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BULLETIN No. 25.

U. S. DEPARTMENT OF AGRICULTURE

OFFICE OF EXPERIMENT STATIONS

DAIRY BACTERIOLOGY

BY

H. W. CONN, Ph. D.

PROFESSOR OF BIOLOGY IN WESLEYAN UNIVERSITY

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LETTER OF TRANSMITTAL.

UNITED STATES DEPARTMENT OF AGRICULTURE,
OFFICE OF EXPERIMENT STATIONS,
Washington, D. C., August 5, 1895.

SIR: I have the honor to transmit herewith for publication Bulletin No. 25 of this Office, on Dairy Bacteriology, by H. W. Conn, Ph. D., of Wesleyan University. This bulletin is supplementary to Bulletin No. 9 of this Office, on the Fermentations of Milk, by the same author. It covers the three years intervening since the publication of that bulletin, and gives a review of the present status of our knowledge of dairy bacteriology and its application.

Respectfully,

A. C. TRUE,
Director.

Hon. J. STERLING MORTON,
Secretary of Agriculture.

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DAIRY BACTERIOLOGY.

By H. W. Conn, Ph. D.

The advance in our knowledge of dairy bacteriology has been very rapid in the last few years and the bulletin on the Fermentations of Milk,[1] published in 1892, no longer expresses our knowledge of that subject. The last three or four years in particular have seen a more extended application of bacteriology to practical dairy matters until to-day it is becoming recognized on the part of our dairy schools that a knowledge of bacteriology is one of the essential features of a dairy education. In the following pages will be given chiefly the advances in knowledge on the subject of dairy bacteriology which have occurred since the publication of the above bulletin, although occasionally for the sake of completeness some articles may be referred to which antedate the publication of that bulletin.

I. BACTERIA IN FRESH MILK.

It has long since been ascertained beyond question that pure milk drawn from a healthy cow contains no bacteria, and that all bacterial contamination of the milk comes from external sources. While this fact has been redemonstrated by the most recent work, it has appeared that the statement must be for practical purposes considerably modified. In the first place, the difficulties which lie in the way of obtaining milk from the cow without bacterial contamination are extremely great and sometimes seemingly insurmountable. Of the many attempts which have been made to obtain milk which shall from the first be sterile most have proved failures. Enough of them have met with success to demonstrate the general position, but so many of them have been unsuccessful as to show the extreme difficulty of obtaining sterile milk in this way. In spite of cleanly methods, of sterilized vessels, and of the greatest care to prevent dirt and dust from falling into the milk, the milk when first drawn from the cow has in the vast majority of cases contained bacteria. This fact is explained by the ease with which the milk is contaminated in the milk duct. The milk duct is, of course, open to the air and will contain at the close of the milking a considerable amount of milk adherent to its walls. Bacteria from the air have no difficulty in making their way into the duct, growing there and becoming extremely numerous. The result is that by

[1] U. S. Dept. Agr., Office of Experiment Stations Bul. No. 9.

the time of the next milking the milk ducts contain bacteria in great numbers and these will inevitably contaminate the milk. It follows that the ease of obtaining uncontaminated milk from the cow varies very much with the different periods of milking. There should be the greatest difficulty at the beginning and the least difficulty at the close of the milking.

Several sets of experiments upon this line have demonstrated this position, although leading to some rather interesting and slightly contradictory results. Schulz[1] has made a large number of tests of milk at the first of the milking, at the middle of the milking, and at its close, and the results were uniform. The first milk contained many bacteria, in some cases as high as 83,000 per cubic centimeter as soon as it was drawn; at the middle of the milking period the number was considerably less—an average of about 9,000 per cubic centimeter—and at the close of the milking period it was very small, in some cases there being no bacteria present. This of course is in accordance with the idea that the milk gradually washes out the milk ducts. A similar set of experiments, more extensive, has been performed by Gernhardt,[2] and while in some respects his results agree, in others they are somewhat surprisingly different. He finds in most of his tests the number of bacteria at the first is high (sometimes as high as 600,000 per cubic centimeter), then rises rapidly, and at the middle of the milking becomes very great, sometimes as high as 7,000,000 per cubic centimeter. Toward the close it falls again to a small number, and the milk is sometimes sterile. This result has been obtained in quite a number of experiments and is one that is somewhat difficult to interpret. The suggestion which he made was that the bacteria not only enter into the milk ducts between the milkings, but that some of them actually make their way into the very depths of the milk gland itself and there grow and multiply. These internal masses of bacteria are not detached at the first of the milking, but only get into the milk after considerable milk is drawn. Thus the first milk would be heavily impregnated because of the washing of the outlet of the duct. The extent of this contamination would increase as the deep-seated channels are washed out. At the close the milk would be purer and perhaps sterile. Gernhardt found great irregularities in his figures, and was inclined to explain them by the supposition that occasionally a whole colony of bacteria would be detached from the milk duct and find its way into the milk. This of course would very greatly impregnate certain lots, while others that did not happen to be thus contaminated with the bacteria colonies would be comparatively pure.

From these results it will be seen that our understanding of the relation of bacteria in fresh milk must be somewhat modified. Undoubtedly the milk gland of the healthy cow produces milk which is uncontaminated with bacteria, but the large caliber of the milk ducts

[1] Arch. Hyg., 14 (1892), p. 260.

[2] Inaug. Diss. Jurjew, 1893.

makes it possible for bacteria to grow in the duct to considerable extent, so that it becomes a matter of extreme difficulty to obtain milk from the cow, even with the greatest precautions, which shall not be contaminated.

Essentially the same facts have been demonstrated in regard to human milk. It had been earlier claimed by Escherich that woman's milk is sterile when taken with sufficient precautions. This claim has, however, been disproved by several independent investigators. Honigmann,[1] Knochenstiern,[2] Ringel,[3] and Palleske,[4] have all independently found that it is impossible to get human milk fresh from the mammary gland in such a way as to be sterile. Not a very large number of bacteria have been found, and the different results of early experiments are doubtless due to the fact that only a small amount of the milk was tested, while in the later experiments large quantities have been taken. The source of the bacteria in these cases has been recognized to be external and not the mammary gland itself, at least in the cases of healthy women. Here, as well as in the cow, the bacteria gain entrance to the milk ducts and multiplying there give rise to contamination of the milk.

GERMICIDE POWER OF MILK.

The suggestion which was made by Fokker that freshly drawn milk was a germicide, surprising as it might be at the time it was made, has been abundantly verified by more recent work. The experiments of Freudenreich, as already pointed out, confirm the position advanced by Fokker, and in more recent years others have reached the same conclusion. Indeed, we have learned to recognize that animal secretions in general have more or less of a germicide power and it is no longer a surprise to us to find this true of milk. The germicide property of freshly drawn milk has, however, been more recently investigated by F. Basenau,[5] who is inclined to question the matter, finding that for a certain pathogenic germ which he studied, milk has no germicide power. Any practical value to this germicide power does not as yet appear. It is known that fresh milk is a very poor medium for the growth of certain pathogenic bacteria; for instance, the cholera germ is quite rapidly destroyed in fresh milk. To what extent this germicide property destroys the cholera germ, however, we do not yet know. According to recent work it appears to be due rather to the multiplication of the lactic organisms.

SOURCES OF MILK BACTERIA.

Pure milk of healthy cows is undoubtedly free from bacteria, and all the contamination is external. We have in the last three years obtained a closer knowledge of the common sources of such contamination. In

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[1] Ztschr. Hyg., 14 (1893), p. 207.
[2] Inaug. Diss. Dorpat, 1893.
[3] Münch. Med. Wochenschr., 1893, p. 513.
[4] Virch. Arch., 130 (1892), p. 185.
[5] Arch. Hyg., 23 (1895), p. 44.

the first place we have learned to look upon the contamination from the air as of less importance than was formerly believed. When bacteriological work was first begun the air was commonly thought to be the great source of bacterial contamination. It is of course true that milk does receive some bacteria from the air during the milking. In an illy ventilated stall, filled with dust from disturbed hay, bacteria will be floating in the air. During milking quantities of dirt and dust are brushed from the under sides of the cow's body and fill the air in the immediate vicinity with bacteria. But such contamination is to be charged to the hay or the dirt on the cow rather than the air. With such exceptions the air appears to be of little significance in this matter, and if we exclude the dust from the food and the dirt from the cow we may almost disregard the air as an important source of contamination. At all events other sources are of much more importance. There are several of the more common sources. The milk vessels themselves are a significant source of contamination. Experiments by Bitter have shown that the number of bacteria in milk is materially decreased if sterilized vessels be used instead of the common milk vessels under similar conditions. We still regard the hands and clothing of the milker as another important source of contamination. The milker seldom makes a cleanly toilet before milking, and any dirt upon his hands or upon his clothing will have abundant chance to get into the milk vessels. We also regard the ordinary water in which the milk vessels are washed, and especially with which the milk is too frequently diluted, as a very important source of bacterial contamination, especially in connection with certain pathological germs like those of typhoid fever. But more careful consideration of the work of the last few years shows us that the great source of bacterial contamination is the cow herself; not, as we have seen, from internal, but from external sources.

Anyone who has noticed the uncleanly condition in which the cow is kept on the ordinary farm will readily appreciate this possibility. As we have just seen, one of the very great sources of contamination, possibly the greatest, is the large number of bacteria which grow in the milk ducts. The other sources of dirt are very great. The hairs of the cow are always covered with dirt and dust, and it is impossible for the milker to prevent a considerable amount of this dirt falling into his milk pail. Every one of these hairs which finds its way into the milk will furnish large quantities of bacteria for contamination. Milk drawn into a sterilized vessel was found to contain 520 bacteria per cubic centimeter, but when drawn into a flaring pail, with considerable disturbance of the udder and bedding, the number rose to 30,000 per cubic centimeter. When we examine the amount of solid material which finds its way into the milk we are amazed at the results. If ordinary milk is allowed to stand for a number of hours, a sediment may be collected which is wholly extraneous matter, and must be regarded as dirt contamination. Different estimates have set the amount of this dirt from 12 to 74 milligrams per liter (Renk[1]). This dirt is largely composed of manure, but the microscope has also revealed undigested hay, molds, hair, shavings, woolen threads, linen threads, earth, cobwebs, particles of skin, human hair, pieces of insects, and down from birds (Grotenfelt[2]). Most of these must be recognized as coming directly from the cow, and they certainly show the great opportunity for contamination to which milk is subject from uncleanly cows.

