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FERMENTED MILKS

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INTRODUCTION

In late years there has been a steady increase in the consumption of buttermilk and other fermented milks. These milks are very wholesome and refreshing as beverages, and their value as foods is unquestionable. Many popular and scientific articles on the therapeutic value of sour milk have appeared from time to time. Such publicity is, no doubt, responsible in a large measure for the increasing sale of these products. The recent development of *Lactobacillus acidophilus* milk is phenomenal, and the beneficial results ascribed to its use have served to stimulate a new interest in the subject of fermented milks.

It is the aim of this bulletin to give the reader a brief résumé of the present knowledge of fermented milks. The literature relating to this subject is already voluminous, and few persons are so situated as to have access to all of it. For the benefit of those having a professional interest in the subject information of a somewhat technical nature has been included.

All the familiar fermented milks are the result of an acid fermentation in which the sugar of the milk is split up into lactic acid and other products. This may be brought about by the presence in the

¹ Mr. Albus resigned from the department June 16, 1928.

milk of varieties of the common lactic-acid group of bacteria, or, as in the case of yogurt, by special organisms; or a yeast may be present, adding an alcoholic to the ordinary acid fermentation.

THERAPEUTIC VALUE OF FERMENTED MILKS

Fermented milks have been used since very early times, but it is only within recent years that physicians in this country have become interested in the possibilities of their use for therapeutic purposes. This interest was stimulated in a large measure by the work of Metchnikoff (60)² and his associates. His views, which are set forth in some detail in Chapter V of his book entitled "The Prolongation of Life," are looked upon by conservative investigators in this country as overdrawn and as unsupported by experimental evidence. In his book great stress is laid on the longevity of the peoples of certain countries in which fermented milks are an important part of the diet. In considering evidence of this kind, however, it should be remembered that many other things may contribute to the general health and vigor of a people and that these factors can not be excluded in drawing conclusions. People who habitually consume large quantities of fermented milk usually live a simple life, largely in the open air. There is no means of knowing how much this may have contributed to the vigorous old age to which they frequently attain.

Sour-milk therapy has recently undergone a new development. Evidence already indicates that *Lactobacillus acidophilus*, which is a normal inhabitant of the intestinal tract, is a definite and efficient therapeutic agent. *Acidophilus* milk has been adjudged a practical means of bringing about the predominance of this organism in the intestinal tract. Upon the advice of physicians, large quantities of *acidophilus* milk are being consumed to combat intestinal disorders.

The use of fermented milks as a therapeutic agent is based on the assumption that they are able to combat the so-called auto-intoxication caused by the undue accumulation in the body of toxic substances emanating from the intestinal tract. The theory of auto-intoxication may be stated briefly as follows: The digestive tract of human beings is at birth free from bacteria; but in various ways, chiefly through the food, many kinds of bacteria are introduced into the alimentary canal. In the intestines, particularly in the large intestines, some of them find favorable conditions for growth and become established there in large numbers. In the normally nourished infant the bacterial varieties are limited in number and for the most part consist of acid-forming types which, by the active fermentation of the milk sugar furnished in large quantities in the food, produce conditions under which bacteria of the putrefactive type are unable to multiply to any extent. The predominance of an acid fermentation in the large intestine produces an acid stool with a characteristic but comparatively unobjectionable odor. As the child gets older the variety of food is greater, and the relative proportion of carbohydrate to protein is much reduced. In place of the acid fermentation there is a decomposition of the protein by other

² Figures in parentheses refer to the bibliography at the end of this bulletin.

bacteria, intestinal gas is produced, and the stools become alkaline and frequently have a very objectionable odor. In the bacterial decomposition of the predigested protein it is supposed that products of a more or less toxic nature are produced. When the quantity of these products is relatively small they are disposed of through normal channels and have no appreciable effect. If the excretory system fails to do its normal work, or if the protein decomposition is unusually active, toxic substances accumulate, and the symptoms of autointoxication are produced. The production of toxic substances in abnormal quantities may be caused by a combination of circumstances promoting an unusual activity of putrefactive bacteria normally present, or it may be because the bacterial flora of the intestines changes and new bacteria are introduced.

The method of treatment by the use of sour milk is based on three conditions, which may be stated as follows: (1) It assumes as correct the theory of the production of toxic substances in the intestine by the action of bacteria in quantities sufficient to cause the symptoms of autointoxication; (2) the putrefaction or fermentation through which these toxic substances are produced can be suppressed by other bacteria; and (3) the bacteria which it is proposed to use in suppressing the putrefactive bacteria may be introduced into the intestines and will be able to establish themselves there and to multiply and persist, and the objectionable types are suppressed or driven out.

The standing of the theory of autointoxication mentioned under the first condition can not be discussed in detail in this bulletin. It may be said, however, that the question of autointoxication, in its broadest sense, is not nearly so simple as is stated here. It is at best only a theory, and much investigation of details will be necessary before its position can be determined.

