore, at least three kinds of bitter tastes developing in milk products from bacteria growth: (1) A dairy infection which affects tolerably fresh milk, and is rare; (2) a bitter taste that develops slowly in sterilized milk as the result of the action of bacteria which have resisted the heat of sterilization; (3) a bitter taste in cheese.

These three phenomena are produced by microörganisms. The bitter infection of fresh milk, in the one case studied, was found to be due to a micrococcus which developed rapidly and rendered the milk bitter and somewhat slimy. Washing the cows' udders with two per cent. soda and the milk duct with three per cent. boracic acid cured one such trouble (80). The bitterness in sterilized milk may be produced by a considerable variety of bacteria, but is a phenomenon of comparatively little importance. The bitter taste in cheese has been found to be due in one case to a bacterium, which was isolated and experimented with (79), and in another case to the development of a species of yeast. This phenomenon will be considered in a later chapter.

The source of the bacteria which produce the bitter taste in milk has been made out in one case, where it was traced to bacteria present in connection with the milk ducts of a cow. A single cow was found to be infested with a bacterium which produced a bitter taste and the trouble disappeared from the dairy as soon as this cow's milk was separated from the general supply. The milk of this cow became free from the trouble after her teats were disinfected. At present little further is known in regard to the infection of bitter milk.

Soapy Milk.—This is an infection which occurs rarely, only one or two instances having been recorded (82). It is characterized by a frothing of the milk, a soapy feeling, a peculiar taste, and has been traced by Weigmann to the presence in the milk of a special bacterium. Its origin appeared to be from the straw and hay of the stable, or from the pastures. It is such a rare incident that it needs no further reference.

COLORED MILKS.

There are a number of somewhat rare fermentations in milk, each due to the action of microörganisms, each characterized by the development in the milk of certain pigments which change its color. These occasionally appear as dairy infections, but in ordinary dairying they will rarely be met. Inasmuch as they do occasionally appear, however, a brief reference to them must be made.

Blue Milk.—The oldest known form of such fermentation is blue milk. This was carefully studied as far back as 1841, by Fuchs (84), and it is interesting as being the first fermentation that was proved to be associated with and caused by bacteria. Milk affected with this trouble does not sour normally, but, about the time it begins to become acid, blue spots develop in it, extending rapidly through the milk, and by the time the milk has become thoroughly acid it has also turned to a sky-blue color. The blue coloring material does not appear to be at all injurious, or even to render the milk unwholesome, but, of course, it spoils the milk for all commercial purposes. This trouble has been found to be due to a definite species of bacterium called *B. cyanogenes,* a species capable of producing this infection when inoculated into milk along with lactic acid bacteria. A second species has in more recent times been discovered with similar properties and called *B. cyanofluorescens* (87).

The blue milk infection occasionally appears in dairies and may spread from one dairy to another. It has been traced to individual cows in a herd and checked by removing the milk of these cows. The only remedy is the general one for all such troubles, namely, cleaning and sterilizing all vessels, the dairy and everything that comes in contact with the milk. The blue milk organism is an unusual one, and with cleanliness and disinfection the dairyman may hope to get rid of it. In one case the trouble was cured by washing the teats of a cow with weak acetic acid. In America no examples of spontaneous blue milk infection appear to have been recorded. The bacterium which produces this trouble, however, has been found in this country, and is capable of rendering milk blue under proper conditions. A blue milk epidemic may sometimes break out, but it is not one of the common dangers of dairying.

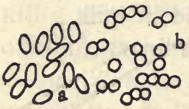
Red Milk.—A fermentation by which the milk becomes red is as rare as that of blue milk. Milk is not infrequently somewhat red when freshly drawn, but this is not due to a fermentation. Milk is occasionally turned red by the presence of blood, which comes from some inflamed or irritated condition of the udder. At times cows give milk of a red color after they have fed upon madder root, or occasionally some other plants. In all of these cases, however, the red color to the milk is at its height at the moment the milk is drawn from the cow. In the rare case of milk turning red from fermentation the red color is not visible at first, but appears after a few hours and increases greatly as the microörganisms develop. A few instances of such red milk have been described in literature in the last fifty years, and two or three species of bacteria have been described as capable of producing red milk (88, etc.). *B. erythrogenes* has been most studied, but a species of *Sarcina* has also been said to produce a spontaneous infection of red milk, *S. aurantiaca*. So rare is this trouble that there is very little chance that one will run across it in common dairy experience, but if such trouble does arise it must always be attributed to the growth of microörganisms, and the remedy will be found along the general line of cleanliness and disinfection of milk vessels and cow stalls.

Yellow Milk, Green Milk, etc.—Certain other colors are sometimes imparted to milk by the growth of bacteria. **Yellow milk, green milk, black milk** and **chocolate milk** have been described. These phenomena, however, can hardly be regarded as dairy infections for they occur under conditions of bacteriological experiment only. Quite a number of the bacteria found in milk are capable of producing different pigments if they have a sufficient time for action. By inoculating tubes of sterilized milk with different species of bacteria we can obtain in the tubes all the colors of the rainbow, and all by the action of bacteria found in milk. But the bacteria do not grow sufficiently in ordinary milk to produce these effects. None of these types of milk, unless possibly it may be yellow milk, has ever been known to occur as a dairy infection. They are phenomena of interest to bacteriologists, but do not occur in ordinary dairying and we need not, therefore, dwell upon them.

BACTERIA WITH NO VISIBLE EFFECT ON MILK.

In addition to the types of bacteria mentioned there is another extremely large group which for the dairyman is of very little importance. It consists of bacteria which have **no action** upon milk so far as concerns sight, taste or smell. Some of these bacteria apparently do not grow in milk. Others, although they grow rapidly, fail to produce any changes which affect the physical nature of the milk. The milk does not sour or become alkaline, nor does it become curdled, even though these bacteria are present and grow in abundance. These bacteria are very abundant in the dairy and in fresh milk. They are found in great numbers in the various forms of contaminating filth in the dairy, and the bacteria that inhabit the milk ducts belong, many of them at least, to this type. Many species of such bacteria are common in the dairy and sure to find their way in milk. A majority of the bacteria in *fresh* milk very commonly belong to this type. This group of bacteria, from the dairyman's standpoint, are of little importance. Whether some of them may not be of importance from the standpoint of the wholesomeness of the milk as a food product, is not as yet known. There is no necessity for further reference to them here, because they never make themselves apparent in dairy processes, and probably play no part in general dairying. It is well to remember, however, that they are commonly present in abundance and that they *may* have some relation to the power of milk bacteria to produce diarrheal diseases.

FIG. 18.



Bacteria with no effect on milk. *a*, a bacterium; *b*, a streptococcus. Both are very common.

There are some other types of bacteria present with more or less constancy, but which, under ordinary circumstances, appear to be of no significance. (1) There are present in milk, apparently in all cases, certain species that are strictly anaërobic and grow only in the absence of oxygen. These organisms are not usually found by ordinary bacteriological study, because they require special methods and special apparatus for their detection. There is no reason for thinking that they grow in the milk under ordinary circumstances; certainly they do not produce any of the usual changes which occur in milk. If they produce any action upon normal milk we are not acquainted with the fact at the present time. It is possible that they are associated with the intestinal troubles attributed to milk, but this is purely hypothetical. It has been thought that they may have something to do with the changes that take place in butter, causing it to become rancid, but this has been rendered doubtful in recent years. Some of them undoubtedly produce butyric acid from milk if they get an opportunity to grow sufficiently. So far as known, these anaërobic organisms are of little significance in the dairy since they grow slowly and rarely make themselves evident. (2) A type of milk bacteria of which we know little has the character of growing only at high temperatures. These are bacteria that will grow only when the milk is warmed to a temperature of about 140°F. Where they come from, or how they can possibly find conditions in nature which are warm enough to enable them to grow, we do not know. There is no reason for thinking that they have anything to do with the changes which occur in milk, and for dairy purposes they may be neglected. Indeed, practically nothing is known about them at the present time beyond the curious fact of their growth at high temperatures only.

It should finally be noticed that there are other less definite troubles arising in milk which must be attributed to bacteria. Dairymen sometimes speak of "tainted milk," "foul-tasting milk," "turnip-tasting milk," "putrid milk," etc. Little is known about the cause of such troubles. A turnip taste seems to be due partly to turnips used as food, and partly to bacteria (*B. fœtidus lactis*). Tainted milk has been traced to bacteria action. These phenomena are, however, rather indefinite and, though probably bacterial fermentations, exact information concerning them is yet lacking.

ALCOHOLIC FERMENTATIONS.

If common *yeast* is added to a solution of *cane sugar* it will produce an alcoholic fermentation. Indeed cane sugar, if left exposed to the air, is very likely to undergo an alcoholic fermentation due to the action of yeast derived from the air. *Milk sugar,* however, does not readily undergo an alcoholic fermentation, even if inoculated with yeast. It is readily changed into lactic acid under the influence of bacteria, but it cannot be converted into alcohol except under peculiar conditions. It is possible to produce an alcoholic fermentation in milk, but not by simply inoculating it with yeast. Under these circumstances other changes take place, but the alcoholic fermentation does not commonly occur.

There are, however, several kinds of beverages known which have as their foundation an alcoholic fermentation of milk. These are found in different parts of the world and are manufactured from milk by different processes. Those which are best known are as follows:

Kumiss.—This is a material which has long been prepared by the Arabs, and commonly made from mare's milk. The milk of this animal will undergo an alcoholic fermentation much more readily than the milk of the cow, and the material that is formed is a favorite beverage. It is characterized by the development of alcohol, but along with alcohol a quantity of lactic acid is produced and the beverage obtained is a drink that is very much enjoyed by Arabs. This kumiss is produced by a combination of microörganisms acting together. There is present a species of yeast, a common lactic organism, thought to be the common *Bact. lactis acidi,* and in addition a third bacillus which is known as the *kumiss bacillus* (97). What the relation of the three organisms is in the production of kumiss is not known, but it is doubtful whether the special kumiss bacillus has anything to do with the process.

Although kumiss was made originally from mares' milk, a close imitation of the product is now frequently manufactured artificially from cow's milk. The method of manufacture is usually to add to the milk a certain quantity of cane sugar and then to inoculate it with some common species of yeast. The yeast begins quickly to produce an alcoholic fermentation of the cane sugar, and the lactic bacteria present produce lactic acid from the milk sugar. The total result is a combination of lactic and alcoholic fermentations and a product which closely resembles kumiss, and which commonly goes by this name.

Matzoon.—This is a material somewhat resembling kumiss and having its origin in Armenia. It is produced out of ordinary cow's milk which is fermented by placing in it small white, fatty, cheese-like grains which can be used over and over and preserved a long time. These grains start up a fermentation that is the result of the action of several organisms. The bacterial study of the product shows the presence of a species of *yeast,* two species of *cocci,* one species of *sarcina,* a *bacillus* which is probably the common hay bacillus, and a widely distributed milk organism, *Oïdium lactis* (94). The relation of these different microörganisms to the production of matzoon is quite unknown, although it is probable that some of the bacteria bring about changes in the ingredients of the milk which make an alcoholic fermentation possible, after which the yeast produces the alcoholic fermentation.

Kefir.—This is an alcoholic beverage which was found originally in the Caucasus Mountains where, from time immemorial, the peasants have used an alcoholic fermentation of milk going by this name. The method of preparation is simple. Milk is placed in leather flasks or sacs and then there is added to the milk a material called *Kefir grains.* These Kefir grains (Fig. 19) are small solid kernels which

FIG. 19.

A large-sized kefir grain and the three species of bacteria of which it is composed. (*Freudenreich.*)

are kept in families and handed on from generation to generation. The flasks are hung up in the sun in winter and in the shade in summer and shaken occasionally. The milk undergoes a fermentation of which alcohol is one of the products, and in the course of a couple of days it contains a quantity of alcohol and carbonic acid and is ready to drink. During this time the Kefir grains increase in size. After the fermentation they are removed and either dried for preservation or placed again immediately in other flasks of milk for a repetition of the process. The grains are very carefully preserved as great treasures. The growth of the grains makes it possible to distribute them, and thus the material is carried from place to place. The production of Kefir is, therefore, the result of the action of the Kefir grains.

Although these grains have been much studied there has been no agreement as to their real nature. That they consist of a variety of microörganisms is easily proved, and that they contain *yeasts, bacilli* and *cocci* has also been uniformly recognized. But there is lack of agreement as to the relation of these microörganisms, and which of them are important and which purely incidental. The most recent, and probably the most reliable work, indicates that there are in Kefir grains four different microörganisms, a species of *yeast,* two species of *streptococci* and a peculiar bacillus called *B. caucasus* (95). The last, which was originally described as especially characteristic of the Kefir grains, probably has no special relation to the production of the alcoholic fermentation. At all events it has been found possible to produce the Kefir fermentation by the use of the yeasts and the streptococci without the aid of the *B. caucasus.* The rôle of this bacterium in producing this fermentation is, therefore, at present unknown. Kefir can be produced by adding the Kefir yeast to milk with a little citric acid.

Leben.—An Egyptian Fermented Milk.—There has recently been described an alcoholic fermented milk, used to some extent by the peasants in Egypt, called **"leben"** (96). It has been known for centuries and is prepared from milk of the buffalo, cow or goat. The fermentation is propagated by adding to the fresh milk, after a brief boiling, a little of an old sample of fermented milk which is kept on hand in the same way as the liquid baker's yeast was preserved thirty years ago. The fermentation is a rapid one, only about six hours being required. In general character it resembles closely the other alcoholic fermentations. A study of the product shows that here, too, is a growth of a variety of microörganisms. There is a *yeast* present and at least three species of *bacteria* producing lactic acid and rennet, and one species of *mold*. These growing together produce the alcoholic fermentation, but it is unknown exactly what relation each species has to the peculiar fermentation that occurs. A material of the same name, but prepared differently, is made by the Algerians, and the Don Cossacks have a fermented milk called *aryan*.

It will be noticed that in all of these types of alcoholic fermented milk a *yeast* is present and a number of other organisms, among which there are, in all cases, *lactic* bacteria. Probably the lactic organisms in some way change the character of the milk sugar by so altering the chemical figuration of its molecule that it is easily capable of undergoing alcoholic fermentation, and that the yeast then produces such a fermentation.

There are a number of commercial products resembling those mentioned which, in recent years, have been placed upon the market. The method by which they are prepared is frequently a commercial secret, but they are always the result of the growth of microörganisms, and probably in all cases the fermentation is produced by a combination of the action of microörganisms and yeasts; the latter producing the alcoholic fermentation proper, after the former have prepared the milk for such a change. They are not as yet very important products and little is known to science of their nature.

The value of these alcoholic milks is not great. They furnish an alcoholic beverage which is enjoyed by some people. It appears that the changes that have taken place in the milk render it somewhat more digestible than the raw milk. As the result, these forms of fermented milk are frequently recommended as a dietetic food, and are used by invalids. They offer a refreshing beverage, with a small quantity of alcohol, and a large quantity of easily digestible foods, and are frequently recommended to persons with weak digestive powers as better than ordinary milk.

CHAPTER IV.

GROWTH OF BACTERIA IN MILK.

THE number of bacteria in any sample of milk is at first dependent upon the extent of the original contamination, but the number found at later hours is more dependent upon the rapidity of growth. The original contamination may be a few thousands or perhaps many thousands per c.c., but the milk distributed in our cities often contains them by hundreds of thousands and frequently by millions. These larger numbers are the result of the multiplication of the original bacteria, and their size depends upon two factors: (1) The *temperature* of the milk, (2) the *age* of the milk. Of these two factors the temperature is the more important. Indeed, the temperature at which the milk is kept is of more importance in regulating the number of bacteria than the extent of the original contamination.

GERMICIDAL PROPERTY OF MILK.

Milk furnishes a most excellent food for dairy bacteria, and common bacteria are capable of growing and multiplying rapidly in milk. Milk, when drawn from the cow, is at a high temperature, a temperature at which most dairy bacteria grow very rapidly. Strange to say, however, the bacteria that find entrance into the milk during the milking do not at once begin to increase in numbers (102). On the contrary, if the number of bacteria in fresh milk be determined and then analyses be made of the same milk at intervals of two or three hours, it is found that the number usually slowly *declines,* and for some time practically never increases. The following three experiments may serve as an illustration (101).

	Fresh Milk.	Same Milk after 6 Hours at 70° F.
No. of bacteria per c.c.	1,950	1,900
	32,000	23,600
	13,000	9,175

After a time this decrease in bacteria ceases and then begins an increase, which soon becomes very rapid. The length of time during which this initial decline, or failure to increase continues, is variable and depends, among other things, upon the temperature. At a high temperature (90°F.), it is short, being not more than three hours. At 70° it is longer, lasting from six to nine hours or even more. At about 50° it lasts longer yet, and milk at this temperature will remain frequently for forty hours without showing any increase in bacteria, and commonly at thirty hours will actually contain fewer bacteria than at the start. In all cases, however, this period of initial decline eventually comes to an end, and the bacteria begin to grow more or less rapidly.

To what this initial decline of bacteria is due is not known. It has been attributed to what has been called the "germicidal power" of milk, the assumption being that there is present in milk some substance which acts unfavorably upon the bacteria, a substance akin to the similar supposed substance existing in fresh blood. By others the phenomenon has been attributed simply to the fact that the bacteria which get into the milk are placed at once in a medium for which they are not immediately adapted. Some of them are so unfavorably affected that they do not grow at all and soon die, while others require some little time to adapt themselves to the new medium. There is consequently a short period during which a few poorly adapted forms disappear while others are adapting themselves to the new conditions before they begin any rapid growth. During this time there is no noticeable growth of any species, but an actual disappearance of some of the poorly adapted forms. If this is the explanation, it is not proper to speak of any germicidal property of milk, but better to speak of it as a period of preliminary adaptation of bacteria to the new conditions. Practically it makes no difference to what we attribute the phenomenon. The fact is that, for a period ranging from two to forty hours, according to conditions, there is no appreciable growth of bacteria but, on the contrary, frequently an actual decline. This phenomenon is probably universal, although it varies with the milk of different cows and has not always been observed. In all recent work where a careful study has been made this initial period of bacteria decline is very apparent.

EFFECT OF TEMPERATURE UPON BACTERIA GROWTH.

Effect upon Numbers.—After the initial period has passed the bacteria begin to increase rapidly. In general terms, the longer the milk stands the larger the number of bacteria which it contains. But this general statement is true only within limits. As already noticed they do not grow at all for a few hours, and at the other end also we find that, after they become very numerous, they cease to grow. At about the time of the curdling of the milk, or a few hours after, the bacteria reach their maximum, and from this time decline in numbers. Old milk contains fewer bacteria than milk of less age, and after several days they are greatly reduced in numbers. Such old milk plays no part in dairy processes except cheese-making, and for the period covered by the time in which milk is handled by the dairyman as milk it is quite true that the older the milk, other things being equal, the greater the number of bacteria.

But the number is most extraordinarily modified by temperature. Life processes in general are more rapid at high temperatures, but in regard to the growth of milk bacteria the influence of temperature is surprising. The following figures show this influence of temperature. The figures at 70° and 50° were obtained from the same sample of milk, while those at 96° were taken from a different sample, which at the outset contained about the same number of bacteria.

	Fresh.	8 Hours at 96°.	19 Hours at 70°.	52 Hours at 50°.
No. bacteria.......	13,000	240,000	34,900	62,000

The significant conclusion from these figures is that temperature is a greater factor in determining the number of bacteria than the extent of the original contamination. In eight hours, if kept at warm temperature, the number of bacteria may be larger than it is in twenty hours at 70° or in sixty hours at 50°. At 50°, indeed, milk may sometimes be kept for forty hours without any appreciable increase in bacteria, a fact which explains the possibility of transporting milk in ice cars for a distance of 500 miles, and its delivery in a city like New York in a condition, so far as bacteria are concerned, actually better than many a sample of milk which is much fresher but has not had so much attention given to artificial cooling. If kept at 45° F. the milk need not contain more than 2,500 bacteria in thirty-six hours. If cooled to this temperature within three hours after milking the number should never be more than 1,000,000 to 2,000,000 even in two days, and usually far less. Hence, if milk shows larger numbers than these it indicates that it has not been properly cooled. The lesson to be emphasized by this is that by the use of a low temperature a milk dealer may keep his milk for a long time, and that the secret of furnishing satisfactory milk lies in the matter of temperature. The effect of temperature is shown graphically by Fig. 20.

There are other factors besides temperature concerned in the increase of bacteria, the nature of which is not understood. It may happen that the increase of bacteria shows quite unintelligible variations. Two samples of milk may be placed side by side under identical conditions, and yet the development of bacteria in one will be far more rapid than in the other, and in the end the sample that contained the fewer may have far the larger number. The following two experiments illustrate the point, the two samples being

FIG. 20.

Showing the effect of temperature upon bacteria growth. *a*, a single bacterium; *b*, its progeny in twenty-four hours in milk kept at 50°, 5 bacteria; *c*, its progeny in twenty-four hours in milk kept at 70°, 750 bacteria. Figures taken from an actual experiment.

milk from the same cow obtained upon different days but kept in identical conditions. Here it will be seen that the sample that started with the smaller number finally ended at fifty hours with far the larger number. To what such idiosyncrasies are due it is quite impossible to say.

	FRESH MILK.	FIFTY HOURS OLD.
November 23, Number per c.c....	3,600	2,090,000,000
November 25, Number per c.c....	7,400	49,600,000

Effect of Temperature upon Different Species of Bacteria.— Different bacteria are very differently affected by temperature. Some species grow best at low and others at high temperatures. Of the dairy bacteria those which develop in milk at 50° are commonly different from those that grow at higher temperatures. At 50° the *Bact. lactis acidi* type hardly grows at all, and the bacteria which multiply in the milk at the close of the initial germicidal period belong to other types than the common lactic bacteria. As a result the milk can be kept at this temperature for a long time without a typical souring and curdling. It is sometimes possible to keep the milk for five or six weeks without curdling if it be retained at 50°, simply because, at this temperature, the common lactic bacteria do not grow. Such milk contains in the end great numbers of bacteria, but they do not belong to the lactic type. At 70° *Bact. lactis acidi* develops readily, and from this temperature up to 90° or over, this and other lactic species multiply rapidly. At 86° and above, however, some other varieties of lactic bacteria are more especially stimulated, and if milk is kept at the higher temperature it is likely to be soured by some of the other species of lactic organisms. Some milk bacteria, as we have seen, require a temperature of 140°–150° for their proper growth.

From these facts it is evident that the temperature will greatly modify the types of bacteria which grow in milk, and that the number is not the only factor affected by cooling. By cooling, milk may be kept for a long time without souring or curdling, but this does not mean that the milk will be fit to use, for it will be almost sure to contain great quantities of other species of bacteria which do not make themselves evident by any visible action on the milk, but are probably more deleterious to the persons using the milk than the more common lactic bacteria which develop at the higher temperatures. Milk which is old cannot be regarded as reliable simply because it has not soured or shown any other visible change.

Comparative Growth of Bacteria in Normal Milk.—For three or four days there is a great increase in the numbers of bacteria in normal milk. But this does not mean that all of the species which are found in fresh milk increase equally and that an increase from 2,000 to 1,000,000,000 is a uniform one of all species. On the contrary, some of the species grow very rapidly, some grow moderately, some grow slowly, and some fail to grow at all and even seem to disappear. The analysis of the kinds of bacteria in milk of various ages shows, therefore, a constant change in percentage of the different species. During the three days following the milking there is a struggle of the different species of bacteria in the milk with each other, and the results of such a complicated struggle cannot be predicted with any degree of certainty. The action of the milk bacteria upon the milk, and still more upon cream in the cream ripening, will be largely dependent upon the results of this contest, and the question whether favorable results are obtained in cream ripening will depend not wholly upon the numbers and actual kinds of bacteria present *at the start*. It is dependent even more upon which of them develop in abundance and which are prevented from growing by the conditions of the struggle. While the results of this complicated interaction of bacteria can never be predicted with certainty the usual course of this growth is as follows:

The general growth of the bacteria in normal milk may be divided into two stages. While the two stages are not sharply separated it is more easy to understand the whole process by recognizing these two periods. The first period is the time from the start up to the time when the milk has about 10,000,000 bacteria per c.c. The second is from this point until the souring and curdling of the milk. The first period thus includes the whole time before the milk is commonly distributed to the consumer, at least in cases where the milk is delivered fresh. The second concerns the later changes of the milk in the consumer's possession and those which effect cream ripening (101).

First Period.—At the outset the number of bacteria is not very high, but the variety is likely to be considerable. A sample of milk from a single cow is likely to have eight to a dozen or even more species of bacteria, each present in varying numbers, the number of each species depending of course upon the extent of the original contamination. Commonly the larger number of these milk bacteria are those which we have classed together as producing no noticeable action on the milk, although liquefiers may be abundant. *The lactic bacteria are usually very few.* Indeed, it frequently happens that in clean milk the number of lactic bacteria is so small that they cannot be found by ordinary methods of bacterial analysis (see Chapter IX.). Sometimes, however, the lactic bacteria are more numerous even at the outset, due probably to extra secondary contamination.

After the initial germicidal period of three to thirty hours a multiplication begins which, hour by hour, affects nearly all of the bacteria present. But they do not all increase with equal rapidity. Some develop slowly, and during this first period only multiply two- or threefold. Others seem to multiply not at all, or very slightly, while others grow much more rapidly. The result is that, while there is an actual increase in numbers of nearly all species, some few —those which grow the fastest—comprise each hour a larger and larger per cent. of the whole. The *liquefiers* frequently, though not always, increase quite rapidly and form a larger per cent. than at the outset. The group which produces *no effect visible* in the milk also increases very rapidly, and at the close of the first period they may be vastly more numerous than at first. But the most striking fact is the growth of the *lactic bacteria.* These, if present at the outset in small numbers, steadily increase in numbers and percentage. The *Bact. lactis acidi,* which may not have been detected at the outset because of their small numbers, become numerous enough in a few hours to form an appreciable percentage, at first perhaps 2% to 4%. The other types of lactic bacteria also frequently increase in percentage, although this does not occur with such constancy as in the case of *Bact. lactis acidi.* The lactic bacteria increase with absolute constancy during the whole of the first period.

The length of time during which this period lasts depends upon the temperature and upon the number and kinds of bacteria originally present. If milk is kept at 70° it is commonly about twenty-four hours before the bacteria reach 10,000,000 per c.c. If the temperature is higher this number is reached more quickly, and if lower it takes longer. At its close the lactic bacteria are numerous, perhaps 30% to 50% of the whole, and the other species are also abundant, most of them being more numerous than at the start, but always comprising smaller percentages, owing to the great growth of the lactic bacteria.

Second Period.—The second period is characterized by the extremely rapid development of lactic bacteria, in most cases of the *Bact. lactis acidi* type. During the first period these bacteria have been accommodating themselves to the milk and increasing in vigor, so that by the beginning of the second period they are actually capable of more rapid growth than at the start. So vigorous are they, that not only do their actual numbers increase rapidly, but they seem to exercise a checking action upon other bacteria. Their percentage increases, at first because they grow more rapidly than any other species, but eventually because the other species actually decrease in numbers. Some of the other types disappear, while others remain alive but without further growth. It commonly happens that the liquefiers are crowded into the background and disappear, and although at the close of the first period they may compose 20% to 50%, they may not be found at all at the close of the second period, after the lactic bacteria have had the opportunity to develop. By the time the milk has distinctly soured, the lactic bacteria may be 70% of the whole, and later, when it curdles, they frequently comprise over 90%, sometimes 99% of all the bacteria present. In other words, the lactic bacteria, and particularly the first type including *Bact. lactis acidi* have shown such exceptional vigor in the milk as quite to have distanced all other species, and sometimes seem to destroy many of them. By the time the milk has become curdled it is filled with bacteria in enormous quantities (1,000,000,000 or more per c.c. being frequently found), but most of them are of the lactic type and *Bact. lactis acidi* is usually by far the most abundant.

It will be clear that the chemical changes which occur in milk must be different during these two periods. In the first period the general growth of the bacteria will produce a variety of chemical changes characteristic of the different species of bacteria. The nature of these changes is little known, but the development of lactic acid is not one of them. During this first period the acidity of the milk does not increase, remaining, as shown by careful tests, exactly what it was at the start. Of the other changes which occur we can say little or nothing, except that the increase of the many species from a few thousands to a few millions per c.c. must result in some chemical changes in the milk. During the second period it is probable that, for a time, these miscellaneous changes continue, at least so long as the miscellaneous bacteria continue to grow. But the distinctive characteristic of the second period is the development of lactic acid. This begins to increase very rapidly, more rapidly, in comparison, than the bacteria growth. The amount of acid increases until it is sufficient to curdle the milk, and even then does not stop, but may go on accumulating for some time longer. Eventually, however, the abundance of lactic acid is sufficient to stop the growth of bacteria so that they begin to die, and finally, some days after curdling, they almost disappear. The milk may now remain without further change for a long time, although eventually moulds will grow upon it and, finally, a further putrefaction may occur. But with these later changes we are not concerned, since they have nothing to do with ordinary dairy phenomena.

It will be manifest that the most striking feature of this bacteria growth in milk concerns the relation of the lactic bacteria. The lactic bacteria are the milk bacteria *par excel-*

lence, not because they are most common around the dairy, but because, of all species of bacteria, they seem to be best adapted to a life in milk and capable, in ordinary milk, of taking possession of the milk to the final more or less complete exclusion of other types of microörganisms. Though other species have the start of them at first, the lactic organisms soon overtake the others, surpass them in influence and, finally, cut off the action of other types of bacteria. The lactic organisms may, therefore, be looked upon as protecting the milk from the action of other bacteria. This fact is of the utmost importance in understanding dairy processes and the relation of milk bacteria to the public health. It will be best, however, for us to consider the bearing of these conclusions upon various dairy problems where they come up naturally, and the subject will, therefore, be postponed to be taken up again in succeeding chapters.

CHAPTER V.

MILK BACTERIA AND HEALTH.

It has been long recognized that poor milk is a source of a considerable amount of sickness, especially among children. It was the poor quality of the milk from a chemical standpoint that was at first regarded as responsible for most of the sickness resulting from its use. If milk is highly adulterated so as to contain only a small quantity of food products, or adulterated with materials that are themselves injurious, the milk becomes unwholesome; and it has generally been believed that such causes have been at the bottom of much of the sickness that results from use of milk. The development of the system of milk inspection and the setting of a chemical standard which milk must reach to be sold on the market, has raised the chemical purity of market milk, so that, speaking in broad terms, it may be said that against the adulterations of milk by water or chemicals the public is, on the whole, quite well protected.

It has been a growing belief in the last ten years that the larger portion of the sickness that comes from the use of bad milk is attributable, not to adulteration or chemical impurities, but to the bacteria present in the milk. We have already noticed how numerous these bacteria sometimes are in city milk, and the recognition of the fact that many diseases are caused by bacteria has led to the belief that milk bacteria are the cause of much of the sickness attributable to milk. The ground for this belief is more than a simple inference, since with quite a number of diseases the direct connection between the disease and the bacteria in the milk has been traced.

It will simplify our study of the relation of milk bacteria to health if we divide the diseases attributable to this source into two classes.

1. **Definite Diseases Due to Specific Organisms.**—These consist of a few clearly marked diseases, supposed to be produced by distinct microörganisms which are capable of living in milk. In some cases the bacterium is well known to bacteriologists; in others it is not yet known. The important diseases under this head are *tuberculosis, typhoid fever, scarlet fever, diphtheria,* and one or two other rare diseases to be mentioned later.

2. **Indefinite Diseases.**—These are generally characterized by intestinal disturbances, a *diarrhea* being an almost universal symptom. These diseases are more obscure in their nature and quite obscure in their origin. Their cause has never been traced to any distinct bacterium, and they are probably not caused by any one kind of microörganism. *Cholera infantum* and various forms of *summer diarrhea* belong to this class.

DEFINITE DISEASES.

Tuberculosis.—During the last twenty years there has been a very voluminous discussion over the problem of the relation of human tuberculosis to milk, and at all times during this period and even to-day, there has been wide difference of opinion as to the facts in the case. The subject is so complex and many of the points are so obscure that it is, at the present time, almost impossible to make any statements upon the subject which cannot be disputed. Nearly all of the facts and all of the conclusions in regard to the danger of the distribution of tuberculosis from cattle by milk are still under discussion. In the following outline the attempt is made to indicate, as closely as possible, the facts that are definitely known and the conclusions which are legitimately drawn in regard to this matter, but it must be recognized that the subject is as yet so far from final settlement that the conclusions here given may require modification in the light of further experiment. The relation of milk as a distributor of tuberculosis may be briefly summarized as follows:

1. Bovine tuberculosis and human tuberculosis are essentially the same disease, although there is a certain amount of difference between them. The identity of these two diseases has long been suspected, but was not demonstrated until the two diseases were shown to be caused by the same microorganisms. Although the disease in man and cattle shows more or less differences there is practically no question to-day that they are to be regarded as the same.

2. Both forms of this disease are produced by the well-known *Bacillus tuberculosis,* a bacillus which is identical, in most respects, whether it be found in the human being or in the cow (Fig. 21). There has, however, within the last two

FIG. 21.

Tuberculosis bacillus. *a,* in a bit of animal tissue; *b,* showing irregularities resembling spores; *c,* typical appearance of the bacilli from ordinary cultures.

years appeared a vigorous discussion whether the bacilli from the two sources are strictly identical. Professor Koch, in a famous address in London in the summer of 1901 (122), insisted that the two bacteria were different from each other, and so decidedly different that there was little likelihood of the disease being carried from man to cattle or from cattle to man. The experiments that followed Professor Koch's paper have in a measure borne out his conclusions, but not the inferences which he drew from them. It is at the present time recognized that there are differences between the bacilli derived from mankind and from cattle. The differences show themselves in a number of points, both in their method of growth in laboratory experiments and in their power of producing disease. For experimental animals the bovine bacillus proves to be more virulent than the human bacillus. Experiments upon mankind have not as yet been definite enough to determine whether or not this holds good in regard to the bovine bacillus when inoculated into man. But while these two types of bacilli are recognized to-day as showing variations, it is generally believed that this indicates that they are not different species of organisms, but merely varieties of the same one produced by the different conditions under which they live. The bacillus growing in the body of the bovine animal develops slightly different characteristics from the organisms developing in man, and these differences are generally looked upon to-day as simply variations in the characters of the same organism, the two types being regarded as essentially identical. If this conclusion is correct it would follow that the inference drawn by Professor Koch, that the disease cannot pass from cattle to man or from man to cattle, is incorrect. Indeed, it is positively proved that the human bacillus can produce tuberculosis in cattle, though commonly of a mild character.

3. It has been demonstrated beyond peradventure that if a tuberculosis infection is located in the mammary gland of a cow, the milk of the animal is quite sure to be infected with the tubercle bacillus. The location of tuberculosis in the udder is not very common but it does occur in a small

percentage of the cases. There is thus a considerable opportunity for the contamination of market milk by tubercle bacilli.

4. If the tuberculosis infection is located elsewhere in the body, and not in the mammary gland, the contamination of the milk is uncertain. If the infection is a slight one and involves only a few small glands, it is quite certain that the milk will not be infected with the tubercle bacilli. If, however, the infection is a generalized form of tuberculosis, distributed more or less through the body, it is more likely that the milk will be infected. It is not yet positively known whether an animal that has not tubercular infection in the mammary gland, ever produces milk infected with bacteria, except in cases of generalized tuberculosis. This, however, is a matter of little practical importance, because it is certain that many an animal with no *visible* infection of the udder may have minute tubercles in the mammary gland so that her milk will be liable to be contaminated with the bacilli, Consequently, many an animal with no external signs of udder tuberculosis may give milk which is infected with the tubercle bacillus.

5. Abundant evidence demonstrates that market milk frequently does contain violent pathogenic tubercle bacilli. These bacteria can be detected by microscopic study of the milk, although this means is very difficult and uncertain. They can also be found by experiments upon animals, for market milk has in many cases been proved to be capable of producing tuberculosis in experimental animals. The percentage of market milk which contains the tubercle bacillus cannot be stated, since it varies widely with different localities. Similar virulent tubercle bacilli are found in market butter.

6. It has been demonstrated that not only can the bacillus from mankind produce tuberculosis in animals, but that the bacillus from animals may produce at least a mild form of the

disease in man. This has been proved by a number of instances which have been collected where men have been accidentally inoculated directly from animals. For instance, butchers have become inoculated with tuberculosis through cuts in the skin which were infected with material from tuberculous animals that they were dissecting. Direct experiments of inoculation of human beings have also been made in the last few months with positive results. Enough instances of this sort have been collected—though they are really few—to prove beyond question that bovine tuberculosis may give rise to an infection in man. The question whether tuberculosis in mankind may come from the use of milk as food has not as yet been directly proved, although a number of instances have been advanced which indicate pretty strongly that such may be the case.