We must, then, regard external conditions connected with the cow as the great and the most important source of bacteria that contaminate milk. We must also recognize that occasionally diseased udders are a source of milk contamination. Where a cow has an inflamed udder it is probable that there will be in the interior of the gland active sources of bacterial multiplication, and consequently abundant internal sources of milk contamination. This has been demonstrated by a number of observations, prominent among which may be mentioned a series of experiments by Guillebeau,[3] in which an examination was made of the milk of 76 cows suffering from udder inflammation. In all cases he found the milk contaminated with bacteria, and experimental evidence showed him that the bacteria which were in the milk had come from the inflamed condition of the udder, and were pathogenic in the sense that they would produce similar inflammation when inoculated in healthy animals. Now, udder diseases are not uncommon, and we must look upon them as a somewhat important source of milk contamination. We have also learned in the last few years that there are a few other diseases to which the cow is subject that may furnish bacteria to the milk. Prominent among these stands tuberculosis. There is now abundant evidence for the conclusion that the milk of tuberculous cattle, at least in certain stages of the disease, may be contaminated with the tuberculosis germ. This appears to be true at all events after the disease has attacked the milk glands and possibly before. The great prevalence of tuberculosis among cattle makes this source of milk contamination one of considerable importance.

Klein[4] has shown that cows sometimes suffer from diphtheria, and when the disease attacks the milk glands the milk may become impregnated with the diphtheria germs. While this conclusion has been questioned by Abbott[5] and Vladimirow,[6] nevertheless the positive experiments of Klein give us no room to question that this dreaded disease does affect the cow, and that at least under some conditions the cow thus affected may serve as a source of diphtheria contamination to the

[1] Münch. Med. Wochenschr., 1891.
[2] Princ. Mod. Dairy Prac., 1894 (Woll).
[3] Landw. Jahrb. Schweiz, 1892, p. 27.
[4] Jour. Path. Bact., 1894, p. 428.
[5] Ibid., II, 1893.
[6] Arch. sc. biol. Imp. Inst. Med. and Expt., St. Petersb., p. 84.

milk. Several observers (Klein, Cameron) have also given us reason for believing that the same is true to some extent in regard to scarlet fever. There seems to be little question that cattle are sometimes attacked by a slight illness which makes itself apparent in the milk gland as well as elsewhere, and that this disease is closely allied to scarlet fever. A few instances have been given where epidemics of scarlet fever have been traced with some degree of accuracy to such contaminated milk, and, while there is yet possibly a doubt as to whether the disease is true scarlet fever, there is no doubt that there is a certain disease somewhat like scarlet fever which attacks the cow and through the milk gland may impregnate its milk. Lastly, it has been abundantly shown that the foot and mouth disease may in a similar way impregnate the milk. While as yet we do not definitely know the organism that produces this disease, there is no question that it is a germ disease, and the fact that it is transmitted through milk leads us to conclude that the milk of animals suffering from this disease becomes directly contaminated with microorganisms. Beyond these instances we have at present no facts tending to the suggestion of the contamination of milk directly through the diseased cow.

It will be seen in general, then, that in dealing with healthy herds the greatest source of contamination is the bacteria in the milk duct and the dirt on the hairs of the cow. The next greatest source is the milk vessels and the milker himself. Lastly, and to a very slight extent we must look upon air as a source of contamination, but if care be taken of the food and of the dirt on the cow little need be feared from the air itself.

There are other sources of milk contamination after the milking. Probably some of them are of considerable importance, although none of them for practical purposes have so much importance as those mentioned. The distribution of typhoid fever by milk has thus far been always traced to some subsequent contamination of the milk, for we have no reason for thinking that the typhoid germ can come from the cow. Of course one can readily see where such sources of contamination may arise by use of impure water in washing, or by handling of milk by persons who help nurse typhoid patients, and we need not dwell upon them here.

NUMBER OF BACTERIA IN MILK.

The work of the last few years has emphasized the fact that the number of bacteria present in milk is of very little significance. The widest possible variations in these numbers seem to be found under almost identical conditions. While it is true that the general purity of the milk can be estimated by the number of bacteria that it contains this is only true to a limited extent, and not infrequently the presence of large numbers of bacteria is possible even in a very good quality of milk. At no time in the history of the milk can anything like uniformity in the numbers be given. Immediately after milking the number may vary from zero, as we have already seen, to 7,000,000 per cubic centimeter the latter number having been obtained by Gernhardt[1] in some of his experiments. The number ranges between these two figures, dependent upon various conditions of cleanliness, the number zero being found only exceptionally under especially peculiar conditions of care, while the other is found in experiments where no care was taken at all to insure cleanliness. After the milk is drawn the bacteria begin to multiply rapidly, and the number present at any moment subsequently will depend simply upon the temperature at which the milk is kept and upon the species of bacteria present. Some species seem to multiply rapidly and to reach higher numbers than others.

Some experiments have been made by Watson and Loveland[2] to determine the number present at the time when the curdling of milk begins. No constancy is found here, the number varying also very widely. The figures that they obtained in a series of experiments varied between 50,000,000 and 1,000,000,000 per cubic centimeter. This at first seems a little surprising when we remember that the milk is supposed to curdle when the acid reaches a certain per cent, and it might be thought that this per cent should have a definite relation to the number of bacteria. But when we remember that there are present in the milk not only acid-producing but alkaline-producing species, we can see that the actual number required to produce a given per cent of acid will depend upon the relation of these two classes. It was found, moreover, that not a little depended upon temperature, for in experiments where the temperature was kept low the number of bacteria rose very much higher before the milk curdled than in cases where the temperature was more elevated. This fact, of course, simply indicated that the acid-producing species grow more rapidly at high temperatures, while at lower temperatures the species that do not produce acid had a better opportunity for growth. It has been shown, moreover, by Timpe[3] and Kabhrel[4] that the casein has certain powers of neutralizing the acid produced. There is no question that some constituent of the casein neutralizes the acid that is formed, and this will evidently materially affect the number of bacteria present before the milk begins to curdle.

Another line of investigation of the last few years of some little interest is the number of bacteria present in ordinary milk as it is delivered in the milk supply of our communities. This line of facts is especially interesting as giving an idea of the comparative purity of milk in different places. This has been determined in quite a large number of European cities by Renk, Lehmann, Escherich, Claus, Uhl, Cnopf, Schulz, Gernhardt, and others. In American cities the only estimations have been by Sedgwick and Batchelder[5] in the city of Boston, Loveland and Watson in Middletown, Conn., and Russell in

[1] Inaug. Diss. Jurjew, 1893.
[2] Conn. Storrs Sta. Rpt. 1894.
[3] Arch. Hyg., 18 (1893), p. 1.
[4] Ibid., 22 (1895), p. 392.
[5] Bost. Med. and Surg. Jour. 1892, Jan.

Madison, Wis. The number of bacteria in the milk thus delivered is found to vary widely. In the city of Boston in the spring it was found to have an average of about 2,300,000 per cubic centimeter, varying from 30,600 to 4,500,000. In European cities in general the number of bacteria is very much higher than in American cities, judging by Boston as a sample. The numbers there have ranged from 10,500, in some special cases (Giessen), to 169,600,000 per cubic centimeter (also Giessen). It is impossible, however, to attempt to give any average of value even for a single city. The number varies so widely with different months, with different milkmen, with the different ages of the milk, whether morning's or night's milk, and with other conditions, that the figures given by the same observers for the same city show such wide ranges that an average appears to be of no significance whatever. The milk supply of the European cities has in a few cases been found to contain less than 5,000,000 bacteria per cubic centimeter. In many other cases the bacteria content reaches 10,000,000; not unfrequently we find 20,000,000, and in some cases the numbers run up even higher than this, 180,000,000 per cubic centimeter having been found in milk in the city of Giessen at the time that it was delivered to the customers. In Middletown, Conn., and Madison, Wis., we have the only instances given of small communities where the milk is delivered within a few hours (two or three hours) from the time it is drawn from the cow. Here the results were superior to anything found elsewhere. The number found in the milk of a number of milkmen in Middletown varied from 11,000 to 300,000 per cubic centimeter, with an exceptional instance of a higher figure. In the majority of cases the number of bacteria fell below 100,000 per cubic centimeter, which, as will be seen, is much superior to the numbers found in the European cities or in the city of Boston. Russell found 35,000 to 275,000 in April, and 380,000 to 2,000,000 per cubic centimeter during May and June in the milk supply of Madison.

The number of bacteria thus found varies partly with the freshness of the milk, but chiefly with the conditions of milk distribution. In Europe the milk that is distributed in the cities is seldom more than seven hours old, while in American cities it is very much older. Much of the milk delivered in the cities of Boston and New York is forty-eight hours old before it reaches the consumer. It would be natural to suppose that for this reason the American city milk supply would be inferior to that of the European city, but as shown by the above figures the reverse is the case. The explanation, however, is not difficult to find. In our American cities, at least in the Northern cities, which alone have been studied, there is an abundant and free use of ice. The milk is cooled quickly by ice, and is kept in a cooled ice car at a temperature not very much above freezing until it is ready to be taken for final distribution. In European cities, however, ice is too expensive to be used[1] in this way except in rare instances, and the milk is not iced at all during transit.

From these facts it will be seen that very little significance is to be placed upon the number of common bacteria in milk. A first-class milk may contain several millions of bacteria per cubic centimeter, and, judging from tests thus far made, city milk which contains not more than three or four million bacteria per cubic centimeter may be regarded as exceptionally good for European cities. No general average of American cities can yet be made, but the probability is that the milk supply delivered in our large cities by the free use of ice is in general superior to that of the milk supply of European cities. The milk of large cities contains more germs than that of small communities, but is probably no more harmful.