The second condition is easily demonstrated, not only by scientific observations, but also by many instances in daily life. For example, vinegar, which is used in pickle making, owes its preservative action to the acetic acid produced by bacterial fermentation; and when milk sours spontaneously, the acid-forming bacteria develop acid so rapidly that in a short time all other bacteria are inhibited. Observations of this kind could be multiplied almost indefinitely. In fact, in the bacterial world, as among the higher plants in their natural state, there is a constant struggle for mastery, in which the types best suited to their environments, or, perhaps more correctly, less sensitive to the unfavorable conditions which they themselves produce, gain the ascendancy and more or less completely suppress other forms.

The question of implantation is most important. Unless the bacteria to be used in sour-milk therapy are adaptable to the complex conditions obtaining in the human intestinal tract, their implantation is obviously impossible.

Metchnikoff (61) proposed to combat intestinal putrefaction and its attendant disorders by the acid produced by the lactic-acid bacteria found in milk. In an endeavor to make his results more certain he chose the bacterium *Lactobacillus bulgaricus* because of its ability to produce a high acidity. *L. bulgaricus* has since been found to be an active member of the microflora of all curdled milks. Attempts to demonstrate the successful implantation of *L. bulgaricus* in the intes-

tinal tract by the ingestion of pure cultures of *L. bulgaricus* have given inconsistent results.

Cohendy (14, 15, 16, 17), a confrere of Metchnikoff, in a series of papers, claims to have recovered considerable numbers of *L. bulgaricus* from the feces of patients who had been given daily 250 cubic centimeters of a 24-hour milk culture of *L. bulgaricus*. He reports implantation to have been established by the third to the fifth day and to have persisted at least 12 days after treatment was stopped. It was found that the volume and weight of the feces increased. The feces were slightly acid or neutral in reaction.

Belonovsky (4) fed milk cultures of *L. bulgaricus* to rats and claims to have recovered the organism from the droppings 15 days after the last feeding. He states that the bacteria in the droppings, especially the gas-forming bacteria, were very much reduced as a result of feeding *L. bulgaricus* but were not affected when sterile milk or milk curdled with lactic acid was added to the basic diet.

Wejnert (90) conducted an investigation in which he employed 10 subjects who received sour milk administered rectally, 8 subjects who received sour milk by mouth, and 8 subjects who received an ordinary diet and *L. bulgaricus* milk. Bacteriological examinations of the feces from the patients receiving sour milk showed a reduction in bacterial count, which returned to normal in three or four days. Examination of the feces from patients receiving *L. bulgaricus* milk, however, showed a much greater reduction in bacterial count, which returned to normal much more slowly than when sour milk was given.

Other investigations tend to disprove the implantability of *Lactobacillus bulgaricus*. Luerksen and Kühn (58) found only a few organisms in feces three to five days after discontinuing treatment. They had ingested one-third of a liter of *L. bulgaricus* milk per day for about two weeks. They repeated the experiment with the same results and concluded that implantation of *L. bulgaricus* was impossible.

Distaso and Schiller (22) fed white rats *L. bulgaricus* cultures with lactose, and *L. bulgaricus* milk cultures. They came to the conclusion that foreign bacteria can not become acclimatized to the intestinal tract. Herter and Kendall (45) found that in the digestive tract of a monkey killed after being fed *L. bulgaricus* milk for two weeks, the organism was abundant in the upper part of the small intestine only. In the lower part of the small intestine and in the large intestine *L. bulgaricus* was found in only moderate numbers as compared with other bacteria. Herter and Kendall (46) concluded, after prolonged administration of *L. bulgaricus* milk, that *L. bulgaricus* can not be implanted in the human intestinal tract.

Rahe (72) made a critical investigation of the implantation of *L. bulgaricus*. His work tends to show that although *L. bulgaricus* may be found in the feces during the period in which *L. bulgaricus* milk is ingested, it disappears very soon after ingestion has stopped. This investigator also points out a very significant fact—that the difference between *L. bulgaricus* and certain acid-forming bacteria which are known to occur normally in the intestines is so slight that they can be distinguished only with difficulty. He suggests that the belief on the part of some investigators that *L. bulgaricus* becomes

established in the intestines was caused by their inability to distinguish between these two types.

Rettger and Horton (76) were unable to implant *L. bulgaricus* in the intestines of rats by the ingestion of milk or broth cultures.

Kopeloff (51) concludes, after a critical review of *L. bulgaricus* therapy, that "it is evident that the question of the implantation of *L. bulgaricus*. . . is the crux of the whole problem of *L. bulgaricus* therapy."

The researches of Rettger and his associates with respect to sour-milk therapy are comprehensive and outstanding. The excellent treatise by Rettger and Cheplin (74) presents the work of Rettger and his associates and contains also an abstract of the literature pertaining to the subject.