The facts thus outlined will probably be recognized by all as demonstrated. But beyond this point the question of the distribution of tuberculosis from cattle to man by means of milk is subject to dispute. It is quite uncertain to what extent the milk of tuberculous cattle is dangerous to mankind when used as an article of food. That it is a possible source of danger seems quite sure, and enough instances have been advanced to suggest that it is sometimes an actual source of the disease; but it is still quite vigorously denied that the extent of the danger is anything more than extremely slight. The reasons for doubting the magnitude of this danger are as follows:

The tubercle bacillus does not multiply in milk. Since market milk is usually mixed milk, the milk from a tuberculous cow, even though at the outset quite highly contaminated with tubercle bacilli, will be diluted by milk from many other cows before it reaches the customer. The result of this would be that each individual who used the milk would take into his body only a small number of tubercle bacilli. The danger of infection increases with the number

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of bacilli taken into the body, and hence this dilution of the milk very much decreases the probability of distribution of the disease by this means.

The milk used as food must pass through the digestive organs and be subjected to the action of the digestive fluids. These will have a tendency to destroy the vitality of the tubercle bacillus and thus still further reduce the danger of infection.

It is urged that the tubercle bacilli which came from milk would find entrance most naturally through the intestines, but that in mankind the intestine is not the most easy source of entrance of this dread disease. For man the lungs furnish the most ready entrance of this infection, and whereas the disease may occasionally come through the intestine, this is comparatively unusual. The bacilli that are present in the milk might lodge, of course, in the throat and possibly find entrance into the lungs, but this would be a rare case, for the milk is swallowed rapidly and most of the bacilli would pass to the stomach. If, therefore, the milk were a source of tuberculosis it would naturally be expected that the disease would start in the vicinity of the intestinal organs. The study of human tuberculosis shows, however, that primary intestinal tuberculosis is not common, and although it is by no means sure, even if the intestine was the means of entrance, that the first traces of the disease would be in the vicinity of the intestine, still the rarity of primary intestinal tuberculosis in human adults is an indication that the intestine is not a common source of infection.

It is urged that statistics in regard to human tuberculosis indicate pretty conclusively that, at least for adults, milk cannot be looked upon as a very important source of this disease. During the last quarter century the use of milk has increased and a large amount of statistical evidence has shown that the amount of bovine tuberculosis has also rapidly increased. During that same period, however, the amount of human tuberculosis, at least for adults, has been undergoing a constant decline. The increase of bovine tuberculosis and the decrease of human disease is not consistent with a belief that milk is a very great source of human tuberculosis.

The question in regard to the relation of milk to tuberculosis in children stands somewhat differently. It is beginning to be recognized that this disease is the cause of quite a considerable portion of the deaths of young children, and there is no reliable statistical evidence which indicates that the amount of tuberculosis in children is decreasing. Some evidence seems to indicate that it is slightly on the increase, although the matter is uncertain because of the difficulty of diagnosing this disease in children. Moreover, it is claimed that in childhood the amount of primary intestinal tuberculosis is quite large. These facts would apparently suggest that milk, which is, of course, a very common food for children, is the source of considerable tuberculosis during the early years of life.

As the result of all the evidence which we have at present, it seems that the tuberculosis bacillus in milk may be a cause of tuberculosis in children, and the safest course is to avoid the use of raw milk for young children, unless one is absolutely sure that the source from which it is obtained is reliable. For adults, however, this is probably not necessary. While it is quite possible that milk is an occasional source of tuberculosis for adults, evidence indicates that this danger is small. The danger for adults is certainly not so great as the danger of taking the disease from some other source, especially through breathing, and if we are to look upon milk as a source of tuberculosis in the adult at all, it must be regarded as one of comparatively small importance. There are some excellent authorities who insist that this is also true in regard to children.

It may seem somewhat strange that it is not positively known whether milk is a source of tuberculosis in mankind. The reason is the difficulty of getting direct evidence. The direct experimental evidence of feeding men with highly tuberculous milk is hardly feasible. To determine whether any individual case of tuberculosis is due to milk is practically impossible. The disease is one of very slow progress and is not recognized until after it has existed in the individual for some time. When it is recognized that a person has the disease it is practically impossible to determine its source. It is known that all persons are subject to tuberculous infection from various sources, and that they are liable at any time to be exposed to the infection by breathing. If, therefore, a person develops the disease it is quite impossible to determine whether the disease came from breathing contaminated air or from using as food milk which contained tuberculous material. In a few instances cases of tuberculosis have developed in a family and subsequently it has been learned that the family has been using as food milk from a tuberculous cow. While this is presumptive evidence that the cow may possibly have been the source of the disease it is by no means demonstrative, for other chances of infection are abundant, and there are thousands of cases of families using milk infected with tubercle bacilli without any evidence of tuberculosis developing. As a result of these facts, the conclusion that milk is a means of distributing tuberculosis rests upon indirect and uncertain evidence.

There is no practical method of protecting the public milk supply from the presence of the tuberculosis bacillus. It is possible, by microscopic means, to detect the presence of the bacilli in milk, but the process is so slow and so uncertain that it is quite impracticable to apply it generally to market milk. It is, also, impossible to tell by a physical examination of a cow whether her milk will be contaminated with tubercle bacilli or not. If she has the disease in her udder the contamination is sure, but if the disease is located elsewhere the question cannot be answered. An attempt has been made to determine whether animals that react to tuberculin, but show no visible signs of tuberculosis, are likely to produce milk that contains tubercle bacilli. The results, though somewhat in conflict, indicate that, in the majority of cases, the milk of such animals contains no bacilli. But in some instances the bacilli have been found, and hence, in order to protect market milk absolutely from tuberculosis, it would be necessary to exclude from the public dairy herd every animal that reacts to tuberculin. This is quite impossible, and it follows that there is no practical means of protecting market milk absolutely from this contamination. Nevertheless if all tuberculous animals that are suffering from visible udder tuberculosis are excluded from the dairy herd, as well as all cases of advanced, generalized tuberculosis, the danger of contamination of the milk is vastly reduced, and would probably be very slight.

DIPHTHERIA.

The cause of **diphtheria** is a well-known bacterium which is capable of growing readily in milk (Fig. 22). That milk has been a means of distributing this disease has been demonstrated by quite a number of epidemics satisfactorily traced. There have been, in the last twenty years, about a dozen such diphtheria epidemics traced, with more or less definiteness, to milk as its source. The method by which the milk becomes contaminated with the bacilli is possibly twofold.

FIG. 22.

B. diphtheriæ. Several forms of the bacterium that causes diphtheria.

The most common means of contamination is one that occurs subsequently to the milking. A person who is suffering from a mild case of diphtheria, or is recovering from such an attack, is allowed to work in the dairy. He impregnates the milk with the bacilli from his throat, and these serve as a source from which the disease may be transmitted through the milk to the consumer. Such a cause of a diphtheria epidemic has been definitely traced. It is possible also that there are other secondary sources of infection, but not much is known about them at present. It has not yet been definitely settled whether the cows themselves are subject to diphtheria and may have the disease located in such a way that they produce milk which, from the outset, is contaminated with diphtheria organisms. Experiments have indicated that the injection of diphtheria bacillus into cows produces an infection of some sort, and that the infection may show signs of itself in the udder. There is, however, no good reason for believing that the milk produced from such udders contains the virulent diphtheria bacillus. It has been claimed that such milk may produce diphtheria, but the most recent evidence indicates that such a danger does not exist (138, 139).

Milk may certainly become contaminated with diphtheria bacillus from secondary contamination, and the bacilli are capable of growing and becoming abundant in the milk. Although it is possible, under some conditions, to detect diphtheria bacillus in milk, it is a difficult task and quite impracticable for ordinary purposes of milk testing. That these bacilli have caused certain diphtheria epidemics has been clearly proved, although there is no reason for believing that many of the diphtheria epidemics are traceable to such a cause. It is also uncertain to what extent the milk may be a source of the miscellaneous cases of diphtheria which are found in communities. We must simply look upon milk as one of the possible means of distribution, and in the case of a diphtheria epidemic this must be looked at as one of the possible sources of infection. In the majority of diphtheria epidemics the infection doubtless comes from some other source (direct contagion, school books, etc.), and milk must be regarded as an unusual, rather than a common, source of such epidemics.

TYPHOID FEVER.

Typhoid fever is probably more frequently distributed by milk than any other definite disease. The disease is easily recognized, the bacillus which produces it is well known, and it has been demonstrated by conclusive evidence that typhoid epidemics have been frequently distributed through the medium of milk. There have been over one hundred such epidemics of typhoid traced to a milk supply. The force of the evidence that the milk supply has been the cause has varied; sometimes the facts are practical demonstrations, and in other cases only suggestive.

It is important to recognize at the outset that the only source of typhoid bacillus in milk is from external contamination. The cow, so far as known, never has typhoid fever or any symptoms of disease produced by the growth of this organism. Milk is, therefore, never contaminated with these bacteria when first drawn, and all contamination comes from secondary sources.

Typhoid bacilli find their entrance into milk from two common sources. The first is from some employee of the dairy who has the mild form of typhoid fever known as "walking typhoid." In such cases the disease is so mild as to fail to confine the person to his room, and allows him to continue about his work as usual. Such persons working in a dairy and handling the milk or milk vessels are an immediate danger to the neighboring community. The second source is the water. Where milk is watered the possibility of such infection is easily seen. But it is by no means necessary that the milk should be watered in order that the water may be a source of typhoid contamination. Milk cans which are washed in well water or brook water, or rinsed in water from these sources, may occasionally become infected with typhoid bacteria, provided the source of the water itself has been infected. The well water upon a farm where there is a case of typhoid fever is always subject to suspicion. The typhoid dejecta thrown upon the soil may drain into the surface of an open well or percolate through the soil, passing through the earth for many feet, and reach the well. The natural drainage of the land is toward the well and there is always, therefore, a chance of contamination of the well water by typhoid bacillus in case of typhoid fever upon the farm. Water from such a well is quite likely to contaminate the milk vessels. The typhoid bacilli, unlike the tuberculosis organism, can multiply in milk, and a few bacteria thus finding entrance multiply rapidly and eventually become a source of trouble. It is, therefore, unsafe for any dairyman to use well water or common brook water for washing or rinsing milk cans unless he is *absolutely confident of the purity of the source.* To avoid this danger it is only necessary to wash the milk cans in water that has been boiled, for the boiling of the water destroys the typhoid germs, and milk cans that are washed in such water only will never be contaminated with the typhoid bacillus from the water. If the milkman will use only boiled water for washing milk vessels, and will isolate his dairy and his milk cans absolutely from all possible connection with typhoid patients, he will practically avoid the danger of distributing typhoid fever to the community by means of milk.

SCARLET FEVER.

There have been a larger number of epidemics of **scarlet fever** attributed to the milk supply than of diphtheria, the last thirty years having brought to light about thirty such epidemics where the evidence is tolerably conclusive. The cause of this disease is yet unknown, although it is doubtless a microörganism of some kind and is probably capable of growing in milk. The lack of knowledge as to the cause of the disease has made it hitherto impossible, by bacteriological means, to trace this disease to its source in the dairy. It is uncertain whether the scarlet fever infectious material may come directly from the cow or only from secondary external sources. One or two instances have been recorded where epidemics resembling scarlet fever have been traced with considerable certainty to a peculiar udder infection occurring in a dairy herd, and this had led to the conclusion that scarlet fever is a disease that may attack cows, producing a mild infection in them, and then, through their milk, may be distributed to the milk consumers (145). There is, however, some uncertainty in regard to the matter. It has been questioned whether the disease in the cases referred to was true scarlet fever. It is, therefore, at present an unsettled question whether milk may be contaminated by the primary source of the cow's udder. But it is beyond question that secondary contamination is perfectly possible. Scarlet fever is, at certain stages, a very contagious disease and the infectious material is, beyond question, eliminated from the body of the patient. Such persons, if they are employed in a dairy in milking, in handling milk or milk vessels, may undoubtedly cause an infection of the milk with the specific agent of scarlet fever, and thus be a source from which the disease may be distributed.

As in the case of diphtheria, milk is not the common source of scarlet fever. Most epidemics are distributed by direct contagion of individual with individual. But it is always well to remember, in trying to trace the source of scarlet fever, that there have been at least thirty tolerably well-authenticated instances of the distribution of this disease by milk.

Miscellaneous Diseases.—It is only necessary to mention, in a word, a few miscellaneous diseases which are occasionally distributed by milk. Certain forms of throat infection characterized by *throat inflammation* have been thus traced.

Gastritis has been attributed to milk. One or two cases of *epizoötic* have been thus located, and at least one epidemic of *foot-and-mouth disease* has been traced to milk of infected cattle. All of these diseases are, moreover, quite rare and do not, except under special circumstances, enter into the problems that arise in connection with the use of milk. *Asiatic cholera* has also been shown to be capable of distribution by this source. Instances are excessively rare, however, and most cholera epidemics are traceable to the water supply.

METHOD OF DETECTION OF MILK EPIDEMICS.

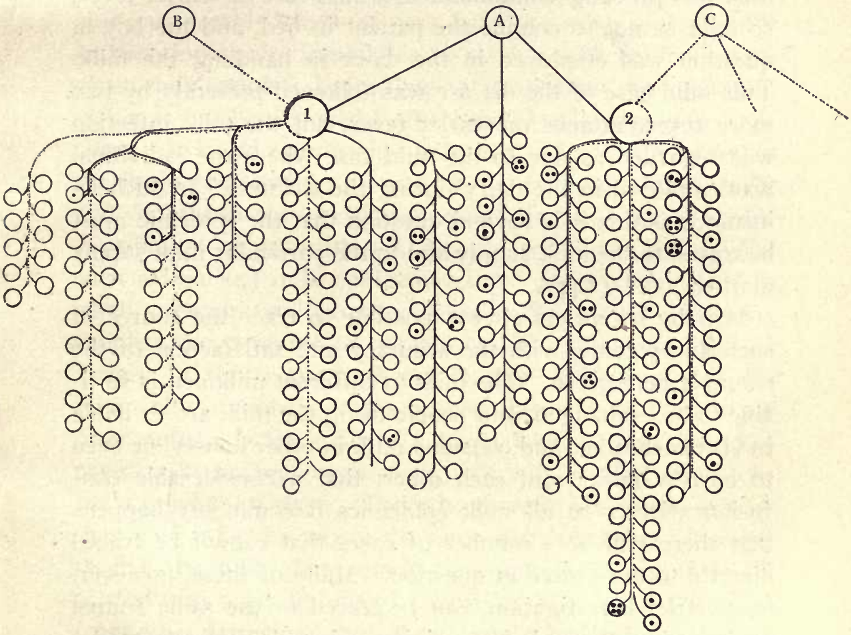
With the exception of tuberculosis the other definite diseases known to be associated with milk usually make their appearance in the form of epidemics, and these epidemics, by a careful investigation, can, in most cases, be followed to their source. It is usually not difficult to trace a "milk epidemic" of a contagious disease. The general characteristics of an epidemic of typhoid, scarlet fever or diphtheria, due to milk, are as follows: They are usually somewhat violent and rapid, a number of cases appearing almost simultaneously or within a few days of each other. They are also commonly quite fleeting, for, after a week or two, the cases cease to develop and the subsequent history of the epidemic is only one of recovery of the patients, with some cases of secondary contagion. The violence of these epidemics varies with conditions. Sometimes there may be only a small number of cases due to the milk supply, not over eight or ten, and in other cases the number may run up to several hundreds, all appearing within a very short time, many of which are so far isolated from each other that no explanation of direct contagion from case to case can be given.

Since these epidemics are so violent and usually so definitely located, it is commonly not difficult to detect their cause. If they are really due to milk it requires only a bit of careful detective work to trace them to their source. This, however, cannot be done by bacteriological means. The cause of scarlet fever is unknown and, of course, can not be detected in milk. The bacteria that produce typhoid fever and diphtheria are, however, well known, but it is as yet practically impossible to study market milk with the expectation of detecting the presence of these organisms. The large number of bacteria in ordinary milk makes the detection of these two bacilli practically impossible. But, aside from this difficulty, there is another one that makes it quite hopeless to use bacteriological methods in determining the source of such an epidemic. When milk has been the cause of a typhoid epidemic the disease does not follow until one to four weeks after the infected milk has been distributed. It requires some time after this for the persons who are investigating the matter to trace the epidemic to a milk supply. Consequently the milk is not thrown under suspicion until some weeks after the milk has begun to distribute pathogenic germs. By that time it is most likely that the milk from the same source will no longer be contaminated with the typhoid bacilli. The contamination of the milk of any farm with the typhoid bacillus is commonly a fleeting one, due to exceptional conditions, and the milk is thus infected with the germs for a few days only. How long the contamination may continue cannot be stated, but it will rarely last many days. The examination of the milk from such a source after the epidemic has appeared would usually be quite fruitless; for the milk, by the time it is placed under the observation of a bacteriologist, will probably, in the majority of cases, be quite free again from the special contaminating bacteria. As a result, detection of milk epidemics by bacteriological methods is, at present at least, quite impossible.

The evidence, therefore, that milk is the source of an epidemic must be derived from other methods of investigation. The method by which an investigation is carried on is tolerably simple. If there is a violent epidemic of typhoid or scarlet fever, or diphtheria, in a community the first thing to do is to determine the locality of all of the cases. Inasmuch as water is a means of distributing typhoid the next task is to exclude, if possible, the common water supply. If it is found that the epidemic is universally distributed through the whole city the common water supply may be thrown under suspicion, but if the epidemic is confined to certain parts of the city, or to certain streets, the common water supply must be excluded. The next problem is to determine the source of the milk in all of the families in which there is a case of the disease. If it is found that all, or nearly all, of the families attacked take milk from the same milkman the milk is, of course, thrown under very grave suspicion at once. In determining the possible source of a milk epidemic it must always be borne in mind that, in ordinary communities, the milk of each source is liable to be distributed by more than one milkman. Milkmen freely interchange milk with each other, either upon certain days or regularly, and it is always necessary, in pursuing such an investigation, to inquire whether the milk from the source of suspicion has not been distributed by two or more milkmen. It is frequently the case, therefore, that the milk from two or three milkmen is placed under suspicion in this way, although only one source itself is at fault.

A method of tabulating the results and obtaining a graphic illustration of the relation of the epidemic to the milk supply is shown in the accompanying figure, which represents an actual instance of a scarlet fever epidemic due to a milk supply (Fig. 23). It was found, in the community in question, that there were 6,922 households taking milk from 85 milkmen, and in all these households there were only nine cases of scarlet fever. It was found, however, that 269 households (represented by circles in the figure) took milk from three milkmen, and in these households there were 65 cases of the disease (represented by the black dots). But when the relation of the milk of the three milkmen was examined and tabulated by the graphic method shown in the figure, it became evident that it was only one of these three sources of milk that caused the infection. As can be seen

FIG. 23.



Graphic representation of a milk epidemic of scarlet fever. A, B and C are milk producers. 1 and 2 are milk distributors. The circles represent households, the inclosed dots cases of scarlet fever. Upon farm A there was a case of scarlet fever. The diagram shows that milk from farm A gave rise to scarlet fever even when mixed with other milk.

from the diagram the milk from the farm in question (shown by the large circle *A*), not only produced cases of scarlet fever where it was distributed directly from the dealer *A*, but it also gave rise to the disease in the routes of the other two milkmen where it was mixed with their milk and distributed to their own customers. The figure shows conclusively that, in this case, the scarlet fever epidemic was to be traced back to the source *A,* which was, therefore, immediately placed under suspicion and was investigated by those in charge of the work. In this particular epidemic it was found that upon the farm *A* there was, at the time when the milk was probably contaminated, a mild case of scarlet fever, so mild as not to confine the patient in bed, and the boy in question was employed in the dairy in handling the milk. This mild case of the disease was followed presently by two more severe attacks of scarlet fever, but the milk infection was regarded as due to the mild case where the individual was employed in the dairy during the sickness. In such an instance as this it is beyond question that the epidemic must be traced to the milk supply and to the particular milk supply marked as *A* (144).

It is by no means always possible to trace the source of such an epidemic with the accuracy and satisfaction of the example here given. The milk of different milkmen is so liable to be exchanged, and people using the milk are so liable to visit each other and consume milk in other houses, or even to borrow milk from each other, that a considerable confusion arises. In all milk epidemics it commonly happens that there will be a number of cases that cannot be traced directly to the source in question. Many of these, however, upon strict investigation, can be traced to the same source of infection through the mixing of milk or some other similar method. It is not to be expected, therefore, that every case of the disease in such an epidemic can be traced absolutely to the milk supply in question, but where the large proportion, ninety per cent. or so of the cases, are found to use milk from one source, the inference that this source has been the cause of the epidemic is emphatic.

It is also evident that such a method of tracing the epidemic, while perfectly feasible in small communities, where the milk is distributed from individual farms by individual milk dealers, is quite impracticable in large cities. In a large city, like New York or Boston, the milk is drawn from a very wide territory, and is more or less mixed before it reaches the consumer. Moreover, the large milk distributors in such cities draw milk from so many different sources that it rarely occurs that the same consumer will receive milk for any considerable length of time from the same source and it is, therefore, quite impossible to trace a milk epidemic to a given milk supply. Indeed, up to the present, no such milk epidemic has been traced to the general milk supply in large cities. It is quite probable—indeed almost certain—that, in the large communities, a part of the typhoid, scarlet fever and diphtheria may be due to milk infection; but the wide distribution of milk through the city will make it rare that such a localized milk epidemic should occur as is found in small communities, where a given milkman day after day supplies the same set of customers with milk from his own farm. In this respect, therefore, the milk supply of the larger city is probably more reliable than that of the smaller community. If the milk of a given farm becomes impregnated with pathogenic bacteria this will be likely to occur for a number of days. There is, therefore, a much greater chance of infection where the same customers day after day receive the milk than where the milk from any farm is poured into a general supply, more or less mixed with that from other farms and then distributed, a little of it to one set of customers on one day and to another set on another day. The intensity of the infection in a large city is slight, although the possibility of infection extends over a wider circle of customers. In the small community the intensity of the infection is great, although the number of people affected is comparatively small.

The detection of the source of such a milk epidemic is useful in two ways. In the first place, it is a means of satisfaction to the community to know the source of the infection. It is also useful as a means of education, because the more widely can we extend the knowledge of the possibility of the distribution of these diseases by milk the greater will be the care which the milk producer will take to keep his milk from possible sources of infection. The distribution of knowledge is thus the most efficient means of protecting the community from a repetition of such epidemics. But the discovery of the source of such a milk epidemic is rarely of any immediate value to the community. The infection of the milk is, in most cases, a very temporary one, commonly ceasing before the milk has been placed under suspicion, and certainly before it has been definitely proved that milk from a given source is the cause of the epidemic. The exclusion of the milk in question from the public milk supply is like locking the door after the horse is stolen, for milk at this later date is probably just as free from infection as any other milk that is brought into the city. To discover its source is, therefore, rarely of practical value in allaying any individual milk epidemic. The value of such investigations comes in the more general way of producing, little by little, a greater and greater protection of the milk supply from such possible contamination.

DIARRHEAL DISEASES.

A somewhat extended consideration is necessary of the miscellaneous diseases characterized by intestinal disturbances, of which a diarrhea is one of the most common symptoms. These occur particularly in warm weather, and are especially common in children. This class of diseases, including *summer complaint, cholera infantum* and various other less sharply marked intestinal disturbances, is the one that causes the largest amount of sickness and death among young children in warm weather. They are, therefore, of primary importance in considering the problem of milk bacteria.

The cause of this class of diseases is unknown. That they are produced in general by some bacterial agency is extremely probable, though hardly as yet capable of absolute demonstration. The extreme probability that milk bacteria are the cause of this general class of troubles is indicated by several series of facts.

1. They are more common at those seasons of the year when the milk bacteria are most abundant. In the summer months the number of bacteria in milk rises commonly to many millions per cubic centimeter, and with this rise in number of bacteria the cases of intestinal disease among children increase. To what extent the hot weather contributes directly, and to what extent the increased number of bacteria are concerned in producing these troubles cannot be positively stated.

2. The number of cases of such diseases is roughly proportional to the purity of the milk. Breast-fed children, it is true, do frequently have similar troubles during hot weather; but in breast-fed children they are very much less common than they are in children fed upon cow's milk. From what has already been stated it is evident that milk drawn directly from the mother's breast will be far less contaminated with bacteria than cow's milk.

3. Treatment of milk which destroys the bacteria has a decided influence in checking these intestinal diseases. The method which is commonly adopted is sterilizing or pasteurizing (see Chap. VI.), and this results either in the complete destruction of the bacteria or in a great reduction in their numbers. It has been found by a great amount of experience that such sterilizing or pasteurizing reduces the amount of intestinal sickness. In cases of cholera infantum or similar diseases the sterilizing of the milk, which causes the destruction of bacteria, makes it possible for the physician to control the disease very much more readily than he can with-

out such treatment of the milk. Moreover, it is found that the amount of intestinal sickness in children has been noticeably reduced in communities where a general sterilizing or pasteurizing of milk has been adopted. This has been done quite largely in European communities, and in some American communities, during the last fifteen years, and wherever accurate statistics have been obtained it has appeared that the introduction of such treatment of the milk is accompanied by a reduction in the amount of intestinal sickness and in the number of deaths from such causes. These various facts together have led to the conclusion that the bacteria in the milk are associated with this class of troubles.

Cause of Diarrheal Diseases.—Probably this class of diseases is not produced by any single bacterium. Indeed, the troubles referred to do not form a distinct disease, like typhoid or scarlet fever, but represent a general class of diseases characterized by intestinal disturbances. This being the case, it would not be expected that any single bacillus would be found to be the cause of them all. At all events, at the present time no distinct bacterium and no particular group of bacteria can be mentioned as being positively the cause of all such intestinal disturbances.

Moreover, it is evident that milk bacteria may produce trouble in either of several ways. They may produce toxic products in the milk, either before it is swallowed or after it is taken into the stomach. If the bacteria have a chance to multiply in the milk before it is used as a food they will produce their toxic secretions *in the milk*. These might act as a direct poison upon the body of a person drinking the milk, and in such a case the intestinal disturbances would be due to the toxic products developed in the milk before the milk is used as food. On the other hand, it is possible that the bacteria may grow after reaching the stomach and intestine and produce their toxic products *in the intestine*. In such a case the diarrheal troubles would be due to growth of bacteria in the intestine. It is also possible that both of these methods may be concerned. The bacteria may develop in the milk both previous to and after its consumption, and the toxic products may be produced in both places. Lastly it is possible that the bacteria may themselves invade the tissues of the person and develop in some organs outside of the intestinal tract. We do not yet know which of these methods is the more common, although it is probably not the last.

Type of Bacteria Producing these Diseases.—Our knowledge in regard to the actual cause of such intestinal troubles is thus rather meager. It is true that large numbers of bacteria have been found in the intestines of children suffering from such troubles. These have been described by bacteriologists, but the results of such study have not been to disclose the cause of the diseases. Nevertheless, some few general facts are suggestive of the type of bacteria which are most likely concerned.

It seems probable that the intestinal disturbances must be in part attributed to that class of bacteria which we have called the *putrefactive bacteria* or *peptonizing bacteria;* the group that produce albuminoid decomposition and give rise to a variety of by-products. On the other hand, it is almost certain that the common lactic bacteria (*Bact. lactis acidi* and its allies) cannot be accused of producing these troubles. In earlier years it was thought probable that the immense development of lactic bacteria in the milk rendered the milk unwholesome. They cause the milk to become acid, and this acidity has frequently been regarded as the cause of intestinal troubles. At the present time, however, the evidence is against such a view and there is good reason for believing that lactic bacteria, so far from being the cause of such intestinal disturbances, must be looked upon as protecting the milk from development of other bacteria that would be more injurious to the consumer.

This conclusion is dependent partly upon the effect of the growth of lactic bacteria in the milk, as described in Chapter IV. The growth of the common lactic bacteria checks the development of other bacteria. We have seen that, after the lactic bacteria begin to become abundant, the other forms gradually become less abundant, and in the course of time practically disappear. The great class of peptonizing bacteria, characterized by their power to liquefy gelatin, are almost universally prevented from developing in milk as soon as the lactic bacteria become abundant. If sterilized milk is inoculated with these putrefactive forms it will in a few days become badly decomposed and putrefied. If, however, lactic bacteria are present the putrefaction of the milk by these peptonizing bacteria is quite prevented. Indeed, milk which contains lactic bacteria may be kept for many days or even weeks after it has curdled, without any further appreciable change taking place. The development of the acid not only checks the growth of the lactic bacteria, but also prevents the other forms of microörganisms from growing. In short, the lactic bacteria actually serve to protect the milk from the decomposition changes which the class of putrefying bacteria will produce if the lactic organisms are not present. Pasteurized milk, in which the lactic bacteria have been destroyed by heat, will putrefy, while unpasteurized milk sours but does not commonly putrefy.

Somewhat similar evidence has been obtained in regard to the relation of lactic bacteria in the intestine (154). The presence of lactic bacteria or other acid germs in the intestine seems to be needed for carrying on the normal digestive processes. The intestine normally contains a certain number of putrefactive bacteria, which, if allowed to act by themselves, produce a putrefaction of the intestinal contents distinctly disturbing the digestive functions. This putrefaction develops in the intestine if it does not contain acid bacteria. The presence of lactic organisms in the intestine has apparently somewhat the same effect that it has in milk growing in the laboratory, preventing the development of putrefaction. If this is true the lactic organisms in the intestine, so far from being the cause of the derangements, are very likely means of checking them.

A somewhat similar inference may be drawn from recent experiments which indicate that, without the presence of bacteria, normal digestive processes cannot take place (161). If chicks are hatched and fed in such a way as to keep their intestines totally free from bacteria the birds are not properly nourished, cannot handle their food, do not gain in weight and eventually die of poor nutrition. The absence of bacteria apparently makes it impossible properly to use their food. If there is given with their food a culture of one of the common intestinal bacteria (an *acid* species) the nutritive functions recover and the birds develop as normal birds. This fact again suggests the beneficial rather than the injurious agency of the lactic bacteria in the intestine, and enforces the conclusion that such bacteria are not only not injurious, but are beneficial, and probably even necessary, in carrying on the normal function of digestion.

It does not follow from these facts that the presence of great quantities of lactic bacteria in milk is desirable, nor that the larger the number of lactic bacteria in the milk the better the results. We need not draw even the conclusion that the lactic bacteria of milk are necessary for proper digestion, for these experiments may have slightly different interpretations. But it is a safe and legitimate inference that the common lactic bacteria cannot properly be looked upon as the agents for producing the digestive disturbances which are associated with milk bacteria. Diarrheal troubles, if they are due to bacteria of milk, cannot, in the light of the facts known, be attributed to the common lactic bacteria, and it is quite probable that milk may be filled with bacteria in large numbers, and still be perfectly wholesome as food, provided the bacteria that are present are of the normal, harmless, lactic type.

What kind of bacteria are associated with these digestive troubles? As already stated, this question cannot yet be positively answered. It has recently been alleged that the cause of the common form of summer complaint in infants is a bacillus called *B. dysenteriæ* (Shiga), which is also the cause of certain forms of dysentery. This organism has been found in a large number of cases of summer complaint and may be their exciting cause. The organism in question is one closely related to the cause of typhoid fever; it is not easily distinguished from this latter bacillus and, therefore, not easily separated from *B. coli.* This organism belongs to the second of our groups of lactic bacteria, described on page 69. Whether this bacillus is really the cause of such troubles is not yet proved, and the source of the organism in nature is as yet unknown, though it is possibly derived from the water. Further evidence is needed before it can be determined whether this organism is really the cause of summer complaint.

It is probable, moreover, that another class of bacteria, those that we have referred to as the *putrefying* or *peptonizing* bacteria, may have some close relation to this same general class of troubles. Digestive disturbances are practically always accompanied by some form of putrefaction. Inasmuch as the intestinal contents in these diarrheal troubles almost always undergo putrefaction, it is quite probable that putrefactive bacteria must have something to do with the phenomena. Such changes in the contents of the intestine must be attributed to putrefactive bacteria. Moreover, it is well known that putrefaction is commonly accompanied by the formation of toxic products. The decomposition of albuminoids, taking place in the absence of oxygen, gives rise to a large variety of decomposition products of more or less high chemical complexity. Among these there are some which possess a violently poisonous nature. Such toxic products are developed in laboratory experiments when putrefactive bacteria act upon albuminoid materials, and it must be inferred that similar products arise in the intestines under similar conditions. The conclusion that digestive derangements are, in part, due to the absorption of such toxic products is extremely probable. It is suggestive to notice that Flügge (192) has proved that there exist in milk, after the lactic bacteria have been killed by heat, certain species of microörganisms that develop toxic products, to which he attributes some of the digestive derangements caused by milk. Some of these bacteria belong to the putrefactive class.

At the present time, however, the data in our possession are quite insufficient to enable us to state with positiveness whether these putrefactive bacteria are primary or only secondary agents in connection with the intestinal disturbances. It is quite certain then that the presence of large numbers of putrefactive bacteria in the milk must be looked upon as extremely undesirable, and as, in all probability, rendering the milk unwholesome. Such milk would certainly be likely to produce digestive disturbances; but whether any of the numerous forms of intestinal troubles are directly attributable to the putrefactive organisms cannot be positively stated.

The growth of bacteria in normal milk is somewhat suggestive in this connection. As already pointed out the lactic bacteria ordinarily soon obtain a mastery over and prevent the growth of the putrefactive forms. As the lactic bacteria become more abundant the liquefying species gradually become less numerous, and finally seem to disappear. As a result the milk that is on the verge of souring ordinarily contains none of these putrefactive bacteria. But, occasionally, quite a different result is found. Sometimes, for unexplained reasons, the lactic bacteria do not overcome the putrefactive organisms, and the latter continue to develop for many hours, until the milk is finally filled with them in large quantities. Such milk will remain *without souring for a longer time than usual,* but evidently is more dangerous than ordinary milk which has soured. The presence of such large numbers of putrefying bacteria cannot fail to make such milk unwholesome. It is evident, of course, that such milk would be used freely, and it is certainly possible that a part of the digestive disturbances attributable to milk are due to the milk having become filled with organisms of putrefaction instead of the more harmless lactic bacteria.

The practical question how the milk can be guarded against such organisms and, therefore, against the likelihood of producing these diseases is difficult to answer. There are two methods that may be adopted. The first is by guarding the source of the milk, and consequently reducing the number of bacteria. The adoption of the various devices for preventing contamination of milk, pointed out on a previous page, is the only method that can be suggested for preventing the milk from becoming contaminated with such organisms. The alleged cause of summer complaint comes from some external source, possibly from water. The putrefying bacteria come mostly from sources external to the cow, and the greater the cleanliness in the dairy the less will be the chance that the milk will be filled with these unwholesome bacteria. The second method is to adopt sterilization or pasteurization. If pasteurizing or sterilizing can be applied to the milk at an early stage, the bacteria may be destroyed and thus the subsequent development of their toxic products can be checked. This method materially reduces the number of bacteria and, therefore, reduces the danger of the development of intestinal disturbances.

TOXIC POISONING FROM MILK PRODUCTS.

There have been quite a number of instances reported in the last twenty years of toxic poisoning which comes directly from the consumption of milk. A larger number of cases of ice cream poisoning have been reported, and quite a number of instances of poisoning resulting from the use of cheese. In all of these cases the phenomena are somewhat similar. The symptoms of the poisoning are violent and develop with great rapidity. They are usually characterized by intense intestinal disturbances, accompanied by diarrhea and vomiting, and develop within a very short time after the food has been consumed. There is no doubt that they are due to the presence of **toxic poisons** in the food material. Indeed, Vaughan has isolated from such poisonous ice cream and cheese certain toxic materials which are capable of producing, when inoculated into animals, symptoms quite similar to those developed in man (167). More than one such toxic substance has been thus found. The poison that has been isolated from cheese has been called **tyrotoxicon**, and it is one of the highly complex organic compounds, though its exact chemical nature is not yet known.

It is certainly not the same substance that produces the poisoning in all cases, but all forms of such poisoning from milk products, are, beyond question, due to toxic products. The effect they have upon the consumer, though violent, is fleeting. They sometimes result in death in a comparatively short time, but, if they do not result in death from the first violent attack, the patient gradually throws off the poisons by excretion and recovers. The phenomena, then, are not due to the development of microörganisms in the body, but simply to a direct poisoning from toxic products in the food.

To what these toxic products are due we have only a partial answer. That they are the result of the growth of fermentative organisms cannot be doubted, inasmuch as no other source of such toxic products is known. Moreover, in a few cases there have been isolated from the poisonous material certain bacteria capable of developing toxic products similar to, if not identical with, those in the original sample of ice cream (166). In one case of milk poisoning, also, there has been isolated a bacterium producing, when used in laboratory experiments, products which give rise to symptoms similar to those of the original milk poisoning (168). Such instances, however, are few at present because of the rarity of the examples and the difficulty of obtaining decisive results; but the fact that, in one or two cases, the phenomenon has been positively traced to the development of bacteria makes it almost certain that the whole series of cases of poisoning from milk products must be attributed to the development, in the milk, of certain unusual species of bacteria which produce violent toxic poisons, and thus render the milk unwholesome.