VARIETY OF SPECIES.

In regard to the question of the variety of the species of bacteria found in milk we have accumulated a vast deal of information without, however, obtaining any very satisfactory settlement of the questions that arise. The most puzzling problem with which the bacteriologist has to deal is to determine what is meant by species. The whole question of specific distinctions among bacteria is recognized to-day as in the very greatest confusion. We know that morphological distinctions are rarely sufficient to enable the separation of species, and we are dependent very largely, therefore, upon physiological characters. We have learned, however, as the result of the experiments of the last few years, that the physiological characters are very variable in the same species under different conditions. Two bacteriologists attempting to describe the physiological characters of the same species would be almost certain to give different descriptions in some respects, so much depending upon temperature, the percentages of the materials in culture fluids, method of inoculation, and upon the age of the cultures. All of these factors produce so much confusion in the characterization of species that it becomes a matter of impossibility at the present time to determine what we mean by species. Now, among dairy bacteria very many different forms have been described. Considerably over 200 distinct types of dairy bacteria have been described in literature up to the present time, and all of these will be found, in the descriptions given, to differ from each other. Most of them have been named or numbered by the persons who describe them. They have been found in various dairy products—in fresh milk, in old milk, in butter, cream, and cheese. To what extent the individual members of this long list are to be regarded as distinct species it is at present impossible to say. A careful comparison of their descriptions with each other shows that they all differ from each other in some respects, but whether these differences would not in many cases disappear if the same observer had the 200 species for comparison with each other is by no means clear. Whether the members of this long list are identical with the species found elsewhere in nature or whether they are all distinct is again impossible to state.

At the present time in our descriptions of species one of the important characters is the locality where the species is found, and the species referred to having all been found in dairy products have been only in rare cases carefully compared with species of bacteria that are found elsewhere. When we add to these difficulties the fact that many of the species of bacteria found in milk have been very inadequately described—some of them having been described only by their microscopical characters, some simply by this character with the addition of their effect upon milk, and others by a few other isolated points—it becomes evident at once that at the present time it is utterly impossible to make any statement in regard to the number of varieties of dairy bacteria. It will require a number of years of careful work of comparison and more careful study before we can get any conception of the number of bacteria belonging to the dairy.

The only data of very great interest in this connection at present will be those from the study of the bacteria in a given locality by single observers. We have not very much information along this line, but several experimenters have found in cheeses numbers of bacteria ranging from half a dozen to 20 species in the same locality. Adametz found 19; Henrici[1] found 69 in a series of cheeses. In creameries in Cromwell, Conn., there have been found by Conn some 50 species of bacteria which show undoubted differences in their physiological characteristics. But to what extent these physiological characters may vary with conditions must be left to the future to decide. In many cases the varieties differ from each other only in the slightest characters. Two species, for instance, were found to be identical in all morphological and physiological characters, the only difference being that one, when used in ripening cream produced a pleasant-flavored butter, and the other produced butter with a sharp, sour taste. Since this was the only difference that could be found between these two very closely allied species, it is uncertain whether this was enough to separate them as varieties, or as species, or as individual peculiarities. The whole matter of species is thus in most inextricable confusion. We can not at the present time identify American species with European species with any degree of certainty. We can not positively identify species found in different localities, and it is at least difficult for a person to identify a species of bacteria found one year in a given locality with another species found the following year in the same locality. Up to the present time the examination of the species of bacteria found in dairy products has simply resulted in increasing the list of forms known and described, without as yet coming to any conclusion as to the extent of the variety or to the actual condition of our bacterial fauna. The problem is a large one and can only be settled as the result of work of many years.

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[1] Inaug. Diss. Basel, 1893.

Among the species found in milk we have the greatest variety. Many of them are aerobic, many are anaerobic; many of them liquefy gelatin, perhaps a larger number fail to liquefy gelatin; many of them curdle milk by the production of lactic acid, many of them produce acid in quantities insufficient to curdle the milk; many of them produce a distinctly alkaline reaction without otherwise affecting the milk; many of them curdle the milk by the production of a rennet ferment; many of them appear to have no effect upon milk at all; some grow best at low temperatures, not very many degrees above freezing; others at moderate temperatures, from 15 to 25° C.; others again at high temperatures, 35 to 40° C , while one species described by Leichmann prefers a temperature of 50° or over; some of them are rapid growers, some grow very slowly; some produce a very pleasant flavor in the milk and cream, others produce decidedly unpleasant flavors in milk and cream; some of them are excessively troublesome to the milkmen, but favorable to the butter maker; others are troublesome wherever they appear. In short, the effect of the various bacteria upon milk is extremely varied, so that the forms of fermentation are almost as numerous as the numbers of varieties of bacteria themselves.

Although the discussion of pathology does not lie within the purpose of this article, it is impossible to avoid certain aspects of the question of the relation of pathological germs to milk. Most dairy bacteria are entirely harmless, but there have been found in milk a few species which are known occasionally to have pathological characters. Four such species have been found quite common in various kinds of milk namely, *Bacillus coli communis*, *Staphylococcus albus*, *S. pyogenes aureus*, and *Streptococcus pyogenes aureus*. These species of bacteria, as well known, are sometimes associated with pathological troubles, but there is no reason for thinking that their presence in milk is in the slightest degree detrimental to the healthfulness of the milk, and it is certain that they are external contaminations from the milk ducts. Infants do not appear to be in any respect affected by them. These species are common everywhere, and their pathological characters seem to depend rather upon conditions of the organism than upon their mere presence. The *Bacillus coli communis*, as is well known, while it is occasionally pathological, is a universal inhabitant of the human intestine, and its presence in milk, therefore, is of no special significance. Beyond these few forms, that seem to have no detrimental effect, we have learned that milk contains no pathogenic germs which do not come directly from some diseased individual. Sometimes it may be diseased cattle or sometimes it may be diseased men. As already noted, cattle may suffer from tuberculosis, diphtheria, scarlet fever, and the foot and mouth disease; and if so, the milk may contain the germs of these diseases. It is also a fact that indirectly milk may become contaminated with the typhoid germs; but beyond these few forms the bacteria of milk, so far as can be stated at present, do not appear to be pathogenic. This matter is, of course, of extreme importance in our understanding of the healthful properties of our milk supply, but a further discussion of it is not our present purpose.

One further point, however, in this connection must be noted. While it is true that none of the common milk bacteria are pathological, it is also true that there are some of them that may, under conditions of excessive multiplication, produce poisonous toxins which are directly injurious to the weak stomach of the infant or of the invalid. These germs are not directly pathological, producing no disease by their multiplication in the body, but if they are allowed to multiply sufficiently in the milk the toxins that they produce may have direct poisonous effect upon the infant who drinks the milk. Probably most of the cases of cholera infantum and of other similar intestinal troubles in infants are from such causes (Baginsky[1]). This is not only a theoretical conclusion but an experimental one. Flügge[2] has made a study of the bacteria in milk which are liable to resist the temperature of boiling, and has found among them several different varieties which were demonstrated to produce poisonous toxins—that is, to produce, when growing, secretions which poison small animals. If these bacteria therefore were allowed to grow unduly in the milk, they might in warm weather produce a sufficient quantity of these products to give rise to intestinal disturbances. This class of bacteria therefore is not strictly pathological, and is not to be feared in milk that is fresh, but in milk that is stale it may be suspected, especially in summer months, as giving rise to many of the cases of intestinal troubles which are so common in infants fed with cows' milk.

DAIRY INSPECTION.

One of the subjects which has been little by little attracting more and more attention among bacteriologists and dairymen is the desirability of some form of dairy inspection. Our laws have furnished us with a milk inspection which protects our pocketbooks, but we are learning that the adulteration of milk by water is really of far less importance to the public in general than the contamination of the milk by improper types of microorganisms. We have learned, as already pointed out, that milk is occasionally contaminated with pathogenic germs, but that such organisms come only from cases of disease—either diseased cattle which directly contaminate the milk, or diseased persons which indirectly are a source of such contamination. We have learned that if we exclude such sources of contamination, milk drawn from healthy cattle, even though it may contain a large number of bacteria, is perfectly good and wholesome—the microorganisms producing not the slightest injury to the human body, and in many respects being directly beneficial to the dairyman. While the patho-

[1] Berl. klin. Wochenschr., 1894, No. 43 and 44.

[2] Ztschr. Hyg., 7 (1894), p. 272.

genic organisms may be guarded against by the sterilization of the milk before it is used as food, we have learned in recent years that this method is unsatisfactory. Such sterilization slightly injures the value of the milk as a food, and secondly, it appears to be impossible to get it universally adopted owing to the difficulty and the trouble of sterilization. It is impossible to depend upon universal sterilization to prevent distribution of disease through milk. If the public is to be freed from the possible effects of pathogenic organisms in milk, the proper point to apply precautions is at the sources of milk supply, and to prevent its contamination by pathogenic organisms. This is perfectly possible, provided a proper knowledge of the necessity for care can be distributed among our milk producers. There is no need of milk becoming contaminated by any of these pathogenic organisms, with a possible exception of tuberculosis, if care and scrutiny are used by the milk producer to prevent the milk from diseased cattle being distributed, and to prevent its being placed under conditions for secondary contamination from diseased persons.

With all of these facts in mind it becomes plain that the proper public precaution against the evils resulting from such contamination consists in some system of dairy inspection, some system by means of which the public may have a direct knowledge and direct control over the conditions of its milk supply. Already such a dairy inspection in one form or another has been attempted in several places. In certain regions in Switzerland a dairy inspection is enforced. In Germany and in England there are laws regulating the method of production and distribution of milk. They are as yet largely in their infancy and have been of little practical good up to the present time. In America little has been done in this line, although in some States an attempt has been made in this direction. In New Jersey a system of furnishing "certified" milk has been established, by which a committee of experts certify to the healthful condition surrounding the production and distribution of a certain grade of milk which has their certificate. Such "certified" milk is reliable, but of course demands a higher price than ordinary milk.