Rettger (73) fed milk cultures of *L. bulgaricus* to chicks in an effort to combat bacillary diarrhea, but no greater advantage was observed in using it than in using ordinary sour milk. He extended the experiments to rats by feeding them large numbers of *L. bulgaricus* grown on agar, but no *L. bulgaricus* was found in the droppings. Feeding sterile milk or lactose to rats on the same basic diet, however, quickly brought about in the feces an aciduric type of flora. In numerous other investigations, Rettger and his colleagues confirmed these findings and extended their observations to human beings. These experiments demonstrated that by sufficiently augmenting the carbohydrate of the diet a change in the fecal flora is brought about. The usual mixed flora is replaced by an aciduric type of flora in which *L. acidophilus* is the predominating bacterium. It was further demonstrated that this transformation of the intestinal flora can be brought about by the daily administration of lactose (milk sugar), dextrin, or live cultures of the bacterium *L. acidophilus*, with or without added lactose.

With respect to the influence of lactose and dextrin on the intestinal flora, Rettger states that a relation appears to exist between the rate of absorption of the utilizable carbohydrate and its tendency to effect a transformation. Only those sugars which reach the cecum and colon unchanged are able to bring about the transformation. He demonstrated that the transformation of the intestinal flora of an adult can be brought about by the daily administration of from 100 to 300 grams of lactose.

Rettger and Cheplin (75) applied the findings of previous investigations to a series of cases. They report favorable results in the treatment of chronic diarrhea, dermatitis, colitis, and sprue. These investigators administered live cultures of *L. acidophilus*, in some cases augmented by added lactose. Cheplin, Post, and Wiseman (13) treated nine cases of gastrointestinal disorders with *acidophilus* milk and obtained favorable results.

According to the work of Kopeloff (51) as reported in his book entitled "Lactobacillus Acidophilus," *acidophilus* milk is of therapeutic value in the treatment of certain intestinal disorders, particularly constipation and diarrhea. He demonstrated the implantation of *L. acidophilus* and showed that the beneficial results therefrom are essentially bacteriological phenomena.

Many other investigations that support the value of *L. acidophilus* therapy have been carried out. Persons desiring to make a further

study of the subject are referred to the treatise of Rettger and Cheplin (74) and to Kopeloff's book entitled "*Lactobacillus Acidophilus*" (51).

To summarize, it is generally accepted that *L. bulgaricus* can seldom, if ever, be implanted in the intestinal tract. The beneficial results ascribed to *L. bulgaricus* milk might well be due to the milk sugar, of which normal cows' milk contains about 5 per cent. On the other hand, *L. acidophilus* is a normal inhabitant of the intestinal tract and under the influence of the ingestion of lactose or dextrin can be made to predominate the intestinal flora. The ingestion of *L. acidophilus* milk, with or without added lactose, brings about the transformation of the intestinal flora more quickly and is generally conceded to be the most logical and practical method of bringing about the preponderance of the *L. acidophilus* type of bacteria in the intestines. By administering *L. acidophilus* milk implantation and proliferation of the organisms in the intestinal tract are most rapidly accomplished, for then there is direct implantation of large numbers of viable organisms. The lactose of the milk induces the multiplication of the desired type of bacteria.

FOOD VALUE OF FERMENTED MILKS

The high food value of milk is too generally recognized to need discussion here; fermented milks also have a high food value, except that in some cases the fat is partially or entirely removed. Otherwise the food value of the fermented milk differs little from that of the fresh milk from which it is made. Any increased digestibility of the fermented milk is due not so much to change in the chemical nature as to the fact that the casein is furnished in a precipitated and finely divided condition. In none of the fermented milks is there any material cleavage of the casein resembling the digestion in the stomach. The fat is practically unchanged, and only a part of the sugar is converted into acid, alcohol, or gas. In certain gastric troubles in which it is difficult to find any food that can be retained by the patient, fermented milks are frequently used with good results. Kefir and kumiss especially are used under such circumstances, as the stimulating action of the carbon dioxide which they contain is believed to aid in their digestion. To the physician the value of a highly nutritious food which can be digested when other foods are rejected is obvious.

Many questions should be very carefully considered before a fermented milk is introduced as an important part of the diet. As Herter (44) has pointed out in the paper already cited, the addition of fermented milk to the diet may change very materially the ratio of protein to other classes of food. If the milk is taken in place of other food, the daily protein ration may be so reduced that intestinal putrefaction, which is dependent on the protein part of the food, is diminished. On the other hand, if milk is added to the usual food, the protein ration may be increased rather than diminished. In many cases the condition of the mucous membranes will not permit the presence of organic acid, and sour milk can not be retained. It is also possible that symptoms of autointoxication are not caused by unusual bacterial activity in the intestine but by functional failure of

certain organs. This point could be determined only by a physician. It would be very unsafe to consume large quantities of milk, fermented or unfermented, under certain pathological conditions. In any case an important change in the diet should be made only upon the advice of a physician.