Up to the present time no means have been suggested of avoiding such occasional dangers. We do not know the conditions under which they develop. Why one sample of cheese will develop such poisons under conditions apparently similar to those under which other wholesome cheeses are kept; why some samples of ice cream are poisonous, and why some cans of milk develop unwholesome qualities, we cannot say. In a few instances the trouble has been attributed to the fact that the milk has been kept for a considerable time in a damp, somewhat unwholesome room, where there has been opportunity for contamination with unusual bacteria and for a long period of growth. As has been shown elsewhere, when milk products are kept at a low temperature the lactic bacteria do not develop readily, while other forms are more capable of growth. The cream which is used for ice cream is occasionally kept for a considerable time at a low temperature, and thus there is an opportunity for a development of some of the organisms which ordinarily do not grow. But beyond such general suggestions nothing is known as to why poisonous products do occasionally develop in milk. Nor is there any method of guarding against them. The fact that we do not know the source of the organisms or the conditions under which they grow makes it quite impossible to give, at the present time, any practical suggestions for allaying the danger of an occasional poisoning from milk such as has been here described. The general suggestion of cleanliness and the use of cream for ice cream purposes *only when fresh* is, of course, a useful one, but fails to solve the problem, and no suggestion can yet be made for efficiently guarding against these poisonous products in ice cream and cheese.

CHAPTER VI.

HOW SHALL THE CONSUMER OF MILK PRODUCTS BE PROTECTED?

RECOGNIZING the variety of troubles that may arise from the use of milk, the practical question arises as to the best method of protecting the milk consumer from them. The possible methods divide themselves into two distinct classes which are, however, usually associated together. The first is the *protection of the source* of the milk, and the second is the *treatment of the milk* itself in such a way as to destroy any infectious material that may, by chance, be present. The first method must be directed toward the dairy, the dairyman and the milk distributor; the second must be directed toward the milk itself.

If the milk could be protected at the source from all undue primary contamination, and could be guarded from all secondary contamination with specific disease germs, it would be a perfectly safe product. It is believed that such a protection is not only possible but practicable, and that the public can be furnished with milk which is obtained under such strict precautions as to be quite reliable and of a character that can be used without hesitation. The recognition of this fact has resulted, in recent years, in a greater or less modification of dairy methods which we may consider under three heads.

PROTECTION OF THE SOURCE.

Sanitary Dairies.—The recognition of the value of extreme care in dairy matters has led to the establishment, near large cities, of quite a number of institutions where milk is obtained under conditions that are absolutely irreproachable.

These special dairies have the dairy buildings constructed with plenty of air and plenty of light, and with particular attention directed to the sanitary conditions for the cows. The cows themselves are tended with extreme care and kept in an irreproachable condition of purity. The attendants are kept under the most strict regulations. Special attention is given to the cleaning and the sterilizing of the milk vessels, and, indeed, every precaution is adopted, which science and experience has suggested. The result is that milk from such dairies is far cleaner than that of the common supply. By the use of the precautions adopted the number of bacteria in the fresh milk is reduced to a very low point, the milk from such dairies having at most only a few hundred bacteria per c.c., and frequently less than this. Such milk can be preserved for a much longer period than ordinary milk. If kept cool it may be preserved from souring for many days, and, indeed, in some cases, the milk may be kept in an apparently good condition for several weeks. In such dairies it is expected that a strict veterinary oversight should be given to the cattle to prevent the use of diseased animals in the dairy herd, and exceptional care is taken to prevent the milk from secondary contamination with infectious material from any source.

The milk from such special dairies is as reliable as care can make it. But even such care does not remove all danger. In at least one instance milk from such a well-kept dairy has been, with considerable certainty, accused of distributing diphtheria. But the chance of such a trouble arising from "sanitary milk," is very much less than from the ordinary milk supply, and, in general, the milk is of a far better quality and more thoroughly reliable.

Such dairies commonly sell their milk under special names, with a special brand upon the milk bottles, and they must of course obtain a higher price than ordinary dealers. The high price which it is necessary to charge in order to keep up these precautions in the dairy makes such milk quite outside of the reach of the ordinary consumer, and the milk from these special dairies is only bought by customers who have plenty of money, or in special cases for the nourishment of invalids or infants. These extreme precautions are so expensive that it is hardly possible to hope that the general milk supply of our communities will ever be produced under similar circumstances. These special dairies must be looked upon as isolated attempts to protect the milk supply and capable, at best, of protecting only a small portion of the total. They produce *milk for the rich but not for the poor.*

Certified Dairies ; Inspected Milk.—A less expensive plan has been adopted in the establishment of what are called **certified dairies.** In this case there is appointed a board, composed of experts, whose duty it is to visit certain dairies and carefully examine the conditions surrounding the milk production. A chemical and bacteriological analysis of the milk, and an inspection of the conditions under which the milk is produced, is made by the central board, and, if they find that the conditions are of a high character and the milk comes up to a high standard in purity, chemically and bacteriologically, they give to these dairies their *certification.* The milk from these dairies, therefore, called **inspected milk,** has the stamp of the certifying board, and this certification indicates that the conditions under which the milk is produced are strictly hygienic. Such a certification is valuable to the dairy in question, and enables the certified dairies to obtain a better price for their milk or to obtain a wider sale for the same. The matter is wholly voluntary on the part of the dairyman. The expense of the central board is shared by the dairies that receive the certification and must, of course, be reimbursed to them by the customers who receive the milk. This method of certifying dairies has not been very widely adopted, but is one of the means which promises to be useful in bringing about a more careful control of the public milk supply, and in rendering this extremely valuable food product of a more reliable nature.

Certified Milk.—A third method, which has been recently adopted, is a modification of the last. Here a certificate is given to the product rather than the source. In the city of New York, for example, a board of experts has been organized and agree, under certain conditions, to give to milk distributors a certificate of the character of their milk. The milk companies that wish the certificate of this board submit their milk to its inspection and examination. The milk is tested chemically and bacteriologically and a careful examination is made of the condition of the milk distribution. In this case the examination of the milk by the experts is the basis of the certificate and a direct inspection of the dairies from which the milk has been obtained is not necessarily made, although the dairies may be visited and a general set of rules for conducting them is formulated and is given to the dairymen for their guidance. If it is found that the milk has a proper chemical quality and the number of bacteria falls below a certain point, the milk of the distributor in question is given the certificate of the board. In order that the number of bacteria should be no greater than the somewhat small maximum allowed by the board, it is quite necessary that the conditions of production and distribution should have been satisfactory, that the milk must have been kept cool, and must not be too old. In general, therefore, such certified milk will be reliable. The expense of the organization of the board of inspectors must, of course, be borne by the distributor who receives the certificate, and must naturally be reimbursed to him by the consumer of the milk. Consequently, the price that is demanded for such certified milk is higher than the price obtained for the ordinary milk supply. The difference in price of the certified milk and that of the ordinary milk supply is not sufficient to prevent the wide use of such milk should this method prove, in other respects, to be satisfactory.

There is one objection to such a certification, however, in the fact that milk from any source is likely to be irregular in quality. The inspectors that test the milk cannot make examination of the milk of a given distributor very frequently. They must give a certificate, not upon any individual lot of milk, but upon the general average of milk of a certain milk distributor. The great irregularities in the numbers of bacteria in milk have already been mentioned. It inevitably follows that the milk distributed by any dairyman will not always reach the same grade, and the milk which comes from a given distributor may some days contain few and upon others vastly greater numbers of bacteria. Inasmuch, however, as the certificate cannot be given for an individual lot of milk, but simply for the general average of the product distributed by a certain dealer, it will happen occasionally that the certified milk will be below the standard of certification. Indeed, it has been found by practical experience that, in some cases, certified milk sours very quickly because of the presence of great quantities of bacteria. This irregularity is inevitable from the fact that the grade of milk produced under apparently identical conditions is extremely variable, and because it is impossible to do more than to give a certificate of the general grade of milk. Hence the method of certifying milk is only of general utility. Beyond question the milk that has the certificate of such a board will, on the average, be of a higher grade than that of the general milk supply, but the certificate can never be absolutely relied upon, and samples of certified milk will sometimes be sold, in entire innocence and good faith on the part of the distributor, which are below the standard demanded by the board that gives the certificate. In spite of these imperfections, however, there is no doubt that the person who purchases certified milk will obtain milk which, in general, is more reliable than that from the ordinary source of supply, and this method of dealing with the subject has much in its favor.

All of these plans, however, involve expense, and the expense must, in the end, be borne by the purchaser of the milk. Practical experience has shown that anything that increases the price of the milk is sure to decrease its consumption, and the fact faces the milk supply companies that any of these methods designed for protecting the quality of the milk but involving extra expense will not be popular on the part of the general milk consumer. The extra expense appeals more strongly to the majority of milk consumers than does the extra reliability of the milk. The methods outlined for improving the quality of the milk cannot, therefore, be looked upon as applicable to the general milk supply, but will probably always be confined to a comparatively small part of the supply unless they can be enforced by public law.

DAIRY INSPECTION.

The question, therefore, arises whether it is not possible by public statute and legislation to exercise a control over the general milk supply, which will have the same relation to the wholesomeness of the milk from a bacteriological standpoint, that the existing statutes already have to the chemical nature of the milk. In other words, is it not possible that a public **dairy inspection** should be organized to control the milk production? Such a dairy inspection has been frequently proposed and rules and regulations for such inspection have been suggested. Hitherto, there have been serious difficulties in the way of organizing such public inspection.

Dairy inspection has, however, been adopted by private enterprises in quite a number of places with considerable success. In some cities large milk supply companies have organized their own dairy-inspecting corps and employed men at considerable expense to travel among the dairies furnishing milk to the company. These inspectors have the authority to make such suggestions and changes in the conditions of the dairies as may seem necessary, and the different dairies are obliged to conform to the directions that are given by the inspectors, under the penalty of having the company refuse to receive their milk. In Switzerland, also, the dairymen themselves have combined together for the purpose of introducing some such general dairy inspection. This is done for their own protection and for insuring the production of an especially good quality of dairy product. In a few other instances private dairy inspection has been adopted, but nowhere at the present time has such an inspection been made obligatory by public statute.

There is a manifest difficulty in obtaining legal statutes for a similar public inspection and even greater difficulty in their enforcement. In the first place, there is in general a lack of appreciation of the value of such requirements. The public has been educated to believe that milk that does not contain a certain quantity of fat is not good food, and that they are being cheated if they purchase milk with a smaller quantity of fat than the standard. People generally are fully in sympathy with the rules for milk inspection which involve the chemical analysis of milk. The public, however, has not as yet become fully familiar with the fact that the bacteria in milk are a source of even greater danger than the falling off of the fat percentage. This lack of appreciation would make it difficult to carry out any regulations which might be suggested.

Again, the manifest possibility of abuse in the application of such rules will be an obstacle in the way of their adoption. At best, the enforcement of regulations that may be adopted by a dairy inspection will depend upon the judgment of individual inspectors. The requirements as to the conditions of the dairy are not like those of chemical composition, capable of *exact* formulation. It would be impossible to make a definite standard of cleanliness which can be defined with accuracy, and to insist that all dairies should come up to a standard. It might be directed that proper attention should be given to cleanliness, but manifestly the determination of what is a proper cleanliness would depend upon the individual notions of each particular inspector. Nearly every other rule that might be suggested for dairy inspection would, in the end, come back upon the judgment or caprice of the inspectors. This, of course, allows a great irregularity and makes it extremely difficult to suggest any rules for dairy inspection that could be satisfactorily accepted.

A third difficulty consists in determining what rules and regulations such dairy inspectors could or should try to enforce. The bacteriologists may suggest a large number of precautions to be adopted in the dairy, but if they were all enforced it would either force up the price of milk to a prohibitory point or would force dairymen out of the business. Exactly what compromise could be adopted, so that really practical and satisfactory rules could be obtained, is extremely difficult to determine.

Lastly, it must be always difficult to enforce such rules. It is quite possible to have a statute saying that milk falling below a certain chemical standard will subject its distributor to a fine, but it would be very difficult to enforce a law that a dairy that falls below a certain indefinite standard of cleanliness shall be prevented from distributing milk. Private concerns can do this with ease, for a milk-distributing company can easily refuse to take milk from a dairy which its inspectors regard as not up to a proper condition in regard to cleanliness. To enforce such a cleanliness, however, by public law is almost certain to be attended with difficulties which will be nearly insurmountable. It is not easy to enforce the rules in regard to the chemical analysis of milk even though extreme accuracy can be obtained by analysis. It will be manifestly extremely difficult to get conviction in any case where the conviction must depend simply upon an individual inspector's word that the conditions in the dairy are uncleanly. For all of these reasons it has not hitherto seemed feasible to adopt a public dairy inspection backed up by public law.

Nevertheless, public milk inspectors in our cities have accomplished a great deal in the last few years in the way of improving the quality of the milk. This has been done partly by enforcing laws, and partly by advisory suggestions. The chemical purity of the milk has been raised, until now there is not very much to be desired in this respect, and, along with the increased chemical purity, there has been a general recognition on the part of milk producers and distributors that the milk must be more carefully treated. Indirectly this has produced a greater cleanliness and a greater care. Moreover, in many cases the regulations which have been adopted have tended directly toward increasing the precautions under which the milk is distributed.

The rules and regulations in the European cities are, in these respects, considerably ahead of those in America and are somewhat more stringent. They are under the control of the public officers and are enforced with more or less strictness. These rules for milk production and distribution vary much in different localities; differ in strictness and in details in different cities. But they all have a general similarity, and in nearly all of the cities, where such regulations have been adopted, the directions that are given take into consideration a variety of factors which are closely connected with the purity and the quality of the milk supply.[1] They give attention to the following factors:

Chemical Standard.—There is almost universally put in force, with more or less rigidness, a series of rules as to the **chemical standard** of milk for public distribution. There are, however, quite generally recognized at least three differ-

[1] The regulations adopted in many European cities may be found in the last five years of the *Zeitschrift für Fleisch und Milch Hygiene.*

ent grades of dairy products that may be sold, and sometimes more. *Whole milk* is required to respond to a certain lactometer test, somewhere in the vicinity of 1.028 and also to show about 3% of fat, although the standard varies slightly in different places. *Skimmed milk* is recognized as a salable article which, however, must always be plainly labelled and must be sold in such a way as to be easily recognized. The skimmed milk is also required to come up to a certain standard, usually somewhere about .5% of fat. In addition to this there is frequently sold what is called *half milk,* intermediate between skim milk and whole milk. **Cream** is occasionally sold, though not so much in Europe as in America.

Physical Quality.—In regard to **physical quality** of milk the rules forbid the sale of milk that has certain unusual appearances. For instance, if it is *red* or *blue* or *green,* if it tastes *bitter* or *salty,* if it has a *bad smell* or shows a *sliminess,* or if it gives evidence of containing *blood,* or if it is covered with *moulds,* the sale is in all cases forbidden. The milk inspector is supposed to examine milk frequently upon the streets, and has the right to condemn the sale of milk that shows any of these imperfections.

The Milk Vessels.—There is usually an attempt made to regulate the **vessels** in which milk shall be kept and from which it shall be sold. Certain kinds of materials are forbidden, as for example, *copper, lead* and *zinc.* It is directed also that the vessels should be of such a character as not to require any handling of the milk in its distribution. In some cases the shape of the milk vessels is suggested, a shape being recommended which will facilitate the cleaning of the vessels. Sometimes even the method of cleaning is included in the general directions which the inspectors give to the distributors, and the requirement of covering the milk to keep out dirt and further contamination is also usually included in the rules.

Storing.—Special attention is given to the method and locality in which milk shall be **stored.** It is not allowed to be kept in a damp room, in a dwelling room of any house, in a sleeping room, nor is the milk allowed to be placed in a room where there is any form of sickness.

The Milk-producing Animals.—So far as possible the milk inspector endeavors to prevent the use of milk from animals suffering from diseases. A long list of animal diseases is commonly given in the rules for milk inspection, and the milk from animals suffering from any of these diseases is not allowed to be sold in the public milk supply.

Health of Attendants.—A special attempt is made to prevent the distribution of contagious diseases from the attendants or those who handle the milk. In some localities a notification to the authorities is required for all cases of contagious diseases occurring among those employed in the dairy or in distributing milk. After the notification of such sickness the authorities themselves are supposed to look into the case and to determine whether it is of a character which will make it necessary to exclude the milk from the public milk supply, or whether the conditions are such that there is no need for such exclusion.

Adulterations.—The presence of **any form of adulterants** in milk is commonly forbidden, and the public authorities have various means for recognizing these adulterants. Sometimes even the amount of dirt present in the milk is included among the adulterants, and milk is not allowed to contain more than a certain quantity of such dirt. At the present time, however, in no place is an attempt made to regulate the number of bacteria which may be present in the milk when it is distributed. This has been a practical impossibility. It has been tentatively suggested that the number may possibly be limited, and then the milk supply forced to come up to the requirements. As has already been pointed out, for certified milk or for certain special dairies, such a bacteriological standard has been set and adhered to, but for the public milk supply it has not hitherto been attempted. Whether such a method of general milk analysis will ever be adopted is at least doubtful.

Special Milk.—In many cases the sale of special milk for children's feeding is allowed and regulated by extra precautions. It is recognized by the public authorities that for infant feeding the milk should be of a higher character, so far as concerns purity, than for the general milk consumption. For this reason the milk designed for infant feeding must be obtained under more stringent rules. The dairies which produce such milk are regulated even more strictly than others; frequently even the food which the cow is allowed to eat and the personal habits of the attendants in the dairy are considered in the rules made for the production of such milk.

Of course these various rules are usually only advisory and not mandatory. Indeed, it is quite impossible to enforce many of them. While the public authorities may make rules for the conditions in the dairy, and may insist that no cow suffering from a certain disease should be allowed to furnish milk for the public, it is quite impossible for the milk inspectors to enforce these rules. It could only be done by constant visits and veterinary examinations upon all farms, and this has been practically impossible. Indeed, the inspection usually ends with the examinations of the milk supply and is rarely if ever followed up by a dairy inspection. Without such dairy inspection the enforcement of these rules must be left to the voluntary coöperation of the milk producer. The only instances where such dairy inspection has been successfully adopted have been where large distributing concerns have, from their own initiative, organized such a milk inspection for the purpose of protecting their milk, and in cases where the milk producers themselves combine to welcome such an inspection for the purpose of being able to obtain a higher price for their milk. Nevertheless, although these various rules are incapable of absolute enforcement they become much more forcible when they are a part of the public statute, as they are in many European cities, than when they are only a matter of advice on the part of a few private persons. In America, where such rules are only suggested, they are not so likely to be followed, as they would be if they were a part of the public statute, and were put in printed form in the hands of every milk producer. The general conditions under which milk is produced are doubtless improved by the numerous regulations in regard to the details of milk production which are adopted by the European cities.

PURIFICATION OF MILK.

Although it is impractical to avoid the entrance of bacteria in milk and difficult to prevent their growth, it is possible to get rid of them later. The problem of how to purify milk from its bacterial contamination has occupied a large amount of attention, and resulted in a large number of devices and special forms of apparatus. None of the methods suggested is thoroughly satisfactory. Most of them are only partially successful in reaching the desired end, and even these have manifest disadvantages.

The Use of Disinfectants.—A simple means of accomplishing this end is to add to the milk some material which will prevent the bacteria from growing. There are a number of chemicals that can be used for the purpose. It is, of course, necessary to use such as are comparatively harmless to man, and which do not give a noticeable taste to the milk. To be of any use a disinfectant or preservative must be of a nature such that a quantity insufficient to give any taste must have a decided effect in reducing bacteria growth. Only a few of the known disinfectants are feasible for use in milk and those most commonly used are the following (177, 178):

Boric acid, which may be used in proportion of 1 gm. per quart milk.
Borax, which may be used in proportion of 5 gm. per quart milk.

Salicylic acid, which may be used in proportion of .3–.2 gm. per quart milk.

Formalin, which may be used in proportion of .02–.01 gm. per quart milk.

Hydrogen peroxide, which may be used in proportion of 5–6 c.c. per quart milk.

There are some other substances occasionally employed, but these are the basis of most of the products sold for the purpose of preserving milk and going under various commercial names. The use of all such disinfectants is open to several objections.

1. They are all adulterants and, therefore, all are forbidden by legal statutes.

2. To be really efficient they must commonly be used in quantities large enough to produce a noticeable taste in the milk.

3. The effect of these adulterants upon the health of the consumer is an important consideration. They are all drugs of a more or less poisonous nature, and it is certainly not desirable to consume them constantly in considerable quantity. That it is undesirable to consume them in large amount is certain, but whether, in such small quantities, they are liable to produce any appreciable injury in the consumer has been vigorously disputed. It is claimed that the presence of boracic acid in milk is responsible for a considerable amount of the digestive derangements and malnutrition which can be found in countries where boracic acid is used quite freely, but it is difficult to prove this fact and it is still under dispute. Whether any of these materials can be used without any danger we do not yet know. The quantity of formalin required to protect milk from souring is extremely minute, and it is doubtful whether this extremely minute quantity of formalin is sufficient to render the milk any less wholesome. Hydrogen peroxid appears also to be harmless and capable of being used without interfering with the wholesomeness of the milk,

but it is too expensive to be used for practical purposes. At the present time, therefore, it cannot be stated that any of these adulterants can be safely used, although it may be that we shall find that some of them can be used without danger.

One of the chief objections to their use is that each person who handles the milk is liable to add a little of the disinfectant, and thus, by the time the milk reaches the consumer, a large amount may be present. Instances of serious illness from such a cause are known, especially in the use of formalin.

4. All of these forms of disinfectants are a means of covering up a lack of cleanliness and proper care in the dairy. As long as the dairyman knows that keeping the milk depends upon his care in handling it he will be more cleanly and more careful in his methods than when he feels that, by adding a small amount of a certain adulterant, he can protect his milk from souring. The use of these disinfectants will thus invite a greater carelessness on the part of the milk producer and milk distributor. In this respect, therefore, their use is unfortunate, tending to decrease rather than increase the conditions of wholesomeness surrounding milk production.

5. The value of these disinfectants is simply to protect the milk from souring but *not to render the milk any less liable to distribute disease.* None of the pathogenic bacteria which find their way into milk are killed by the small quantity of disinfectants used. The only reason why such disinfectants are used is for the purpose of protecting milk from souring for a few hours until it can be distributed, and they are not to be looked upon as surrounding it with any safeguards or of aiding at all in preventing its distributing disease.

Taking these facts together little can be said in favor of the use of disinfectants. They do not render the milk any more wholesome or any less liable to distribute disease; they simply encourage carelessness on the part of the dairyman and most of them certainly make the milk unwholesome.

Centrifugal Force.—When milk is passed through a separator for the separation of the cream there is deposited upon the drum of the machine a thick slimy mass which contains a considerable quantity of dirt. This has suggested that centrifugal force may be used to free milk from its impurities. Recognizing that the bacteria find their entrance into the milk with the various kinds of filth, it is natural to expect that centrifugal force, in removing the filth, will, at the same time, remove many of the bacteria. The plan of passing milk through a centrifugal machine for its purification has been adopted in many places and great claims have been made for special machines used for this purpose.

In such a purification of milk it is, of course, not desired that the cream should be separated from the milk. Consequently the machines devised for this purpose are run at a speed lower than that of the ordinary separator, but sufficient to remove the heavier particles of dirt.

Such machines quite effectually remove the dirt and filth particles from the milk. A long series of tests, for example, has shown that the milk drawn directly into an open pail may contain as much as .4 gm. per liter of various kinds of dirt (183). Nearly all of this dirt is removed from the milk by passing it through an ordinary cream separator, and, the larger part of it at least, is removed by passing it through the machines with a lower rate of revolution. The removal of the dirt improves the quality of the milk. It takes away the so-called animal odors, and both the taste and the odor of the milk is benefited by the treatment. A device which will thus remove most of the filth and improve the taste is certainly a useful device for the dairyman.

But the effect of the treatment upon the bacterial content is very slight (180–183). Experimental tests have, indeed, shown that the number of bacteria in milk which has passed through such a centrifugal is sometimes slightly reduced, or occasionally quite materially reduced. Other tests, and these comprise a majority, indicate no material reduction of the bacteria, and sometimes there is an apparent increase (283). When such milk is placed side by side with a sample of the same milk not treated, it is found that the centrifugalized milk does not keep longer than the control. Indeed, in many experiments the centrifugalized milk sours and curdles an hour or two before the untreated sample (183). The centrifugal treatment is quite unreliable, therefore, as a means of removing bacteria, and is of no use whatsoever in rendering less dangerous milk which contains pathogenic bacteria. The fact that such treatment removes filth and improves taste is certainly sufficient to recommend the employment of the centrifugal force where feasible, but it does not, to any appreciable extent, remove the bacteria.

Filtering.—The plan of filtering the milk has been frequently adopted. Various kinds of filtering apparatus have been constructed. In most of the machines the actual filtering material is some form of sand of a fine texture through a tolerably thick layer of which the milk is forced to pass. In some machines the milk passes through from its own weight as in ordinary filtering, while in others the milk is by pressure forced upwards through the filtering layers. In all of the machines there is a convenient device for removing the sand filter for washing and sterilizing. Without such a sterilization the filter would be worthless, since the sand would soon become filled with filth and bacteria. These filtering machines have been sometimes adopted by milk companies and used on a large scale to filter great quantities of milk for general distribution. But smaller machines are also on the market. They have not come into very general use and most of them, indeed, are inventions of recent date, whose efficiency is not yet tested.

Concerning the efficiency of the filtering machines, almost the same is to be said as concerning centrifugalizing. The filters are quite efficient in removing the dirt and filth, although perhaps not quite so efficient as centrifugal force (187, 188). Filtered milk certainly tastes cleaner and sweeter than unfiltered milk. In removing the bacteria, however, the filter is practically of no value. In many tests there has actually been found an apparent increase of bacteria after the filtering. This fact is probably due to two causes. First, many of the bacteria in the milk are likely to be clinging together in masses, and each mass, when planted upon a gelatin plate (see Chapter IX.), will appear as a single colony, even though there be several in the mass. The passage through the filter tends to break up these masses so that each bacterium may float freely. Each of these would produce a colony, and there would appear to be an increase in numbers, although there is really none. Second, it is extremely difficulty to clean and sterilize thoroughly the sand in the filters. If some bacteria and a minute amount of organic matter are left in the filters, the bacteria are quite sure to grow and presently become numerous in the sand. The next lot of milk to pass through is actually contaminated with bacteria during the filtering. If the sand is thoroughly sterilized by heat after use this apparent increase in bacteria during the filtering process becomes reduced or disappears. But even then the filtering process cannot be looked upon as a means of reducing the bacteria or increasing materially the keeping property of the milk. The removal of the filth is certainly desirable; but filtering, by any device yet invented, has no value in rendering milk less dangerous from the standpoint of disease bacteria. Nor indeed does it seem possible that such should be the case. The bacteria are very much smaller in size than the fat globules, and a filtering machine that would take out the bacteria would also take out the fat, leaving only the poorest of skim milk. If we wish the milk to pass the filter as milk, the pores in the filter must be large enough to allow fat to pass, and then the bacteria will pass through even more readily. Filtering thus renders the milk more appetizing, but not more wholesome or less dangerous.

APPLICATION OF HEAT.

Heat may be employed to destroy bacteria and, if it is properly applied, it will kill *all* bacteria. The possibility of its use for the purpose of purifying milk is modified by the action of the heat upon the milk itself. In considering the applicability of heat for this purpose we must bear in mind the several purposes which it may be desired to accomplish. It may be the purpose:

1. Simply to destroy the strictly pathogenic bacteria so as to remove the danger of milk epidemics.

2. To destroy other bacteria so that the milk will be protected from souring or from other fermentative processes.

3. To destroy or reduce the bacteria which are associated with the indefinite class of intestinal troubles which are attributed to milk.

4. To remove the bacteria so as to leave a free field for the action of other bacteria to be inoculated, as in the use of pure cultures.

Sterilization.—A sufficiently high temperature destroys all bacteria and, of course, accomplishes all these purposes at once. But to do this requires a temperature so high that it produces certain changes in the nature of the milk. It *cooks* the milk, and the feasibility of the process depends upon the nature and significance of these changes. Since some of the common milk bacteria are spore-producing forms which resist high heat, to destroy them completely requires a heat higher than that of boiling water. To accomplish such a sterilization it is necessary to heat milk in closed vessels under a steam pressure sufficient to give a temperature of several degrees above boiling, the exact temperature being dependent upon the length of time the heating continues. A temperature of 220° F. continued for half an hour is commonly, though not always, sufficient for the purpose.

To accomplish this sterilization effectually various machines have been invented and patented. Although different in construction, they are all based upon essentially the same principle. Milk is placed in bottles and subjected to a steam pressure of a certain definite amount, for a proper length of time, and then the bottles hermetically closed without being exposed to the air. The milk in these closed bottles is then kept indefinitely, the assumption being that the sterilization is so complete that it removes all possibility of subsequent bacterial growth in the milk. Bottles are kept sealed until used.

Such a method of furnishing a sterile milk was adopted several years ago and, for a time, promised to be widely adopted. In European cities such sterilized milk has been on sale for some years. The process, however, has never been very popular anywhere, has hardly been tried in America and, at present, seems likely to be abandoned, at least for ordinary purposes. It is safe to say that such a method will not likely be adopted for the general milk supply. There are objections against such milk more than sufficient to counterbalance the value of having milk free from bacteria. These objections suggest so much information concerning our general subject that they will be briefly considered. They are chiefly as follows:

1. The expense of such a process is considerable and the milk must be sold at a price higher than that of ordinary milk. This might not be sufficient to prevent its use among wealthy families, but the poorer people could hardly adopt it.

2. The taste of the milk is altered, the cooking which the milk thus receives imparting to it the well-known taste of boiled milk. The intensity of this boiled taste varies with the different methods. Some samples of sterilized milk show this new taste very slightly, while others show it very strongly; but it is always noticeable. Now it may be that the public might learn to enjoy such cooked milk, and it is

stated that children brought up on such milk learn to enjoy its taste and will not drink raw milk. The public taste is certainly subject to education and with little doubt could be educated to the use of cooked milk. But the fact remains that, in America at all events, the public in general do not enjoy cooked milk and will not drink it, while raw milk is a widely used food. Most people prefer not to drink milk at all rather than to drink boiled milk, being willing to use the latter in cases of sickness, but not as a beverage in time of health. Hence the adoption of sterilization of milk would greatly reduce the amount consumed, a result to be greatly deprecated from the standpoint of both the dairy interest and public health, inasmuch as milk is one of the cheapest and best of foods.

4. The high heat produces chemical changes in the milk of considerable importance. It modifies the condition of the *fat* emulsion. It produces a partial caramelization of the *milk sugar,* frequently resulting in a slightly brownish color. It modifies the nature of the *casein* so that the rennet ferment of the stomach, although it will curdle it, produces a curd of a different physical nature from the curd of raw milk. Heat coagulates the *albuminoid* present in the milk, the scum which appears on the surface of the milk being in part such coagulated albumen. Heat destroys some of the phosphoric proteids. The heat also destroys the *ferments* or *enzymes* which are in the milk, the *galactase,* for example, being rendered quite inert by the action of sterilization. Some of the soluble calcium salts are converted into insoluble calcium phosphate. These changes render sterilized milk quite a different material from natural milk. While they do not interfere with the chemical value of the milk as food they do somewhat interfere with the ease of its digestion and assimilation. From the first use of sterilized milk the question of its digestibility has been disputed. Numerous experiments have been carried out to compare the relative digestibility of sterilized

milk and raw milk. These have been performed upon various animals, and incidentally numerous observations have been made upon children fed with sterilized and with raw milk. The results have been very conflicting. Some have concluded that sterilized milk is as readily assimilated by the body as raw milk, and others have reached the opposite conclusions (189, 190). But there has been gradually accumulating more and more evidence that there is a difference between the ease of digestion of the two. Sterilized milk, it is true, can be digested by the vigorous stomach and can be readily absorbed. It is quite possible for an animal or a man to assimilate plenty of nourishment from sterilized milk. But such milk is not so easy to digest nor so readily assimilated as raw milk. Hence, it follows that for infants, or for those whose digestive organs are weak and delicate, sterilized milk is a less useful food than raw milk. Indeed numerous observations have indicated that the constant use of boiled milk by some children is liable to produce a condition of malnutrition, and, in some cases, certain diseases of children which have been traced to the use of sterilized milk disappear when raw milk is substituted. Too much weight should not be placed upon such facts. Cooking other foods, like eggs, makes them more difficult to digest, but we do not condemn cooking eggs for this reason. Nevertheless, although many children are brought up in a well-nourished condition by the use of boiled milk, it must be recognized that the practice of sterilizing milk decreases the ease of its digestion and thus lessens, to a slight extent, its value as a food. A constant diet of sterilized milk is not advisable for children with weak digestive powers.

4. Sterilization, as ordinarily practiced, does not sterilize. Milk is liable to contain some bacteria with resisting spores, and the high heat of sterilization does not always absolutely destroy them. It is true that the sterilization does, in the large majority of cases, totally destroy all bacteria. But it has been

proved in recent years that, frequently, a few living spores may be left in some of the bottles after sterilization (192, 197). So-called sterilized milk from many sources has been tested, and no establishment has been found whose sterile milk is always sterile. Now, while a few bacteria present are of no great importance in raw milk, the presence of a *single* living spore in sterilized milk may be a matter of serious import. The sterilized milk is commonly preserved for days, or even weeks, before it is used, this possibility of keeping being one of the chief reasons for using it. During this period a single spore left alive in the milk has a chance to grow and, by the time the milk is used, it may be filled with bacteria in enormous quantities, especially since there will be no lactic bacteria present to check the growth of other species. It has also been shown that, among the bacteria thus left in sterilized milk, there are some which produce toxic poisons, and thus the milk in which they have been growing may be actually poisonous. Lastly, it happens that some of these species produce no visible effect upon the milk, and the milk would thus be used unhesitatingly as a good food for children, although containing bacteria in vast numbers. These facts make sterilized milk unreliable, and while it is only here and there that a bottle of such milk will undergo these subsequent changes, the fact that such an event does occasionally occur removes from sterilized milk its reputation of reliability, and makes it doubtful whether it is much less liable to produce trouble than raw milk. Raw milk must be used fresh, otherwise it will sour. The putrefying bacteria do not have the chance to develop so abundantly in fresh milk, for the lactic bacteria soon check their growth. Hence raw milk, though full of bacteria, will be pretty sure to be protected from the excessive growth of these toxic bacteria which are found in the sterilized milk. This fact, that sterilized milk is not absolutely reliable, has helped to render the method of treatment unpopular, and its use has declined rather than increased in recent years. Of course, a remedy for this would be to use it fresh, but this would take away the chief advantage of sterilization, viz., its furnishing a kind of milk which will *keep*.

Boiled Milk.—A practical method of *nearly* sterilizing milk by the simple process of boiling is very widely adopted. Boiling milk does not sterilize it and the milk cannot be preserved indefinitely, but it does destroy most of the bacteria. The simplicity of the process makes it the most convenient of all methods of treating the milk in ordinary households, and for this reason it has been used very widely. It has most of the disadvantages of sterilizing, but has the one very great advantage of being convenient of application. It is used far more widely than any other method of treating milk and the ordinary households in the large cities, especially among the poor people, adopt the plan of boiling milk very extensively, doing it, however, chiefly to keep it from souring rather than for the purpose of rendering it more wholesome.

Pasteurization.—The disadvantages associated with sterilization have created a dissatisfaction with this method and have led to a different treatment of milk which, though theoretically less satisfactory, proves to be practically more useful. This treatment, originally devised by Pasteur for the treatment of wine, consists in heating the milk to a temperature of 140–175° F., and then rapidly cooling. In choosing the temperature two facts are considered:

1. It is desired to use a temperature which produces a minimum change in the chemical and physical nature of the milk, and does not give the taste of boiled milk. The highest temperature to which the milk can be subjected without acquiring these tastes is about 155°, although if the heating lasts only a very short time a higher temperature produces no noticeable effect.

2. It is desired to use a temperature which will destroy most of the bacteria. Pasteurization does not destroy all the

bacteria; but we must remember that the two chief objects to be accomplished are to increase the keeping quality of the milk and to remove the danger of disease bacteria. Will the pasteurizing temperature produce these two results?

Such temperatures certainly do increase the keeping property of the milk. Although 155° does not destroy bacteria spores, it does destroy most of the non-spore-bearing bacteria. We have already seen that the lactic bacteria, which are the cause of the souring, do not produce spores and, since these will be killed by the pasteurizing temperature, the total number of bacteria in milk is immensely reduced, and its keeping quality correspondingly increased. Experiment shows that pasteurization kills from 95% to 99% of the bacteria present, and such milk will frequently remain good for two days longer than similar milk not pasteurized. Pasteurization, therefore, does accomplish one purpose, for although the milk will not keep indefinitely it will keep sweet much longer than usual.