The chief advantage in such dairy inspection is to the milk producer. In Switzerland and in the United States the plans started with the dairymen. Of course the benefit is also largely to the public, but the producer of the milk will in the end gain the greatest advantage in the increased confidence which the public may have in his product. At the present time the public is rapidly becoming suspicious of the healthful qualities of our milk supply, and just so far as this suspicion can be allayed by the inauguration of a proper dairy inspection, just so far will the milk industry be benefited.

It must, however, be noted that even such a dairy inspection will not render milk absolutely sure. The insidious disease tuberculosis may, and frequently will, with our present knowledge, escape the notice of even an expert. To what extent tuberculosis may be stamped out by the use of tuberculin can hardly be stated, but at the present time many an early stage of tuberculosis will escape notice. Instances have been noted of fancy herds of cattle, kept under the best possible conditions, infecting their milk in such a way as to produce the disease in those drinking the milk. We can not therefore expect that the dairy inspection will render milk absolutely reliable unless the tuberculin methods eradicate the disease among our cattle. But such a dairy inspection will probably remove entirely the dangers from the distribution of typhoid, scarlet fever, or diphtheria, and probably of all diseases with the exception possibly of tuberculosis; and as knowledge is extended the demand for such dairy inspection will come not only from the dairyman but also from the public. It is in some places already being demanded by State boards of health.

II. VARIOUS TYPES OF FERMENTATION.

Lactic fermentation.—As pointed out in the previous bulletin, the lactic fermentation is produced by a large variety of bacteria. This fact has been confirmed over and over again in recent years and the list of lactic-acid bacteria has been constantly growing. At the present time a very large number of species (over one hundred) have been described as producing the acid fermentation of milk. To what extent, however, they are all to be regarded as producing the lactic fermentation we can hardly say, because in most cases no attempt has been made to determine chemically the presence of lactic acid, the investigators being ordinarily content with the determination of the production of an acid reaction. Moreover, it is impossible to state to what extent these different species are really different from each other. The list includes many pathological forms, as well as the distinctively dairy bacteria. The first-described species, *Bacillus acidi lactici*, has been found to assume many varieties. When it has been kept under cultivation in a bacteriologist's laboratory for some time certain modifications have taken place in its physiological characters. In addition to this there have been isolated from milk many forms of bacteria which resemble this organism closely enough, and at the same time differ from the characteristics as described by Heuppe, so that we recognize among them quite a number of varieties of this organism. Adametz mentions 15 varieties of this species known to him. It has further appeared that the organisms which produce the normal spontaneous souring of milk in different localities are by no means identical. While it is true that *Bacillus acidi lactici* is quite common in European dairies, it is also true that it is by no means the universal cause of milk souring. Fokker discovered a micrococcus as the cause of spontaneous souring in Wiesbaden and a bacillus in Groningen. Leichmann[1] has

[1] Milch Ztg., 23 (1894), p. 523.

found an entirely different species as the most common in a large series of dairies in Königsberg.

In this country spontaneous milk souring has been found to be produced by different organisms. Quite a variety have been described by Conn, none of which appear to be identical with the European organisms, but some of which certainly show strong resemblance thereto. We see, then, that while the spontaneous souring of milk is an almost universal phenomenon it is not always produced by the same or even very closely allied organisms. Occasionally a large series of dairies in one locality will be found to be soured by the same species of bacteria, while in other cases the spontaneous souring may be produced by different species in dairies at no great distance from each other. Sometimes, indeed, this spontaneous fermentation is absent. Certain herds of cattle have been noted whose milk does not sour, but will after a time undergo other types of fermentation. It is also a fact that not infrequently in the winter months milk is found not to undergo the souring spontaneously, but may be kept for a long time without curdling, and when it does show signs of fermentation the type is entirely different from that of normal milk souring.

As stated above, there have not been many chemical studies of the type of acid produced by the lactic organism. At the same time we possess more knowledge of this type of fermentation than of any other one. Although the lactic fermentation has been studied more commonly in other fluids than milk there is no doubt that the results give us a pretty good knowledge of the general type of fermentation. The most exhaustive work in this line has been done by Kayser,[1] but there have been smaller investigations in the same general line by Mayer, Perè, Haydruck, Gosio, Nencki, Frankland, MacGregor, Delacroix, and others. The foremost fact which has resulted from this work is that different lactic species produce very different effects, not only in the rapidity but in the type of the fermentation. Lactic acid is seldom, if ever, the only acid produced, and the term lactic fermentation is therefore not strictly correct. The acid produced is partly volatile and partly fixed. The fixed acid appears to be chiefly, if not wholly, lactic acid, while the volatile acid is largely acetic and formic. The ratio between the amount of fixed and volatile acid is widely variable with the different species of bacteria. In some cases the amount of volatile acid is equal to the fixed acid, while in most cases it is considerably less, and with some species no more than one-sixtieth of the fixed acid. It has been found, further, that the total amount of acid and the ratio between these two types varies with conditions, with the age of the bacteria culture, with the temperature of the fermentation, and with the extent of time which the fermentation may last. The amount of acid actually increases with the length of time up to a certain point and

[1] Ann. Inst. Past., 8 (1894), p. 737.

may then decrease. The increase may continue after the growth of the bacteria has practically ceased.

In some cases it has been found that if the fermentation continues long enough the fixed acid entirely disappears and the volatile acid alone remains at the end. Furthermore, the chemical properties of the lactic acid produced do not appear to be uniform, or in other words, not the same type of lactic acid is produced in all kinds of fermentation. Ordinary fermentation lactic acid (the α acid) exists in three forms, characterized by their optical properties, the one turning the plane of polarized light to the right, a second to the left, and a third being inactive in its effect on polarized light. Each of these three types of lactic acid is produced by the lactic bacteria. The most common is that turning the plane of polarized light to the right. The inactive form is quite common, and quite a number of species produce levorotatory acid. Some appear to produce a mixture of two of these types. Some of the species produce left or right handed lactic acid when growing in one culture medium, and the inactive acid when growing in another; and different kinds of acid may be produced by young and old cultures. It was formerly supposed that oxygen was necessary for the lactic fermentation, which was regarded as a sort of oxidation. But oxygen is used only in small amount in any case, and probably as a support to the life of the bacteria and not as a part of the fermentation proper. Indeed some anaerobic species produce a typical lactic fermentation, of course in the absence of oxygen (Kayser[1]).

Little is yet known of the by-products of the fermentation, although alcohol, butyric acid, carbon dioxid, and free nitrogen appear to be common, and marsh gas is occasionally produced. That other by-products are formed is evident from the flavors and tastes which the different species impart to milk, cream, and butter. In the lactic fermentation, however, these by-products appear to be small in amount, the chief product being the acids, quite contrary to the relations in the butyric fermentation. The curd of spontaneously soured milk does not appear to be simply the casein precipitated by the acid, for Storch[2] shows that sour-milk curd has different chemical properties from curd produced by precipitation with pure acids.

As seen in a later page some of the types of milk fermentation are directly produced by chemical enzymes formed by the bacteria. Many attempts have been made to prove the same to be true of the lactic fermentation. All such attempts have, however, been failures, and thus far no one has obtained any evidence that such enzyme is produced. The fact above mentioned that the fermentation continues after the bacteria growth has practically ceased appears to require some explanation which has not yet been reached.

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[1] Ann. Inst. Past., 8 (1894), p. 737. [2] Bot. Centbl., 1893, No. 23.

Electricity.—The action of lactic organisms appears to be still the only known method of spontaneous milk souring. It is true that careful investigation has shown that when milk is drawn fresh from the cow it is commonly very slightly acid, an acidity dependent upon certain conditions in the milk gland, but no other secondary causes of milk souring are known except that of microorganisms. Experiments on the relation of electricity to milk have verified the conclusion mentioned in the previous bulletin, that electricity has no direct influence in producing the souring of milk. The only recent work on this subject has been that of Liebig,[1] whose conclusions are that the souring of milk during a thunderstorm is simply due to the rapid growth of bacteria, produced by the same conditions which produce the thunderstorm, a conclusion which was announced in the previous bulletin.

Butyric acid.—We know very little more in regard to this type of fermentation than at the time of the writing of Bulletin No. 9. Butyric acid fermentation is a very common type. At the present time about a dozen organisms are known to produce this fermentation and have been described by the following bacteriologists: Gruber, Beyerinck, Hueppe, Conn, Botkin, Perdrix, Kedrowski, and Loeffler. There are many others which have been less thoroughly studied, but which produce butyric acid as a by-product. They have been found in various milk products (milk, butter, and cheese) and in other places in nature as well.

The butyric-acid fermentation does not appear to be so simple a one as the lactic fermentation. In the lactic fermentation we have what we regard as nearly a smooth splitting of the milk-sugar molecule into carbonic acid and lactic acid. It is true that there are always produced other by-products, and these sometimes in considerable amount, but these secondary products are not very important in the lactic type of fermentation. In the case of butyric acid, however, the reverse is the case. Butyric acid is to be regarded probably in all cases as an end product of a long series of fermentative changes. It is always accompanied by many by-products, and usually the butyric acid is slow in its appearance and is not the chief product in the fermentation. There is therefore no distinct butyric acid fermentation type comparable to the lactic fermentation, but this acid is simply to be regarded as an end product of a large number of other fermentative types. As would be expected, therefore, the butyric-acid organisms are very variable. Many are anaerobic, but some of them are aerobic. Most of them produce spores, and all of them produce many other products in the fermentation besides butyric acid. With some of them the butyric acid produced is very small in amount, in other cases it is very great in amount. In small quantities it is probably a by-product in large numbers of fermentative types. This type of fermentation is of comparatively little importance in dairy matters. In the milk itself

[1] Inaug. Diss. Heidelberg, 1891.

butyric acid never appears in sufficient quantities to be noticeable. In ripening cream, butyric acid has not yet been detected, and probably is not formed in any considerable quantity. In the rancidity of butter this type of fermentation plays an important part and possibly also in the ripening of cheese.