VARIOUS KINDS OF FERMENTED MILK

BUTTERMILK

Buttermilk, properly speaking, is the milk remaining after the fat which collects in granules during churning is removed. If cream is churned when sweet the buttermilk does not differ from ordinary skim milk, but if it is churned when sour (the usual practice) the acidity is sufficient to coagulate the casein in the cream. In the churning process this curd is broken up into very fine particles. These curd particles settle very slowly, and if the buttermilk is agitated occasionally it will retain its milky appearance. When the buttermilk is allowed to stand undisturbed for several hours the curd particles sink to the bottom, leaving an opalescent whey at the top. At the present time a large part of the so-called buttermilk sold in cities is not buttermilk, properly speaking, since it is not made by churning cream, but is simply sour skim milk which has been churned or stirred in order to break up the curd.

The souring of milk or cream is brought about by the activity of certain bacteria which form lactic acid by decomposing the milk sugar (lactose). The ability to form acid from lactose and other sugars is possessed by many kinds of bacteria but is so characteristic of a certain group that they are commonly spoken of as the lactic-acid bacteria. These bacteria have been described as distinct species or varieties under many names. Among them may be mentioned *Bacterium guntheri*, *Bacillus acidi lactici*, and *Streptococcus lactis*. In spite of the confusion in nomenclature it is evident that the term "lactic-acid bacteria" includes a fairly well-defined group of closely related varieties possessing in common several definite characters. Variations from the type in minor characters produce an almost infinite number of varieties. These variations may be in the ability to ferment different sugars, in the tendency to grow in chains, in the kind of flavor formed in milk, in the intensity of acid formation, and in the ability to produce pathological conditions in animals.

In many creameries the cream is allowed to sour spontaneously. In this case many bacteria other than the true lactic-acid bacteria will take part in the acid formation, and in addition to lactic acid the buttermilk may contain small quantities of acetic, succinic, and formic acids and sometimes traces of alcohol. The lactic-acid bacteria form lactic acid, with only slight traces of other organic acids, no alcohol, and no gas. In well-managed creameries the acid fermentation is assisted and controlled to some extent by the use of a starter. This may be milk allowed to sour spontaneously or buttermilk from the previous day's churning, but careful butter makers build up starters from commercial cultures sold in the form of powders, tablets, or fluid cultures, as varieties of lactic-acid bacteria selected with special reference to the production of a desirable flavor in butter. The butter maker puts this culture into about a quart of

milk which has been steamed for an hour or more to reduce the bacteria to the lowest possible number. After standing overnight the milk will usually be curdled, but gas bubbles and other evidences of contamination may be observed. A small portion of this milk is transferred to another bottle of milk prepared as before, and this process is continued until the acid fermentation has become sufficiently active to eliminate the contaminating bacteria and the milk curdles with a clean, acid taste and without signs of gas or the separation of a watery whey. This small starter, called "mother starter," is carried along indefinitely by daily transfers to freshly steamed milk. If reasonable precautions are taken to prevent contamination after a thorough heating of the milk, this culture will remain pure and vigorous for an indefinite time.

To prepare the starter actually used in ripening the cream, a larger lot of milk—25 to 50 gallons or more, according to the amount of cream—is heated for an hour or more. This is usually done in a special apparatus (sold by creamery supply houses) which consists of a large can inclosed on the sides and bottom by a steam jacket and fitted with a belt-driven stirrer. Milk either skimmed or unskimmed is heated by turning steam into the jacket; during the heating the milk is stirred constantly. After the pasteurization is completed cold water is run into the jacket and the milk is cooled to about 24° to 27° C. (75.2° to 80.6° F.). A bottle of the mother starter is added and the can is covered and allowed to stand overnight. This gives a large and active pure culture of lactic-acid bacteria to start the acid formation in the cream. Better results are obtained if the cream is first pasteurized.

When lactic-acid bacteria grow in milk the lactose is converted into lactic acid with slight traces of certain other organic acids. This acid precipitates the casein as a firm, jellylike mass. When this occurs in cream the fat globules are entangled in the precipitated casein. In the process of churning the casein is broken into fine particles and the fat globules are collected into large granules that float on top of the buttermilk. Buttermilk, then, is the water of the milk holding the sugar, acids, ash, and other soluble constituents in solution and the finely divided particles of precipitated casein in suspension. The amount of fat in the buttermilk depends on the completeness with which the fat is removed in the churning. Even with the best methods a little of the fat in the form of very small globules remains in the buttermilk. On standing, the suspended casein settles slowly to the bottom.

The composition of an average buttermilk is about as follows (20, p. 119):

	Per cent
Fat.....	0.5
Casein.....	2.4
Albumin.....	.6
Lactose.....	5.3
Ash.....	.7
Total solids.....	9.5

Chemically, buttermilk differs but little from skim milk. Only a slight rearrangement is necessary to bring about the physical change in the casein. If the milk has been pasteurized at a high tempera-

ture, the albumin is precipitated and the larger part lost. A small part—less than one-fifth—of the milk sugar is converted into acid. This acid combines with the ash constituents, probably converting the triphosphates into diphosphates and monophosphates and the diphosphates into monophosphates. It is obviously not necessary to make butter in order to obtain a perfect substitute for buttermilk. Sour skim milk has all the chemical properties of buttermilk, and if it is thoroughly agitated in order to break up the curd it has the same appearance and flavor as buttermilk obtained by churning cream.