Most of the disease germs which are liable to be distributed by milk are destroyed by the temperature of pasteurization. Typhoid fever and diphtheria bacteria are killed by this temperature, and the same is probably true of scarlet fever. The tuberculosis bacillus, however, will, under some conditions, stand a higher heat without injury, and it has therefore been claimed that, to free the milk from the danger of distributing tuberculosis, a temperature as high as 185° F. is necessary. At this temperature, however, the cooked taste appears. There has been much dispute over the question whether lower temperatures will destroy the tubercle bacillus. Into the details of this matter we cannot enter. The present conclusion seems to be that, if the milk is heated in such a manner as to avoid the formation of a scum on its surface, a comparatively low temperature is sufficient to destroy the virulence of the tubercle bacillus (113, 134). A temperature no higher than 140°, if continued for twenty minutes, has been found sufficient to reduce the virulence of the tubercle bacilli which are in the milk so as to render them harmless. It follows from this that, although the tubercle bacilli in milk are not absolutely destroyed by a pasteurizing temperature, they are rendered less harmful. Hence pasteurizing has two advantages. It removes from the milk the danger of the distribution of contagious diseases, and it accomplishes this in such a way that the pasteurized milk, after cooling, can hardly be distinguished from fresh milk. Lastly, the milk does not keep long and must be used fresh, thus avoiding the above mentioned danger occasionally experienced in old sterilized milk in which the bacteria have not been wholly killed.

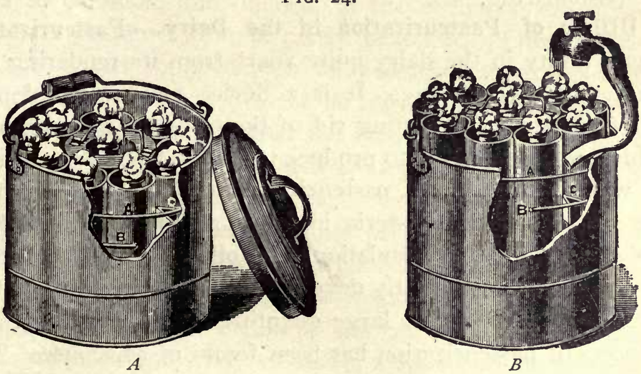
It is believed also that pasteurization reduces the likelihood of milk being a cause of the diarrheal diseases. Inasmuch as we do not positively know the bacteria which produce these troubles, it is difficult to say how pasteurization of milk actually affects them. Pasteurization greatly reduces the number of bacteria in the milk, destroying the larger part of the lactic bacteria and many of the peptonizing and putrefying class. The milk is, therefore, freer from bacteria and, perhaps for this reason, less liable to produce intestinal troubles. If the diarrheal troubles are produced by toxic products in the milk, and a sample of milk is old so that the bacteria have had a chance to grow and produce in it their toxic products, it is hardly likely that the pasteurization can have an influence upon the poisons themselves, and thus wholly remove the danger of intestinal troubles. Pasteurization could be a remedy only for such troubles as are due to the actual growth of bacteria in the intestinal tract. But the best proof of the utility of pasteurization is the result. It has been found from a large amount of experience that such treatment of milk does have a marked tendency to reduce diarrheal troubles among children, and that cases of intestinal diseases yield much more readily to treatment if the milk is pasteurized. While the process is not an absolute protection against digestive troubles it is to-day recognized as a useful means of reducing them.

Pasteurization on a Large Scale.—The pasteurization of milk has proved to be practical as a method of treating the milk supply from distributing centers. A variety of machines have been invented for pasteurizing milk in large quantities. Some of these, called "continuous pasteurizers," are so constructed that a stream of milk flows into the machine at one end, is heated and cooled, and flows out at the other end in a constant stream of pasteurized milk. Another class of pasteurizers receives a considerable quantity of milk in a large chamber, heats it to the required temperature, and holds it at this temperature for any desired length of time. Of these two methods the latter has proved to be the most efficient in its action. Either of these types of pasteurizer is applicable for the treatment of large quantities of milk, and their use makes it possible for a milk dealer to furnish his customers with a supply of pasteurized milk.

Such methods of treating the public milk supply have been adopted in several cities. In Europe quite a number of cities are now supplied by certain milk companies with pasteurized milk, which is sold for a price either the same as that of ordinary milk, or at a slight advance. This kind of milk has been quite popular, and it has appeared that this is, perhaps, the most satisfactory method of treating the general milk product. In America the same thing has been tried in a number of places, but, for various reasons, has not been very successful. There appears to be a difficulty in America to obtain a market, for the American public have not been convinced that pasteurized milk is sufficiently superior to the ordinary milk to warrant paying an *advanced* price for the same. While, then, in our own cities pasteurized milk is occasionally to be found upon the market, the industry of furnishing such milk has not as yet been thoroughly successful upon this side of the Atlantic.

Home Pasteurization.—It frequently happens that a housewife or nurse feels obliged to treat milk in some way which will remove the danger of distribution of diseases. Pasteurization of milk is not an easy process because it usually involves the use of a thermometer, and those employed in the kitchen of an ordinary household can hardly use this instrument intelligently. To avoid this difficulty there have been devised some apparatus which make possible the pasteurization of milk in small quantities by a plan so simple that it can be used by any one. The method consists of placing milk in bottles of certain size and then immersing them in a vessel containing boiling water, allowing the water to warm the milk and the milk to cool the water. If the size of the bottles of milk and the amount of boiling water are properly proportioned the milk will be heated to the desired temperature, of about 150–180° F., and will then

FIG. 24.



A *B*

Apparatus for home pasteurization, arranged for heating, and also as connected with water faucet for cooling.

slowly cool. To accomplish this satisfactorily a simple little device has been placed upon the market, shown in Fig. 24.

The milk to be pasteurized is placed in the bottles, the bottles placed in the vessel and the vessel filled with boiling water and set aside at once to cool. The practical result is that the milk is heated to the proper temperature. After about half an hour cold water is run into the vessel to cool the milk as in Fig. 24, *B*. Such a plan of pasteurization is not strictly accurate, but is satisfactory for practical purposes. It is also possible, by an even simpler device, to accomplish the same purpose with less accuracy. If the milk is placed in quart bottles and these placed in a pail which is filled with boiling water and set aside to cool, the result is that the milk is warmed to about the proper temperature by the time that the water is cooled to the same temperature. If allowed to stand in this pail for half an hour and then subsequently taken out and cooled, the milk is practically pasteurized, although the method of course is by no means accurate. It has the advantage, however, of being within the easy reach of any common household, and probably accomplishes the purpose about as well as more expensive methods of pasteurizing.

Utility of Pasteurization in the Dairy.—Pasteurization has a utility in the dairy quite apart from its rendering the milk more wholesome. It is a device frequently adopted for the purpose of getting rid of the ordinary milk bacteria which would be liable to produce trouble. In butter-making, as will be noticed later, pasteurization is very widely adopted for destroying the bacteria in cream in order to make the cream free for the inoculation with other desirable bacteria. In the somewhat recently developed industry in this country, of supplying cream in large quantities for city custom, the process of pasteurization has been found of great use. The cream must sometimes be shipped for long distances and may be three or four days old before it is consumed. In preserving it this length of time and thus enabling it to command a market, pasteurization has been of great service, and considerable amounts of cream are now subjected to pasteurization and subsequently shipped to a distant market. In cheese-making the pasteurization has not yet been much used, for the heat appears to produce changes which make it difficult to procure a normal cheese from the milk.

As a means of treating skim milk for the purpose of protecting from tuberculosis animals to whom it is fed, pasteurization has come to be very widely used, and is becoming more popular each year. Especially in the northern countries of Europe where tuberculosis is very common, pasteurization of milk in creameries is becoming almost universal. In Denmark there is a law requiring this treatment for all creamery skim milk that is to be returned to the farm to be fed to live stock. Practically all creameries in Denmark, are, therefore, supplied with pasteurizing apparatus, and the pasteurization of the milk and cream is one of the everyday routine parts of the work which is rarely omitted. In other European countries, also, the process of pasteurization is being rapidly adopted. In America the practice is by no means so common, and, indeed, in the ordinary American creameries pasteurization is rarely practiced. Our creameries are not yet supplied with the necessary apparatus and pasteurization would at present be hardly a possibility. It seems probable, however, that the method of treating milk and cream by pasteurization will before long become much more widely extended in America.

Although pasteurization does not produce great chemical changes, it does have some slight effect upon milk. One of its results is that the cream thus treated loses some of its viscosity. Pasteurized cream is not so thick as ordinary cream and does not whip so easily, a result which injures its market value. The viscosity and whipping power can be restored to the cream by a simple chemical device suggested by Babcock and Russell (199). This they accomplish by a material called *viscogen* which is prepared as follows: 2½ parts of cane sugar are dissolved in 5 quarts of water; 1 part of quick lime is slaked in 3 parts of water, the coarse particles removed and the liquid is added to the sugar solution. The whole is allowed to settle and the clear liquid is viscogen. This viscogen is added to the pasteurized cream slowly, about two-thirds enough being added to neutralize the acidity of the cream. To determine this amount there is added to a small measured quantity of the cream enough viscogen from a burette (see Chapter IX.) to neutralize the acidity. The amount of viscogen thus employed for the small sample of cream makes it possible to calculate the amount needed per liter to neutralize the acid. About two-thirds of this calculated amount of viscogen is added to the cream and this is found to restore its viscosity.

Of course, the addition of such material to the cream is a manifest adulteration and would come under the condemnation generally given to adulterants. It is to be remembered, however, that the viscogen is perfectly harmless, and, indeed, that lime water is frequently recommended to be added to milk. But if viscogen were used in the public supply it would be condemned as an adulterant, and its use is too troublesome to be adopted by a small household. Viscogen has, therefore, not proved to be of much practical value.

Milk Thermophore.—A somewhat ingenious application of heat for protecting milk from the growth of bacteria has been recently invented. It is called the **thermophore,** and is an apparatus for keeping the milk warm, depending upon a peculiar property of acetate of sodium to give off much heat while solidifying (209). The thermophore consists of a double-walled vessel, in the inner compartment of which the milk is placed in flasks. The space between the inner and outer walls is filled with acetate of sodium. For use the whole apparatus is heated to a temperature of boiling water, which melts the acetate of sodium. It is then placed upon some good non-conductor, the milk deposited inside and allowed to stand. As the apparatus cools the acetate of sodium solidifies at about 58° C., and in solidifying it gives off its heat to the milk within the inner chamber. The milk may be kept by this means at a temperature of from 40° to 55° C. for ten hours. It might be expected that at such high temperatures the bacteria would grow very fast, but this is not found to be true. On the contrary, the bacteria begin to disappear and, after several hours, are perhaps less numerous than at first. If two samples of the same milk are placed, one in an ice chest and the other in the thermophore, at the end of ten hours the sample in the thermophore will contain the smaller number of bacteria.

The chief advantage of the thermophore is that it is a convenient device for keeping milk warm. In feeding young children with milk it is necessary to warm the milk and this is a great inconvenience, especially during the night. The thermophore keeps the milk warm for ten hours, and at the same time prevents the growth of bacteria. The device is, however, so new and so little is known about it that it is impossible at present to say whether or not it will really prove a practical means of treating milk. It is not yet upon the American market.

Use of Ice.—Nothing has as yet proved of such practical value in preventing the growth of bacteria as the use of low temperatures. Different localities, however, differ much in their methods of the use of cold. In this country there has been a very wide adoption of the use of ice for the purpose of keeping milk cool during transportation. This has resulted in the invention of the *ice car,* in which the milk is kept surrounded by ice, making it possible to retain the milk for two days before distribution, and yet furnishing the consumer with milk which has comparatively few bacteria and keeps well. This is easy to understand, since, as shown in Chapter IV., at a temperature of 50° F. the bacteria hardly multiply at all for nearly forty hours, and for fifty hours multiply so little as to be still comparatively few in number. If milk is produced under cleanly conditions and is kept at a temperature of 50° F. it is possible to preserve it many days without any apparent changes in it due to bacteria growth.

In Europe ice is not used so freely as in America. This is partly due to its being more expensive, and partly to the fact that the milk companies have not been accustomed to its use. The milk for European cities is commonly cooled by water or otherwise, but without the use of ice, and is shipped into the city and delivered as quickly as possible. In Berlin most of the milk is delivered before it is seven hours old, and the methods adopted in American cities are unknown. The use of ice in America makes it possible to furnish milk in large quantities from a radius covering 500 miles, and yet delivered in the city in a better condition, so far as bacteria are concerned, than is found in many European cities where the dairy farms are only a short distance from the city. The milk of New York city in cold winter weather will average about 250,000 per c.c., while in summer it frequently runs up to 500,000–1,000,000 or more. In European cities the numbers which are present in market milk run considerably higher than this. The free use of ice is the foundation of the American milk industry.

Frozen Milk.—If milk is cooled below 32° it is frozen into ice and in this form it may be protected absolutely from the action of bacteria. Since milk bacteria do not grow at temperatures below freezing, such frozen milk will keep indefinitely. Within the last few years the plan has been adopted of furnishing milk in a frozen condition for city consumption (210). This plan has several practical advantages. The milk is commonly frozen in special cans which are easy to handle, may be packed in very small compass, shipped as freight, and transported for a long distance without danger of melting. Milk may be transported in this form for many hundreds of miles and may, if properly treated, be kept for many weeks, and subsequently, when delivered to the customer and allowed to thaw, be in as fresh condition as at the outset.

The method of freezing the milk is not always the same. The low temperature necessary is produced by one of the artificial ice-making machines. The milk is frozen in special cans, which are commonly cubical in shape and thus conveniently packed away, and easily allow the frozen milk, subsequently, to be slipped out of them. These cakes of frozen milk can be packed in large numbers in a car for shipment. When milk is frozen under ordinary circumstances there is more or less of a separation between the water and the solid parts of the milk, so that a cake of frozen milk is not a uniform product throughout. This is in a measure avoided by some of the methods of freezing which have been devised. The details of these methods, however, cannot be given here. The general result is that the milk may be frozen without materially changing its physical nature, and when subsequently thawed out the product cannot be distinguished from fresh milk.

This method of distributing milk has been attempted only in very recent years, too recently to determine whether it is practical and likely to extend. It has been already adopted in quite a number of cities of Northern Europe, appears to be becoming popular, and is extending with some rapidity. The manifest advantages of such a method, particularly in warm weather, when milk is liable to be ruined by bacteria before reaching the consumers, makes the plan a very promising one, and it is by no means impossible that this method of furnishing large communities with milk from a distance may be adopted more and more widely. It may, perhaps, in the end become the most popular method of handling this dairy product. From the standpoint of the bacteriologist it is almost an ideal method, for it furnishes a means of placing in the hands of the consumer milk that contains no more bacteria than were in the milk at the time it was taken from the dairy; an advantage too manifest to need further emphasis. At present, however, experience as to its practical utility is too slight to make it safe to predict its future, but it is certainly a very promising plan.

OTHER METHODS OF FURNISHING BACTERIA-FREE MILK.

Condensed Milk, etc.—Several methods have been devised of treating the milk in such a way that it can be preserved indefinitely. For this purpose it is necessary to bring the milk to a condition in which the milk bacteria will not grow. One method widely adopted is to condense the milk, which simply means to remove a considerable quantity of its water. The methods of condensing the milk need not concern us, but they consist essentially of evaporating the water of the milk, usually in a vacuum, and thus reducing its bulk. In common condensed milk there is subsequently added to the product a considerable proportion of sugar, the purpose of which is to prevent bacteria growth, for bacteria do not grow in a material which contains great quantities of sugar. The condensed milk of the market, therefore, is made up of the solid material of the milk, together with large quantities of sugar, and though the specimens are not in all cases absolutely sterile, they are of such a nature that the bacteria do not grow in the product.

Other forms of prepared milks, either condensed or not condensed, are prepared without the addition of sugar. In these cases it is necessary to adopt some other means of preventing bacteria growth, and the materials thus prepared are usually subjected to a high heat which sterilizes them. The various market products, usually sold in sealed cans, commonly represent preserved milks, not adulterated with sugar, but preserved by sterilization and showing the peculiar taste of cooked milk. None of the methods of preserving milk yet devised gives a product exactly similar to the original milk.

Powdered Milk.—Within the last few years methods of preserving milk by reducing it to a powder have been invented and perfected. There are several methods of accomplishing this powdering of the milk, some of which make use of a vacuum and others evaporate the milk without a vacuum. In any case the milk, usually without the fat, is thoroughly dried and subsequently passed through a mill and ground into a fine powder. The material thus obtained is much like flour, but is far more nutritious than flour, inasmuch as the amount of proteid it contains is very high, being very nearly 40%. The powder thus obtained can be kept indefinitely if it is protected from moisture. The bacteria that were present in the original milk are not killed by the process, and they remain in the powder for a long time in the condition for growth, but they cannot develop in the powder, since it is dry and most of them soon die. The milk powder which has been kept for some months contains very few bacteria. The presence of these bacteria, however, is a matter of no special significance, inasmuch as they cannot multiply, and when the powder is subsequently dissolved in water for use it is sure to be consumed so quickly that the few bacteria left in the powder thus find no opportunity to do any mischief. The milk powder thus produced is an extremely valuable product, and can be used for a great variety of purposes for which fresh milk has hitherto been used. The material made by dissolving this powder in water is not, however, exactly like fresh milk and will never take its place. This method of preserving milk is particularly applicable to warm climates, for milk powder, if kept dry, can be sent to any climate and preserved indefinitely, being used anywhere and at any time that it is needed. If the fat is retained the milk may be similarly dried, but the product is somewhat different in character. It makes a more balanced food, but the fat is apt to become rancid after a time.

PRACTICAL CONCLUSIONS.

After having thus noticed the various dangers occasionally found in milk and the methods of meeting them, the question arises to each consumer how he shall personally furnish his own family with milk.

In Europe the plan adopted almost universally is the simple one of sterilizing or pasteurizing all of the milk. This is sometimes done before the milk is distributed to the consumer and, if not, it is almost universally adopted in the household as a means of freeing the milk from its supposed dangers. The use of cooked milk has become well-nigh universal in European households. This has not been adopted so widely in England or America, and in this country the use of raw milk is still very widely continued.

If one lives in a small community, where the milk is distributed by individual dealers, the best method of dealing with the question is to choose from among the milk producers one who seems to be the most cleanly and the most reliable. It is not always easy to determine this, but a little thought put in the matter, and perhaps a visit to one or two dairies, will usually enable one to select a dealer whose milk may be ordinarily relied upon. In choosing a milk dealer one should be careful not to choose one whose milk is cheapest, for this will in most cases mean one whose milk is poorest, produced under uncleanly conditions, and, therefore, most liable to distribute contagious or other diseases. The cheapest milk is usually the worst and most dangerous. The so-called " grocery milk " of cities is the cheapest, the poorest and most dangerous type of city milk (28).

In large cities where the milk supply cannot be traced to individual producers the question must be answered differently. If one is able to do so, the best means of furnishing a family with milk is to procure it from some of the sanitary dairies, such as already referred to, that produce their milk under ideal conditions and charge for it a high price. The milk from these dairies is the best in every respect, but the price is high. The purchase of certified milks or milk from certified dairies is almost sure to furnish milk which is reliable, although less so than the milk from sanitary dairies. The slightly advanced price of the certified milk should not deter one from obtaining milk from these sources, for the small advance in price is more than compensated for by the extra reliability of the milk. If, however, one is not ready to give the advanced price required for such milk, it is necessary to depend upon the general milk supply. The general milk supply of large cities may be purchased with a confidence that it is usually chemically reliable, but will not be reliable from the standpoint of bacteria. It contains bacteria in large quantities, sometimes in excessive quantities, and must be regarded as a possible source of danger.

In the use of milk, according to the data in our possession at present, it seems that, for adults, provided we can have a source of supply that is tolerably reliable, it is quite unnecessary to go to the trouble of sterilizing or pasteurizing. It is true that the use of raw milk is a possible source of certain diseases; but it is quite impossible to avoid all danger of contagion in this world, and the danger from the use of raw milk is probably one of the small rather than one of the large dangers. If one uses raw milk he must do so with the understanding that it is a possible source of danger, and may occasionally produce trouble; but the danger is not a great one and it is doubtful whether it is sufficient to warrant the extra trouble of sterilizing the milk. In using milk for feeding young children a different attitude must be taken. The danger to young children of the distribution of certain contagious diseases, especially tuberculosis, diphtheria and the diarrheal troubles by raw milk, is considerable. For young children fed upon milk it is probably wisest to adopt one of two courses: Either purchase milk from some of the special dairies that furnish an absolutely reliable product though at a high price, or adopt the process of pasteurization of the milk after it reaches the household. In general, then, the consumer should choose the most reliable source of milk which he can find. For the use of adults the milk may be consumed raw, but for young children it should be treated by some means for destroying the pathogenic bacteria.

CHAPTER VII.

BACTERIA IN BUTTER.

THE relation of bacteria to butter, the second great dairy product, divides itself into two heads: (1) The influence of bacteria upon cream in the preparation for butter-making by the process of cream ripening; (2) the relation of bacteria to butter itself, and the changes which they produce therein. More is known in regard to the former subject than the latter, and the question of cream ripening will be first considered.

CREAM RIPENING.

Butter is made from cream which has been separated from the milk either by gravity or by the centrifugal method. The shaking which it receives in churning causes the fat to adhere together in little masses which are finally bulky enough to be removed from the liquid in which they float. In most processes of butter-making the cream is, however, not churned immediately after it is separated from the milk, but is allowed to undergo a process of *ripening*. It is true there is a certain demand for sweet cream butter, but the demand has hitherto been a very slight one, and even at the present time there is very little market for this product. Whether the future taste of the butter consumers will change so as to increase the demand for sweet cream butter cannot be predicted, but at the present time butter is made almost universally from cream that has been allowed to undergo the process of ripening.

The origin of the process of cream ripening was probably purely accidental. Until within comparatively few years butter has been made only upon individual farms and in most cases the amount of cream at the disposal of the butter-maker has been too small to make it possible to churn daily. As a result, the cream, after being separated from the milk, has been kept for two to four days, usually in a cold place, until enough has accumulated to make a conveniently sized churning. This keeping of the cream for several days, even though it is kept cool, has of necessity resulted in ripening. This is doubtless the origin of the process of ripening.

But, although its origin was thus a necessity of the conditions of churning in earlier years, the process has been kept up and is adopted almost universally to-day, even though the butter-making has been concentrated into large creameries and the churning takes place daily. Cream ripening has continued till the present time because of its utility; for it produces certain changes in the character of the cream which result in a considerable modification of the process of butter-making and a noticeable change in the nature of the butter. Indeed, the more the butter-making industry becomes concentrated the greater is the necessity, not only of the ripening of the cream, but of the more and more careful attention given to this phase of butter-making. In earlier years butter was made on the individual farm, a few pounds at a time, and it made little difference whether the ripening was properly conducted or not. If the ripening was not satisfactory in any churning it affected only a few pounds of butter, and was a fact to which the farmer paid little attention. When, however, a creamery makes hundreds or even thousands of pounds of butter per day, and is subjected to the competition of modern industry, it is much more important that every detail in the butter-making should be carefully considered. It has become more and more necessary, as the creameries have become concentrated, that the ripening of the cream should be placed under as strict control as possible. It is only as the ripening is controlled that the concentration of butter-making in large creameries is thoroughly successful.

To understand this we will notice first the purposes of cream ripening.

PURPOSES OF CREAM RIPENING.

1. Ripening has been found to *increase the yield* of butter, for numerous experiments have shown that the yield of butter from a given quantity of cream is greater if the cream is properly ripened than from the same quantity of unripened cream. This is certainly true for cream separated by the old-fashioned gravity method, though less significant if the cream is separated by the modern separator. A *greater ease of churning* is associated with this greater yield, for gravity cream, while sweet, is found to be difficult to churn, whereas it will churn readily after proper ripening. This again does not hold so true of separator cream. It is a matter of considerable significance in a large creamery that the yield of butter from a given lot of cream should be as large as possible, and for this reason alone the ripening would be a matter of importance, for it might mean a saving of hundreds of pounds of butter in the course of a single year.

2. It is claimed that butter made from properly ripened cream has *better keeping properties* than butter made from unripened cream. Whether this is true, however, is not definitely settled, for experiments upon this matter have been and still are in conflict. Moreover, whether or not it be true it is a matter of comparatively little importance, for a large creamery usually disposes of its butter when it is tolerably fresh.

3. By far the most important reason for cream ripening is to develop in the butter the desirable *flavor* and *aroma.* By flavor is meant the taste of the butter, and by aroma the odor. It has been demonstrated over and over again that butter made from unripened cream lacks the peculiar flavor and aroma which are characteristic of high grade butter. These flavors appear during the ripening. Sweet cream butter tastes almost like fresh cream, and the peculiar butter

flavor is quite lacking. The production of these flavors is the chief reason why cream ripening has been almost universally adopted in butter-making.

The importance of flavor is greater than is frequently recognized by the butter-makers themselves. Though the market price of butter is not dependent directly upon the flavor, it is largely controlled by it. Butter with a poor flavor will command a very low price, or if the flavor is very bad the butter may be quite unsalable, whereas butter with a good flavor will command a higher price, and exceptionally fine-flavored products bring very high prices as fancy butter. Certainly the flavor counts for two or three cents a pound in the price of butter. It is true that the condition of the butter industry is not such as to allow each sample of butter to sell upon its merits, for the "market price" of butter may be obtained by products varying very widely in flavor. This is one of the reasons why many butter-makers feel no stimulus to produce a very highly flavored product. But, nevertheless, it is equally true that butter having poor flavor will not bring market price, and especially good products will bring a price above the market. In concentrated dairying the success or failure of a business depends very largely upon the small differences in the price that may be obtained for the product. A difference of a cent or even half a cent a pound upon the yield of a large creamery may make the difference between financial success and failure. A creamery that makes great quantities of butter and fails to obtain a proper ripening, so that the desired flavor is not obtained, must be content with a price somewhat below the market, whereas another creamery in which the ripening is satisfactory will obtain market price, or may be able to obtain even a fancy price for the butter if the flavor is exceptionally good. While other factors are concerned in the market price besides flavor, there is no other one that is of more importance than this, and no other one which is so liable to fluctuate with the conditions. The conditions that cause the variations in flavor and aroma are largely, though not wholly, the conditions of the ripening of the cream. For these reasons butter-makers have been, in recent years, more and more recognizing the significance of the cream ripening, especially in the larger creameries.

It must be further noticed that the question of the proper flavor of butter is in a measure dependent upon the caprice of the public. The public taste has become educated to a certain kind of butter flavor and now demands this particular kind. This public taste, moreover, is very capricious and, indeed, it is constantly undergoing modification. A few years ago, when creameries began first to be built, the public taste in America did not like the creamery butter, claiming that the flavor was not quite so strong as that of the ordinary farmer's butter. For a time the creamery butter was not so popular as the old-fashioned stronger butter made on individual farms. This taste, however, has changed, and at the present time the stronger flavored farmer's butter is generally regarded as inferior to the more delicate but less highly flavored creamery product. On the continent of Europe the change has gone still farther, and the public taste in Europe demands a flavor in its butter considerably less prominent than that which is demanded by the American market. The European butter would not at present meet the approval of the American public, and the American more highly flavored butter does not meet the approval of the European public. European visitors frequently complain that they cannot find any butter that is fit to eat in America, insisting that it is all too strong, and on the other hand, many an American has experienced the fact that the butter upon the European table seems to him to have no taste at all. These facts indicate that the public taste is capable of change, and the butter-maker should bear this in mind and recognize that the demand for highly flavored butters may disappear in the next few years.

If the butter-makers of this country should place upon the market a milder and milder flavored butter there is little question that the public taste would adapt itself to the new conditions and the demand would in time be for a milder flavor.

What is Cream Ripening?—The chief agency in cream ripening is the growth of bacteria. The bacteria of milk collect in the cream and multiply very rapidly at the temperature at which the cream is kept during the ripening. The growth of the bacteria may be best illustrated by two examples of ripening cream, both representing the process in winter weather when the number at the outset was small and the ripening slow.

At Beginning of Ripening.	Half Ripened.	Ripened.
2d, Fresh, 44,000	300,000,000	1,500,000,000
1st, Fresh, 309,000	303,000,000	1,300,000,000

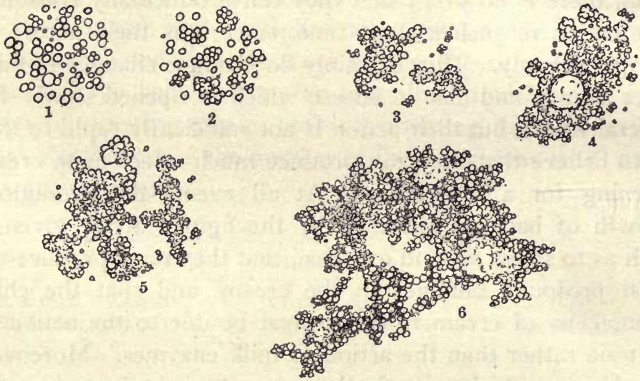
The numbers here given are somewhat larger than those found in the ordinary ripened cream. A large series of tests has shown that in a normally ripened cream the number of bacteria present will vary from 100,000,000 to 1,500,000,000 per c.c., about 500,000,000 being an average number.

This enormous growth of bacteria, occurring within a day or two days, will produce profound changes in the chemical nature of the cream. The bacteria, having multiplied to this great extent, must not only have consumed a certain quantity of material for food, but must also have produced a series of by-products, either as excretions or as decomposition products, and the chemical nature of the cream will consequently be very much changed.

That this bacteria growth is the chief cause of the changes in ripening cream is beyond question. Whether it is the only cause of the changes has not yet been demonstrated. We have already learned that there is present in milk, even when freshly drawn, at least one of the so-called chemical ferments or enzymes capable of producing chemical changes in the milk entirely independent of the agency of bacteria. There is no proof that these enzymes may not have a part to play in the cream ripening, and the changes which occur during this process may be in part due to their agency. On the other hand, there is no proof that they can produce any considerable action in such a short time, since it is their nature to act quite slowly. They certainly do produce changes in milk after a time, and also in cheese which is ripened slowly for several weeks, but their action is not sufficiently rapid to lead us to believe that they can produce much effect upon cream ripening for a few hours. At all events the prodigious growth of bacteria indicated by the figures above given is such as to show, beyond question, that they must produce the most profound changes in the cream, and that the chief phenomena of cream ripening must be due to the action of bacteria rather than the action of milk enzymes. Moreover, the changes which occur in the cream are exactly such as we have learned are produced by the growth of bacteria. The cream becomes acid from the development of lactic acid; it also becomes thick and acquires a peculiar odor, indicating a variety of decomposition products. Such changes are the very ones found to be produced by the growth of bacteria, and thus the general nature of the changes during cream ripening is quite in accordance with the belief that bacteria are the primary agents in the change.

Why Ripening Affects the Yield of Butter.—There is no difficulty in understanding how the growth of the bacteria increases the yield of butter. A microscopic study seems to show that churning is a process of shaking the fat drops into contact with each other, causing them to adhere in larger and larger lumps. This process can be understood from the examination of Fig. 25, which is taken from cream at different intervals in churning. When the lumps become large enough to be removed from the liquid the churning is stopped, and the butter is taken out in the form of the butter granules. The ease of the churning will depend upon the readiness with which these drops of fat can be shaken together until they fuse.

FIG. 25.



Fat globules of cream in the process of churning. 1, in cream before churning. The successive figures show the gradual fusion of the globules into larger and larger masses.

In fresh gravity cream the fat drops do not float around separately in the liquid, but are bunched together in little groups, as shown in Fig. 25, 1. There is apparently some invisible material holding the bacteria together, although the exact nature of this material is not positively known. It has been thought by Babcock (211) to be similar to fibrin which forms in blood, and this author assumes that there occurs in milk, after it is drawn, a process somewhat similar to the clotting of blood. Whether this is correct has not been demonstrated, but it is certainly a fact that there is something of a proteid nature connected with the fat drops, which keeps them from floating freely (212). As long as they are held apart by such fibrin-like material they will not easily be shaken in contact with each other, and the churning will consequently be difficult. The action of bacteria growing in the cream has a tendency to soften this material. The lactic acid formed will tend to soften protein material which may be holding the fat drops, and the growth of peptonizing bacteria would further tend to dissolve such substances. The result is that, after a few hours' ripening, the fibrin substance which prevents the ready fusion of the fat drops is softened and dissolved. The globules are then readily shaken together, churning becomes easy and the yield is greater. When cream is separated from milk by the separator this protein-like material is thrown out of the milk and collects on the drum of the separator; the fat drops are, therefore, more free in separator cream, and fuse with readiness, thus explaining the fact that centrifugal cream churns readily enough without previous ripening.

The explanation here given is the most plausible account of the phenomenon, but it is by no means demonstrated. The existence of the fibrin is not proved. Storch believes each fat drop is coated with a thin mucin-like layer and doubts the existence of fibrin. The exact physical condition of the fat in the cream is, in short, not yet positively known, and the account given here must be regarded only as a tentative explanation.

Ripening as Affecting the Keeping Property.—As already stated, it is not definitely settled that ripening does affect the keeping property of butter, for some experiments seemingly indicate that this is not true. The majority, however, have shown that butter made from ripened cream does keep better than butter made from the cream that is not ripened. It is easy to understand why this should be the case. There is present in cream a considerable quantity of albuminoid material and milk sugar capable of being acted upon by bacteria. If the bacteria be allowed to grow for a short period before churning, the lactic bacteria produce acid and this, as we have seen, has a tendency to check the development of other bacteria. The growth of lactic bacteria will actually stop any further fermentative changes due to bacteria. If, however, the cream is churned without ripening, the butter will contain various bacteria which can grow in the butter, inasmuch as the lactic bacteria do not check them. The lactic bacteria do not grow so readily in the butter as in the cream, and the result is that other forms of fermentative bacteria which may injure butter have a greater chance of developing in butter made from unripened cream than in that made from ripened cream.

Effect of Ripening upon Flavor.—The most important effect of the ripening process is upon the flavor. The growth of bacteria produces a variety of new chemical products. Some of these may be excretions, and some of them by-products of chemical decomposition. Of these products, some are known and others are as yet not understood by chemists. Prominent among them, of course, is lactic acid, but there is a long series of others. The products of fermentative decomposition always have very strong tastes and smells. These new chemical products which are developed rapidly in the ripening cream, as the result of the wonderful growth of bacteria, will give to the cream a variety of new flavors and odors. The butter made therefrom will show a flavor not found in butter which is made from cream before such decompositions have taken place. The butter flavor is that of incipient decomposition.

While this is certain, the problem of what kind of bacteria produces the typical flavors is one that has not yet been satisfactorily settled. We have noticed that two prominent types of milk bacteria are those producing lactic acid and those producing albuminoid decomposition. Since these are the chief types in ripening cream there can be little question that to one of them must be attributed the butter flavors. But which is primarily concerned? It has been generally assumed by bacteriologists that it is the lactic bacteria which are responsible both for the flavors and the acid. This conclusion is a natural one, since the lactic bacteria greatly predominate in milk when it is ripened, making over 90% of the bacteria of ripened cream. The natural inference is that these lactic bacteria are concerned in the ripening and, therefore, produce the flavors. The first of these inferences is certainly true, but the second does not necessarily follow. That the lactic bacteria have an important part to play in the cream ripening cannot be doubted, but it is not so sure that they produce the flavors. Lactic acid in itself does not have the peculiar flavor of butter, and when the experiment is tried, of making butter by the adding of lactic acid to the cream, it is found that the distinctive butter flavors are wanting.

Different species of bacteria acting upon ripening cream have quite different effects on the flavor of the butter. Many experiments have been tried with different species of milk bacteria to determine their action upon cream and their influence upon resulting butter flavors (213, 214). These experiments have commonly been performed by isolating different species of bacteria from milk or cream and then adding pure cultures of these bacteria to cream which has been deprived of most of its microörganisms by pasteurizing. The inoculated bacteria grow rapidly, produce their effect upon the cream, and the cream, when subsequently churned, yields a butter the nature of which has been modified by the growth of the bacteria in the cream. By such experiments a large number of bacteria have been tested and the results have shown wide differences. The flavors of the butter produced vary widely; sometimes it is *strong,* sometimes it is *bitter,* sometimes it shows a *burning taste,* sometimes it tastes *tallowy,* occasionally it may develop a *turnip taste,* etc.

These results, however, although interesting and useful, do not represent the exact condition of things in ripening cream, because the experiments are performed under unusual conditions. In a normally ripening cream a variety of bacteria are contending with each other for mastery; the result of this is a complex struggle, in which some species are rapidly crowded into the background and have little or no effect on the character of the ripened cream. Laboratory experiments carefully exclude all of the miscellaneous species of bacteria, and especially the lactic forms which develop in normal cream. The results of such experiments, are, therefore, quite unlike those of normal cream ripening. These experiments do show, however, the different effect of different species of bacteria upon butter, and prove beyond question that different species of milk bacteria have the power of producing different flavors in the butter.