Bitter milk.—Only a slight addition to our knowledge of this type of fermentation has occurred in the last three years. Two organisms have been previously described as producing bitter milk, one by Weigmann and one by Conn. Freudenreich[1] has recently added to the list two new organisms producing this peculiar trouble, one of which he obtained from bitter cheese, the other from bitter cream. The experiments of Freudenreich not only show that his organisms are new bitter-milk species, but they also tend to show that the explanation of bitter milk given by Hueppe is erroneous. Hueppe thought that the bitter taste in milk was caused by peptones produced by peptonizing germs. As pointed out in the previous bulletin, this seemed to be hardly applicable to many of the facts, and Freudenreich has now disproved the claim by separating the bitter-tasting material in a partially free condition. By treating with alcohol he obtained a product which has the characteristic bitter taste, but is not a peptone. The bitter taste in the four described species is therefore due to a special bitter substance produced by these organisms. Vandenhoydonck[2] has found in one instance that bitterness in milk was produced in a whole herd by feeding to the cattle turnips which had been washed in foul water. Whether it was due directly to the turnips or to the water in which they were washed he did not ascertain, but the conditions were such as to point plainly to microorganisms as the direct cause.

Rennet and tryptic forming bacteria.—In the previous bulletin it was pointed out that a large class of bacteria probably produces chemical ferments, and by these enzymes affect milk. The class of bacteria in general which liquefy gelatin has been found to have tryptic characters, and many of them also curdle milk without rendering it acid, but subsequently dissolve the curd. While it has been for a number of years believed that the curdling was due to the formation of a rennet-like enzyme, and the solution of the curd to a trypsin-like ferment, this has been recently demonstrated by experiment. In the first place, Fermi succeeded in isolating from bacteria cultures a tryptic ferment which had the power of peptonizing proteids. Later Conn[3] isolated from milk cultures of several species of bacteria a ferment having all of the essential characteristics of rennet, and separated it from the simultaneously formed tryptic ferment. This observation was subsequently confirmed by Gorini.[4] This formation of rennet is quite

[1] Land. Jahrb. Schweiz, 1894.
[2] Ztschr. Fleisch- und Milchhyg., 4 (1893), p. 55.
[3] Conn. Storrs Sta. Rpt. 1892.
[4] Hyg. Rund., 1893, No. 9.

a common characteristic of liquefying bacteria, and is usually at its maximum at a moderately low temperature. The rennet and tryptic ferments are commonly produced by the same species of bacteria, though the quantities do not run parallel. Some species produce a large amount of the rennet and a smaller amount of the tryptic ferment; others the reverse. There are, on the other hand, some of the trypsin-producing bacteria which appear to produce very little, if any, of the rennet. In some the tryptic ferment has been found to be produced in such quantities as to peptonize the casein before the rennet accumulates in sufficient quantity to curdle it, and the milk therefore becomes transparent from the dissolved proteid without curdling; but proper methods of isolation have shown that even in these cases the rennet ferment is produced, although more slowly than usual, and therefore the curdling does not occur. In other trypsin-producing species, however, the milk in a similar way is rendered transparent without curdling, but appears to produce little or none of the rennet ferment (bacillus of swine cholera).

This class of enzyme-producing bacteria is quite abundant in dairy products, but does not appear to be so abundant as the lactic class. In some specimens of milk there will be found none of the enzyme-forming class, while in other cases the number of enzyme-producing bacteria may be very large. The relation of these two classes in different specimens of milk is indeed highly variable, sometimes the one and sometimes the other being in excess. Where the enzyme-producing class is in excess we may with certainty expect abnormal characters in the milk, for the normal dairy milk contains a majority of the lactic organisms. It is stated by Grotenfelt[1] that these enzyme-producing bacteria are not very abundant in Denmark and northern Europe, although they are abundant in southern Europe. This class of organisms is of practically little importance in dairy matters. They sometimes interfere with bacteriological experiments because most of them produce spores and are with difficulty killed by heat. For this reason also they are of great significance in the processes of sterilizing milk, their presence frequently rendering it impossible to produce the complete sterilization. It is possible also that they are of considerable significance in the ripening of cream and cheese, although this is doubted by Freudenreich.

When this class of organisms acts upon milk there is produced, after a time, a moderately clear liquid which contains the dissolved proteids. While it is usual to regard them as in a condition of peptones, Duclaux questions this, and has regarded this dissolved proteid as of a special form which he calls *casecone*. Bernstein[2] has suggested the manufacture of a new healthful drink by the use of such organisms. He inoculates milk with a tryptic-producing bacteria, which after a time digests the casein, and then by filtering he removes the bacteria and obtains a

[1] Princ. Mod. Dairy Prac., 1894 (Woll).

[2] Milch Ztg., 24 (1895), p. 85.

clear, nutritious, healthful liquid which contains all of the digested proteids.

Blue milk.—The only important addition to our knowledge of blue milk is from a paper by Gessard.[1] This paper has explained some of the seeming complexities of earlier work. It still appears that there is only one species of bacteria producing blue milk. But as this species has been described by one and another bacteriologist, very great differences have been found—differences not only in general bacteriological characters, but even in the property of producing the blue pigment. That these differences were due to variations in the same species appeared probable, and has been demonstrated by the work of Gessard. He has found it possible to obtain several distinct varieties of the organism by cultivating it under different conditions. These varieties differed widely in the type of pigment they produced, one producing no pigment, another producing a gray color, and another producing a green pigment. The varieties thus obtained agreed with the forms hitherto known, which shows more conclusively that the blue-milk organism is a single species which may exist under several varieties. Gessard also experimented upon the chemical problems connected with blue milk, and found a number of artificial media in which the pigment is readily produced. He finds it necessary not only to have present an acid, but, in order that the pigment be a typical blue, the acid must be lactic acid, and it must be present during the formation of the pigment. He infers that the pigment is the result of synthesis into which lactic acid enters.

Alcoholic fermentation.—Very little that is new has been learned concerning the alcoholic fermentation of milk. The Kefir grains have been somewhat carefully studied by Beyerinck,[2] who has, by means of sections, found the relation of the various bacteria and yeasts in the grains. The alcoholic beverage produced by Kefir has in recent years become somewhat popular in European countries, and Kefir grains have become to a certain extent an article of commerce. Kefir grains very similar to the European forms have been found in the United States.

As to the chemical nature of the alcoholic fermentation we are not very much further advanced. The only paper of importance has been that of Mix.[3] From a careful review of the work of others and by means of experiments of his own he concludes that the alcoholic fermentation is really produced by the yeast present in the Kefir grains, and that the bacteria present had nothing to do with the alcoholic fermentation proper. The bacteria present produce lactic acid and subsequently dextrose, while the yeast ferments the dextrose into carbonic acid and alcohol. Quite a number of yeasts have been found capable of producing this alcoholic fermentation. Those that are known up to the present time to have this power are the following: *Saccharomyces*

[1] Ann. Inst. Past., 5 (1891), p. 737. [2] Arch. Neerl., 1888–89, p. 428.
[3] Proc. Am. Acad. Arts and Sc., 26 (1891), p. 102.

lactis, *S.* of Duclaux, *S. kefir*, *S. tyrocola*, *S. galacticola.* The alcoholic fermentation of milk is so well recognized now as a normal phenomenon that it has been suggested that whey may be economically used in the formation of alcohol by inoculating it with the proper species of bacteria (Spiro[1]).

Slimy milk.—Our knowledge of slimy milk fermentation has not advanced much in the last three years. One or two new species of bacteria producing this peculiarity have been described by Guillebeau,[2] but we are no nearer to a proper solution of the process. The new bacteria which have been described have not been found under conditions which lead us to believe that they can cause the normal spontaneous slimy milk sometimes occurring in dairies.

Pigments.—Recent investigations have shown that the production of pigments in milk is by no means an uncommon occurrence, and that a large number of species of bacteria produce pigments of one kind or another. No new pigments have been recently produced, but we have now knowledge of bacteria producing pigments corresponding to all of the primary colors. We have red milk produced by *Bacillus erythrogenes;* orange milk by *S. auranticum;* yellow milk by *B. xynxanthus;* green milk by *B. flourescens;* blue milk by *B. cyanagenes;* and violet milk by *B. violaceous.* The pigments in most cases do not appear very rapidly, and it is somewhat misleading to speak of red milk and violet milk in the same category as blue milk. Blue milk is a dairy infection which attacks the milk rapidly and produces in a short time a very prominent pigment. In the case of the other types mentioned, the pigment is slow in appearing, sometimes a few days and sometimes several weeks elapsing before it makes its appearance to any appreciable extent. None of these forms, therefore, except that of blue milk, is rapid enough in its action to become a distinct dairy infection, and the production of the various pigments in milk is a phenomenon which is interesting to the bacteriologist only

Miscellaneous.—Among the miscellaneous types of fermentation a few have been distinguished as different. Weigmann and Zirn[3] have studied a type of dairy trouble which they have called soapy milk. This has been found due to a species of bacterium which they isolated from straw of cow stables and which produces a peculiar soapy taste in the milk accompanied by considerable frothing. It is a troublesome infection, rendering the cream difficult to churn and practically destroying its value. A different type of "tainted" milk has been found to be caused by a distinct species named *Bacillus fœtidus.* Beyond these two types the miscellaneous forms of fermentation still remain an unexplored field, and one from which we may expect in the coming years now and again a bacteriologist will isolate some distinct type, and thus little by little we shall gain a further knowledge of this obscure subject.

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[1] Milch Ztg., 20 (1891), p. 900. [2] Ann. Micr., 1892.
[3] Milch Ztg., 22 (1893), p. 569.

III. RELATIONS OF BACTERIOLOGY TO GENERAL DAIRYING.

The greatest advance in dairy bacteriology which has occurred in recent years has been in regard to the practical application of some of the scientific discoveries. The treatment of each of the three chief dairy products—milk, butter, and cheese—is being rapidly modified by modern bacteriology.

MILK.