CULTURED BUTTERMILK

In making cultured buttermilk from milk the same procedure should be followed as in making a starter for cream ripening. A good, clean-flavored mother starter should be carried along with every possible precaution to prevent contamination. Good commercial cultures can be obtained, but if it is not convenient to use one of these a natural starter should be prepared. For this purpose the following procedure may be followed:

Milk should be selected from several sources. About 1 pint of each lot of milk is put into clean glass jars or bottles and allowed to stand in a warm place until the milk has curdled.

When this occurs about 1 pint of milk is put into each of an equal number of bottles and held in steam or boiling water for one-half hour, after which it is cooled.

About 1 teaspoonful of milk is transferred from each of the bottles of sour milk to one of the bottles of heated and cooled milk.

These samples are allowed to curdle and the process is repeated until one sample is obtained which curdles in at least 8 or 10 hours with a smooth curd free from whey and gas bubbles and with a pleasant acid taste. Gas bubbles or the separation from the curd of a milky or straw-colored whey show that the lactic-acid bacteria are still mixed with other kinds of bacteria. Considerable variation in flavor can be found in different cultures, and care should be exercised to select one that gives a clean, sharp taste.

This culture is propagated in the same way from day to day. The amount of this mother starter which should be carried will depend somewhat on the quantity of cultured buttermilk to be made. One quart should be enough for 20 to 30 gallons.

(1) The mother starter is added to the milk to be used for cultured buttermilk, or (2) the milk is pasteurized in a continuous pasteurizer at 180° to 185° F. (82° to 85° C.), or preferably it is held in water-jacketed vats or cans at 180° F. (82° C.) for 30 minutes to an hour; cooled to about 70° F. (21.1° C.); and the mother starter added. The most desirable temperature for this fermentation is 70° to 75° F. (21.1° to 24° C.).

When this milk has curdled, it is cooled at once to about 50° F. and churned thoroughly to break the curd into fine particles.

The cultured buttermilk should be smooth, free from lumps, and show a separation of whey and curd only on long standing.

Milk to be used for making cultured buttermilk should be fresh and clean flavored. Good cultured buttermilk can not be made from

milk that is tainted or too old to be used for other purposes. Skim milk, partly skimmed milk, or whole milk, as desired, may be used.

A more nearly uniform product is obtained if the milk is pasteurized. The scorched taste which results from pasteurization at a high temperature is not objectionable as it is obscured by the acidity of the sour milk. The time of the inoculation may be arranged to suit the convenience of the maker and can be determined by experience in each case. By using the same culture and holding the temperature uniform, the amount of the starter can be adjusted to bring the acidity to the curdling point at any definite time within narrow limits. The temperature of the milk should be between 21° and 24° C. (70° to 75° F.). More rapid development of acid can be obtained at higher temperatures, but at the lower temperatures the lactic-acid bacteria are more successful in checking the growth of digesting and gas-forming bacteria. At lower temperatures and with a slower development of acid the casein is precipitated in a finer and more friable curd than at temperatures inducing a more rapid acid production. As soon as a fine curd has been formed the milk should be cooled promptly to below 50° F. to prevent the contraction and toughening of the curd.

Buttermilk made in the usual way as a by-product of butter making, and especially buttermilk obtained by churning pasteurized cream, is improved by mixing with it about 10 per cent of a skim-milk culture of the *Lactobacillus bulgaricus*. Directions for the preparation of this culture will be found on page 12.

This culture not only gives the buttermilk a sharper and more agreeable flavor, but on account of its viscous nature also gives it a smoother texture and prevents the separation of the curd from the whey.

MAKING CULTURED BUTTERMILK IN THE HOME

A more nearly uniform product can be obtained if the cultured buttermilk is made on a large scale. If good buttermilk can be purchased from a reliable milk dealer at a reasonable price it is not advisable to attempt to make it on a small scale. However, it is possible to make cultured buttermilk in the home by following in a small way the directions for making cultured buttermilk on a commercial scale. It is necessary first to obtain a culture or starter, which is merely milk containing the lactic-acid or sour-milk bacteria free or very nearly free from other kinds of bacteria. Lactic-acid or sour-milk bacteria are present in any normal milk, and it is only necessary to provide conditions favoring their growth to obtain them in a state of purity. This may be done by following the directions on page 9 for obtaining cultures for making cultured buttermilk on a large scale. When the culture is obtained it will not be necessary to carry a small culture to inoculate the larger quantity.