The Growth of Bacteria in Ripening Cream.—In order to understand properly the phenomena of cream ripening it is necessary to study the actual growth of bacteria in cream under normal conditions, rather than under these rather unusual conditions of laboratory experiments. This has been done in recent years with considerable satisfaction, and we now know tolerably well the essential facts. The development of bacteria in cream is very similar to that which we have described in an earlier chapter as the growth of bacteria in milk (101). It is essentially as follows:

Fresh cream contains commonly a very large variety of bacteria. This is especially true in the case of creameries, for here the cream is collected from a somewhat wide territory, and consequently receives a varied stock of bacteria. The number of species that may be found in such ripened cream is sometimes comparatively few and sometimes very great. Moreover, the number found under these circumstances is dependent, in a measure, upon the age of the cream, for the older the cream the smaller is the number of kinds of bacteria which are found by bacteriological analysis. In cream that is brought into the ordinary creamery the character of the bacteria will, therefore, be different in accordance with the age of the cream.

For the first few hours there is a gradual growth of bacteria, all species probably increasing somewhat in numbers. The lactic bacteria are commonly few in numbers in the perfectly fresh cream, but these grow more rapidly than any other species, and slowly increase in proportion until they become as numerous as all of the other species put together. After this the lactic bacteria grow more rapidly still; the other species are gradually crowded into the background, and either disappear entirely, or become relatively so few as to play practically no part in the further cream ripening. Fully ripened cream contains little besides lactic bacteria.

It is evident, therefore, that the study of the growth of bacteria in cream shows that the ripening of the cream is divided into two distinct stages. The first period consists of the first few hours after the cream is separated from the milk, the exact number of hours depending chiefly upon the temperature, but also upon the other conditions. During this time there is a development of many different species. In all cases the peptonizing bacteria increase in numbers, and the same is true of most of the bacteria which were found in the original cream. At the close of this first period of ripening, although the lactic bacteria have become relatively more abundant than at the outset, the peptonizing and the many miscellaneous species have also increased rapidly and have become very numerous. The second period in cream ripening is that in which the lactic organisms gradually replace the others, becoming more and more abundant and producing lactic acid which sours the cream and slightly curdles it. When this occurs the cream is ready for churning.

These two periods are passed through in all cases of cream ripening. Sometimes the first period is completed before the cream has been brought to the creamery, and sometimes, indeed, the cream is fully ripened when brought to the creamery. In other cases the whole of the process may go on in the creamery vat.

Both of these stages of cream ripening must have an important influence upon the product. That the production of lactic acid has an important influence is beyond question. The presence of lactic acid is one of the essential factors in the churning, and the slight acid taste due to this acid is one of the factors of the butter flavor. But it is extremely probable that the first phase of cream ripening has also an important influence upon the final product. The decomposition products developed by the bacteria growing during the first few hours, and which cause more or less of a destruction of the albuminoids are, at least, very likely to contribute to the final product of the butter flavor. Indeed, there seems to be very good *a priori* reason for this. The butter flavor, when it becomes a little too strong, has a very decided tinge of a putrefactive taste. People accustomed to the mild-flavored European butter tell us that the American butter frequently tastes decayed. Now the lactic bacteria do not produce these odors and flavors of decay but simply lactic acid. It, therefore, follows that the peculiar flavors, of American butter at all events, must be attributed in part to the action of the type of bacteria that produce albuminoid decomposition. In other words, it seems that the flavor of butter is not a simple phenomenon, but a somewhat complex one. It is dependent partly upon the presence of lactic acid, which undoubtedly imparts a flavor, but, at least in the American butter, it is dependent also in part upon some of the other ingredients in cream, produced by some of the bacteria that develop in the first period of ripening, during which the miscellaneous bacteria grow rapidly and become abundant. To the bacteria producing albuminoid decomposition, the peptonizing bacteria, we must attribute a part of the butter's flavor.

Why Ripening is Ordinarily Satisfactory.—The facts just outlined explain why the ripening of cream is ordinarily satisfactory in spite of the fact that the cream is almost sure to be filled with a miscellaneous variety of bacteria. Sweet cream contains many species of bacteria, and among them are some that would produce mischief in the butter if they had a chance to develop. But, in spite of this, the ripening of cream usually produces a satisfactory product. The reason appears to be threefold. First, of the various species of bacteria in milk or cream the majority either produce good results in the butter or have no appreciable effect. Consequently, if care is taken to keep the dairy in good condition, the cream will usually be supplied with bacteria which produce good results, and only under improper conditions will the unfavorable species be abundant. Second, the species which give rise to a good product are probably more vigorous than the others. At all events, the lactic organisms develop more rapidly and more vigorously than any other types; and ordinarily before the other forms of bacteria have had a chance to produce any very unpleasant flavors their further growth is checked by the development of the lactic organisms. The miscellaneous bacteria have a chance to develop slightly, they give rise to very small quantities of decomposition products, and then their further development is checked by the growth of the lactic bacteria. The development of the lactic bacteria must be regarded as the primary factor which checks the development of unfavorable decomposition in the cream and, therefore, prevents the development of improper flavors. Third, the temperature used for ripening cream is one that has been selected because it favors the development of the lactic bacteria at the expense of the other species, so that the lactic bacteria, in the majority of cases, prevent the excessive growth of those forms which might otherwise produce trouble. All of these things together ordinarily give rise to a type of ripening which produces a good-flavored butter. Nevertheless, while the cream ripening usually proceeds in a normal fashion, occasionally abnormal results are obtained. Butter-makers are sometimes troubled by the development of improper flavors and an unsatisfactory type of butter.

Control of Cream Ripening.—The butter-maker has little or no control over the kinds of bacteria which find their way into his cream. This is especially true of a creamery that receives cream from a wide territory; for such a creamery must take the cream that is brought, and this will be filled with bacteria of numerous varieties derived from many sources. If it chances that the cream which reaches the creamery contains the proper species of bacteria, the ripening will go on in a normal fashion and the results obtained will be satisfactory. If it chances, however, that the cream brought to the creamery contains species of bacteria which are unfavorable to the development of proper flavors, the butter is likely to fall off in quality, and no care which the butter-maker may take in the subsequent preparation of his butter can remedy this particular defect.

It is, of course, quite evident that it will be an advantage to have some method of controlling the growth of bacteria in the cream. The revolution which has been produced in the brewing industry by the use of pure yeast cultures has led to the suggestion that a similar control might be possible in cream ripening, which is also a fermentative process, and that the whole process of butter-making would be improved if a method could be devised of controlling the growth of the organisms that produce cream ripening. According to this idea bacteriologists have developed, in the past fifteen years, a process of butter-making which has been spoken of as the use of *pure-culture starters.*

The Use of Starters.—This process was suggested about fourteen years ago by Professor Storch, of Copenhagen, who first conceived the idea of obtaining from cream those species of bacteria which produce the best results, and then furnishing them to the butter-maker for the purpose of cream ripening, somewhat as yeast is furnished to the brewer (229).

The plan suggested by Professor Storch was put into practical use in Denmark. In one sense this plan was not new, having been in use for some time. For many years butter-makers have known that, in winter weather, the cream does not ripen very rapidly, and in order to produce satisfactory ripening in a convenient length of time it has been necessary to hasten it with what was called a **"starter."** A starter was simply a lot of already soured milk or cream. The bacteria present in such cream are already growing under favorable conditions, and, if added to a new lot of cream, hasten the ripening process. The use of such starters was adopted only in cold weather or in the winter season, since cream ripened rapidly enough in warm weather without them. The new feature of the method suggested by Storch was the artificial preparation of such a starter by the use of pure bacterial cultures obtained by bacteriological methods. This method was soon adopted by the Denmark butter-makers and extended through the country. From Denmark the method was adopted in North Germany and distributed itself more or less in various other butter-making countries. Nowhere, however, has this method been used to a very great extent except in the countries of northern Europe. At the present time the use of such cultures is practically universal in Denmark, common in North Germany and occasionally used in other butter-making countries, especially at times when the butter of a creamery shows some of the "faults" which are the results of improper ripening of the cream.

For using this method of controlling cream ripening, it is necessary, first, to obtain a proper culture for the purpose. This involves bacteriological work, and has proved to be extremely difficult, and, at least for some places, quite impossible. To obtain such a culture bacteriologists have naturally had recourse to ripening cream and have selected some type of bacteria that was found to be most common in ripened cream which produced a high-grade butter. There is no difficulty in obtaining from such cream the kinds of bacteria that are present in the greatest abundance. For reasons already pointed out, the organisms that have been obtained in this way and used for this purpose have been, in practically all cases, *lactic organisms,* since they are by far the most abundant in well-ripened cream. Consequently, lactic organisms have been practically always selected as the types of pure cultures to be used for cream ripening. But it is almost certain that the chemical changes occurring in normal cream ripening are not produced by the growth of any one bacterium. It is not to be expected, therefore, that any one species of bacterium can be found which would produce normal ripening with the typical results. Consequently some of the cultures which have been used have been *mixtures* of different species of bacteria, obtained according to the idea that such mixtures would be more liable to produce normal results than any single *pure* culture. Mixtures that have been hitherto used, however, have been purely accidental ones consisting in most cases of such mixtures as would be found by simply drying a mass of ripening cream. Thus far, no practical attempts have been made to prepare artificial mixtures of species for the purpose of giving a normal ripening.

Quite a number of such commercial cultures are upon the market to-day. They are for sale in the chief butter-making countries by dealers in dairy supplies. They go by various names, sometimes by the name of the person who makes them, and again by some commercial name. They differ from each other more or less, and the results obtained by the use of them are slightly variable. In general, however, they are cultures of one species of lactic bacterium, pure cultures, though some are mixtures.

It must be noticed, further, that the problem of a proper culture for cream ripening will be different in different countries. Since the public taste for butter is different in different sections of the world it is evident that no type of ripening, even though satisfactory in one country, will necessarily meet the demands of another. The use of pure cultures has been satisfactory in Denmark and produces a butter which exactly meets the demands of the European public; but the same cultures do not meet the conditions at present existing in the United States. The demand for a highly flavored butter in this country makes it necessary, if pure cultures are to be used for this purpose, that some form of culture should be obtained that will give a stronger flavor than that which is developed in Denmark. Hence it happens that the use of pure cultures may be quite successful in one locality and unsuccessful in a second. But with the possibility of change in the public taste, it is quite possible, or even probable, that the continued use of such cultures would, in time, develop a taste for the peculiar kind of butter which they produce.

Use of Pure Cultures.—In the use of these cultures the first step necessary is that of **building up** the culture. The bacteria culture is furnished to the purchaser in quite small quantity. It is commonly in the form of a powder or a liquid, and although it contains many billions of bacteria, the number is quite insufficient to inoculate properly a large quantity of cream. It is, therefore, necessary to increase the supply. This is usually done by a simple method. A small quantity of cream or milk, perhaps a quart, is sterilized by heating to a temperature of boiling, and then to it is added the culture purchased from the market. The material is covered to keep out the dust, and placed in a moderately warm locality for a couple of days. During this period the bacteria increase in numbers, and there is obtained a quart of culture well impregnated with the bacteria. Then a larger quantity of milk or cream, five or six gallons, is heated to a pasteurizing temperature of about 155° and cooled rapidly. After it has cooled to a temperature of 70-80° the quart of prepared culture is poured into it, stirred thoroughly and the whole set aside, carefully covered up, for a further growth of the microörganisms. The bacteria develop, and in the course of two days there are obtained several gallons of a culture well impregnated with the bacteria in question. This material is used as the "starter" for inoculating the body of the cream which is to be ripened.

This process of building up is, of course, somewhat troublesome. It requires a more or less constant attention on the part of the butter-maker, and has had much to do with preventing the wider extension of the use of pure cultures. For this reason there has been, in recent years, an attempt to put upon the market a form of pure culture which can be used directly in the cream, without this previous building-up process. One or two such commercial products have been introduced to the butter-makers in Europe, the makers claiming that they can be added directly to the cream without any previous cultivation. The tests of such cultures, however, have not been satisfactory. They appear to be made up largely of lactic acid, and the bacteria are not present in sufficient abundance to accomplish very much. At the present time there is no reason for believing that such pure cultures for direct use are of any practical value, and the use of pure cultures at the present time still depends upon this building-up process.

The starter prepared by this building-up process is added to the cream to be ripened, in proportion of about 10%. After the cream has ripened and is ready for churning a few gallons are removed, to be used as a starter for the next day's churning. In this way the starters are carried from day to day for several days. About once a week it is commonly found desirable to build up a new starter from the commercial pure culture.

Natural Starters.—The starters thus prepared are called **pure culture starters,** and though they are widely used, a more common method of accomplishing the same end is by the use of what are known as **natural starters or home starters.** These are prepared by the butter-maker himself, without the necessity of purchasing a commercial product. A natural starter is nothing more than a lot of naturally soured milk or cream obtained under proper conditions. It may be prepared in a variety of ways, but the following will illustrate, in general, the method of making it. A perfectly healthy cow is selected from a cleanly, well-kept dairy. After the under parts of her body are carefully brushed and the udder moistened with a damp cloth, the first few jets of milk from the teats are rejected, and the rest is drawn into a sterilized vessel. This vessel is then covered at once, taken to the dairy, heated to a proper temperature and passed through a separator. The skimmed milk is collected in a sterilized vessel, covered, and set aside to sour. The material sours in the course of a couple of days, and after it has become distinctly acid, but before it has become curdled, it serves as a starter for the cream ripening.

There are, of course, many other methods by which such starters can be prepared, for a natural starter is nothing more than a lot of milk or cream which has soured naturally.

It is quite impossible for the dairyman to be sure that the natural starter which he prepares in this way contains the kind of bacteria that he is most desirous of putting into his cream. He has, indeed, no means of knowing what bacteria are present, or of controlling them. Logically, then, the method of the use of natural starters is incorrect. The practical experience of dairymen shows, however, that the natural starters, as a rule, are quite successful, and produce a ripening of the cream that is satisfactory. But they are not uniform, and occasionally are not reliable. Even though produced under similar conditions, they do not always give rise to the type of ripening that is desired. Such irregularities, however, are comparatively few. The dairyman is interested in the practical results rather than the logic of his method, and so long as he finds that, in the great majority of cases, he can in this way easily obtain a starter that produces satisfactory results, he is quite satisfied with the method. This use of natural starters began years ago for the purpose of hastening the souring during cold weather; but in recent years it has extended more and more, until now it is widely adopted by butter-makers who desire to make a high grade of butter.

When we recognize the great variety of bacteria that may find their way into milk from ordinary sources, we are forced to ask why natural starters should ordinarily be satisfactory. The answer has been already given in the facts regarding the growth of bacteria in milk.

1. The bacteria which commonly find their way into milk are mostly of the types which produce either favorable action upon the cream or no action at all. The probability is, therefore, that any sample of milk obtained under proper conditions will be filled with bacteria, most of which are favorable to the purpose for which the starters are wanted.

2. Soured milk is commonly nearly a pure culture of lactic bacteria. If allowed to sour at a temperature of 60–70° the lactic bacteria rapidly get the upper hand of the other species present, and by the time the milk is distinctly soured it will contain so nearly a pure culture of lactic bacteria that the other species must be regarded as of comparatively little significance. Hence, the natural starter prepared as described will be a quantity of milk filled with typical lactic bacteria in great quantities, commonly a single species of lactic organism, though frequently there may be two or even three such species. It will not always be the same type that has obtained the upper hand, but some lactic bacteria are always in great pre-

ponderance. When such a starter is added to the unpasteurized cream the effect will be almost the same as if a pure culture of some of the commercial products had been added. Such starters allow the miscellaneous species of bacteria already present to grow for a little, but the lactic germs, being so numerous, soon get the upper hand and check the further growth of the miscellaneous microörganisms that might produce trouble in the cream.

The Use of Starters in Pasteurized Cream.—In the use of starters two methods have been adopted. The first is to prepare the cream to be ripened by pasteurization. The cream is heated to about 155–160°, sometimes higher. If the heat is too high a slight cooked taste appears in the butter, which, however, disappears after two weeks. This temperature destroys a large portion of the bacteria that are present in the cream. Not all of the bacteria are destroyed, but such a large proportion, that, when this cream is subsequently inoculated with the several gallons of the starter, the inoculated bacteria are capable of developing, unhindered by the organisms that may have originally been present. The cream is thus ripened through the agency of the inoculated bacteria and not through the agency of the bacteria which were originally present.

This was the original method of using pure cultures, and it has been generally recommended wherever pure cultures have been used. It is clearly the logical method, for if we wish to ripen cream by the agency of a pure culture it would first seem to be necessary to get rid of the bacteria which are present in the cream. The use of pasteurization, therefore, has been adopted almost everywhere that pure cultures have been introduced. In Denmark the method here outlined has been adopted universally through the result of a somewhat recent law passed in that country. Denmark suffers from a very large percentage of tuberculosis among its cattle, this disease having become so abundant as to be a threat to the very existence of the dairy industry. This disease has been distributed over the country by the agency of the central collecting stations. A creamery receiving milk from a wide territory will return skimmed milk to the various farms. No farmer, however, receives back the skimmed milk of his own farm, but an equivalent amount of skimmed milk from the same creamery. If there chances to be any tuberculosis in the neighborhood the infected tuberculous milk will be widely distributed over the territory from the creamery. It is the general belief that this has been one of the chief sources of distribution of this disease. To meet this danger regulations recently passed require that all milk and cream which is brought to a creamery shall be pasteurized at a sufficient temperature before it is returned to the farmers. Thus forced to pasteurize their cream the butter-makers are also forced to use starters, since the cream, after pasteurizing, will not ripen normally without them. For this purpose they find commercial cultures most convenient and Denmark now almost universally adopts the use of pure cultures for the ripening of cream.

The Use of Starters in Unpasteurized Cream.—Pasteurizing cream, which is the essence of the method described, has not been very generally adopted by the butter-makers in America, nor, indeed, has it as yet been very widely adopted by butter-makers outside of northern Europe. Pasteurization is a matter that requires considerable time, trouble and expense. To heat several hundred gallons of cream to a temperature of 160°, and then to cool it rapidly, so as to inoculate it subsequently with pure cultures, is a procedure which involves considerable extra work, and butter-makers hesitate about adopting it unless they see good reason for doing so. The use of pure cultures which depends upon such pasteurization has for this reason not extended widely among butter-making countries.

The desirability of some means of controlling the cream ripening has, however, been clearly recognized. A second plan of using starters has been adopted which does not involve the previous pasteurization of the cream. The method consists of building up the cultures by the method described and then adding it in quite large percentage to unpasteurized cream, allowing the ripening to take place by the combined action of the bacteria originally present and those that were added by the inoculated culture. This method is undoubtedly illogical. The cream which has been brought to the creamery is already filled with bacteria from a variety of sources and sometimes in very great numbers. To inoculate such a lot of cream with bacteria in such a way that the inoculated bacteria shall produce the ripening would clearly require that the cream should previously be treated in such a way as to get rid of the bacteria already present. In the method of use of cultures now considered such a previous treatment is not adopted, but the starters are added at once to the cream already filled with bacteria.

Although this method is theoretically incorrect there are certain reasons for believing that it is not quite so illogical as might at first sight appear. In the first place the majority of species of bacteria present in cream do not succeed in growing very much and do not become very abundant in the cream, provided there be present a sufficient number of lactic bacteria. Such being the case it is quite clear that, if a large culture of vigorous lactic bacteria be added to a lot of cream already filled with miscellaneous species, the addition of the lactic organisms might soon check the growth of the miscellaneous bacteria and prevent their producing the unfavorable decomposition changes which they would produce if they developed abundantly. If the cream were allowed to ripen normally the growth of lactic organisms would, after a time, check the development of the miscellaneous species, but this might not be until after such a large abundance of decomposition products had been produced as to affect injuriously the quality of the butter. The addition of the large quantity of lactic organisms as a starter would check this growth more quickly, and would thus prevent the development of too great an abundance of unfavorable decomposition products. For these reasons it is quite clear that the addition of a culture, even to unpasteurized cream, may be useful in butter-making.

THE VALUE OF ARTIFICIAL STARTERS.

In determining the value of the methods of butter-making above outlined, several distinct problems come up which may be considered in turn.

Pure Culture Starters Compared with Spontaneous Ripening.—Experimental tests of the value of butter made with and without pure cultures have shown that the best quality of butter made with pure cultures is apparently not quite equal to the best quality of butter made by spontaneous ripening. In other words, under the best conditions a better butter may be made from cream which ripened spontaneously than from cream which is ripened by the use of pure cultures. But these exceptionally fine grades of butter are not the ordinary yield, and the result of general experience is that *culture* butter is, on the average, somewhat better than butter that is made in the normal fashion. The explanation of this is simple enough. When butter is made in an experiment station or upon an individual farm special care may be taken to produce cream in the best condition. Under these circumstances a pure culture may be of no advantage. In general dairying, and especially in creameries, all sorts of cream, good and bad, must be accepted. While cultures cannot correct these imperfections they are of some use in counteracting the effect of the miscellaneous organisms in the mixed cream. Under such conditions cultures may produce an improvement, though individual tests do not show the same advantage.

The advantage of the use of cultures is along three distinct lines.

1. Pure cultures enable the butter-maker to handle his cream more uniformly. He can regulate the ripening in such a way that the cream will always be of the proper grade of ripeness at a certain time of day; for experience soon tells him how much culture must be added to the cream in order to make it ready for churning at the particular time of day which is most convenient for his business. The advantage of this uniformity in the conduct of a creamery is sufficient to warrant the use of these starters in many places where, otherwise, they would not be adopted.

2. The use of pure cultures has produced a greater uniformity in the product. Under the ordinary system of butter-making the butter is apt to show fluctuations in quality, sometimes being very good and sometimes falling off in grade for days or weeks. The butter-maker, by pasteurizing cream and subsequently inoculating it, can with certainty depend upon obtaining a product of a uniform grade. The general practice of butter-making in Europe in the last twelve years has warranted the conclusion that the use of pure cultures with pasteurization, puts into the hand of the butter-maker a method of producing an almost constant uniformity in his product. There will be slight variations at different seasons of the year due to variations in the character of the cream, but the irregularities which are common in ordinary dairy practice tend to disappear with the use of pure cultures. This is perhaps the most distinct advantage of the pure-culture method, and is the one which has gradually brought about the introduction of this method of butter-making throughout northern Europe.

3. It is pretty definitely agreed that, on the *average,* the flavor of the butter is somewhat improved by such cultures. It is difficult to obtain any proof of this, owing to the uncertainty in the grading of flavors. The general belief is, how-

ever, that the flavor of the butter is somewhat improved, at least for those markets that demand a clean, mild, acid flavor to the butter and do not desire the more highly flavored product which is characteristic of the American markets.

Pure Cultures Compared with Natural Starters.—A very good natural starter is equal to and sometimes possibly superior to the commercial cultures furnished by bacteriologists. At all events, where the matter has been tested it is found that the results obtained from the use of natural starters, at least in America, rank equal with those obtained from commercial cultures, and in some experiments are somewhat superior. Moreover, the natural culture is inexpensive and has the manifest advantage of being capable of preparation by any ordinary butter-maker who has at his command a clean dairy. On the other hand it requires more time and trouble to prepare a natural starter than it does to use one of the commercial cultures, and in a large creamery the extra care and trouble required for the frequent preparation of natural starters is more than sufficient to compensate for the slight expense of purchasing the commercial products. Moreover, a natural starter cannot be relied upon so strictly as can the commercial culture. In the early years the commercial products were liable to be impure, since bacteriologists had difficulty in furnishing cultures of bacteria that were not contaminated with other undesirable organisms. These difficulties, however, have been overcome and the commercial cultures which may now be purchased can almost always be relied upon as pure. If they are pure cultures their action may always be relied upon as being strictly uniform. Natural starters cannot be thus relied upon. It is true that, in the majority of cases, if the milk is obtained and allowed to sour under proper conditions, the result will be a good, properly soured milk, which will be an ideal natural starter; but it is equally true that sometimes such a result does not appear. Some dairies will be impregnated with bacteria that produce undesirable results. In some dairies the lactic bacteria which are present are gas-producing organisms, and these do not produce so satisfactory a natural starter as the type of lactic bacteria that does not produce gas. Consequently, in the preparation of a natural starter the butter-maker cannot be absolutely confident that the starter will be satisfactory. When it is satisfactory it is probably equal, if not slightly superior to, an artificial starter; but it is not always of a character that can be used.

The question which kind of starter is most practical for use in ordinary butter-making will be answered in each case, to a large extent, according to convenience. In large creameries it will, perhaps, be found less expensive and more satisfactory to use commercial cultures; in small ones, where the dairies are more directly under the control of the butter-maker and close at hand, the use of natural starters will probably continue, and it is doubtful whether commercial cultures will ever be introduced. At all events, in this country natural starters have hitherto proved to be more practical than the commercial cultures. Natural starters are used very widely by butter-makers; commercial cultures are used, as yet, only in a comparatively few isolated cases, although their use is increasing. What the future will be would be rash to predict.

The facts above given indicate that the commercial cultures are never quite equal to the best type of natural starter, and that the butter made by the use of pure cultures is never quite of the high grade of flavor that is found in the best kind of spontaneous ripening. This apparently indicates that the bacteriologists have not, as yet, succeeded in putting into their cultures the proper kind of organisms. If the explanation of cream ripening given above is correct, it is easy to understand why this is true. If the ripening of the cream is a twofold process, involving first the growth of miscellaneous bacteria with a production of flavor and, later, the growth of lactic bacteria and the consequent cessation of the growth of miscellaneous forms, it is quite evident that no form of pure lactic bacteria culture could ever produce a normal ripening, especially if used in pasteurized cream. To produce the normal ripening it would be necessary to inoculate the cream in such a way that the miscellaneous bacteria should grow first and subsequently be replaced by the lactic germs. Such a commercial culture bacteriologists have not yet been able to obtain. Until, therefore, bacteriologists have succeeded in furnishing a culture that will do something besides produce lactic acid, we cannot expect that the pure cultures of the market will ever give a product which is equal in flavor to that developed by spontaneous ripening or perhaps by natural starters.

The Use of Pasteurized Cream Compared with Unpasteurized Cream.—There is a difference of opinion as to whether the better results are obtained by the use of starters in pasteurized or unpasteurized cream. This lack of uniformity of opinion is partly a matter of locality. In experiments which have been carried on in Europe the conclusion has been very general that the butter produced from pasteurized cream with the use of starters is superior to butter produced from unpasteurized cream (203, 226, 228). The pasteurization removes the bacteria which would produce many of the butter "faults," and gives an opportunity for the starter to produce its normal results. Tests that have been made in America, however, do not seem to be so favorable to the pasteurized cream.

Comparative experiments (221) have shown that the flavors produced by unpasteurized cream are commonly somewhat stronger and, therefore, better adapted to the American market than the milder flavors produced from pasteurized cream, for a reason not difficult to understand. Unpasteurized cream is already impregnated with miscellaneous bacteria which are sure to grow and develop flavors. The simple addition to the cream of lactic organisms will, in a few hours, check the growth of these bacteria, but it will not do so immediately, and during these few hours the flavoring products may develop to a considerable extent. Pasteurization of such cream kills the bacteria at the outset, so that the ripening which subsequently took place would be due to the action of the culture (lactic) organisms alone, and these do not produce high flavors.

For these various reasons the method of using starters for controlling the cream ripening, without the use of pasteurization, has been more readily adopted in America than their use with pasteurization, and, indeed, with certain modifications, is very widely adopted by butter-makers who have a desire to make a high quality product. The experimental tests of the comparative value of the butter made from pasteurized and unpasteurized cream have been borne out in the general experience of dairying. The pasteurization of the cream does not seem to produce an improvement in flavor, at least for the American market, and butter made from unpasteurized cream with a starter is certainly not inferior, and perhaps on the whole a trifle superior to the butter made from the same cream pasteurized and subsequently treated in the same manner. For butter designed for export this does not hold. The European public wants a milder flavor than our markets demand and if an American butter-maker wishes to make butter to be exported the best plan is to pasteurize the cream and then inoculate it with 10% of a lactic starter.

GENERAL SUMMARY OF THE VALUE OF STARTERS.

The ripening of cream is the factor in butter-making which most closely concerns the butter flavors. If not controlled, this ripening, although commonly successful, shows irregularities which frequently injure the quality of the butter. If the butter-maker could regulate the condition of each dairy he might be able to depend upon obtaining a proper ripening. But, being unable to do this, his most practical method of controlling the ripening is by the use of starters. Butter-makers, at the present time, who are acquainted with modern methods, recommend the use of some kind of starters for controlling the cream ripening, at least at those seasons of the year when the cream can be obtained by the creamery in a tolerably fresh condition. If the cream is already tolerably sour and nearly ready to curdle, *i. e.*, already ripened, by the time it reaches the creamery, of course the use of a starter is impractical.

Both pure cultures and home starters are useful, and it is difficult to say which is better. The former are less troublesome and more reliable, the latter less expensive. Pure cultures are especially valuable in a creamery at times when trouble is experienced in producing a normal product, for they will frequently remedy the trouble at once. Whether they should be used in pasteurized or unpasteurized cream depends upon the market. For the American market the use of starters in unpasteurized cream has proved fully as satisfactory as their use in pasteurized cream, but for the European market pasteurizing appears to be necessary to produce the desired product.

BACTERIA IN BUTTER.

What becomes of the immense number of bacteria after the churning? Do they have any further part to play in the changes that take place in the butter? A large part of them are removed from the butter with the buttermilk and many more are washed out of the butter by the washing that occurs during the working of the butter. But these processes do not by any means remove them all, and the completed butter still contains great numbers of bacteria. In this butter, however, the bacteria do not find favorable conditions for growth. The butter is very compact, contains only a comparatively small amount of moisture, and into it oxygen cannot readily penetrate. The conditions in the butter are certainly unfavorable to the continued existence of bacteria, for they begin to die very quickly and they diminish with great rapidity. Freshly made butter will contain millions of bacteria per gram, but if the same butter is examined from day to day the number is seen to diminish rapidly. The following figures of a single sample of butter illustrate this rapid decline.

Number of Bacteria per Gram of Butter.

Two hours old	50,000,000
One day old	26,000,000
Four days old	2,000,000
Thirty days old	300,000

This rapid decline of bacteria is found in practically all samples of butter that have been tested and may, therefore, be looked upon as the normal history of the bacteria after the butter is made. It is, of course, evident that a large part of the butter will be consumed before the bacteria have become very greatly reduced, and even butter which has been preserved for many months is found to contain small numbers. The consequence is that in consuming butter the purchaser is always swallowing bacteria in comparatively large numbers.

The salting of butter has a checking action upon bacteria growth and aids in preserving butter from their action. Butter that is made without salting does not keep so readily as salted butter, and the evidence indicates that this is due to the fact that, under these circumstances, bacteria grow more readily.

Certain pathological bacteria may possibly find their way into butter, and can remain alive for some time. The typhoid bacillus and the tuberculosis bacillus are capable of living for many days in butter and this product, therefore, may possibly be a source of these two diseases in the consumer (124). Although this is certainly a theoretical possibility, there is no direct evidence at the present time that such a course of infection ever occurred, no examples having ever been observed where there was even a strong suspicion of the distribution of typhoid or tuberculosis, or any other infectious disease, by means of butter. While, therefore, we must look upon butter as a possible source of the distribution of some infectious diseases, it must be looked upon as a source in regard to which there is still room for doubt. This possible danger, however, has so strongly appealed to European scientists that they have urged the legal requirement that all cream for butter-making should be pasteurized before it is ripened, for the purpose of removing the possibility of the distribution of tuberculosis and other infectious diseases by means of butter made therefrom. The absence of direct evidence of such danger has prevented this suggestion being anywhere received with favor, but it is still being urged.

DETERIORATION OF BUTTER.

Butter does not keep indefinitely, but undergoes deterioration due to several different distinct changes. The first of these is simply the disappearance of the peculiar delicate flavor and aroma which is found in fresh butter. This takes place very rapidly, and even within a few days after the butter is made there is a very appreciable falling off in the flavor of the butter. The explanation of this is, doubtless, that the flavor and aroma are due, in a measure, to volatile products which pass off from the butter with considerable rapidity. This effect, however, is not very appreciable, and the butter, for a considerable time, remains of a high quality, though not quite so delicate in flavor as at first.

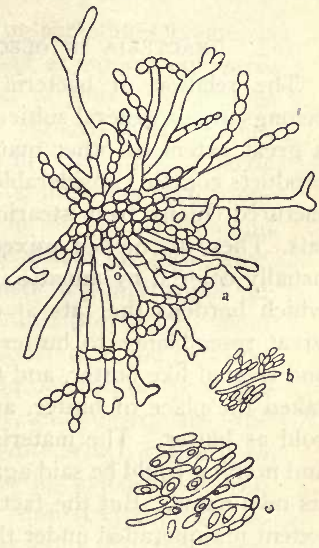
After this there occurs a series of changes that are much slower in their action and which depend upon a variety of conditions. They occur most readily if the butter is kept moderately warm and exposed to the light and the air, whereas if it is kept cool, in the dark and protected from too great an exposure to the air, the butter may be retained for many months without any appreciable deterioration. The changes which are here concerned are profound and very complex. The butter becomes somewhat acid from the development of both volatile and non-volatile acids, the best known of these products being *butyric acid,* although others are found. The butter also has in the end a tendency to become somewhat tallowy; but the most familiar change is the development of *rancidity*. A very large amount of study has been devoted to determining the nature and the cause of the rancidity of butter, which is the most noticeable characteristic of old spoiled butter. While as yet the subject has not been wholly explained, the general phenomena associated with rancidity appear to be somewhat as follows:

Rancidity of Butter.—The rancidity of butter is due to a complicated series of chemical changes. The butter fats are split up chemically, and there is formed a considerable quantity of volatile acids, fixed acids and ethers. The development of butyric acid is a universal phenomenon and has been frequently regarded as the distinctive characteristic of rancidity. Indeed, in the earlier studies on the subject the rancidity has been measured by the amount of butyric acid developed. But it has become evident that, besides the formation of butyric acid, there appears in the butter a peculiar penetrating, unpleasant flavor, and that this rancid taste and smell is not parallel to the production of butyric acid, or, indeed, to the production of acids in general. No chemical tests can yet give an accurate measure of rancidity, because this phenomenon is really one that appeals to the sense of taste, and is not detected by any chemical analysis. The development of rancidity in the butter is dependent, to a large extent, upon the presence of oxygen, for if the butter is kept out of contact with the air the rancidity may be largely prevented, and in the change that takes place in any sample of butter the rancidity is found to progress from the surface toward the interior. The rancidity is also stimulated by the action of light, takes place more readily at high temperatures, and occurs more quickly in butter than in the oleomargarin products.

To what this rancidity is due has been long disputed. It was at first regarded as a purely chemical process of direct oxidation or decomposition of glycerids, but when the significance of bacteria was recognized it was suggested that the development of the rancidity was due to the growth of certain species of bacteria in the butter. Butyric acid was regarded as a necessary factor, and it has long been known that certain milk bacteria produce butyric acid (see Chapter III.). Pasteur first proved this, but later others have shown that this acid is an important by-product of a number of species of milk bacteria. The inference that these bacteria cause rancidity was a natural one. In more recent years rancidity has been distinguished from butyric acid production, and as the significance of enzymes has been recognized it has been claimed that rancidity is due, not to the growth of bacteria in the butter, but rather to the action of certain enzymes that produce a slow chemical change.

A crucial experiment to settle the question seems to be extremely difficult to devise. The most recent work by Jensen (239), however, has rendered it almost certain that bacteria, rather than the presence of any chemical enzymes, must be looked upon as the agents producing this change. This fact has been indicated by a number of lines of experiment. In the first place, the rancidity has been found to be checked by anything that checks the growth of bacteria. Butter can be made from pasteurized cream without the presence of bacteria and is practically sterile. Such butter, if protected from the contamination by bacteria, does not become rancid, though it will become rancid if inoculated with certain species of bacteria. The fact that the rancidity develops from the surface inward is an indication that the trouble is due to bacteria which grow in the butter near the surface, where there is the greatest contact with oxygen. The details of these experiments we cannot consider here, but the evidence is quite conclusive that the rancidity is actually due to the development of bacteria or other allied microörganisms, and Jensen has even pointed out the probable species which produce the rancidity. The three most prominently associated with the phenomena are *Oïdium lactis* (Fig. 26), *Cladosporium butyri* and *B. fluorescens liquefaciens.* Jensen believes that these come from external localities, and he regards the air and the water as the chief sources of the organisms that produce the rancidity of butter. His experiments indicate that if butter can be made without contact with water or air and without the presence of bacteria the phenomenon of rancidity will not occur.

Fig. 26.



a, *Oïdium lactis*, a common milk organism frequently causing the rancidity of butter; *b* and *c*, other bacteria associated with rancidity.