Probably the most important practical result connected with our milk supply has been a thorough investigation of the subject of milk sterilization and pasteurization, but this subject is not to be considered in this bulletin.

Next in importance stands the recognition of the sources of bacterial contamination of milk. As soon as Lister had shown that fresh milk contained no bacteria it became at once evident that the bacteria present in ordinary milk are due to contamination from the exterior. Further study has shown also that the usual types of milk fermentations are due to usual conditions, while the uncommon types of milk fermentation, which are commonly troublesome, are due to uncommon conditions affecting the barn or dairy. It has also appeared that the remedy against these unusual conditions is to be found in cleanliness. This has been emphasized for a number of years. If there has been anything taught in the last three years it has been the extreme necessity for cleanliness. Poor milk, poor butter, and poor cheese are in a vast majority of cases to be attributed to uncleanliness in the barn or dairy. Not only so, but we have been learning more definitely where we may expect to find the sources of mischievous bacteria, and therefore to what point to direct our special attention in the way of cleanliness.

The great source of bacterial contamination of the milk is the cow herself. This does not mean the bacteria from the mammary gland, but those connected with the exterior of the cow. It is true that there are other sources of importance. The food that the cow eats (indirectly), the cow stall itself, the water with which the cans are washed or with which the milk is adulterated, the hands of the milker as well as his clothes are all occasionally the sources of bacterial contamination. But after all we must look upon the cow herself as the cause of the most trouble. We thus learn that the important point toward which to direct the cleanliness is the cow herself. The farmer never appears to feel that it is necessary for him to keep his cows as clean as he does his horses; but there is very much more real need for cleanliness in the case of the cow. Upon such cleanliness will depend his ability to obtain a pure, wholesome kind of milk, while so sure as he allows his cow to become covered with dirt and manure, so sure will he be liable to have trouble in the quality of his milk supply.

A most valuable gift of recent years to our knowledge of bacteriology is a more definite understanding as to what we may fear in the way of diseases being distributed by milk. We have now acquired a pretty definite knowledge of the kinds of disease distributed by milk, and of the sources from which the milk gets these pathogenic germs. As has been shown in previous pages the diseases which may be directly transmitted from the cow to man are tuberculosis, diphtheria, scarlet fever, and the foot and mouth disease. Diseases which may contaminate the milk by carelessness subsequent to the milking are typhoid fever, diphtheria, and possibly scarlet fever and cholera. The cases of typhoid fever distributed by milk (of which many are on record) have been commonly traced either to contaminated water used in washing the cans or in adulterating the milk, or to some individual handling the milk who also served as nurse to a typhoid patient. Diphtheria has also with certainty been carried through milk from secondary contamination. Scarlet fever and cholera are as yet somewhat uncertain. We have learned further that undue multiplication of some of the common forms of milk bacteria may, through their poisonous toxins, produce summer diarrhea and cholera infantum.

Now, after recognizing these facts, we are in a far better condition to guard against such troubles. To guard against tuberculosis is the most difficult, and our State governments having awakened to the danger are trying to meet this problem. But with healthy cows and a proper care of milk, there is no need that our milk supply should be the means of distributing either this or any other disease. There is no difficulty at the present time in giving a series of general rules to the milk producers and milkmen which will enable them to avoid these sources of difficulty. They should keep their cows healthful; they should keep them clean; they should keep careful watch upon the cows and exclude from the milk supply any one which shows signs of sickness, especially any form of udder inflammation or infection; they should be especially careful about the source of water which is used in washing milk vessels, and should never allow for such purposes the use of well water near a residence in such a position as to make a contamination from sewerage or privy vaults a possibility; they should see that no persons handle the milk or milk cans who have any connection with a sick room of any contagious disease. Such precautions will enable the milk producer to avoid the danger of distributing any of the infectious diseases which have been traced to milk, and will at the same time usually enable him to avoid troublesome fermentations.

Another fact of importance which has been emphasized is the value of cooling the milk as thoroughly as possible. When we compare the results of the study of the milk supply of American and European cities, the facts show at once. In American cities milk that is two days old is actually in a better condition than that of European cities which is delivered within a few hours after milking. The reason is because of the free use of ice in this country, and the fact emphasizes more than ever the extreme value to the milkman of its use. In recent years one of the sources of milk supply for England is Denmark, the milk being frozen for transit. In this way it can be kept perfectly fresh for many days, and can easily be delivered in a country as far away as England.

BUTTER.

In Bulletin No. 9 the prediction was made that "the time will come when the butter maker will always make good butter, and the cheese maker will be able in all cases to obtain exactly the kind of ripening that he desires." This prediction in regard to butter appears to be just on the eve of fulfillment. Previous to the publication of Bulletin No. 9 the use of pure cultures, artificially inoculated into the cream, had been suggested and applied to a certain extent, though little beyond preliminary experiments had been made. At that time little or nothing was actually known in regard to the action upon cream of the different kinds of bacteria that are liable to get into it from ordinary normal sources. Upon these general subjects the last three years has seen a very rapid advance. Storch, Weigmann,[1] and others have experimented to some extent with different dairy bacteria for the purpose of finding, if possible, some kind that would produce the proper desirable aroma and flavor in the butter. Adametz and Wilckens[2] have tried the effect of a series of bacteria, eight in number, upon the ripening of cream, without any special attempt to obtain a good butter, but merely to find out the effect upon the butter of the different species. The most extensive series of experiments of this sort have been by Conn.[3] He has experimented with nearly fifty different species of dairy bacteria, using them for ripening cream and making careful study of the butter obtained from the cream ripened by this large series of bacteria. These bacteria were normal dairy bacteria, and are species, therefore, which may be expected normally to find their way into the cream.

The general results of these experiments have been very interesting. In the first place it has appeared that many of the types of abnormal butter are unquestionably due to improper species of bacteria being present in excessive abundance in the ripening cream. For instance, "foul smelling butter" (Jensen[4]), "cowy" butter (Pammel[5]), "tallowy" butter (Storch), "oily" butter, "turnip-tasting" butter, "dappled" butter (Grotenfelt[6]), have all been found to be caused by definite species of bacteria. Of the various species experimented with by Conn not a few have been found which produce bad-tasting butter, and are therefore to be regarded as causes of abnormal cream ripening and abnormal butter. It has appeared, in the second place, that the majority of the common dairy bacteria are rather inert in their effect upon cream, and when used for ripening the cream have no decided

[1] Milch Ztg., 19 (1890), p. 224.
[2] Landw. Jahrb., 21 (1892), p. 131.
[3] Conn. Storrs Sta. Rpt. 1894.
[4] Centbl. agr. Chem., 21 (1892), p. 628.
[5] Iowa Dairy Assoc., 1892.
[6] Princ. Mod. Dairy Prac., 1894 (Woll)

influence upon the butter, neither giving it a good nor a bad flavor, and having practically no influence upon the process of butter making. Their presence in the milk and cream will therefore be of no importance to the butter maker. Thirdly, it has appeared that there are a few species of bacteria whose growth in the cream produces a pleasant, desirable aroma and flavor. These species are seemingly fewer in number than the others, but it is to their presence that a good butter is due, and it is with little doubt largely the presence of these species in June cream and their absence in January cream that gives June butter a better flavor than winter butter.

When we recognize, then, that the species that produce the proper flavor are comparatively few in number, the question arises as to why butter makers commonly produce good butter. The answer is simple enough. Cream itself without any ripening will produce a moderately good butter, though one without any very decided flavor. Now the butter maker in ripening his cream will always produce in it a certain amount of acid from the lactic organisms, and even if he has no proper flavor-producing species present, the butter that he obtains will be a moderately good product, provided he does not happen to have any of the mischievous species present. During certain seasons in the year he can obtain a butter that has no very bad taste, and yet that does not have the desirable flavor. No method at his disposal will enable him at these times to give to his butter the flavor he desires. Under such conditions his cream is affected by the neutral class of bacteria, while mischievous ones are absent as well as the desirable flavor-producing species. Now, by proper care in barns and dairies, the mischievous species may be in general kept out of the cream. By the use of cleanly methods in the cow stall and dairy we may depend upon the milk and cream containing a small quantity of bacteria and only wholesome ones. The careful creamery superintendent may have some control over the farmers that supply him, and can by proper care prevent the improper species of bacteria from reaching him. But hitherto he had no means of assuring a proper supply of the species which produce the best flavor. The fact is that during the different seasons of the year the dairy bacteria vary. Conn has found that winter cream and June cream contain a distinct bacteria flora in the same creamery; that the species of bacteria in different creameries differ at identical dates; that the species furnished a creamery by different patrons differ; and that, in short, the bacterial flora of the creamery is undergoing constant change. Now it is, of course, largely a matter of luck whether the cream at a given creamery happens at a certain time to have the high flavor-producing species present.

Various attempts have been made to control the bacteria in the cream, and furnish artificially the flavor-producing species. Starters from creameries where good butter is made have been taken to other creameries where poor butter was being obtained. One of the most recent methods of avoiding the difficulties of the ripening has been to omit the ripening, and simply to add to the cream either lactic acid or hydrochloric acid in proper proportions (Bolle[1]). It has been claimed by those using this method that there is obtained in this way an excellent quality of butter in every respect save one—it does not have the proper flavor. This, of course, is exactly what would have been expected, for while the acid may be needed for the proper churning of the butter, the aroma and desired flavor is the result of bacterial decomposition, and can never be produced by the simple addition of acid to the cream.

The selection of the species of bacteria which have been used for ripening cream has depended upon two different principles. In the first place it is well known that cream ripening always results in cream souring. For this reason it has seemed to some bacteriologists that the souring was perhaps the important factor, at all events an essential factor, and in searching after a species of bacteria to use in a pure culture for artificial inoculation of cream they have sought for those producing lactic acid and thus souring the cream. The species of pure cultures that have been isolated and whose use has been introduced in Europe by Storch, Quist, Hansen, Blaumfeldt, and Weigmann have all been acid-producing species. When inoculated into the cream they sour it rapidly and produce at the same time a desirable aroma. The use of these organisms has extended from the laboratories where they started in Denmark and in Germany, and at the present time they are used somewhat widely in European countries. To a very slight extent a similar culture has been used in this country under the name of Carl Hansen's butter ferment.