When the culture is obtained the following process should be carried out:

One quart of either whole or skim milk is heated in a double boiler for at least one-half hour. The milk is then allowed to cool to about 75° or 80° F. At this temperature the outside of the container will feel warm to the hand. One teaspoonful of the fresh culture is added and the milk is transferred to a bottle or covered

fruit jar and put away in a warm place. One of the vacuum-jacketed bottles will be found very convenient for this purpose, because the milk can be held at a nearly constant temperature favorable to the growth of the lactic-acid bacteria. On the following day the bottle is shaken thoroughly to break up the curd, and the product is put on ice to cool. The process is repeated, a teaspoonful of the freshly curdled milk being used to inoculate the heated and cooled milk.

Butter makers in the Northwest make a very refreshing and nutritious drink by adding sugar and lemons to buttermilk. As the casein is already precipitated, the acid juice of the lemon has no effect. Slightly more sugar and lemon juice are necessary than in making ordinary lemonade, and the mixture should be well iced.

ACIDOPHILUS MILK

It has been shown elsewhere that the possibilities ascribed to fermented-milk therapy by Metchnikoff (61) lacked one important detail. He chose a bacterium foreign to the alimentary tract, and therefore one not adaptable to conditions existing in the intestines. It has been shown further that *Lactobacillus acidophilus* is a normal inhabitant of the intestines, and under the proper influence predominates the bacterial flora of the feces to the exclusion of putrefactive and other undesirable bacteria.

Rettger (73) has shown the following methods to be effective in bringing about the preponderance of *L. acidophilus* in the intestines: (1) The daily use by an adult person of from 100 to 300 grams of lactose; (2) the daily ingestion of relatively large amounts of fresh pure-broth cultures of the *L. acidophilus*, together with appreciable quantities of lactose or dextrin; and (3) the ingestion of a pure culture of the *L. acidophilus* in milk or *acidophilus* milk.

Acidophilus milk is generally conceded to be the best method of implanting *L. acidophilus* in the intestines. Milk is a medium which is especially suitable for the growth of *L. acidophilus*, once the organism has become acclimated. The organism remains viable in milk for a longer time than in any other medium. The product is palatable, has an agreeable mildly acid flavor, and is an easily digested food. It contains about 5 per cent of lactose, which has been shown by Rettger to be so valuable an agent in the transformation of the intestinal flora.

Acidophilus milk has been prescribed by physicians and has been reported to be efficacious in the treatment of constipation, diarrhea, and other intestinal disorders. According to Rettger (75) *acidophilus* milk, to be effective, must contain large numbers of viable *L. acidophilus* organisms of proved therapeutic value. The product therefore should be fresh and should be obtained from a reliable manufacturer. Kopeloff (51) has shown that about 90 per cent of the viable organisms are destroyed after three days at ice-box temperature and recommends keeping the product at room temperature.

The dose is usually about 1 quart of *acidophilus* milk a day and may or may not be reinforced with from 50 to 300 grams of lactose. It is sometimes better to begin with a smaller dose and gradually increase the quantity. Sometimes a considerable amount of gas is produced in the intestines the first few days, but this soon disap-

pears. The dose may be reduced after implantation is established. It has been found possible to maintain the implantation, when once it is established, by the daily use of 100 to 250 grams of lactose taken in the diet. *Acidophilus* milk is best taken between meals so as not to interfere with the appetite, and the daily quantity may be divided into several portions. It is frequently made more palatable by adding orange or lemon juice.

Lactobacillus acidophilus was first isolated by Moro in 1900 from the feces of infants, and he describes it as a gram-positive rod, 1.5 to 2 μ long and 0.6 to 0.9 μ wide. Surface colonies on agar plates have a hairlike periphery, but few threads are seen on deep colonies. Growth is scant on agar slopes. *L. acidophilus* produces acid and is tolerant to high acidity. It produces no gas. Albus and Holm (1) have shown that *L. acidophilus* grows very well in a medium the surface tension of which has been depressed with sodium ricinoleate to 36 dynes, whereas *L. bulgaricus* is unable to grow in the same medium depressed below 40 dynes. This is offered as a means of culturally differentiating between these two closely related organisms.

The commercial production of *acidophilus* milk is practical, and the product is now being manufactured in large quantities in some localities.

The manufacture of pure *acidophilus* milk requires the greatest care, both in the preparation of the milk before inoculation and throughout the entire process of manufacture. It must be remembered that *L. acidophilus* is not a natural inhabitant of milk and must be acclimated to this medium. Even though it be acclimated to milk, its growth is nevertheless such that it is unable to overcome a slight contamination. Laboratory facilities, close supervision, and adequate equipment are essential to the successful production of *acidophilus* milk.