The practical results of these facts are not very great at the present time, for it is probably quite impossible to devise any system of butter-making which shall protect the butter from contamination with these rancidity-producing bacteria. A few practical suggestions are possible. Butter in small masses becomes rancid more readily than butter in large masses, because a larger surface is exposed to the air, and if preserved in large tubs the interior of the butter is, for a long time, protected from the changes which affect its surface.

Butter can also be most easily protected from this change by the use of low temperatures and by keeping in the dark. It is also a fact of practical importance to remember that the presence of salt delays, or may even prevent rancidity. These conclusions have all been reached empirically from entirely independent sources.

BACTERIA IN OLEOMARGARIN PRODUCTS.

The relation of bacteria to the oleomargarin products belongs to our general subject, for, although they are made to a great extent of other materials, most of the modern oleo products contain considerable milk. Oleo products are manufactured chiefly from stearin, lard, cotton-seed oil and other oils. These are melted, mixed together in proper proportions, usually colored by annatto, and then drawn into cold brine which hardens the fats at once into a product that has a great resemblance to butter. The material is then worked and treated like butter, and the result is an article which has taken the place of butter, and been, to a very large extent, sold as butter. The material is a wholesome food product and nothing could be said against it if it were only used under its own name. But the fact that it has to such a very great extent masqueraded under the name of butter, has given rise to the agitation and the laws against its manufacture and sale.

Although the oleo products thus made resemble butter in appearance, they do not resemble it in odor or flavor. In oleo factories different devices are adopted for modifying the flavor of the products so as to make them resemble, as closely as possible, the particular kind of butter which the manufacturer attempts to imitate. The method of imparting the flavors desired is quite varied. It is sometimes done by mixing with the fats a certain quantity of a butter with a tolerably high, strong flavor; but the more common method is by a process which makes use of exactly the same factors which the butter-maker makes use of in manufacturing his butter. The oleo manufacturer, in other words, uses the decomposition products of bacteria growth obtained by a method similar to that which is used in the manufacture of butter. The method is commonly based upon a process somewhat as follows:

A considerable quantity of milk, or sometimes cream, is placed in proper vessels, which are kept at moderately warm temperature and the milk is allowed to sour. Sometimes ordinary milk is simply warmed and allowed to stand until the lactic bacteria grow and sour it; in other cases, a lactic starter is added to the milk. After the milk has been properly soured it is mixed with the fats in the mixing vats and the whole product of the mixture is drawn off into brine. The final hardened product thus consists of a mixture of the various fats, together with a considerable proportion, about one fifth, of real *soured milk*. The souring of the milk has produced in it the same kind of decomposition products which give rise to the flavors of butter, and consequently the oleo product is impregnated with flavors which cause it to resemble butter. The same flavors are imparted to the oleo as would be imparted to butter under similar circumstances.

The oleo manufacturers fully understand that the secret of obtaining a ready market for the oleomargarin is in obtaining the desired flavors in their product, and they are also fully aware of the fact that these flavors are given to them by bacterial action. They, therefore, use the best methods that they can devise for favoring the growth of the desired organisms, for the purpose of developing the flavors. Ordinarily the milk used for the purpose is allowed to sour by normal lactic fermentation; but in recent years some of the oleo manufacturers have not been satisfied with this rather uncertain method, and have endeavored, by scientific means, to control the ripening of their milk through the aid of pure cultures of bacteria. In some cases such manufacturers have even equipped bacteriological laboratories and employed bacteriologists to keep a careful watch of the phenomena of the ripening process in their milk and cream and to prepare the proper bacteria cultures for the manufacture of the desired product. The use of pure cultures by oleo factories has increased somewhat rapidly. The oleo manufacturers have recognized more fully than the butter-makers that the product which they obtain will have a character largely dependent upon whether the proper species of bacteria are at hand. In the last few years manufacturers of oleomargarin have been employing quite extensively pure cultures of bacteria, and bacteriological methods.

It is a suggestive fact that oleo-makers have more fully appreciated the significance of bacteria in their ripening milk than the butter-makers have in the ripening of cream. The reason for this, however, is not difficult to understand. Oleo products made without the addition of proper flavors have been hardly marketable, because their own flavor is so unlike that of butter. The possibility of obtaining a market has, therefore, depended upon the possibility of imparting to their product a butter flavor. To do this they have been ready to expend almost any amount of money, and have learned that one of the factors concerned in producing such a desirable flavor is the action of the proper species of bacteria. Butter-makers, on the other hand, have manufactured butter in very much smaller quantities than any oleo factory, and, moreover, they have been able, as a rule, to obtain a butter flavor without the recourse to artificial bacteriological cultures. The butter-makers have, therefore, generally neglected the phenomena of bacteria in connection with butter-making, whereas the oleo manufacturers have been forced to consider them. If the butter-maker would be as careful in the manipulation of his cream ripening as the oleo manufacturer has found it necessary to be in order to produce flavors in his oleomargarin, it is quite certain that the character of the butter would have assumed a uniformly higher grade with which, in all probability, oleo products could not compete.

Renovated or Process Butter.—This product, which in recent years has come into competition with ordinary butter, is also dependent upon bacterial action for its flavor. It is made from old butter which has become ruined by the development of rancidity or other changes due to long standing. This worthless butter is collected from groceries or markets, and is subsequently melted and washed by a simple process, the result being a pure tasteless butter fat. To make it marketable as butter it must be given a butter flavor, and this is accomplished by a method practically identical with the method used in the manufacture of oleomargarin products. The fat is mixed with a quantity of sour (ripened) milk or cream and then chilled in brine. In the preparation of the sour milk bacilli are at work, and the manufacturers of renovated butter use *starters* just as do the oleomargarin manufacturers. Sometimes they use pure culture and sometimes natural starters, but under all conditions it is bacilli which furnish the flavor and enable them to produce a marketable product. The material manufactured has the appearance and flavor of butter, and is more difficult than oleomargarin to distinguish from legitimate butter.

CHAPTER VIII.

BACTERIA IN CHEESE-MAKING.

In cheese-making, microörganisms are of even more significance than in the manufacture of butter. Butter made from unripened cream will command a market, though a small one, while unripened cheese commands no market at all. The changes which take place in the cream during the ripening are of considerable significance, but the changes that take place in the cheese during its much longer period of ripening are more profound and result in producing in the ripened cheese a series of chemical compounds not present in the original unripened product. The market value of cheese is dependent upon its ripening.

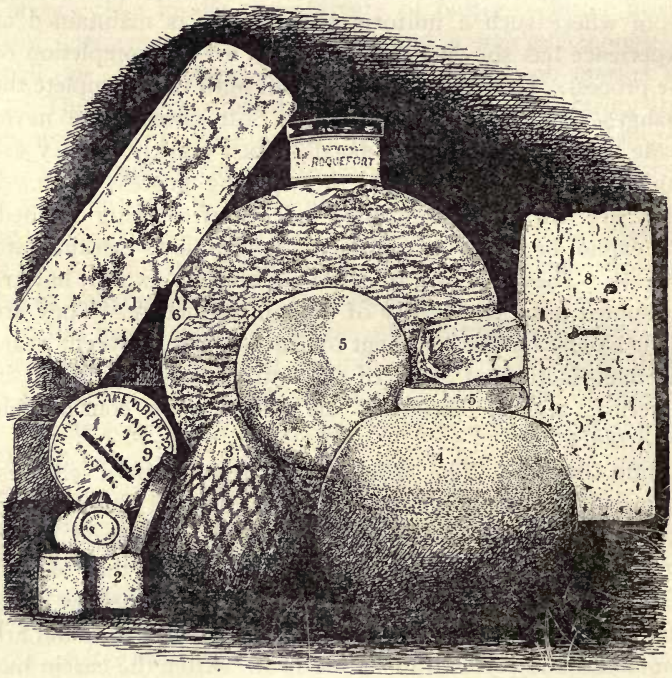
In the manufacture of cheese some means is adopted for precipitating the casein of the milk. This is commonly done by the addition of rennet, although certain forms of cheese, known as sour *milk cheeses,* are made of milk that is simply allowed to sour and curdle, after which the curd can readily be separated from the liquids and made into cheese. The completeness with which the liquids are removed from the curd differs greatly in different types of cheese, and one of the large factors which determine the character of the cheese is the amount of liquid which is allowed to remain in the curd. After the curd is separated from the liquid it is molded into various shapes and then allowed to undergo ripening, a process which results in the final market product.

Soft Cheeses.—There are two quite different types of cheese, depending upon the amount of liquid left in the curd. The first includes the soft cheeses, such as the Limburger, the Camembert (Fig. 24, 9), the Brie cheeses (Fig. 27, 6), etc.

In their manufacture, after the casein has been precipitated, it is commonly simply ladled out of the curdled milk and, after being placed in special shaped forms, is allowed to drain without being subjected to pressure. The forms in which it is placed usually have false bottoms or sides so that a considerable amount of the liquid may drain off in the course of a few days, when the material becomes consistent enough to handle. The cheeses are later placed in a ripening room where such a uniform temperature is maintained as experience has shown to be necessary for the completion of the process. Here the cheeses remain until they complete the changes that constitute **ripening.** Soft cheeses are never made very large, and they usually ripen quickly. They are subject to rapid decay after the ripening process is ended. They are, therefore, consumed quickly after being ripened, and cannot be preserved for such a long time as can the hard cheeses. They are, as a rule, not suitable for export, but are consumed near the locality of their manufacture. They are made chiefly on the continent of Europe, but in recent years the manufacture of some of the soft cheeses has begun in America, and is becoming an industry of considerable importance.

Hard Cheeses.—The manufacture of the hard cheeses, comprising most of the cheeses of the United States (Fig. 27, 8), England, Switzerland (Fig. 28), Holland (Fig. 27, 4), and some other countries, differs from that of soft cheeses chiefly from the fact that they are hardened by pressure which removes a considerable quantity of water, and are sometimes subjected to moderate heat. After the casein has been precipitated by rennet, it is commonly cut into fine bits which allow the liquids or whey to exude from the curd. After this, in the manufacture of some cheeses the whole is subjected to a heat of about 110° F. This heat changes the physical nature of the curd, causing it to become rather tough and elastic. The curd is then removed from the whey, placed in large forms and subjected to heavy pressure. The pressure is increased at intervals for a few days, the cheese being turned from one side to another to make the pressure uniform, and there is thus produced a hard compact mass conforming strictly to the shape of the form in which it has been pressed. These cheeses are then removed from the

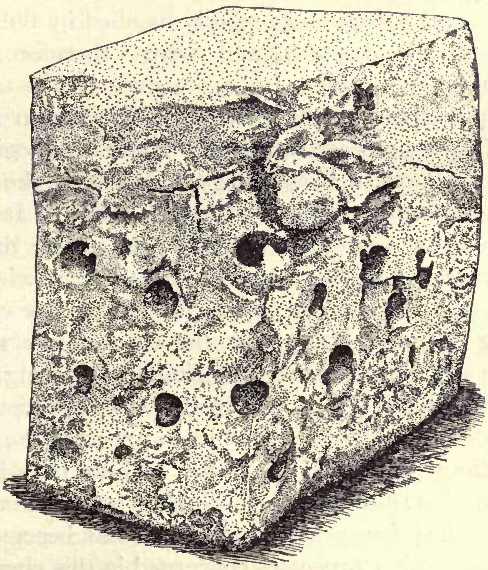
FIG. 27.



A group of cheeses. Nos. 3, 4 and 8 are hard cheeses, the rest are soft cheeses.

forms and left in a ripening room for weeks and sometimes for months to undergo a ripening. The cheeses are not ready for the market for many weeks. Their hard texture makes it possible to manufacture them in large sizes. They are made of many shapes, and form, in the end, a very hard, tough cheese, which does not readily undergo putrefaction and which may be preserved for months, becoming more strongly flavored and improving with age. They can be exported readily, and are carried to all parts of the world and form the chief cheeses of commerce.

FIG. 28.



A Swiss cheese. A hard variety known as Emmenthaler cheese.

In making both hard and soft cheeses, *ripening* is the most essential part of the process. The changes which occur during the ripening are by no means thoroughly understood. They may be best considered under two heads.

1. Changes which increase the solubility of the proteids.
2. Changes which give rise to flavors.

CHANGES AFFECTING THE SOLUBILITY OF THE CASEIN.

The chemical changes that occur in the ripening of cream affect all of its ingredients, but the most significant feature is a modification of the casein. When freshly precipitated, either by acids or by rennet, casein is insoluble in water, and, especially after heating, is a hard, tough mass, difficult of digestion. During the ripening of the cheese this material becomes partly converted into more soluble bodies. The cheese becomes softer, more readily handled by the digestive processes in the stomach, and forms, therefore, a more wholesome food.

In the production of these changes there appear to be two quite different processes at work. Since the beginning of the study of modern bacteriology it has been thought that this ripening was the result of bacteria growth. It was long ago proved that bacteria multiply in the cheese during the ripening, and it was also known that certain species of bacteria found in cheese (the peptonizing class) are capable of producing chemical changes in the casein quite similar to those that occur in the cheese during the ripening. Lastly, it was found that if cheese was treated with antiseptics, or by the action of heat in such a way as to prevent the growth of bacteria, the ripening did not occur. These facts led to the conclusion that the ripening was due to bacteria growth. Within the last few years, however, it has become evident that there are other processes concerned in this cheese ripening besides bacterial action.

Action of Enzymes.—Fresh milk contains a chemical ferment or enzyme known as *galactase* (246). This enzyme is a normal ingredient of milk and, inasmuch as it is present in the milk before bacteria have a chance to grow, it is not produced by the action of bacteria. This galactase has a power of acting upon the casein, producing in it a change similar to, if not identical with, that which occurs in the ripening of cheese. This has been proved by subjecting milk to the action of ether vapor which prevents bacteria growth, but does not prevent the action of the enzyme. Under these circumstances, although bacteria do not develop, a series of chemical changes occur in the casein similar to those of the ripening of cheese. Besides galactase, there is also a second enzyme present in cheese made in the ordinary manner. The rennet, which is commonly used for the curdling of the milk, is obtained from the stomach of young mammals. In the stomach of the same mammals there is always present a second enzyme, *pepsin*. Pepsin also has a digestive action upon casein somewhat similar to that of galactase, forming soluble materials of the insoluble proteids. As ordinarily prepared, rennet will always contain some of this pepsin, which will be added to the cheese.

Cheese thus commonly contains two enzymes capable of slowly changing the nature of the casein. To what extent is the ripening produced by the action of these enzymes and to what extent by the development of bacteria? Although at the present time this question is not fully answered, a somewhat general consensus of opinion appears to have been reached. A brief summary of the conclusion held is as follows:

In the ripening of both hard and soft cheeses enzymes undoubtedly play a part in producing the solubility of the casein, since cheeses will undergo certain parts of the ripening process without the development of microörganisms. On the other hand, it has become evident that the normal ripening of cheese demands, in addition to the enzyme action, the aid of microörganisms. When cheeses are ripened in the presence of chloroform, which prevents bacteria growth, a ripening takes place, but *not a normal one* (250). The invincible conclusion is that the normal ripening requires the development of microörganisms. Moreover, Freudenreich has made cheeses out of milk drawn from a cow under aseptic conditions and containing only a small number of bacteria. Cheeses made from such milk, if they are protected in the whole process of manipulation from the possibility of becoming contaminated with bacteria, will not ripen (268). If, however, the milk in question is inoculated with certain species of lactic bacteria, all other conditions being the same, the cheese ripens normally. These facts show that the ripening of cheese is a double process involving the action of the milk enzymes and also the growth of microörganisms.

Agency of Microörganisms.—The relation of microörganisms to the process of cheese ripening is a subject of immense difficulty. The problems have proved to be complicated and their complete solution will require many more years of study. There are several reasons for this. The ripening is a long process. During the whole of this period the bacteria are growing, and there is a considerable change in the type of bacteria present. Fresh cheese contains quite different types of bacteria from those that are found in an older cheese. This change in species is quite similar to that already seen in butter, but it complicates the problem and makes it quite difficult to determine to what extent any one species found may contribute to the process of the cheese ripening. Secondly, the cheeses which are manufactured in different localities are widely different from each other. The immense variety of cheeses, each with its own character and each with its own flavor, makes it difficult to answer any question as to cheese ripening in general, for each type of cheese is a problem by itself. One experimenter, working with one variety of cheese, will reach a result quite different from that which another experimenter obtains, working with different cheeses. Some kinds of cheese may be ripened more by the enzyme action; others more by the growth of microörganisms. Different students naturally work upon the type of cheeses that are most common in their own locality, and the results that are obtained inevitably differ. Until there can be a large series of studies upon a great variety of cheeses and the whole can be properly compared, it will be impossible to reach general conclusions as to the phenomena of cheese ripening, or make any general statements as to the comparative significance of enzymes and microörganisms.

Nevertheless many facts demonstrate the importance of microörganisms in the ripening. There is a marked increase in bacteria in the cheese during the ripening. The growth of bacteria during cheese ripening, however, is much slower than it is during cream ripening. Cheese contains only a comparatively small amount of water, and is a compact, usually somewhat hard, dense mass, from the interior of which oxygen is, to a large extent, excluded. Under these conditions the growth of bacteria is much slower than in cream. Nevertheless, they do develop abundantly. In the different types of cheeses the growth of the bacteria is not always the same. In some cheeses there is, for a day or two, a marked decline in number of bacteria, followed by a rapid rise, continuing for some days. This is followed eventually by a slow decline, until the number becomes very small. In other cases no initial decline has been found, the bacteria from the beginning multiplying rapidly, rising to a high number in the course of three to four days, and then declining, at first rapidly, and then much more slowly, until, in the end, the number is quite small. In the common Cheddar cheese, for example, the number may, in the course of three days, rise to about 450,000,000 per gram, a number which is about as high as it is in some samples of ripened cream. This number later falls off and eventually reaches a million or so per gram. Usually, however, the number does not rise as high as this, and even these numbers are less than are found in cream which has ripened a couple of days.

In cheese ripening, as in cream ripening, it is chiefly the lactic bacteria that grow (267). The enzyme-forming bacteria are apt to be abundant in the fresh cheese and these organisms, since they digest casein, have been supposed to be the cause of the ripening. But during the ripening period these organisms, instead of multiplying, constantly decline in numbers, never becoming more numerous than at the outset. In nearly all cases they disappear in the course of a comparatively few days. On the other hand, the lactic bacteria, chiefly the first of our types (page 65), increase prodigiously in the first few days, though they afterward decline rapidly, and later more slowly. These facts would seem to indicate that the lactic organisms are more intimately concerned in the ripening phenomena than the enzyme-producing bacteria, at least in the case of hard cheeses.

There has been considerable dispute among bacteriologists as to the relation of microörganisms to the cheese ripening. When the subject was first discussed by Duclaux it was assumed that the peptonizing bacteria were chiefly concerned, inasmuch as these organisms were known to produce a digestion of the casein. Many others adopted Duclaux's position, and, up to very recent times, it has been assumed that this conclusion was correct. But the fact that the peptonizing bacteria constantly *decline* in numbers while the acid bacteria *increase* very greatly, has led recently to the belief that the lactic organisms are concerned in the phenomena rather than the peptonizing types. An objection to this view has been the fact that the lactic bacteria have not been known to produce a digestion of the casein, such as characterizes cheese ripening. Good reasons have, however, been given for believing that, under proper conditions, the lactic organisms may produce a peptonizing of the casein, and recent experiments have practically demonstrated the conclusion that, to the lactic, rather than the peptonizing, organisms is to be attributed the ripening of, at least, the Swiss cheeses.

There is left little room to doubt that, in the case of the hard cheeses, the ripening must be attributed to the action, firstly, of the enzymes, and, secondly, of the lactic bacteria. Without both the normal ripening does not take place. This conclusion, although it has been more or less vigorously disputed, has been so thoroughly tested by the experiments of various bacteriologists in recent years that it stands upon a firm foundation, and must be accepted as almost certainly true. It has not yet been determined what part of the chemical changes must be attributed to the enzymes and what part to the microörganisms, but simply that the ripening, as it normally occurs, is a process in which the enzymes and the bacteria both play a part.

The facts here referred to apply chiefly to the hard cheeses, such as the Swiss and the Cheddar cheeses. The relation of these problems to the ripening of the soft cheeses has not been so carefully studied. It has appeared, however, from recent work, that the chemical changes in the ripening of the soft cheeses are probably more largely to be attributed to the development of microörganisms and less to the action of the enzyme, than are those of the hard cheeses (251).

PRODUCTION OF FLAVOR.

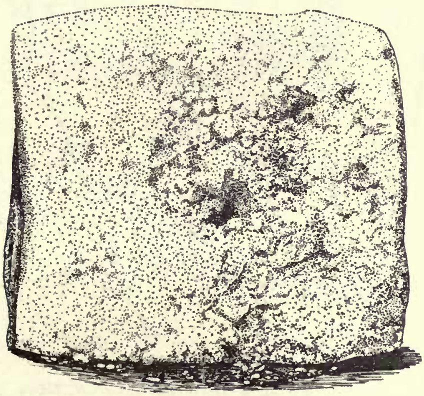
The agency of microörganisms in producing the *flavor* of the cheese is probably of more importance than in the development of the chemical changes. Cheese is a popular article of food, not so much because it is nutritious—although it is one of the very best of foods—as because of the flavor which it possesses. The popularity of cheese among so many races of men is due to the fact that its strong flavor enables it to give an appetizing taste to a variety of coarse, tasteless foods. Physiologists find no food value in flavors, but they realize perfectly well that the flavor is of the greatest significance in diet, inasmuch as it is a powerful stimulation to the digestive functions. A diet of tasteless food cannot be long digested and assimilated. The high flavors of cheese are a priceless boon to the poorer classes, because they enable them, at a small expense, to furnish the necessary flavor for their plain fare. Even the coarsest bit of dry bread may be made palatable and digestible by a little cheese flavor to give it relish. The source and origin of these flavors is, therefore, a matter of great significance.

While the chemical changes which concern the digestibility of the casein may be due largely to the enzymes present in the milk there is at present no reason for believing that these enzymes are the primary causes of the flavors. So far as known, these enzymes do not develop flavors, and certainly they are quite incapable of developing the numerous types of flavors that characterize the large variety of cheeses. The flavors in the ripened cheese are probably due, in large degree, to the action of microörganisms. The high flavors and tastes of decomposition products are well known, and decomposition products of the microörganisms growing in the ripening cheese give the cheese its peculiar flavors. We need simply to remember that bacteria are the only known cause of putrefaction, to be perfectly well convinced that the source of the peculiar flavors and odors of the Limburger cheese must be due to microörganisms, and, although the putrefactive odor is not so prominent in other cheeses, the same general nature is recognized in the whole series of cheese flavors.

Soft Cheeses.—The relation of microörganisms to the ripening of cheeses is very different in the soft and hard cheeses, although in both they play a part. Other microorganisms besides bacteria are concerned in the ripening, particularly of the soft cheeses. In some kinds of cheeses molds are more significant than bacteria; in other cases yeasts may be concerned; while in others bacteria are probably the chief agents.

The importance of microörganisms in cheese ripening may be illustrated by a brief description of the method of manufacture of the Roquefort cheese (Fig. 27, 1). This familiar cheese is filled with green spots, and the peculiar piquant taste of the cheese is associated with these green masses. A microscopic study shows that these green spots are chiefly spores of a common species of mold (*Penicillium glaucum*, Fig. 1), the species perhaps being identical with the common blue mold that grows on bread. The method of manufacture of Roquefort cheese is designed to stimulate the growth of these molds. The first step in its manufacture is to place slices of bread upon shelves in a damp, musty room, and leave them until they have become completely overgrown with a dense mass of mold. The moldy masses of bread are then dried and subsequently ground into a

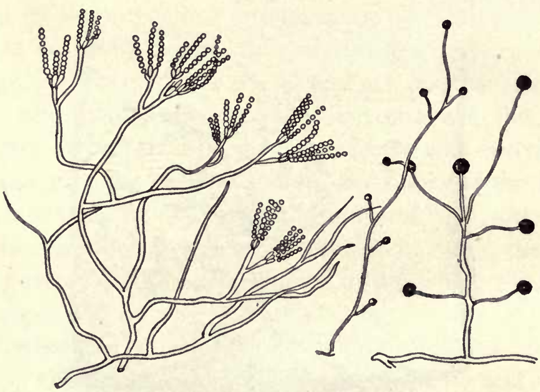
FIG. 29.



Stilton cheese.

powder. In making the cheese, after precipitating the curd by means of rennet, it is mixed with a considerable quantity of this ground, moldy, bread powder and set aside to ripen. In order to facilitate the growth of the molds oxygen is necessary, and to furnish oxygen the cheese is sometimes pricked full of holes by a special machine provided with large numbers of fine needles. This allows air to enter and, when the cheese is placed at a proper temperature, the molds, which are thus brought in condition for growth, develop rapidly and in time the whole cheese becomes filled with them. The development of the molds gives presently the peculiar taste to the Roquefort cheese. We thus have here an example where the flavor of the product is due primarily to the growth of molds. A somewhat similar process is adopted in the manufacture of the Stilton cheese (Fig. 29), although details of the preparation are slightly different. Within recent years a common Danish cheese, known as Gammelost, has been found to be ripened by the growth of molds (285). Two or three species have been separated

FIG. 30.



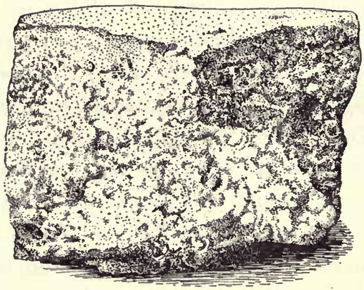
Molds concerned in ripening of Gammelost cheese. (*Johan-Olsen.*)

from this cheese (Fig. 30). They have been cultivated by laboratory methods and used for artificially inoculating cheeses, with the result that a high quality of a marketable product has been produced, thus making it evident that here is a third type of cheese, the essential nature of which is dependent upon the growth of molds. The same is true of Gorgonzola cheese (Fig. 31).

Not all the soft cheeses, however, are ripened by the growth of molds; indeed, it is possible to divide the soft cheeses into three types, which are not, however, rigidly separated from each other. They are as follows:

1. The cheeses in which the ripening is due primarily, or wholly, to the growth of molds which grow throughout the entire cheese. These include Roquefort, Gorgonzola, Stilton and Gammelost.

Fig. 31.



Gorgonzola cheese.

2. Cheeses in which molds and bacteria both are concerned in the ripening process, the molds usually growing first on the surface and extending somewhat into the interior. These include the Brie cheeses (Fig. 25, 6), and some others.

3. Cheeses in which bacteria alone are concerned in the ripening. In these cheeses the ripening occurs chiefly from the surface and extends toward the inside. It includes the Limburger, Camembert (Fig. 25, 9), the Backstein cheeses, and some others.

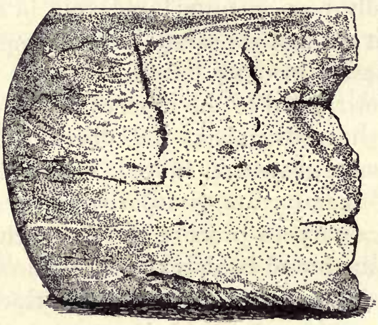
No general description can be given of the method of manufacture of these various types of cheese, because each kind of cheese is made in a special way. The methods of manufacture have all been determined empirically without the cheese-maker having any knowledge of the reasons for the peculiar effects that take place in the cheese. Some of the cheeses are made from whole milk; some of them from part whole milk and part skim milk; some from milk of sheep or goats; some of them are salted, and some not salted; some are partly deprived of water by draining, while in other cases the curd is simply ladled out of the curdled milk; some are ripened at moderately high temperature, some at low temperature, and some are ripened for a while at one temperature and later at a different temperature, and so on. The methods that are devised vary as widely as the different types of cheese. In general the soft curd, without very much draining, is taken from the curdling vat and placed in forms for shaping. These forms, being used over and over again, become thoroughly impregnated with the spores of molds, and the cheeses, while they are shaping in the forms, are at the same time inoculated with the molds. After they have assumed a proper consistency they are then commonly placed in ripening rooms. Here the molds grow luxuriantly over the cheese and in time cover it perhaps completely. The growth is allowed to continue for a time, determined by experience, and then the cheeses are frequently placed in a second ripening room where the temperature and other conditions are such as to check the growth of molds and encourage the development of bacteria. Now a new series of changes takes place, due to the development of bacteria.

The details of the ripening of the different kinds of cheeses are hardly understood in any single case. The ripening of the *Roquefort* cheese is perhaps as fully comprehended as any; but here, although we are familiar with the fact that molds are the chief agents in producing the flavors, we are quite in ignorance of the chemical changes which are produced. *Camembert* cheese is ripened by two bacilli (263), one a lactic species which at first grows only in the center of the cheese, and the second a peptonizing species which grows at the outside, gradually extending towards the center, checking the action of the lactic bacilli. The cheese is fully ripened when the influence of the peptonizing species reaches the center. It is necessary that both species should act together to produce the result. *Limburger* cheese, in the same way, is produced by the combined action of two species of bacilli. *Brie* cheese is said also to be ripened by the combined action of *Penicillium album* and a bacillus of the lactic type. In regard to the long list of other soft cheeses little is known, and we must be content with the general knowledge that the ripening is due in part to the action of chemical enzymes, but in large degree to the development of microörganisms stimulated into growth by the conditions under which the cheese is ripened. Each type of soft cheese must be studied as an individual problem.

The manufacture of these cheeses has hitherto been based upon purely empirical methods, and each of these numerous kinds has usually been manufactured only in the particular locality where it has first appeared. The attempt to manufacture similar cheeses in other localities is only partially successful, a fact attributed to the absence in the new localities of the proper microörganisms for developing the typical ripening. In some instances, indeed, in establishing a new factory for the manufacture of a particular brand of cheese, it has been necessary to procure the fresh cheeses from a factory where the brand has previously been made, and rub them over the shelves and the walls of the ripening room in the new factory, the purpose being to inoculate the new factory with the types of microörganisms that are needed for the proper ripening, and which are abundant in the older factory, though absent from the new one.

The soft cheeses have their home in continental Europe and have not been manufactured very widely elsewhere. There is no reason, however, why any of these soft cheeses could not be perfectly well manufactured in this country, provided their manufacture could be properly studied in their own home and the organisms discovered, transplanted and developed in new factories. Patient study will no doubt in time disclose the peculiar organisms which produce the ripening of most of these types of cheese, and after these have been determined there should be no difficulty in establishing the manufacture of any kind of cheeses in any one locality. Here is an industry ready for development, but its proper development will require, not only a practical knowledge of the manufacture of these brands of cheeses in their own homes, but also a bacteriological study of the microörganisms present in the original localities, and their transfer and cultivation in the new localities. What may develop in this line in the future can hardly be stated, but there seems to be nothing in the way of the manufacture of any type of soft cheese as soon as the problems are properly studied.

FIG. 32.



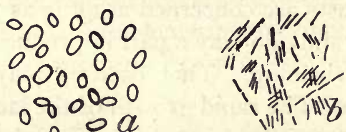
Parmesan cheese.

Hard Cheeses.—We know even less in regard to the ripening of the hard cheeses. The general phenomenon is somewhat the same. The cheese becomes converted into partly digested products and develops certain flavors. The ripening, however, takes place much more slowly than in the soft cheeses, several weeks or even months being required. The final product is quite different from the soft cheese, not only in its texture, but in its flavor (Fig. 32). In the ripening, moreover, it is quite certain that the enzymes play a part of considerable importance, probably a greater part than they do in the ripening of the soft cheeses; but even in the hard cheeses enzymes alone are incapable of producing a typical ripening. They are aided by the development of microorganisms and the typical ripening is thus a twofold process.

It has hitherto been impossible to determine with absolute certainty what kind of microörganisms are concerned in the development of the peculiar character of the hard cheeses. Molds are excluded, since the cheese is so hard that molds cannot grow in its center, and it is commonly salted so highly upon the outside that they are prevented from growing on its surface. Whether yeasts are concerned at all is as yet unknown, although they certainly do play a part in producing certain kinds of abnormal ripening. That bacteria play a part in the ripening is evident. The rapid growth of the lactic bacteria and the disappearance of the peptonizing forms have quite strongly suggested that the former must be regarded as the chief organisms in the process, and most recent experiments have indicated that the lactic organisms certainly do play a very important part in the ripening. Cheese made from "aseptic milk" will not ripen, but when inoculated with certain species of lactic bacteria they ripen in a perfectly normal fashion. Cheese made from pasteurized milk will not ripen, but when inoculated with soured milk, which is nearly a pure culture of lactic bacteria, it ripens in a perfectly normal fashion.

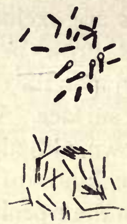
Of the origin of the flavors of the hard cheeses practically nothing can as yet be said. The lactic organisms have not been shown to produce these peculiar flavors. There are bacteria which laboratory experiments have shown are capable of producing the flavors recognized in various types of cheese (Figs. 33 and 34). These organisms, however, are not yet known to develop in cheeses, and it is by no means certain that they have anything to do with the flavors in the cheese. In short, the development of flavors in hard cheeses is as yet an unsolved problem. They are probably not due to the action of the enzyme, and must therefore be attributed to the action of microörganisms; but what kind of microörganisms produce them, and under what conditions they may be most satisfactorily developed are problems that bacteriologists have not settled, and upon which they are now assiduously working.

FIG. 33.



Bacteria producing cheese flavors. (*Conn.*)

FIG. 34.



Bacteria isolated from cheese and supposed to contribute to its ripening. (*Weigmann.*)

The ripening of cheeses thus appears to be an extremely complicated phenomenon. It is due to the formation of new chemical products, formed partly by enzymes and partly by microörganisms. Two enzymes are concerned, pepsin and galactase, and many kinds of microörganisms. Sometimes molds and sometimes bacteria are the most significant. The soft cheeses are ripened more by molds and miscellaneous bacteria, while the hard cheeses more under the influence of lactic bacteria.

Ripening at Low Temperatures.—Reference must be made to the ripening of cheeses at low temperatures which has been recently discussed. Babcock and Russell (248) have shown that American Cheddar cheeses may be ripened at temperatures only a few degrees above freezing, and this observation has been confirmed by others. The ripening, under these conditions, takes a much longer time than at ordinary temperatures, although it may be hastened by the use of a large quantity of rennet. The cheeses are of a milder flavor than when ripened at a higher temperature. Apparently this ripening is due chiefly to enzymes. It has been shown by Freudenreich (249) that Emnenthaler cheese, which has a more pronounced flavor of putrefaction, will ripen at these low temperatures well enough so far as concerns the changes due to enzymes, but it does not develop into a normal production because of a lack of a typical flavor. This is due to the failure of the bacilli to grow at these low temperatures. That enzyme action should go on under these conditions, although somewhat slowly, is not surprising, and it is certain also that some bacilli grow also at the temperature of freezing water. Both actions may, therefore, be concerned. Not enough is known concerning this subject as yet to warrant any positive statements as to ripening under these conditions. It is certain that Cheddar cheese may be ripened at low temperatures, but as yet we are uncertain through what agency, and we do not know whether other types of cheeses can be treated in the same way. If this process should prove to be practical it will be useful in many respects, since it will enable the cheese-maker to have his cheeses ready for market at any season of the year.

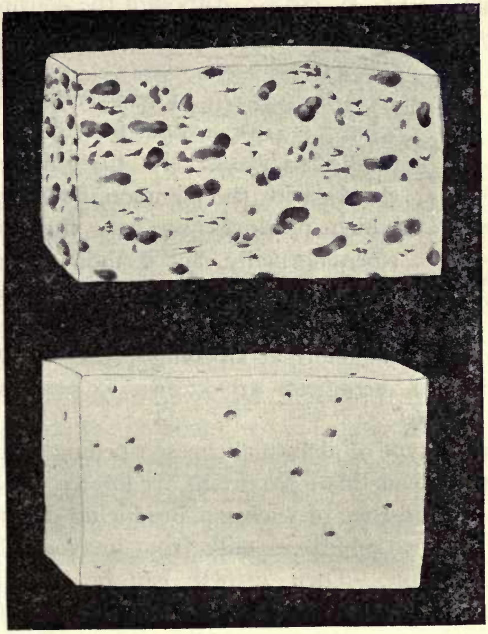
It is even more surprising to find that cheeses may be ripened at temperatures below freezing; but this has also been demonstrated. The ripening is not normal, however. The texture is soggy and crumbly, and the flavors do not develop. It is difficult to understand how chemical changes can take place at these low temperatures under the influence of either enzymes or bacteria. The whole phenomenon indicates that the physical breaking down of the casein is a phenomenon independent of the production of flavors, the former being, likely, an enzyme action, the latter probably due to microorganisms.

ABNORMAL CHEESE RIPENING.