There appears to be no doubt that the use of these cultures has resulted in an improvement in the butter. But the acid ferments have one disadvantage. Being all acid-producing organisms they hasten the souring of the cream, and if added directly to cream already containing lactic organisms they usually cause the ripening to take place too quickly. To get their full effect it has been found necessary to pasteurize the cream before the artificial culture is added (thus getting rid of the bacteria already present). While it has been claimed that these starters improve the cream even when added to ordinary gathered cream, the pasteurization of such cream is recommended, and indeed Grotenfelt concludes, after careful study, that in "a majority of cases it will hardly pay the trouble of applying, if the cream to be ripened be not previously pasteurized." This is especially true of cream that is a little old, and therefore already provided with a large number of bacteria. To produce their own effect they must have the field largely free to themselves.

This process of pasteurizing the cream (heating to 69 to 72° C.) for butter making is, however, somewhat questionable. It has been claimed by many that the heating of the cream to this temperature

[1] Milch Ztg., 23 (1894), p. 624.

injures it for butter making and gives to the butter a slight cooked taste. It has been claimed by others that this is entirely unnecessary, that butter made from such pasteurized cream does not necessarily contain the cooked taste and is equal (perhaps superior) to butter made from unpasteurized cream. Indeed, in some tests butter made from cream simply pasteurized without any subsequent inoculation of pure cultures has rated higher than butter from the same cream unpasteurized. Opinions thus at the present time seem to differ. We may thus agree that unless spécial attention is given, and more care than the ordinary butter maker can ever be relied upon to give, there is danger of obtaining bad results from pasteurization. Careful experiment has shown further, that the pasteurization of cream diminishes the amount of butter that can be obtained from it, the amount of butter falling off from 1 to 2 per cent. It is undoubtedly a matter of a good deal of trouble and expense to pasteurize cream. Where a creamery has 100 or 500 gallons of cream a day it is no little trouble and considerable expense with our present apparatus to heat that cream to 70° C., and especially to cool rapidly afterwards to the proper temperature for ripening. The particular difficulties, then, in the way of pasteurization make it very improbable that this method will ever be adopted by our butter makers generally, except as a last resort, and if the acid starters are, as Grotenfelt says, not worth the trouble unless the cream be pasteurized, it is doubtful whether they will ever be very widely used.

The other method of selecting a species for an artificial culture has been based upon the assumption that the flavor and aroma may be produced in butter by a species of bacteria that do not render it acid. The only culture of this kind that has been used is one obtained by Conn,[1] and named by him "Bacillus No. 41." While Bacillus No. 41 is an acid organism, the amount of acid produced is very small, and it does not noticeably sour milk or cream. When added to cream which is already provided with the lactic organisms it not only does not hasten the souring but it actually delays it considerably, the reason for this appearing to be in the fact that Bacillus No. 41 actually retards the growth of the other organisms present. For this reason this organism can be used in cream that has already become pretty well impregnated with bacteria and without any previous pasteurization, and since this species imparts a flavor, it can be used under conditions where the acid organism can not. In the use of Bacillus No. 41 a large culture of the organism is added directly to the ordinary cream, and the ripening is carried on as usual. The result has been that the souring is delayed, and the ripening may be continued longer, thus the flavor be improved, and a noticeably better quality of product is obtained.

The peculiar effect of this organism appears to be to add to the butter a flavor which the butter maker describes as a "quick grass" flavor,

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[1] Conn. Storrs Sta. Rpt. 1894.

such as he looks for in June butter. The aroma (i. e., the volatile products which appeal to the nose) is not so much affected. The pleasant flavor appears to be added to the butter in all conditions in which the experiments have thus far been tried. It has been tried upon poor cream and upon good cream; upon fresh cream and stale cream; upon separated cream and upon gravity cream; in creameries of the very highest character and creameries of a very much lower grade, and the verdict in all cases has been uniform. Wherever it has been added to the cream for ripening in the proper way there has been an improvement in the quality of the butter made in the individual creamery. The butter of a poor creamery has not, indeed, been brought up to the quality of gilt-edged butter, but it has been improved; and even the gilt-edged butter of our highest class creameries has been pronounced better after the use of this bacillus in the ripening of its cream. Indeed, up to the present time it has been chiefly the better creameries which have adopted its use.

The advantages of this last class of pure cultures over the acid species which have hitherto been used thus appear to be several. First, the method of use is simpler, not requiring for best results any pasteurization of the cream, and requiring no technical knowledge beforehand. Second, when used in cream that has already become pretty thoroughly impregnated with bacteria the use of this organism produces an improvement by checking the growth of these bacteria, and keeping the cream sweet for a considerable time, until Bacillus No. 41 itself has a chance to produce its proper flavor; as a further result of this property the buttermilk keeps sweeter. Thirdly, it has been found that the butter obtained from the cream ripened with Bacillus No. 41 retains its flavor for a longer time than butter made from the same cream at the same time without its use.

There is little question that the method of ripening the cream by pure cultures of bacteria is sure to become popular, for it makes it possible for the butter maker to obtain uniformity all the year round. Both the acid ferments and Bacillus No. 41 are now put up in such a form that they can be readily distributed to the creameries in our country. Their use is rapidly growing, and in the opinion of some of our best butter makers it will not be long before their use will become almost universal; there will thus be produced almost a revolution in the methods of butter making.

It is impossible to say how long any one of these special species of bacteria will hold its characters under the conditions of cultivation to which it is subjected. Bacteriologists have learned that the physiological characters of bacteria species undergo changes with continued cultivation. Possibly, therefore, the species now in use may lose their powers of producing good butter, and thus compel our bacteriologists to obtain others. Nor is there any reason to think that even better forms of such ferments may not be obtained by finding new species with even better qualities or by mixing together two or more species. That the ferments now used will continue to be used is by no means certain, but the method of using pure cultures for ripening cream is based upon such a logically correct principle that there can be little doubt as to its future application.

Keeping property of butter.—As is well known, the peculiar delicate aroma of fresh butter disappears rapidly. It is due to volatile products which quickly pass from the butter. The length of time which it can be retained is seldom more than two weeks, although seemingly by the use of pure cultures in cream ripening the flavor may be retained somewhat longer. After this the butter may remain sweet and wholesome for a time, but eventually is likely to become rancid. As previously pointed out, this rancidity, due largely to the production of butyric acid, may take place entirely independent of the growth of bacteria. Nevertheless, more recent work of von Klecki[1] and Sigismund[2] have shown that bacteria play a part, and a very important part, in the rancidity of butter. While pure butter fat may be oxidized by purely chemical processes, this does not occur in normal butter. When the bacteria in normal butter are studied it is found that they rapidly diminish in numbers (Watson and Loveland)[3]. In the first six hours the reduction in numbers is very great, and in the first two or three days a very large majority of them die and disappear. But some still remain, and while there is after this a constant reduction in the numbers, still some of the bacteria continue to live and grow in the butter for months, and they have been found in butter at least a year old. Now the growth of these bacteria is certainly one of the important factors in the matter of the rancidity of butter. The problem, however, is an excessively difficult one. There appear to be two processes going on—one purely chemical, chiefly oxidation; and the other fermentative, due to bacteria growth. Both may produce butyric acid, but the amount of acid produced is not a measure of the rancidity of the butter. The one process may go on well enough in a cool ice box, while the other requires a higher temperature for its proper action. In the ordinary rancidity of butter, therefore, we must look upon the bacteria as agents of no little importance, but at the present time the subject has presented too many difficulties in the way of investigation to enable us to get a very clear idea of what is going on.

Seemingly, butter made from pasteurized cream has greater keeping qualities than that made from cream without the pasteurization. This is intelligible on the supposition that the rancidity is due in part to the growth of bacteria, but not intelligible when we supposed it was a pure chemical process.

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[1] Centbl. Bakt. und Par., 15 (1894), p. 354.

[2] Arch. An. Nahr., 6 (1891), p. 42.

[3] Conn. Storrs Sta. Rpt. 1894.

CHEESE.

Upon the bacteriology of cheese more work has been done than upon any other phase of our subject. But up to the present time very little in the way of practical application of these facts to cheese making has been found. The subject of cheese ripening appears to be an extremely complex one; and while many investigators have been working at the problem from one side or another, the subjects for research are delusive, and at the present time we can not claim that our knowledge is very satisfactory. The matter of cheese ripening may be divided conveniently into two divisions, although these divisions run into each other. It is customary to recognize the normal ripening and the abnormal ripening of cheese. The normal ripening of cheese is the one that produces a good marketable product, and the abnormal ripening an abnormal product. But when we remember that certain types of normally ripened cheese are very certainly putrefied, as is the case of Limburger cheese, and when we remember further that some types of normally ripened cheese (Roquefort) have a large number of molds through the mass of the cheese, and that the flavor is due largely to this mold, we see that there is no very sharp line of distinction which can be drawn between normal and abnormal cheeses. Nevertheless, this is as good a classification of the types of ripening as any.

Abnormal ripening.—Up to the present time we have a very much better knowledge of the types of abnormally ripened cheese than of normally ripened cheese. In the last few years very many such cheeses have been studied. It is a well-known fact that such abnormally ripened cheeses make their appearance in almost all cheese factories. Sometimes as high as 50 per cent of the cheeses made in a factory are worthless, or comparatively worthless, from the results of abnormal ripening. Many investigators have been studying the various types of spoiled cheese for the purpose of discovering the cause of the trouble. The most common type of abnormally ripened cheese is one in which there is a large accumulation of gas, chiefly carbonic acid, but sometimes ammonia or free nitrogen. This accumulation of gas causes the cheese to swell and produces large cavities. In another special form of a similar infection, known as "Nissler" cheese, the cheese is filled with innumerable small cavities. Between such types where there is an abnormal production of gas, and a normal cheese ripening where the gas is moderate in amount, there appears to be no sharp line. This abnormal swelling has been found to be produced by certain species of bacteria growing in the cheese which develop a superabundance of gas. Quite a large number of species of bacteria have been found to produce this effect by Adametz, Freudenreich, Guillebeau, Weigmann, Duclaux, and others. Some twenty-five species of microorganisms up to this time have been definitely proved to be the cause of such an abnormal swelling of cheese, including both bacteria and yeasts (see Adametz[1]). It

[1] Milch Ztg., 20 (1891), pp. 237, 249; 21 (1892), pp. 205, 221; 22 (1893), pp. 188, 219. 235, 354.

would appear, however, that as much depends upon the conditions in the cheese and the numbers of the organisms as upon their actual presence. It is certainly true that some of these species may be present in small quantity in the cheese and it will ripen normally, while if they are present in large quantities there will be an abnormal swelling of the cheese (Baumann[1]).