The first step in the manufacture of *acidophilus* milk is to obtain from a reliable source a pure culture of the bacterium *Lactobacillus acidophilus* of proved therapeutic value. After a pure culture is obtained it must be kept pure as a starter. All chances of contamination must be avoided. Florence or Erlenmeyer flasks are the most suitable containers in which to carry the starter. The flasks should be about half filled with fresh skim milk and plugged with cotton. They are then sterilized in an autoclave at 15 pounds pressure for 20 minutes. The milk after sterilization has a slightly caramelized appearance. After the milk has cooled, a flame from a gas burner should be passed over and around the mouth of the flask and the culture introduced into the flask after the mouth of the culture tube is passed through the flame. The cotton plug from the flask should be held in the hand during this operation and replaced in the mouth of the flask immediately after inoculation. The inoculated milk is incubated at 98° to 100° F. until the milk has curdled. A starter should show a small quantity of whey on top of the curd which should appear firm with no evidence of gas. The curd is broken up by rotating the flask vigorously. The starter should be of a creamy consistency and possess a clean acid flavor and a characteristic aroma. This starter, or mother starter, is carried on by daily transfers from flask to flask. The same procedure as described above is followed except that usually the first bit poured over the lip of the flask is

discarded as it serves to wash off the lip. The care necessary to maintain a pure starter of *L. acidophilus* can not be overemphasized. The starter should be examined frequently for purity either microscopically or by plates. Should it develop a bad flavor or odor, it must be discarded and a new culture obtained. In any case it is advisable to get a new culture at regular intervals. Kopeloff advises the use of a new fecal culture every two to four months to assure the therapeutic value of the product.

The bulk starter, which is the starter to be used to inoculate the batch, is prepared in the same way as the mother starter; and the same precautions against contamination must be observed. Florence or Erlenmeyer flasks of 2-liter capacity or larger are most satisfactory in which to prepare this bulk starter. These flasks permit the autoclaving of the milk and are most easily handled under the conditions necessary to prevent contamination. The starter should be 18 to 24 hours old when used to inoculate the batch and should possess all the qualities indicating purity previously mentioned for the mother starter.

Equally as great care must be exercised in the treatment of the milk as in the preparation of the starter. Only fresh milk of the best quality should be used in the manufacture of *acidophilus* milk. Whole milk, milk which is partly skimmed, or skim milk may be used. The pasteurizer employed in the preparation of the product must be so fitted that the milk may be heated and cooled without removing it from the vat, which should be equipped with an efficient mechanical stirrer. The vat must be used not only as a pasteurizer but also as an incubator for the milk. The characteristics of the bacterium *Lactobacillus acidophilus* make it necessary that the bacteria in the milk be killed before the starter is added. The milk is heated to about 205° F. for an hour and a half or longer and then cooled to 98°. It should have a slightly caramelized appearance after heating; however, this in itself is not an indication that the necessary destruction of the bacteria has been accomplished. Bass (3) recommends heating the milk to 190° to 195° for one hour; then cooling it to 98°, at which temperature it is held for three or four hours; again heating it to 190° to 195° for one hour; then cooling it to 98°; and inoculating. After the milk has been cooled to 98° to 100° it is inoculated with about 2 per cent of the bulk starter. The greatest care must be observed in the inoculating process to prevent the contamination of the batch. The mouths of the flasks should be passed through a flame before pouring the starter. The cover of the vat is lifted just sufficiently to permit the pouring of the starter and is replaced immediately. After inoculation the milk is stirred for a few minutes to distribute the inoculum, and the batch is then allowed to incubate. The temperature of incubation, 98° to 100°, should be maintained throughout the entire incubation period. The batch is incubated until a firm curd has been formed, which usually requires about 18 to 24 hours. The length of the incubation period is dependent upon the size of the inoculation, the temperature of incubation, and the activity of the starter. After the milk has curdled it is broken up by stirring, cooled to room temperature, and bottled. According to Kopeloff (51), *acidophilus* milk should be held at room temperature to preserve the largest number of viable

acidophilus bacteria. The product should be of a creamy consistency and have a mildly acid taste, absolutely free from off flavors or odors. *Acidophilus* milk has a characteristic flavor and aroma. From time to time the batch should be examined microscopically or culturally in order to be certain of the purity of the product as a culture of the *acidophilus* organism. This affords a means of checking the efficiency of the process. *Acidophilus* milk should always be distributed while fresh.

For the production of *acidophilus* milk on a small scale, the method outlined for the preparation of the bulk starter may be followed. Thus any desired number of flasks of the milk may be prepared. *Acidophilus* milk is being produced in flasks in those localities where the population is small and the demand for the milk limited.

THE PRODUCTION OF ACIDOPHILUS MILK IN THE HOME

It is possible to prepare *acidophilus* milk in the home, but the procedure is so tedious and the possibilities of contamination are so great that few attempt it except when the commercial product is not available. As has been previously stated, the milk to be used in the preparation of *acidophilus* milk must be sterilized before the culture is added. Inasmuch as facilities in the home do not permit the sterilization of milk, canned evaporated milk, which is a sterile product, is used. The culture of *Lactobacillus acidophilus* to be used should be obtained from a reliable source.