The close relation of bacteria to cheese is emphasized by certain types of abnormal ripening. Our knowledge in regard to various types of improperly ripened cheeses is somewhat greater than our knowledge of the ordinary ripening. Cheeses that are set to ripen do not, by any means, produce a uniform product. There is always a certain character which is the type desired in the product, but a considerable proportion of the cheeses fail to come up to the proper standard, especially in flavor. It is sometimes stated that half of the cheeses are injured in ripening. This does not mean that this large proportion is so badly ripened that it is not marketable, but that a large proportion of the cheeses fails to come up to the highest grade. Some of the abnormal cheeses are only slightly "off" in flavor, and will sell as palatable cheeses in the market, while others are so decidedly bad that they are valueless. It is, of course, the desire of the cheese-maker to reduce the proportion of these abnormally ripened cheeses as far as possible, and the bacteriologist's endeavor to enable him to control the ripening process so that he may obtain a uniform product. It is not possible to classify all of these types of abnormally ripened cheeses; even the cheese-maker who is most familiar with them cannot do so. There are, however, some prominent types that are distinct and easily defined, and in regard to quite a number of these the agent which produces the abnormal results is known. Some of these are of sufficient practical importance to require a brief consideration.

Inflated Cheese.—Of the various types of abnormal cheeses there is none better known than that which is called "inflated" or "swelled" cheese. This is characterized by the development in the cheese of large amounts of gas, and the gas accumulating during the ripening causes the cheese to become swelled and filled with cavities (Fig. 35). The amount of the swelling and the size of the cavities is by no means constant. Sometimes there will be a few large

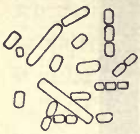
FIG. 35.



Two pieces of cheese curd ripened by different species of lactic bacteria. The upper one would produce a swelled cheese, the lower one a normal, cheese and is ripened with *B. lactis acidi.*

cavities, sometimes an immense number of small ones; sometimes the trouble may be so slight as not to interfere with the value of the cheese, and, indeed, certain types of cheeses are always characterized by the presence of such holes (Fig. 28). In other cases, the development of these cavities is so great as to spoil the product, particularly when the gases and other products developed at the same time give the cheese unpleasant tastes. There is no doubt that this trouble is due to the development of certain species of bacteria in the cheese. We have learned in an earlier chapter that among the lactic bacteria there is one type—of which *B. lactis aërogenes* is an illustration—that has the character of producing large quantities of gas (CO_2, H, etc.). Although the souring of milk is not commonly produced by this group of lactic bacteria, they occasionally become very abundant. It is this group of gas-producing bacteria which is responsible for this trouble of swelled cheeses. If the milk is filled chiefly with the common *Bact. lactis acidi,* which produces no gas, the cheese will ripen normally, but if there chances to be large numbers of the gas-producing group, the cheese is very liable to develop gas and undergo this abnormal ripening process. Other bacteria besides *B. aërogenes* are known to produce swelled cheese (Fig. 36).

FIG. 36.



A bacillus causing swelled cheese (*B. Shafferi*).

There is no type of abnormal cheese ripening that produces so much trouble as this. No means are known for absolutely avoiding the presence of such gas-producing organisms, for they are liable to appear in milk from unavoidable sources of contamination. Their growth can be, in a measure, checked by the addition of salt to the milk, and this is one of the means suggested for avoiding this type of abnormal ripening.

A more widely used plan of avoiding this trouble is by testing the milk of individual patrons, and excluding any which is, in the slightest degree, suspicious. This is done by what is called a **fermentation test,** and is based upon the fact that, at a high temperature, these gas-producing organisms grow rapidly. It is conducted essentially as follows: A small sample of the milk from each patron who furnishes milk to a cheese factory is placed in a vial, warmed up to a temperature of 90° F. and allowed to remain at this temperature for some six hours. At this high temperature the gas-producing organisms develop rapidly, as well as most other organisms which are liable to produce bad flavors. At the end of six hours the samples are examined, and if it is found that the milk has soured and curdled into a smooth hard curd, without improper odors or a considerable production of gas bubbles, the milk is regarded as normal and used unhesitatingly. If, however, any sample of milk should be found to be filled with bubbles of gas, or if it should show any unpleasant and strongly penetrating odors, the milk is regarded as suspicious, and should be excluded from the milk which is to be manufactured into cheese. It is found that milk which sours and curdles into a hard, smooth curd, may be regarded as quite sure to make a type of cheese which will not become unduly swelled, while those samples in which gas bubbles appear in great abundance in this fermentation test are suspicious and must be excluded if the cheese-maker wants to avoid the possibility of obtaining inflated cheese.

An improvement over this is the so-called **curd test** (256). In this test small jars are half filled with each milk to be tested, heated to 98°C. and rennet is added. The curd formed is cut to pieces and the whey allowed to drain off. The curd is then kept at a warm temperature to allow the bacteria to grow. The character of the curd, after a few hours, makes it possible to determine whether the milk contained too many gas-forming bacteria, which show their presence by filling the curd full of holes, as shown in Fig. 35. The application of this test has proved to be of practical value to cheese-makers in locating and remedying the cause of imperfections in cheese ripening.

Finally, there have been some successful attempts at avoiding this difficulty by inoculating the milk which is to be used for cheese-making with a satisfactory culture of lactic bacteria, the proper species of lactic bacteria being capable of developing the proper ripening of the cheese and of preventing the growth of undesirable bacteria.

Other Types of Abnormal Ripening.—There are no other types of abnormal ripening that produce so much trouble as swelled cheese, but a few others may be mentioned. Occasionally cheese develops such a strong **bitter taste** as to be practically worthless. The fact that bacteria have been proved capable of developing a bitter taste in the milk has suggested that they are also the cause of bitter cheese. A few instances only have been studied sufficiently to disclose the cause. In one case the trouble was found to be produced by a definite species of bacterium closely related to one previously found in bitter milk (266). The organism, named *M. casei amari,* developed a bitter taste in milk in two days, and when inoculated into milk which was subsequently made into cheese developed the typical character of the bitter cheese. In another series of cases (273) a bitterness was proved to be produced by a yeast which was widely distributed and found its way into the milk cans which were left standing open by roadsides, or under trees or near barnyards. Sterilizing of the cans and utensils, greater care in handling the milk, and increased cleanliness are the only efficient remedies for such a condition.

A common fault of cheese ripening is a **putrefaction** of the product. This trouble occurs especially in soft cheeses, doubtless because of the larger amount of water. It seems strange that putrefaction does not occur in the soft cheeses more readily than it does, for these cheeses are moist and would seem to offer most excellent material for the growth of putrefactive organisms. Under the conditions of ordinary ripening putrefaction is checked and is not noticeable; but sometimes the cheese, instead of ripening normally, decays into a soft, vile-smelling mass of slime. This is due, doubtless, to the development of bacteria, inasmuch as bacteria are the only known cause of such putrefactive changes. The putrefaction of the soft cheeses is more likely to occur if the cheese is ripened at a low temperature; but why it occurs in some cheeses and not in others, and to what distinct micro-organism it is due, have not yet been ascertained.

Some forms of abnormal cheese ripening are characterized by the appearance of colored spots in the cheese. **Red spots** or **rusty spots** are quite common, occurring chiefly upon the surface of the cheese. **Black spots** and **blue spots** are also sometimes found. The red and blue spots are produced by bacteria which are known; the black spots are caused by a mold-like organism.

Certain forms of cheeses sometimes develop a **sweet, sugary taste,** which is foreign to their normal flavor and injures their marketable product. This is especially true of Neufchatel cheese, and it has been proved to be due to the development of a yeast in the cheese. Other foreign flavors are occasionally developed, as well as various odors; but little is known in regard to them, and the cause of the particular flavors has not yet been demonstrated (271).

It is not our purpose to proceed further into the consideration of the various abnormal forms of cheese ripening. The list is a long one, but beyond the few that have been mentioned, hardly any of them have been sufficiently studied to enable us with accuracy to state their cause, and in a few cases only has it been possible to suggest any remedy. The general remedy for all dairy troubles may be recommended for any cheese factory that has any trouble of any of these kinds. Cleanliness in cheese-making is apparently as requisite as cleanliness in dairying in general. In actual practice it has been found that some of these troubles may be quite allayed by careful disinfection of the cheese factory and all the utensils used therein, and then by giving attention to methods of cleanliness in the dairies that furnish the milk. The improper ripening of cheeses is probably, in most, if not in all cases, due to the presence of improper species of microörganisms, and to the stimulation of their growth by the conditions of the ripening. To find the exact source of the organisms which produce the trouble should always be the first aim in treating such troubles, although this may frequently be difficult. No effective remedy can be applied until this source is determined.

PRACTICAL APPLICATIONS OF BACTERIOLOGY TO CHEESE-MAKING.

Up to the present time the practical application of bacteriology to cheese-making has not been very great, in spite of the fact that the product of the cheese-maker is more distinctly dependent upon the action of bacteria than any other one of the dairy products. The difficulty has been the extreme complexity of the problems, and the fact that each type of cheese is a problem that must be treated in its own peculiar way. Various attempts have been made to use pure cultures in the ripening of cheeses, for the purpose of increasing the uniformity in this process. In some cases the result has been successful; in other cases less satisfactory.

One important application of bacteria cultures in cheese ripening is the use of slimy whey in making *Edam* cheese. This well-known cheese (Fig. 27) is made in great quantities throughout Holland, and in recent years there has been adopted a method of inoculating the milk with a bacteria culture for the purpose of hastening the ripening process and making it more uniform. Some years ago it was found that, by the use of a certain type of fermented milk, known as **slimy whey,** the ripening process might be somewhat modified. Slimy whey is a fermented milk or whey which has become slimy. Slimy whey is by no means a pure culture of any bacterium, but a miscellaneous mixture in which the slimy organism, a streptococcus, is, however, always abundant. The value of slimy whey was discovered by cheesemakers before bacteriologists knew anything about it, and the knowledge of the bacteria concerned was obtained long after the material was in common use. This whey is cultivated something as yeast was in earlier years, and is carried from farm to farm. A quantity added to the milk before it is made into cheese is found to have a noticeable effect upon the ripening. The ripening is hastened, the inoculated cheeses being ready for market in about a third less time than other cheeses not thus inoculated. The ripening is also more uniform. There are smaller numbers of abnormal ripened cheeses than if the cheese is allowed to ripen without such inoculation. The character of the cheese is slightly inferior to the best type of cheese ripened without such inoculation, and the cheeses do not keep quite so well and are less favorable for export. In spite of these disadvantages, the uniformity of the product has made the use of slimy whey very popular in Holland, and its use has extended until quite a large proportion of the cheeses of that country (at least one third) are now manufactured by this method of artificial ripening.

In the manufacture of other types of cheese also the use of pure cultures of microörganisms is being adopted and is slowly extending. The immense financial interests in the cheese industry hold out great promises to any one who can successfully discover a means of preventing the irregularities of the cheese ripening and bring the process under strict control. But great obstacles have appeared in the way of practical results. In the first place, even after the proper microorganisms have been discovered, in order to insure their proper action upon milk, it would seem that there must be some means of depriving the milk of the bacteria already present. This has been accomplished in the ripening of cream by the simple process of pasteurization. In the manufacture of cheeses, however, pasteurization presents a serious difficulty. Milk which has been pasteurized and subsequently made into cheese does not ripen normally, pasteurization appearing to have some influence upon the milk which prevents it undergoing the normal changes. The pasteurization destroys or injures the enzymes present in the milk, and inasmuch as the ripening is produced in considerable degree by the action of these enzymes, it is easy to understand that cheese made from pasteurized milk would not normally ripen.

Nevertheless, the work of most recent times has given more promise of success in these lines. It has been found that certain of the *soft* cheeses and some of the sour milk cheeses can be made in a perfectly normal way by pasteurizing the milk and subsequently inoculating it, either with pure cultures of certain microörganisms or with soured milk, or with freshly made cheese. Such pasteurized milk cheeses undergo a normal ripening. Moreover, in some of the *hard* cheeses more recent experiments have indicated that a proper ripening can be produced by the agency of pure cultures added to the pasteurized cream (270, 284). But, nevertheless, pasteurization does injure the enzymes in the milk and this makes it uncertain whether it will ever be possible to treat milk in this manner and be sure subsequently of a normal ripening by the simple addition of pure cultures of bacteria.

But in cheese-making, as in butter-making, it is becoming apparent that pure cultures may be used successfully even *without* the previous pasteurization, and for similar reasons. Lactic bacteria have a marked power of checking the growth of other organisms. A few years ago Lloyd used certain species of a pure culture of lactic bacteria for inoculating milk to be made into the Cheddar cheeses, and claimed to have obtained satisfactory results both in quality and uniformity.

The extension of this use of bacteria has proved to be one of the most promising applications of bacteriology to cheese-making. In the last few years manufacturers of the Cheddar cheeses (American cheeses) have been learning that the use of lactic cultures enables them to control the character of the ripening far better than in previous years. The growth of lactic bacteria checks the growth of other species in all milk products, and thus protects them from undesired fermentations. A pure culture of lactic bacteria added to the milk to be made into cheese proves to have a valuable influence in preventing abnormal ripening of the cheese. The cultures used are prepared by practically the same method as in making starters for cream ripening. Both *pure cultures* and *natural starters* are used, and they are sometimes propagated in a cheese factory from day to day for months without the necessity of renewal by a fresh culture. Here, as in cream ripening, the pure cultures appear more reliable than the natural starters. The use of these lactic cultures has come to be adopted almost universally in some of the large cheese-making districts in America, and is making a rapid change in the method of cheese-making.

The future development of the use of bacteria in cheese-making cannot be predicted. It is probable that the problems will be solved slowly and that, little by little, as the different types of cheeses are studied, one factor after another will be brought under control. The next few years will probably see the gradual solution of many of the problems connected with cheese-making, and whereas it will not be likely that any one great discovery will be made which will apply to all types of cheeses or which can be used widely throughout the cheese-making sections, it seems very likely that, in different localities, through the agency of bacteriological discoveries, the whole industry will be slowly but completely changed. We may probably look forward to the time when the process of cheese ripening will be much more completely under the control of the cheese-maker than at the present time. This, however, is not likely to come by the wholesale use of pure cultures, but rather by a combination of the use of pure cultures, natural starters and enzymes, and by controlling the conditions of ripening. All of this, however, is a matter of prediction which only the future can determine. It is, however, safe to say that the large amount of study that is being given at the present time to the problems of ripening of both hard and soft cheeses, is sure to produce results looking toward a greater uniformity in the process of ripening, and to place in the hands of the cheese-maker information which will enable him to control this phenomenon more accurately than he has been able to do in the past.

CHAPTER IX.

BACTERIOLOGICAL ANALYSIS OF MILK.

THE recognition of the importance of bacteria as affecting the wholesomeness of milk and as modifying dairy products, has led to the attempt to determine by laboratory analysis the nature of the bacteria in the milk, in order to gain some idea as to its wholesomeness and its fitness for use. Can a bacteriological analysis of milk be made which will enable us to determine whether the milk is in proper condition for use?

The analysis of drinking water has served as an example, since here a bacteriological analysis has proved extremely useful as an assistance in the determination of its healthfulness. The study of the bacteria of water has reached considerable perfection, and it has been quite natural that the methods used there should be transferred directly to the study of milk. The bacteriological analysis of water has, in past years, consisted chiefly in a *quantitative* determination of the number of bacteria in each cubic centimeter, and then the drawing of a conclusion as to the suspicious nature of the water from the number of bacteria that are present. In recent years more attention has been paid to a *differentiation of the species* of bacteria in water, but most of the bacteriological analysis is simply quantitative. As a result, the bacteriological analysis of milk has been of a similar character, and nearly all of the work that has been done hitherto has been confined to the study of the number of bacteria per cubic centimeter, with only here and there an attempt to determine the kinds of bacteria. We may, therefore, notice first the methods of quantitative analysis of milk bacteria.

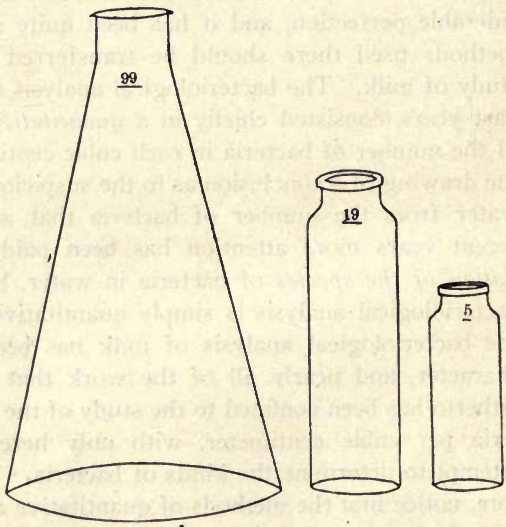
FIG. 37.

1 c.c.-pipettes inclosed in glass tubes for sterilizing.

QUANTITATIVE ANALYSIS OF THE BACTERIA IN MILK.

Glassware.—At the outset there should be carefully washed a number of test-tubes, liter flasks, petri dishes (Fig. 40), cubic centimeter pipettes (Fig. 37), a number of small flasks which are marked to hold 99 c.c. (Fig. 38), together with a considerable number of small vials, like homeopathic vials, marked to hold 5 c.c. and another lot marked to hold 19 c.c. (Fig. 38). These various pieces of glass plugged with cotton, are placed in a sterilizing oven and heated for about an hour to a temperature approximating 180° C. It is well to sterilize a considerable number of them at the

FIG. 38.



Flasks and vials for quantitative bacteriological analysis.

outset, so as to have them on hand. Some of the cubic centimeter pipettes should be graduated to one tenth of a c.c., and it is best to place them in a larger glass tube whose ends are plugged with cotton, as shown in Fig. 38, in which the pipettes can be kept after being sterilized. After this apparatus has been sterilized it should be set aside where it can be kept free from dust until needed.

Preparation of Culture Media.—Any of the methods of preparation of culture media used in bacteriological laboratories are satisfactory for the purpose of milk analysis. The method of making a satisfactory medium for quantitative analysis is as follows:

Peptone-Agar Culture Medium.

A. In a large porcelain cooking dish place the following ingredients:

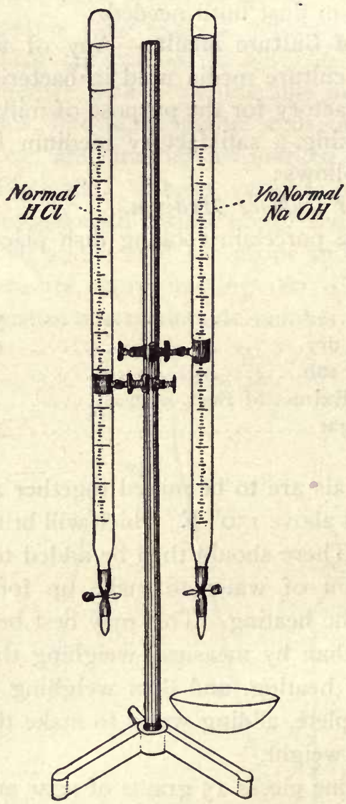
Water	500 liters.
Peptone, dry	10 grams.
Common salt	5 "
Liebig's Extract of Beef	5 "
Milk sugar	30 "

These materials are to be mixed together and heated to a temperature not above 150° F., which will bring the materials into solution. There should then be added to the mixture a sufficient amount of water to make up for that which is evaporated in the heating. This may best be determined by weight rather than by measure, weighing the dish with its contents before heating, and then weighing again after the solution is complete, adding water to make the weight equal to the original weight.

B. Cut into fine pieces 15 grams of agar and dissolve it in 500 c.c. of water. This will require considerable heat. After the agar is dissolved cool to 150° C., add water to restore evaporation, and mix with solution A. The mixture must then be neutralized carefully. This neutralization is the most important step in the whole preparation. A satisfactory method of neutralization is as follows:

Neutralization.—Remove 5 c.c. of the mixture by a pipette and place in an evaporating dish. Add 45 c.c. of distilled water, boil for three minutes and add 1 c.c. of phenol-

FIG. 39.



Two burettes arranged for neutralizing culture media.

phthalein solution (5 gm. phenolphthalein powder in 100 c.c. of 50 per cent. alcohol). Place in a burette having a rubber pinch-cock (Fig. 39), a quantity of one tenth normal solution of sodium hydroxid.[1] Add this solution, drop by drop,

[1] To make strictly accurate normal solution of NaOH and HCl requires familiarity with quantitative chemistry. Solutions accurate enough for bacteriological work may be made as follows:

Normal NaOH.—Dissolve 40 grams of fresh, *dry* NaOH in one liter of distilled water. This will not be a strictly accurate normal solution, but very nearly so. The solution should not be kept in a glass-stoppered bottle. It loses its strength and it is best to use solutions which are fresh. To make a one tenth normal solution dilute any quantity of the normal solution with ten times its bulk of distilled water. This should be made fresh from a normal solution *at the time of using.*

Normal HCl.—To 700 c.c. distilled water add 100 c.c. of concentrated c.p. HCl which, assuming that the HCl is 30 per cent. acid, would give 30 grams of HCl in 800 c.c. of the solution. This gives about the right strength (*i. e.,* 36.5 c.c. per liter), but since the HCl varies in strength, the solution must be standardized with a normal NaOH prepared as described above. To do this proceed as follows: Place 10 c.c. of the HCl solution in an evaporating dish. Add 40 c.c. of distilled water and 1 c.c. of phenolphthalein solution. Fill a burette (see Fig. 39) with some freshly prepared normal NaOH, and allow the NaOH to flow from the burette into the evaporating dish, drop by drop, until the faintest pink color appears in the acid solution and remains. This indicates the neutral point. Read from the burette the amount of NaOH which was required to neutralize the 10 c.c. of acid. If the HCl solution were exactly normal, 10 c.c. would require exactly 10 c.c. of NaOH solution for neutralization. The solution prepared as above described (100 c.c. of HCl in 700 c.c. of water) is usually too strong and requires more than 10 c.c. of normal NaOH for neutralization. It must, therefore, be diluted with water. The amount of water that must be added can be calculated as follows: Suppose there were required 11 c.c. of the NaOH to neutralize 10 c.c. of the HCl solution. This would indicate that the HCl was eleven tenths of its proper strength. To make it normal there should be added to it one part of water to every ten parts of solution. The solution prepared now contains 790 c.c., and $790 \times 1/10 = 79$ c.c. Hence 79 c.c. of distilled water should be added to the 790 c.c. of HCl to give a normal HCl solution. Add 79 c.c. of water and test again to correct any error. If the original HCl solution should prove to be too weak it is easier to make another solution a little stronger than to calculate the amount of acid necessary to bring the solution to a normal strength.

The normal HCl solution once made will keep a long time without deterioration if kept in a stoppered bottle. A one tenth normal HCl solution may be made by diluting the normal solution with ten times its bulk of water at the time of using.

to the material in the evaporating dish until it turns to a very faint pink color. This indicates the neutral point. Treat two other samples in the same way, and if the amount of the sodium hydroxid added is the same in each case it indicates the amount necessary to neutralize 5 c.c. of the culture medium. The average of the three tests should be taken. By calculation, determine the amount necessary to neutralize the whole liter, and add sufficient amount to the whole for neutralization. Instead, however, of adding to the mixture the *one tenth* normal NaOH, add a *normal* solution, which is ten times as strong, and of which, therefore, only one tenth as much should be added as would be required of the one tenth normal. The whole of the medium is thus to be neutralized. It is well to test the accuracy of the neutralization by adding a few drops of phenolphthalein to a little of the neutralized medium. This should give the faint pinkish tinge; if it does not, it means that the neutralization has not been properly affected.

After neutralization boil for five minutes and restore to the original weight, after which the reaction should be tested again and corrected if necessary.

The material thus neutralized is too strongly alkaline for the proper growth of bacteria and must be rendered less alkaline by adding HCl. The amount to be added should be such as to bring the reaction to 1.5% acid. To produce this acidity add to the neutralized medium 15 c.c. of normal HCl for each liter. The acidity thus obtained is found to be that at which common milk bacteria grow most readily.

After adding HCl in proportion of 15 c.c. to each liter of solution, pour into the mixture slowly the white of an egg mixed in a little water. Boil vigorously for a few minutes to coagulate the albumen, and then filter through absorbent cotton or through filter paper moistened with hot water. The material filters rather more easily through absorbent cotton, and if the directions above given are followed closely it will filter perfectly clear. After filtering the material is to be collected in a sterilized flask.

Fill a considerable number of test-tubes with the material from the flask, placing in each test-tube about 10 c.c. of the medium, carefully replacing the cotton stoppers. After the test-tubes have been filled with the medium the whole quantity, both that in test-tubes and in the flasks, should be sterilized in a steam sterilizer for twenty minutes. To produce complete sterilization it is necessary to repeat the steaming upon three successive days. The second and third sterilization require a longer time than the first, inasmuch as it requires some time to melt the agar, and until the agar is thoroughly melted the sterilization is not effective. Upon the second and third days, therefore, the material should be steamed for at least half an hour. After the third steaming the material in the test-tubes is ready for use. If an autoclave is at hand one sterilization for twenty minutes at 120° C. is sufficient.

Method of Making Quantitative Analysis of Milk.—In order to make an analysis of the bacteria in milk it is necessary to have an approximate idea of the number of bacteria which are to be expected. The reason for this is that the bacteria are commonly so numerous that it is necessary to dilute the milk highly with sterilized water in order that reliable results may be obtained. In the quantitative analysis of ordinary market milk it is commonly satisfactory to dilute the milk one hundred times with sterilized water, provided the medium to be used is the agar culture medium above described. If the milk is old and contains large numbers of bacteria a much higher dilution than this is desirable, but for the kind of milk usually found in milk-distributing carts a dilution of one hundred times is usually satisfactory for the purpose here considered.

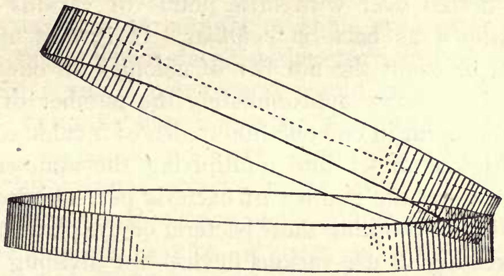
Several of the small flasks marked to hold 99 c.c. are filled to this mark with water and placed in an autoclave for sterilization. If it is desired to dilute the milk more than one hundred times there should, at the same time, be placed in the autoclave a number of the smaller vials filled with water to the 19-c.c. mark and others to the 5-c.c. mark (Fig. 39). All of these vessels of water are to be sterilized for an hour at a temperature of 120° (a steam pressure of ten pounds will do), after which they are to be removed. If an autoclave is not at hand the water may be sterilized by steaming for two hours.

There is now taken from the milk to be tested a single cubic centimeter in one of the sterilized pipettes. *Taking of the sample* of milk is the most important point in the analysis and most liable to introduce errors. The number of bacteria found in different parts of a can of milk is by no means uniform, the surface layers containing different numbers from the deep layers of the milk. To avoid this irregularity it is necessary to give the milk a very thorough stirring or shaking immediately before the sample is taken, so as to distribute the bacteria as uniformly as possible.

A cubic centimeter of milk is transferred by a sterilized pipette into one of the 100-c.c. flasks of sterilized water. The mixture is then to be very *thoroughly shaken,* so as to distribute the milk uniformly through the water. This thorough shaking is extremely important to break up the clumps of bacteria shown in Fig. 9, page 34.

Meantime six test-tubes of the agar culture medium have been melted by placing them in water over a gas flame. The tube should be kept in warm water at a temperature just sufficient to keep its contents from solidifying. A single cubic centimeter of the mixture of milk and water is removed with a second sterilized pipette and placed in each of the test-tubes of melted culture medium. The test-tubes are then to be gently but thoroughly shaken, so as to distribute the inoculated material uniformly. It is necessary to avoid shaking too vigorously, or otherwise bubbles will make their appearance, which will interfere with the accuracy of the test. The shaking should be thorough but not violent.

FIG. 40.



Petri dish for making "plate cultures."

Six sterilized petri dishes (Fig. 40) should have been placed upon a plate of glass which is held in as nearly a level position as possible and cooled artificially. This can be accomplished by taking a large, flat dish, filling it with water and ice and then laying a large plate of glass upon the top. The ice will rapidly cool the glass plate, and the petri dishes placed upon the plate will also rapidly become cooled. The contents of each of the test-tubes inoculated with the diluted milk are now to be poured each into a petri dish and the cover quickly replaced. The culture medium will distribute itself in a thin layer over the bottom of the petri dish and soon harden. The dishes are to be labelled and then set aside in a proper place for growth. If desired to hasten the analysis the dishes may be placed in a culture oven kept at a temperature of 98°. For ordinary study of milk bacteria it is usually most satisfactory to leave the petri dishes at a room temperature of 70°, allowing them to remain for three or four days before the final study is made.

The Study of the Plates.—The solidified culture medium fixes each bacterium at a single point. As the bacteria feed upon the culture medium they grow and multiply, but, unable to move through the solidified medium, the descendants of each bacterium remain together in a mass and, in the course of two days, become abundant enough to produce a spot which can be seen with the naked eye. The plate, therefore, becomes dotted over with little points of various size and shapes known as bacteria *colonies*. It is only necessary, therefore, to count the number of colonies on one of these plates and we know approximately the number of bacteria that were present in one one-hundredth of a cubic centimeter of the original milk; and multiplying the number by one hundred we get the number of bacteria per cubic centimeter of the milk. In counting these bacteria on the plate it is sometimes necessary to use various devices for dividing the plate into areas. If the number is small they can be counted without difficulty, but if the number of colonies on the plate is large it is more convenient to place underneath the plate a piece of black paper with white lines ruled upon it, dividing the plate into a series of sections of equal size. Such cards for aiding the counting can be obtained from dealers in bacteriological material, and a quantity of them should be at hand in every laboratory to assist in the counting. If the numbers are not very great the counting may be done without the aid of these slips, by simply marking the under side of the petri dishes with a waxed pencil, and thus dividing the plate into a series of sections which may be counted individually. The actual determination of the number of bacteria on these plates is not difficult, though it requires a little practice.

The number thus obtained represents approximately the number of bacteria in a cubic centimeter of the original milk, but the number is only an approximate one. Different tests of the sample of milk will show considerable irregularities, and it is for this reason that six petri dishes have been made. Each of the six should be counted and the average result of the six regarded as the average number of bacteria per one one-hundredth cubic centimeter. But, apart from this irregularity in the samples, there are at least three other facts which make the analysis only approximate. First, if in the diluted milk there chance to be several bacteria clinging together, as is quite probable even after thorough shaking, these, when placed within petri dishes, would develop into a single colony and would be counted as one. This will naturally give a number in the analysis somewhat too low. A second and more serious difficulty is the fact that not all bacteria present in the milk will grow in the culture medium as above prepared. While a large proportion of the bacteria will develop on plates and make their appearance in analysis, there are some that do not grow at all, and, therefore, do not appear in the analysis. Third, it is impossible to pour out all of the contents of the tube into the petri dish, for some will inevitably stick to the tube. To obtain the absolute number of living bacteria present in a cubic centimeter of milk is quite impossible by any means at our command.

The number obtained by the method described will always be an *underestimate*. But while it must always be recognized as approximate, the results in different cases may be compared with each other. If two samples of milk show, one ten thousand and the other ten million, it is quite certain that these numbers express approximately the relative number of bacteria in the two samples, though neither expresses the number accurately.

RESULTS OF SUCH ANALYSES.

The results of analyses of milk, as obtained by the above method, show the widest possible variations. If the milk is fresh and exceptionally clean the number may be as low as one hundred per c.c., or sometimes even lower. Fresh milk from an ordinary dairy, however, will contain from 300 to 10,000, and very frequently there will be more than this; 50,000, 100,000 bacteria per cubic centimeter, or even larger numbers, may be found in the milk freshly taken from dairies where the conditions are bad. The examination of market milk as it is distributed to the consumer shows far wider variations. In small communities where the dairy is close to the consumer, the number present in the milk, as distributed, may be no more than 10,000 per c.c. or occasionally even less; though even in such small communities the number may rise to several millions per c.c. In the market milk of large cities the number is more variable and is usually higher, since the distance from the farm to the consumer may be great. In the larger cities of America the number of bacteria found by analysis ranges all the way from 10,000 per c.c., in cases of exceptionally good milk, to many millions; 20,000,000 per c.c. being frequently found in samples of milk in summer weather, and occasionally even this number is surpassed. Some samples of city milk have a bacterial content of over 100,000,000 per c.c., although such extremely high numbers as this are unusual and indicative of some exceptionally bad conditions. The average number in milk of large cities in the winter season may generally be reckoned by tens or hundreds of thousands, while in summer weather the number must commonly be reckoned by millions. The milk furnished by different dealers varies so widely that an average number for the city gives no guide as to the number to be found in any sample.

Interpretation of these Results.—The interpretation of these results is certainly not what would at first be expected. It is evident that the problems of bacteria in milk and in water are quite incomparable. Whereas a sample of water which contains a few thousand bacteria per c.c. is justly regarded as suspicious, many a sample of milk contains bacteria by the hundreds of thousands, and possibly by millions, and yet is perfectly wholesome. Even when compared with sewage the bacterial content of milk proves to be surprisingly bad. It is occasionally stated that the milk of our cities contains more bacteria than the city sewage. This statement is certainly true. It frequently happens that the milk distributed in summer weather contains more bacteria than the ordinary sewage, and in many cases the number of bacteria in the city milk far surpasses that found in the worst sewage.

It is manifest that we cannot interpret the number of bacteria in milk in the same way that we do the number in water. We cannot infer that milk is a more unhealthful product than sewage because it contains more bacteria. The use of sewage for drinking would be disastrous; but milk that contains bacteria in large numbers is constantly used without injury. In short, the determination of the number of bacteria in milk does not determine its wholesomeness. We cannot condemn a sample of milk upon the same bacteriological grounds which would warrant us in condemning a sample of water.

In the first place the presence of a comparatively *small* number of bacteria does not necessarily mean that the milk is wholesome, for that small number may contain certain pathogenic forms, and in this case the milk would be decidedly injurious. On the other hand, the presence of *large* numbers of bacteria does not necessarily mean that the milk is unwholesome. We have already seen that there are no reasons for believing that lactic bacteria are injurious, but, possibly, on the contrary, are advantageous to our digestive functions. If, therefore, of the large number of bacteria found in a sample of milk most of them are of the normal, harmless, lactic type, their presence in great quantity may not render the milk less wholesome.

Although we cannot determine the wholesomeness of milk by a quantitative analysis of the bacteria, nevertheless such an analysis is useful. The number of bacteria in milk is dependent upon three factors: (1) The care in the dairy, (2) the care in transportation, and (3) the temperature of the milk. Milk produced in a filthy barn will contain larger numbers of bacteria than milk produced in a clean barn, and milk which has been subjected to improper conditions in transportation will, in general, show more bacteria than milk that has been properly handled. Hence it follows that the quantitative analysis of milk will enable us to determine approximately the cleanliness of the conditions under which the milk has been produced and transported. If a sample of milk is only a few hours old, and the number of bacteria is large, it indicates that the conditions of its production have been uncleanly. If, however, the sample of milk is older and the number of bacteria is large, it indicates either that the original conditions are uncleanly or that the milk has not been properly treated subsequent to the milking. In other words, if the number of bacteria in a sample of market milk is found to be excessive, the inference is drawn that the milk is either very old, or has been kept too warm, or was produced under conditions of filth and uncleanliness. On the other hand, if the number of bacteria is found to be small it would indicate (leaving out of account the possibility of the presence of antiseptics) that the conditions of production and transportation have been satisfactory. While the presence of a large or a small number will not positively determine the wholesomeness of the milk, the quantitative determination will give us a means of drawing a conclusion as to the conditions under which the milk has been produced.

While it is true that milk containing small numbers of bacteria may contain some pathogenic forms and thus be dangerous, nevertheless milk produced under proper dairy conditions is far less liable to be contaminated with pathogenic germs than milk that is carelessly handled. The dairyman who has taken special care to protect his milk from bacteria will be more likely to have taken care to protect it from the possibility of secondary contamination with pathogenic bacteria. Hence it will follow that milk which by quantitative analysis shows a small number of bacteria, and, therefore, indicates conditions of care on the part of the milk producer, is likely to have been protected from secondary contamination. On the other hand, the presence of large numbers of bacteria suggests carelessness in the production and the handling of the milk, thus giving a greater opportunity for the introduction of mischievous bacteria. Hence it is that, while the presence of large numbers of bacteria does not necessarily render the milk unwholesome, it does render us suspicious of the conditions to which it has been subjected.