The sources of the organisms in this long list are, of course, variable. One important source is the organisms that come from cows suffering from udder inflammations. Several of the bacteria from such cows have been shown to produce such an abnormal swelling in the cheese. Other sources may be in special lots of hay or from sources that are entirely unknown. So varied appear to be the bacteria that no general directions can yet be given for avoiding them; and so little do we know now of the proper conditions, that very little can be done to remedy the trouble. Other types of abnormally ripened cheese that have been studied are red cheese produced by a micrococcus (Adametz); blue cheese produced, according to Vries, by a bacillus; black cheese produced by certain molds (Adametz); bitter cheese produced by a bacillus (Freudenreich); and poisonous cheese, in which the cheese becomes impregnated with a poisonous ptomaine (tyrotoxicon) produced by organisms as yet entirely unknown. In short, all of the types of abnormally ripened cheese which are distinctly recognized in the cheese factory have been studied, and practically all of them at the present time have been traced to an origin in certain microorganisms.

Normal ripening.—In comparison with our knowledge of abnormal cheese ripening, our knowledge of normal cheese ripening is very slight. A study of the bacteria in normally ripened cheese has been made by Adametz, Benecke, Duclaux, and Freudenreich.[2] These studies have consisted in the examination of the bacteria in the cheese at intervals in the ripening, from the first day until the time the cheese is fully ripened. It appears that the multiplication of bacteria in cheese is comparatively slow. In cream during its ripening the bacteria multiply with prodigious rapidity, increasing perhaps six hundred to one thousand fold within twenty-four hours. In cheese, however, while for some weeks the bacteria do increase in numbers, the increase is very slow. In one case Freudenreich found an increase of about sixty fold in eighty-five days; in another, about one hundred and sixty fold in twenty-eight days. After a time, however, this increase in bacteria comes to an end, and later the number of organisms present in a living, active condition becomes less and less, until finally at the end of the ripening the number is very much less than it was during the middle of the ripening period, sometimes coming down to nearly the original number. There are, however, great irregularities. At times the multiplication appears to be very much greater and more rapid than at others; sometimes the number present at a given stage of the ripening is ten

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[1] Landw. Vers. Stat., 42 (1893), p. 181. [2] Landw. Jahrb. Schweiz, 1894.

times as great in one specimen as it is in another, even though the latter had the larger number to start with.

As for the species of bacteria present, this too, is undergoing constant change during the ripening. At the beginning the number of species may be considerable, depending, of course, upon the number that were in the original milk from which the cheese was made. But as the ripening continues the number decreases, and finally at the end of the ripening in many of the cases there has been left a single species or a very small number of species.

Some species originally in the milk disappear at once, and can have no share in the ripening process. According to Freudenreich, the species which liquefy gelatin disappear very rapidly, and probably have no influence in the cheese ripening. This is a little surprising. These species produce a peptonization, and it has been regarded as probable for several years that one of the essential features in the ripening process was the peptonization of some of its proteids. Freudenreich thinks the lactic organisms are the chief agents in cheese ripening, but the whole subject is as yet too obscure to warrant any conclusion.

Whether the normal ripening and the flavor of properly ripened cheese is due to a single species in each kind of cheese, or to the combined action of several, or whether it may not be produced by a number of different species equally well, as in the case of the butter flavor, we do not at present know. There is a growing belief that the flavor of different types of cheese is due to different species of bacteria, and that when we have mastered the problem we shall be able to produce any given type of cheese by simply inoculating the milk with the proper quantity of definite species of bacteria. But this belief is at present based upon general inferences, and not upon demonstrated facts. The fact that such definite types of abnormally ripened cheese can be produced by inoculating the milk with certain species of bacteria shows the great influence of bacteria. The fact that a given locality will produce a uniform product of cheese for a long time, indicates that this locality has probably become impregnated with a certain species of microorganism.

Moreover, it is known that when a new cheese factory is started precautions are frequently taken to carry some of the cheese from an old factory to the new one, and to rub over the shelves and vats and the other appurtenances in the factory with the old cheese, in order to infect it with the proper ripening organisms. Baumann[1] has isolated a species which he thinks plays an important part in the ripening of Emmenthaler cheese, thinking that when it is present in the proper proportion it produces a normal ripening, but when present in too great quantity an abnormal ripening. Freudenreich has, however, doubted the specific agency of this organism. Pammel[2] has isolated and described a species of bacteria which, when inoculated into milk

[1] Landw. Vers. Stat., 42 (1893), p. 181. [2] Iowa Sta. Bul. 21.

to be made into a cheese, produced a strong and sharp but not altogether unpleasant flavor in the cheese, although it was not a typical flavor. Many species of bacteria are known to produce desirable cheese flavors when growing in milk. In some cases the use of pure cultures has been adopted in cheese making, although not from the bacteriological standpoint. One method of making Edam cheese (the method of Boekel) consists in inoculating the milk with a slimy whey, which has been found by Weigmann to contain a very nearly pure culture of a slimy-milk organism.

These facts indicate that the cheese ripening is the result of distinct specific germs, but what they are or how large a variety we do not at present know. Seemingly they must belong to the anaerobic organisms because certainly the interior of the cheeses can get only a very small quantity of oxygen. Duclaux[1] has shown that the bacteria in the rind of the cheese prevent the interior organisms from getting oxygen, and thus protect the interior of the cheese from oxidation. The fact is that the ripening of cheese is probably a process more complex than was at first thought. It is due not unlikely to the action of many organisms together, and the proper ripening will be as much dependent upon the relative quantities of the various organisms as upon the distinct species.

The sources of cheese bacteria are more variable than the sources of bacteria in ripening the cream. Of course we have in the first place many organisms in the milk which is brought to the cheese factory, and these organisms come from the same sources which we have already learned furnish the milk bacteria. But in addition to this the cheese is inoculated with organisms from several other sources. The cheese vats and the various apparatus used in the cheese factory are a prolific source of organisms. The water that is used in the manufacture of cheese is an important source. More important than all of them is the rennet which is used to curdle the casein. Recent studies of rennet have shown that the number of bacteria in it is very great, especially in certain forms of rennet (Pammel,[2] Baumann[3]). The rennet added to the milk in considerable quantities is thus a direct inoculation of the milk with a large number of bacteria. It has long been recognized that it makes considerable difference in the character of the cheese whether one or another kind of rennet is used for curdling, and after we have recognized that the various types of ripening are due to different kinds of bacteria, we see at once that the addition of rennet to the milk is to be regarded as an inoculation of the cheese which will result in a vast modification of its ripening. This agency of the rennet bacteria in the ripening of cheese is only just beginning to be recognized, and is a subject upon which much further work needs to be done.

We have, in short, at the present time an insufficient knowledge of the ripening of cheese to enable us to control the process. We can to

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[1] Ann. Inst. Past., 7 (1893), p. 304. [2] Iowa Sta. Bul. 21.
[3] Landw. Vers. Stat., 42 (1893), p. 181.

a certain extent avoid some types of abnormal ripening by the following simple method: If cheese ripens abnormally it will probably be due to the milk from one patron being impregnated with an unfavorable species of bacteria. By use of a simple apparatus samples of the milk of each patron may be set by themselves and allowed to ferment spontaneously. After two days an examination of the samples, a study of the odor, the taste, and the amount of gas produced enables the cheese maker to judge somewhat accurately whether the milk is safe to put in his cheeses. If there is a superabundance of gas, or if there are produced very vile odors, the milk of the patron in question should be excluded from the cheeses. Of course it takes a day or two to apply this test, but this is a matter of no very great importance in the cheese factory, because the farm that is furnishing an improper species of bacteria one day will probably continue to do so for the season.

A method of preventing the abnormal swelling of cheese has been suggested by Freudenreich;[1] i. e., the salting of the milk. When the trouble appears in a cheese factory all the subsequent cheeses may be treated as follows: After the milk has curdled, about two-thirds of the whey is removed and salt is added to the rest to the extent of 3 per cent. The cheese is then made as usual, although a smaller quantity of salt must be put into it in the end. This use of salt has been found in some cases to be quite efficient in preventing the abnormal swelling. Another method of remedying the abnormal swelling, also based upon bacteriological knowledge, has sometimes been found to be useful. When the cheese begins to show signs of this abnormal production of gas it is at once cooled to a very low temperature, either by putting it into a cold cellar or, if the cheese is a large one, by the use of ice. This lowering of the temperature at once stops the fermentation which is going on, and if the cheese is kept at this temperature for some time the milk sugar will gradually undergo such changes that when subsequently the temperature is elevated the fermentation will not recommence. Beyond these facts, however, little of practical importance to the cheese maker has as yet resulted from bacteriological study.

From this brief outline it will be seen that the most important advances in dairy bacteriology in recent years have been in the application of our discoveries to practical dairying. In guiding the milk producer to his best methods of furnishing pure milk, in aiding the butter maker in obtaining a uniform and desirable flavor, and in helping the cheese maker to avoid some of his difficulties dairy bacteriology has already done much. In the immediate future we can see still further practical results, and can, in the light of our knowledge to-day, feel confident that within ten years the discoveries in bacteriology will produce a complete revolution in almost every branch of the dairy industry.

[1] Landw. Jahrb. Schweiz, 1896.

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