It is probably impossible to fix upon any standard as to the number of bacteria which wholesome milk may contain. Should we condemn milk when it has 10,000 per c.c. or 30,000 or 100,000, or 1,000,000 bacteria? To fix a standard is difficult, because the number is so dependent upon the temperature and the season of the year. It might be possible to set a standard which could be enforced easily in the winter, but such a standard would be quite impracticable for summer weather. The number of bacteria is sure to increase in the warm weather, and no standard which can be adopted for winter would be practicable for the warmer seasons. On the other hand, if a number should be adopted which would be practicable for warm weather it would be so high as to be of no use in winter weather. In some cities the attempt has been made to establish such a standard for certain grades of milk. Special dairies, referred to in the previous pages, set a bacteriological standard and guarantee to furnish milk to the consumer which contains no more than a certain number of bacteria. Sometimes this number has been fixed at ten thousand, in other cases at thirty thousand. This is practicable for small dairies where the dealer wishes to furnish a special product at a special price, and where the dairy is within a short distance of the consumer. A bacteriological standard has also been found useful in testing certified milk, as mentioned on the previous page. But for the general milk supply of a large city it has, up to the present time, been found quite impracticable to suggest any bacteriological standard without excluding too large a portion of the milk which will be brought into the city. Moreover, it seems by no means sure that such a standard would be of much practical value, because, even though the numbers be large, the milk may be perfectly wholesome if they are of the normal lactic type, whereas a much smaller number of bacteria in another sample of milk might make it decidedly injurious if the bacteria should be of a different character.

These various facts raise the question whether a bacteriological analysis of milk which shall differentiate the different kinds of bacteria from each other is possible and practical. Is it possible to devise some means of analysis of the bacteria in milk which shall give the numbers of the different kinds of bacteria, separating the normal forms from those that render the milk suspicious? If we could do this the practical analysis of city milk might be more useful and might become an efficient means in the hands of boards of health in protecting the public from the dangers in its milk supply. There has, hitherto, been no attempt made to develop such a method of differential analysis of milk and, indeed, at the present time we know too little in regard to the relations of the different species of bacteria to the wholesomeness of milk to make such an analysis absolutely reliable. Nevertheless, even an approximate qualitative analysis of the bacteria in the milk may be useful in detecting the quality of samples of milk from unknown sources. Such approximate analysis is at least possible.

SIGNIFICANCE OF A QUALITATIVE ANALYSIS.

We must notice again a few facts in regard to the different types of bacteria. It is practically impossible, by any ordinary means, to detect in milk the presence or absence of the bacteria producing tuberculosis, scarlet fever, typhoid fever or diphtheria. The detection of the bacteria producing these diseases, even when possible, requires so much work and is so uncertain as to be impracticable of adoption in the analysis of milk. Hence, no practical analysis of milk, at the present time, will enable us to detect the presence of the exciting causes of any of these distinctively contagious diseases.

We have learned that the ordinary bacteria in milk may be divided into three chief classes: (1) The *lactic* bacteria, which are most abundant in samples of milk after it is a few hours old. Most of these organisms probably have little or nothing to do with rendering the milk unwholesome, although the bacillus of typhoid fever and possibly that of summer complaint belong here. (2) The *enzyme* bacteria, many of which produce putrefactive products, and are most likely the organisms associated with the production of some of the digestive disturbances attributed to milk. (3) Bacteria which produce *no distinctive action* upon milk. We have, as yet, no knowledge as to whether this last group is associated with the production of intestinal disturbances. There may be among them some bacteria that are distinctively injurious, but at the present time we really know nothing about the matter.

It is possible, by a simple modification of the common bacteriological methods of study, to differentiate these three types of milk bacteria from each other. While such a differentiation will not be sufficient to determine accurately whether a sample of milk is wholesome, it will certainly bring us much closer to such a conclusion than simply counting the numbers. Moreover, this differential analysis will frequently enable us to determine whether the milk has been badly contaminated by uncleanliness in the original dairy, or has simply been kept until normal harmless bacteria have had a chance to multiply.

In fresh milk it is quite rare to find the common lactic bacteria very numerous. If milk is retained at a moderate temperature for a number of hours the few lactic bacteria originally present commonly grow rapidly, and soon come to outnumber all other species put together. Hence, it follows that, if a sample of market milk contains a large number of bacteria and of these a large percentage are lactic bacteria, this is probably due to the fact that the milk has been kept at a moderately warm temperature for a number of hours.

If, on the other hand, the milk should show large numbers of miscellaneous bacteria, either of the putrefactive type or those which have no effect upon the milk, the inference to be drawn is different. We are then led to suspect that there was a greater primary contamination of the milk. If there is much filth around the barn or the cows, the milk is likely to be contaminated with a great variety of bacteria, and hence the presence in a sample of market milk of great quantities of these miscellaneous types suggests an excessive contamination in the original milk. Moreover, if we are correct in the assumption that the common lactic bacteria are beneficial rather than the reverse, it is perfectly evident that this qualitative differentiation of species will be of decided significance in determining the wholesomeness of the milk as an article of diet.

QUALITATIVE BACTERIOLOGICAL ANALYSIS OF MILK.

This requires different culture media from that described above. It is prepared as follows:

1. *Sugar-gelatin.*—Mix together the following:

Water	1,000 c.c.
Peptone	13 gms.
Gelatin	150 "
Milk sugar	30 "
Liebig's Extract of Beef	7 "

These materials are to be dissolved at a temperature, preferably not over 140°. After thorough solution the mixture is to be neutralized by the use of NaOH as described on page 254, and after neutralization the reaction is to be brought to 1.5% by adding 15 c.c. normal HCl for each liter of solution. The white of egg is added and the mixture boiled for half an hour and then filtered through absorbent cotton. The solution thus made is like an ordinary gelatin culture medium, except that it contains milk sugar and has slightly larger proportions of the various ingredients. This medium, after filtering, is to be filled into sterilized test-tubes, *exactly 8 c.c.* of the medium being placed in each tube. These tubes are then to be sterilized by steaming for fifteen minutes on three successive days as usual.

2. *Litmus Solution.*—Weigh out 50 grams of *dry litmus cubes* and add to it 300 c.c. of water. Allow the mixture to steep for a few hours in warm water (four hours at 160°), or allow it to soak twenty-four hours in water at the ordinary temperature of a room. This dissolves the active material from the litmus. The whole is then to be filtered, giving a deep blue solution. This solution is commonly alkaline to litmus but acid to phenolphthalein. The next step is to determine its grade of alkalinity. 5 c.c. is placed in an evaporating dish, diluted with 45 c.c. of water, and one tenth normal HCl is added from a burette as explained on page 256, until the litmus neutral point is reached. The neutral point is recognized by the turning of the blue solution into a faint red. The HCl is added, drop by drop, and the neutral point is reached, when there is the first trace of change in the blue color toward a reddish. The reading upon the burette will show how much HCl must be added to the solution to bring it to the *litmus* neutral point. The calculation is made by the method already described and the litmus solution is neutralized. This solution is now too strongly acid for bacteria

growth and it wants to be brought to the same grade of acidity as the gelatin medium already prepared. When a solution is neutral to litmus it is about 2.5% acid to phenolphthalein, *i. e.*, it requires 25 c.c. normal NaOH per liter to bring it to the neutral point of phenolphthalein. The gelatin solution has been brought to 1.5%, and the litmus solution, in order to be brought to the same grade of alkalinity, must have added to it 10 c.c. (*i. e.*, 1.0% by volume) normal NaOH per liter $(2.5 - 1.5 = 1.0)$. Normal NaOH is therefore added to the litmus in proportion of 10 c.c. per liter, after which the solution is sterilized in steam in a flask closed with cotton in the same manner as the gelatin. It will be evident that the gelatin and the litmus solutions may be mixed in any proportion without changing the reaction.

Milk Whey Culture Medium.—For some purposes a culture medium made from milk is preferable to the one described above. It is made as follows:

To about two quarts of milk there is added enough rennet to curdle it in half an hour. The curd is to be cut with a knife and the whey strained out through cheese cloth. Place the whey in a flask and sterilize one hour in an autoclave at a pressure of twenty pounds. This sterilization kills the spores, after which the whey is to be filtered through filter paper. Then add 13% of gelatin, dissolve by moderate heat, and neutralize to phenolphthalein by adding NaOH from the burette as already described. Bring the reaction to 1.5% acid as before, add the white of an egg, boil briskly and filter through cotton as usual. The filtered solution is to be placed in test-tubes, 8 c.c. in each, and sterilized. In other respects this medium is to be used exactly as the peptone gelatin described above. This milk medium gives a sharper differentiation of the different kinds of bacteria colonies, but does not give quite such high numbers when used to determine the number of bacteria in milk.

METHOD OF QUALITATIVE ANALYSIS OF MILK.

Dilution.—In the use of these media the first thing to be done is to determine upon the proper dilution of the milk. The milk must always be diluted with sterilized water, but the determination of the proper amount of dilution is the most difficult point of the analysis, from the fact that different samples will require different dilutions. The dilution should be such that the plates which are to be made should contain, if possible, from 100 to 300 colonies. The extent of dilution varies with the age of the milk, with the temperature and with the season. In cool winter weather with common milk a dilution of 200 or 600 is commonly satisfactory. In warm weather it must be higher, and it is always best to prepare plates of more than one dilution.

At the beginning of experiments there should be sterilized a series of flasks marked to hold 99 c.c. of water, and some small vials marked at 5 c.c. and at 19 c.c. There should also be one or more large flasks of sterilized water at hand. The first step is to dilute the milk with sterilized water. It is always wise to make two different dilutions in order that the chances of a proper dilution may be greater than if only one should be made. In the study of market milk in ordinary winter weather it is best to choose the two dilutions of 200 and 600. If the milk is old or the weather is warm the dilution must be 2,000–4,000, or even greater. Having selected the dilution to be used, a single cubic centimeter of the thoroughly mixed milk is removed with a sterilized pipette and placed in a flask containing 99 c.c. of sterilized water. This, after thorough shaking, is to be further diluted according to the amount of dilution required. The following table will show the method of obtaining different dilutions.

A. First dilution, 1 c.c. of original milk in 100 c.c. of sterilized water = a dilution of 100.

1 c.c. of A placed in 5 c.c. of water gives a dilution of 600.

2 c.c. of A in 4 c.c. of water gives a dilution of 300.

1 c.c. of A in 19 c.c. of water gives a dilution of 2,000.

½ c.c. of A in 19½ c.c. of water gives a dilution of 4,000.

1 c.c. of A in 99 c.c. of water gives a dilution of 10,000.

Higher dilutions of course can be obtained by simple calculation.

Making Plate Cultures.—Meantime a number of tubes of gelatin (either peptone or whey gelatin, or both) are melted. With a sterilized pipette two c.c. of the litmus solution are added to each tube of gelatin. A single c.c. of the final dilution of the milk, after thorough shaking, is removed by a clean sterilized pipette and placed in each of the test-tubes of gelatin, and then the contents of each tube is gently but thoroughly shaken until completely mixed. There is thus obtained a deep-blue mixture. The contents of the tubes are to be poured out into petri dishes in the ordinary way. At least three plates of each dilution chosen should be prepared, making six plates in all, one half more highly diluted than the other half. It is necessary to have the different dilutions, since if the plates are too crowded with colonies the differentiation which is to follow is not satisfactory. A little experience is required before one can judge of the proper dilution to make, but after a few experiments it is commonly easy to fix upon the dilution which is likely to be satisfactory.

For reasons to be presently mentioned it is desirable to prepare one or two agar plates at the same time with the litmus. It is done in the same way. Ordinary agar tubes (preferably with 2% agar) are melted and to each is added two c.c. of the litmus and one c.c. of the diluted milk. The rest of the procedure is the same as with the gelatin cultures.

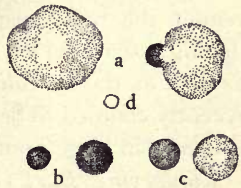
Before these plates can be satisfactorily studied it is necessary to allow them to grow for several days. The reason for this is that not until about the sixth day are the different colonies on the plates well enough developed to be readily distinguished from each other. Sometimes, however, it is impossible to allow the plates to grow for such a length of time. The difficulty with the whole problem of qualitative analysis lies in the presence of liquefying bacteria. It sometimes happens that the milk contains a number of rapidly liquefying bacteria which liquefy the gelatin so as to destroy the plates within a couple of days. Such plates cannot be studied and the differentiation of the bacteria is very difficult, or frequently impossible. It is for this reason that the two agar plates are prepared. The differentiation of the bacteria upon the agar plates is very unsatisfactory, but it is better than nothing, and if the liquefiers should prove to be so abundant as to make the study of the gelatin plates impossible, the agar plates can be used. The presence of a few liquefiers, however, does not necessarily interfere seriously with the study of the gelatin plates. The liquefiers may be quite abundant, but if they are not very rapidly growing forms they may not so far liquefy the gelatin within four days as to prevent the tolerably satisfactory study of the rest of the plate. It is only the presence of large numbers of liquefiers or of a few extremely rapidly growing forms that interfere with the analysis to be made, and experience has shown that this does not very frequently happen. In making a long series of analyses the large majority are satisfactory, but there will be a small percentage which cannot be differentiated because of the abundance of rapid liquefiers. In these cases all that can be done is to depend upon the agar plates which have been simultaneously made. These agar plates will enable us to determine the total number of bacteria and differentiate tolerably well the lactic forms from other species, but a more complete differentiation is not possible upon agar.

The Types of Colonies found in Litmus Gelatin.—This is not the place to attempt any detailed description of the normal milk bacteria. Such a description must be found in more technical works on bacteriology. A few general facts in regard to the nature of the most common bacteria in normal milk may not, however, be out of place. Normal milk should contain a moderate number of *Bact. lactis acidi,* the number and percentage varying, as we have already indicated, with the age of the milk. Fresh milk should contain them in small numbers, while milk that is older should have a larger proportion. Normal milk will also contain large numbers of *neutral forms,* that is, bacteria which produce colonies on gelatin that are neither acid nor alkaline, nor have any other distinctive characteristic. Normal milk will commonly also contain some *liquefiers.* The number of liquefiers, however, will be quite variable. If the number is very high it renders the milk suspicious and suggests that it has been subjected to great contamination at the dairy, or at all events that the protecting lactic bacteria have not developed as they normally should in the milk. While, then, the presence of large numbers of liquefying bacteria will not positively condemn such milk, it does render it somewhat suspicious, and suggests that something unusual has produced a high contamination of the milk with the liquefying and putrefactive forms. Milk which is not strictly fresh should be found to contain the lactic bacteria in considerable proportion, a percentage which will be likely to vary from 40% to 60% or even higher. If the number of bacteria is very high in total numbers, ranging in the millions, and the percentage of lactic bacteria is low, the milk is an abnormal form of milk and is at once suspicious, for normal milk produced under proper conditions will, by the time it has bacteria in great quantities, have a very large percentage of lactic forms.

It may be convenient to give here a brief description of the kind of colonies which the chief dairy bacteria produce in the litmus gelatin. The normal forms of milk bacteria are as follows:

Bact. lactis acidi.—This produces in the litmus gelatin a small, opaque colony, which is red after the growth of a few days, and soon becomes surrounded with a red halo. It *never* *grows upon the surface* of the gelatin, but always below the surface. It can readily be distinguished, with a little experience, by its being opaque and having its edge slightly irregular, as though provided with excessively minute spines (Fig. 41, *b*). It is usually by far the most abundant colony in normal milk a few hours old. Its size varies somewhat, but it is commonly about .3 mm.

FIG. 41.



Colonies of bacteria in litmus gelatin: *a*, *B. lactis aërogenes*; *b*, *Bact. lactis acidi*; *c*, a neutral bacterium; *d*, *Bact. lactis acidi II.*

Bact. lactis acidi II.—This is a bacterium which is closely related to, if not identical with, the former, but upon gelatin it produces a different colony. The colony is excessively minute, scarcely visible to the naked eye, but easily visible with the lens (Fig. 41, *d*). It is perfectly transparent, but is surrounded by a red halo, showing that it develops acid. It is usually found only in milk a day or so old, and is rarely observed in fresh milk.

B. lactis aërogenes and B. coli communis, etc.—These bacteria, which are common in milk, cannot be differentiated from each other by the study of their colonies. They are, however, together easily distinguished from the first type of lactic organisms, from the fact that they produce surface colonies. The colonies are very robust, and grow to the size of one or two millimeters or even more in diameter, raised up in a thick mound of white bacterial matter (Fig. 41, *a*). They are frequently surrounded by a ring of intense red, showing the production of much acid; they commonly produce gas, which sometimes appears in the middle of the colony, raising it into a bubble upon the surface, although more frequently the bubble does not appear. The colony itself sometimes protrudes vertically from the surface of the gelatin for the distance of half a millimeter or even more.

This type of bacteria is common in milk, though not so universal as the first type. Some samples of milk show none of this type of bacteria, while in other specimens they are more common than the first type and, indeed, some samples of milk appear to be soured by the action of these bacteria alone, the *Bact. lactis acidi* being practically absent. The group of bacteria is one whose presence in large numbers may render the milk suspicious, since it contains the gas-producing species which gives rise to such troubles in the dairy, as well as the *B. coli* and *B. typhosus,* and also the bacillus recently claimed to be the cause of summer complaint. Their presence in large numbers is, therefore, undesirable.

Liquefying Bacteria.—These are easily distinguished by the fact that they liquefy the gelatin. It is possible to distinguish among them a large number of species, but for ordinary analysis this is hardly practicable. It is, however, convenient to distinguish between two types of liquefiers. One, which may be called *rapid liquefiers,* grows with excessive rapidity and liquefies the gelatin plate in a short time, so that occasionally twenty-four hours after the making of the plate the gelatin begins to liquefy, and if even three or four colonies are present upon such a plate it is usually so far liquefied in two days as to be beyond the possibility of further study. The second type of *slow liquefiers* is far more common. It grows slowly, producing liquefying pits which do not become much more than half a centimeter in diameter, and do not grow so rapidly as to interfere with the study of the rest of the plates, unless they themselves chance to be excessively abundant.

Neutral Forms.—In fresh milk the large proportion of bacteria are commonly found to produce colonies which must be classed together as neutral. These colonies are small, rather opaque, or of a dirty gray color, producing no acid, and appearing either on the surface or below as gray indefinite colonies; they do not become very large, and are never very characteristic (Fig. 41, *c*). A study of these colonies has shown that there is a variety of bacteria which produce such colonies, and several species would be included under this head of neutral colonies (see Fig. 18). It is, however, impracticable to differentiate them from each other upon the gelatin plates, and it is, therefore, necessary to group them together under one type as neutral forms.

Yellow Colonies.—In some localities it is almost universal to find, upon the surface of the gelatin and just beneath the surface, some bright yellow, or occasionally orange, colonies, which may become of considerable size, and which may or may not show the development of an acid. These are commonly a species of *Micrococci* or *Sarcina.* They are so common that, at least in some localities, they must be regarded as almost universally present in normal milk. They can usually be distinguished at a glance by the bright yellow color with or without an acid reaction.

A further differentiation of the colonies to be found upon gelatin is perfectly possible. Indeed, the colonies of different species of bacteria growing upon this litmus milk sugar gelatin are commonly very sharply marked off from each other and easily recognizable. Probably forty to fifty different species of common milk bacteria can, by careful study, be distinguished from each other without much difficulty. To distinguish them all with accuracy requires long experience, and can only be done after the observer has studied the different colonies for a considerable time and has learned to recognize them. Moreover, it is never possible, with absolute accuracy, to distinguish all of the colonies from each other, and at best the results will be approximate rather than strictly accurate. So far as concerns the chief types, however, the differentiation here described is quite accurate, and it is only in regard to the forms present in smaller numbers that the method described is uncertain.

Study of the Gelatin Plates.—After the plates have grown for about six days, they are to be carefully examined with a lens, and if necessary with a low-power microscope. In this study the following points are to be determined:

1. The total number of bacteria. These are to be counted by the ordinary methods.

2. The number of acid-producing bacteria. These can easily be detected from the fact that every acid colony will be red, and commonly surrounded by a red halo where the acid has turned the blue litmus. This detection of acid bacteria is also possible upon agar. It must always be borne in mind that an alkaline colony in the vicinity of an acid colony may obscure the production of acid, and some of the lactic bacteria will not show signs of acidity if lying close to a colony developing an alkaline reaction. It is for this reason that it is necessary to have several dilutions, so that in one of them the colonies may be far enough apart to have no such influence upon each other.

3. The number of liquefying colonies is to be counted. This is especially important, inasmuch as the liquefying colonies commonly represent the putrefactive organisms, and their relative abundance in milk is a matter of importance.

4. The number of bacteria producing no reaction in the gelatin, or an alkaline reaction, but which do not liquefy, are to be counted. This is very easy to do, provided the plates have been properly diluted and have grown sufficiently. Where it is necessary to study the plates early because of the abundance of liquefiers, the distinction between the acid bacteria and those producing no reaction is less sharp and not always satisfactory.

5. The number of surface-growing acid bacteria of the aërobic type, see page 275.

Calculation of Results.—The method of calculating the results is as follows:

First the total number of bacteria upon any plate is counted. Then the plate is studied and the total number of each recognized type is determined by actual count. The sum of these numbers of each species should give a number equal to that of the total number on the plate, provided all of the colonies on the plates are easily differentiated. It usually happens, however, that the total number of differentiated colonies fails to add up as much as the total number of bacteria on the plate. This, of course, means that some of the colonies are not clearly recognized types and the difference between the total number and the sum of the numbers of the recognized species must be regarded as *undetermined* or *miscellaneous*. The number of miscellaneous colonies is found by experience to vary greatly, according to circumstances. If the liquefiers are abundant, and the plates must be studied before they are four days old, the number of miscellaneous colonies that will be found is commonly large, whereas if the plates can be kept till six days old the number of the undetermined colonies is small. Other circumstances interfere occasionally to prevent the satisfactory differentiation, and whenever this occurs the percentage found in the undetermined column always rises. The number found in this column, therefore, is usually an indication of the success of the proper differentiation.

The results which are obtained should then be tabulated and each table should be a double one. It should contain first, the total *number* of each type of bacteria detected, and second, the *percentage* of each type. The purpose of the latter is to show the relative preponderance of the different microörganisms. The advantage of this is considerable. From facts already mentioned it follows that if the milk shows large numbers of bacteria, but of this large number the great per cent. are lactic organisms, the milk must be regarded as normal though rather old. If, on the other hand, the results should show a large per cent. of liquefiers, or even a large per cent. of the non-acid bacteria, the milk must be regarded with more suspicion. Two actual analyses of milk may be given in the way of illustration.

	Total.	Lactic.	Liquefiers.	Miscellaneous.
	No. 1.			
Number of bacteria per c.c.	6,800,000	6,324,000	68.000	438,000
Percentage		93	.1	6
	No. 2.			
Number of bacteria per c.c.	17,000	500	5,300	11,200
Percentage		2.8	31	66.2

Of these two samples it will be seen that the first contains many more bacteria than the second, but the percentage of lactic bacteria is very great. The second sample contains small numbers but with very small per cent. of lactic bacteria. The second sample of milk is doubtless fresh, but the first is a perfectly normal milk and not suspicious in spite of its large total numbers. If No. 1, with its high numbers, had a large percentage in the third and fourth columns it would have been suspicious.

This method of testing milk is not given here as by any means complete, nor is it assumed that the results obtained will enable us to determine positively the wholesomeness of milk. It is believed, however, that such a method of bacteriological study is an advance over the practice of simply counting the numbers, which has been the common method of the past. The great difficulty of the whole method consists in the fact that it sometimes happens that the milk contains many liquefying bacteria, which grow rapidly and liquefy the gelatin. If the gelatin begins to liquefy rapidly it is necessary to study the plate at once, in spite of the fact that the differentiation is not perfect. If, however, the liquefying bacteria are not numerous and do not grow rapidly, the plates may be kept for six or seven days, or even more before they are studied. The longer the plates grow the better the differentiation which is obtained.

If it is desired to study the different bacteria further, samples of the different types of colonies are to be picked out of the plates with a platinum needle and inoculated into agar or gelatin to be subsequently studied by bacteriological methods. The description of these methods does not lie within the scope of this work.

To Determine the Fermentative Power (Gas Production) of Bacteria.—Fill a considerable number of fermentation tubes (Fig. 42) with common bouillon used for bacteriological work, but containing 3% milk sugar. The closed arm of the tube is to be filled full as shown at Fig. 42, *A*, and the tubes are then to be sterilized as usual by steaming. To determine whether a bacterium produces a fermentation of sugar and develops gas the bacterium from a pure culture or from a colony growing upon a petri dish, is inoculated into the bouillon in the fermentation tube. If it produces gas by fermenting the sugar gas will be found to collect at the top of the closed arm in a day or two as shown at Fig. 42, *B*. If no gas appears in the closed arm it indicates that the bacterium is not a gas-producer. It is always to be remembered that gas-producing bacteria are troublesome in the dairy, and this test of gas production is, therefore, sometimes of great importance. The useful lactic bacteria of the dairy should not produce gas when subjected to this test.

PRACTICAL VALUE OF A BACTERIOLOGICAL ANALYSIS.

A bacteriological examination of the milk promises to be of practical value, at least in two directions. It has already proved itself to be a useful test in some of the modern high-class dairies where the dairyman is interested in obtaining the very best product. These high-class dairies make a frequent bacteriological analysis of their milk, and thus keep a general oversight of the bacterial contamination. By carefully watch-ing these tests day by day the dairyman can discover the sources of excessive contamination and frequently can remove them. The dairyman can also in this way have an accurate knowledge of the kind of milk which he is furnishing to his customers, and he is able much more satisfactorily to con-

FIG. 42.

Fermentation tubes, showing method of testing gas-producing power. *A*, growth of a non-gas-producer; *B*, growth of a gas-producer

trol the dairy processes. Quite a number of our modern dairies thus keep a more or less careful record of the bacteriological condition of the milk which they produce.

The examination of the public milk supply by the health boards of our cities promises to be of considerable value in the future, although up to the present time it has not been made very extensively and, indeed, for reasons already pointed out, the bacteriological examination has hitherto had very little significance. As long as it is impossible to say how large a number of bacteria may be consistent with wholesomeness of the milk, so long is the quantitative analysis of milk by health boards a matter of doubtful value. In the last year or two the problem of a bacteriological oversight of the milk supply has been agitated in various places, and it seems at least probable that the development of the methods of inspection will in future demand a more careful study of this problem. If it should be practical for health boards to detect, by means of a differential analysis, the types of bacteria in milk, it may prove that this method of analysis of public milk supply will give important data for regulating this extremely valuable food product. At the present time, however, the condition of our public statutes is not such as to have made a bacteriological analysis of milk by health boards a matter of much importance, and nowhere has this analysis been turned to much practical use.

ANALYSES OF BUTTER OR CHEESE.

In making bacteriological analyses of these products the methods above described are used with slight modifications. The number of bacteria is given per gram rather than per c.c. To make the tests, a small, accurately weighed bit of the material (butter or cheese) is used. If it is *butter* it may be placed in melted gelatin, and then, by proper agitation, distributed through the culture medium. The rest of the details are the same as with milk. In the analysis of *cheese* there is some difficulty in getting the cheese finely enough divided to distribute the bacteria uniformly. It may be accomplished as follows: A small bit of the cheese is removed with a sterilized knife and placed in a tube containing a known quantity of melted gelatin. The weight of the tube before and after this addition gives the weight of the cheese; or this bit of cheese may be weighed directly upon a bit of sterilized paper. With a sterilized glass rod the bit of cheese is thoroughly rubbed up with the gelatin to make a uniform emulsion. Upon the thoroughness with which this rubbing is done depends the accuracy of the test. After a thorough mixing in this way, by means of a sterilized pipette a certain number of drops of the emulsion are transferred to a second tube of gelatin. By determining the number of drops in a cubic centimeter, and knowing the number of c.c. of the gelatin mixture, it is easy to calculate the exact part of the weighed volume of cheese which is transferred to the second tube. A petri dish culture made of the second tube will give the number of bacteria in the small part of the cheese transferred by the few drops of the emulsion, and from this the number of bacteria per gram may be calculated. It is sometimes necessary, especially if a *qualitative* analysis is to be made, to dilute the emulsion still further. This is done by transferring a definite number of drops of the second tube of gelatin to a third, and making, of course, new calculations as to the amount.

THE DIRECT MICROSCOPIC EXAMINATION OF MILK.

A direct microscopic study of milk for the purpose of detecting the presence of bacteria is rarely of much importance. It is sometimes convenient, however, to make such an examination. This usually is done for the purpose of detecting the presence of *tuberculosis bacteria,* or for detecting the presence of *pus cells.* The method that is most satisfactory is as follows:

It is necessary to make use of an ordinary centrifuge, capable of rotating about 3,000 times per minute. Into the vials of such a centrifuge is placed 10 c.c. of milk to be tested; this is then rotated in the centrifuge from five to seven minutes. In examining for tuberculosis several cover-glass preparations should be prepared from the cream as well as from the sediment. For the study of the sediment the milk is to be decanted from the tubes, leaving the sediment at the bottom; a few drops of sterilized water are added to the sediment to make a uniform emulsion, and then, with a pipette, the emulsion, including the sediment, is withdrawn from the vials and distributed uniformly on the surface of two ordinary sized covered glasses. These are allowed to dry in the air, after which they are fixed in a flame by the ordinary method for microscopic study of bacteria. The cover-glasses are then placed in a covered dish with chloroform or ether, for the purpose of dissolving the fat, after which they are stained for the detecting of pus cells. The stain most satisfactory to use is methylene blue, a solution of which is allowed to act on the cover-glass for a few minutes; it is then washed away and the specimen studied under the microscope. This method of staining will color bacteria a deep blue and will also stain the pus cells. The latter appear as large, irregularly shaped, deeply stained cells as shown in Fig. 9, page 34. It is difficult to distinguish pus cells from the ordinary leucocyte cells which are quite sure to be in the milk. Nearly all samples of milk show some such cells. If the cells are very numerous they indicate the presence of pus. The common method of study is to count the number of such cells in twenty microscopic fields with a one-twelfth-inch objective. If the average number in a field is over twenty it is quite certain that it indicates the presence of pus, and, therefore, suggests some inflammatory condition in the udder; a smaller number of cells per field than this cannot be regarded as necessarily indicating the presence of pus.

To detect the presence of tuberculosis bacteria is not easy, nor are the results certain. For this purpose the sediment prepared as above described, and also some cover-glasses made from the cream in the same way, should be stained by the ordinary stains for tuberculosis bacteria. The directions for such staining can be found in text-books of laboratory bacteriological technique and the details need not be given here. The presence of bacilli which are stained by the methods employed do not certainly indicate the presence of tuberculosis bacillus inasmuch as there are other bacilli liable to be found in milk, which have similar staining properties. On the other hand, the failure to find such bacilli does not indicate the absence of the tuberculosis organism, since it is so difficult to detect its presence that negative evidence has little meaning. The microscopic study of the milk for detecting the presence of the tuberculosis bacillus, is therefore, unsatisfactory and of little value.

TO DETERMINE THE ACIDITY OF MILK.

Although not strictly bacteriological, the test for the amount of acidity in milk is occasionally necessary in connection with bacteriological work, and is extremely useful in many respects. For this reason the method of making this test is here briefly described.

In testing fresh milk it is necessary, if one wishes to be accurate, to boil the sample of milk tested in order to drive off the CO_2. If the milk is diluted with ten times its bulk of water and boiled for a few minutes before testing, the errors introduced by the CO_2 are avoided. The readings will be a little less than without such previous treatment. If the milk is old, such boiling will curdle it and interfere with the ease of the test.

For an accurate test it is necessary to prepare a one tenth normal NaOH solution. A good-sized burette should be filled with this solution.

A carefully measured quantity of milk (50 c.c. is a convenient amount) is placed in a porcelain dish and 1 c.c. of phenolphthalein is added.

The alkaline solution is now to be drawn from the burette into the milk by small additions, the milk being stirred between each addition, and this is continued until the milk retains a permanent faint pink color. This indicates that the acid has been neutralized, and the number of c.c. of the alkaline solution used is read from the burette.

To determine the per cent. of acid in the milk the following calculation is necessary: The number of c.c. of the alkaline used is multiplied by .009 (the amount of acid neutralized by 1 c.c. of this one tenth normal solution) and divided by the number of c.c. of milk tested, the whole multiplied by 100 to give the per cent. of acid, thus:

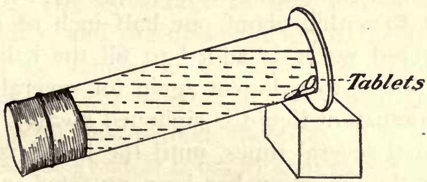
If 50 c.c. of milk are neutralized by 30 c.c. of the one tenth normal solution the result is:

$$\frac{30 \times .009}{50} \times 100 = .54\% \text{ acid.}$$

This method is quite accurate, but a more convenient one, which is fairly accurate, has been devised by which the phenolphthalein and the alkaline are preserved in dried tablets, the Farrington's alkaline tablets. These are prepared with a measured quantity of material such that their use requires no calculation. They are used as follows:

Place in a cylinder 97 c.c. of water and five alkaline tablets. (These may be procured of dealers in dairy supplies.) Cork tightly with a rubber cork and lay the cylinder on the table to allow the tablets to dissolve (Fig. 43). They dissolve

FIG. 43.



Showing method of dissolving alkaline tablets.

slowly and it is best to set them dissolving the night before they are wanted. The solution will not keep, however, more than twenty-four hours and must be used fresh.

17.6 c.c. of milk to be tested is placed in a porcelain dish. The dissolved tablet solution is placed in a burette and drawn off into the milk as in the previous method, until a faint pink color is retained in the milk. The number of c.c. of the alkaline solution gives directly the per cent. of acid. Thus if 13 c.c. of alkaline solution are required the acid present is .13%. This direct reading makes this method very convenient, and it is fairly accurate, accurate enough, indeed, for most work upon the acidity of milk.

DETERMINATIONS OF THE AMOUNT OF DIRT IN MILK.

The dirt which makes its way into milk is a source of bacteria and its determination is, therefore, closely associated with bacteriological problems. There is no very simple method of determining the amount of dirt in milk, and none gives the amount very accurately. The methods in use will give somewhat closely the amount of insoluble dirt that may find its way in the milk, but none of the soluble material is detected. The methods of determining this filth are two, one depending upon gravity and the other upon centrifugal force.

Gravity Method.—By this method a measured quantity of milk, usually a liter, is allowed to stand in a tall cylinder for several hours. During this time the solid dirt will mostly settle to the bottom of the jar and can be seen as a sediment. By means of a tube the milk is carefully siphoned off from the sediment to within about one half inch of the bottom, and then filtered water is added to fill the cylinder. The material is allowed to sediment again for several hours, and again the supernatant liquid is siphoned away. This operation is repeated several times, until the liquid is practically clear. After the operation has been repeated until the dirt has been thoroughly washed, the sediment which has settled in the bottom of the cylinder is filtered from the remaining liquid. The filter paper used should have been previously accurately weighed with a chemical balance, after having been thoroughly dried by being kept for a day or two in an ordinary chemist's desiccator. The sediment is collected upon the filter paper, and the paper carefully dried, first by heat and then by being placed in a desiccator for a day or two. It is then to be weighed upon the chemical balance. After weighing it should be replaced in the desiccator for another twenty-four hours and again weighed. If the two weighings agree, the weight may be taken as the proper weight, but if the second weight is less than the first, it indicates an insufficient drying of the filter paper, and it must be placed again in the desiccator until two successive weighings agree. The weight of the dried filth will be obtained by subtracting the original weight of the filter paper from the final weight.

Centrifugal Method.—This has the advantage of being much more rapid and probably fully as accurate as the first. In most respects it is identical. Filter papers must be dried and weighed as before, and all of the methods of procedure are the same except the method of separating the sediment from the milk. For this purpose a measured sample of the milk is placed in one of the vials of a *centrifuge* and rapidly rotated. The rapidity of rotation should be about 2,000–3,000 times per minute. After the dirt has thus been sedimented the supernatant milk is pipetted off in the usual way, and the sediment, after being washed as before, is collected upon filters. The advantage of this method is its much greater rapidity, and the disadvantage is the fact that it is necessary to use smaller amounts of milk, since the centrifuges in use are not adapted for the treatment of large quantities. With this method, therefore, it is especially important to use the greatest precaution in selecting the sample, for any irregularities in the sampling will produce very great errors in the result. In order that the sampling may be accurate it is, of course, necessary that the milk should be thoroughly stirred and the sample taken should be as nearly as possible an average sample of the whole milk. In all methods of estimating the dirt in milk it is necessary to be especially careful in the matter of selecting a sample.

The results obtained by these methods give the amount of dried dirt, but does not of course represent the original quantity of filth, which was at the outset in a moist condition. To obtain the moist weight of the filth it is necessary to multiply the figures obtained at the end by a certain factor which will reduce it to the weight of ordinary moist material. This, of course, will be a variable one according to the material of which the dirt is composed, but a rough approximation may be obtained by multiplying the dry weight by the factor 7, which will produce a result not very far out of the way.

The amount of filth that is found in milk tested by these methods is subject to the widest variation, as would be expected in accordance with the conditions of cleanliness in the dairy. In some samples tested the amount has been found to be as small as 2 mgs. per liter; on the other hand, in some samples of fresh milk obtained in a moderately clean dairy, but without any precautions and without straining the milk, the amount is sometimes as high as .3 of a gram or more. This number is very high and indicates an excessive amount of dirt.

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