The following are the directions for preparing *acidophilus* milk in the home:

After boiling water is poured over the top of a can of milk the can is cut about halfway around with an opener which has been previously boiled. Neither the can nor utensils to be used should be dried. The plug of the culture tube is removed carefully and the mouth of the tube passed through a flame. After the lid of the can is opened just sufficiently to permit the entrance of the mouth of the tube, the culture is poured into the can of milk. The contents of the can are stirred with a spoon which has been boiled in water. The top of the can is then pressed down and covered with a clean dish. The can is kept at a temperature as near 100° F. as possible for about 24 hours, or until the milk has curdled. The milk may be incubated in a fireless cooker. After the milk has curdled a spoonful of this milk is transferred to another can of milk, the same precautions being observed as in the use of the culture. This process is repeated from day to day.

With great care it is possible in this way to carry on the culture from day to day and to have a fresh supply of *acidophilus* milk every day that will contain a large number of viable *acidophilus* bacteria. The curdled milk may be diluted with fresh milk if the product is too concentrated to be palatable. If the product acquires an off flavor or odor, it is contaminated and should be discarded and a new culture obtained. No harm can result from allowing the milk to remain in the open can.

Some information concerning the relative value of the commercial preparations of *L. acidophilus* now on the market may be found in a recent article by James (46 a).

KEFIR

Fermented milks have evidently been extensively used for many centuries by the people of southern Russia, Turkey, the Balkan countries, and their neighbors. The natives have no records and few traditions of the origin of the milks they use, and it is probable that their preparation and use developed gradually by accident and cumulative experience. One of the first of the fermented milks known to Europeans was the kefir, made from the milk of sheep, goats, or cows in the Caucasus Mountains and neighboring regions. Kefir differs from most of the fermented milks of the Mediterranean countries in that it is made from a dried preparation and contains considerable quantities of alcohol and gas. Kefir is made by many tribes under varying names, as "hippe," "kepi," "khapon," "kephir," "kiaphir," and "kaphir," all of which are said to come from a common root signifying a pleasant or agreeable taste.

For a large part of their food the mountaineers of the Caucasus depend on kefir, which they prepare in leather bottles made from the skins of goats. In the summer the skins are hung outdoors, either in the sun or in the shade, according to the weather, but in winter they are kept in the house. The bags are usually hung near a doorway, where they may be frequently shaken or kicked by each passer-by. Fresh milk is added as the kefir is taken out, and the fermentation continues. During this process foreign bacteria mingle with the essential bacteria of the seeds or grains, which are small, yellowish, convoluted masses in the kefir, and abnormal and frequently disagreeable flavors may result. In order to prevent the escape of gas when the milk is drawn off, a string is tied around the neck of the leather bottle, so that the small part wanted for use is held between the stricture and the opening. In the villages and the low country kefir is made in open earthen or wooden vessels, and most of the gas escapes.

The grains in kefir consist of a central filament of two parts, of which the outer spreads out, forming the convoluted polyplike exterior. These parts are built up one upon another, giving the large grains a corallike appearance. The central part is made up of a mass of bacterial threads. In the outer layer yeast cells are found mingled with the bacteria. When the grains are added to milk they swell and increase in size by forming new grains. At the beginning of the fermentation they settle to the bottom, but in a short time they are carried to the surface by attached bubbles of gas. If the fermentation is active, a thick layer will be formed on the surface, but when shaken or stirred this layer settles again to the bottom.

The biology of kefir was studied by Kern (47) in 1881, but, owing to the faulty technique of that day, his descriptions are evidently erroneous.

Freudenreich (28) describes four organisms that he isolated from kefir grains. One of these was a yeast which he designated *Saccharomyces kefir*; this he found to grow best at 22° C. (72° F.), but not at all at 35° C. (95° F.). It ferments maltose and cane sugar but not lactose. It gives a peculiar flavor to milk but causes no fermentation. The cells are oval, 3 to 5 microns by 2 to 3 microns. It is not identical with the ordinary beer yeasts. Two of the organ-

isms were of the lactic-acid bacteria type but differed from them in forming gas in lactose media. The most interesting of the organisms described is a long, slender bacillus corresponding to one described by Kern as *Dispora caucasica* and to which Freudenreich gave the name *Bacillus causicus*. In morphology, in failure to grow on ordinary laboratory media, and in high acid production in milk, this bacillus resembles very closely the bacillus mentioned later, in connection with yogurt, as *Laetobacillus bulgaricus*. If Freudenreich's description is accurate, *B. causicus* differs from *L. bulgaricus* in forming gas from lactose and in being feebly motile. Gas was formed slowly at 35° C. and still more slowly at 22°. No one of these organisms alone produced kefir, but when the four together were grown in milk typical kefir was produced on the first or second transfer.

According to the investigations of Nikolaiewa (66), three organisms are always present in the fermented milk. One of these, *Bacterium causicum*, which forms the filament of the grain, is evidently identical with Freudenreich's *Bacillus causicus*. This investigator considers this bacterium, with a torula yeast fermenting lactose, dextrose, and cane sugar, as essential to the production of kefir. Other bacteria and yeasts are found in the grains and the fermented milk, but they are looked upon as contamination.

It is probable that kefir is produced under different circumstances by different organisms. Any combination of bacteria or of bacteria and yeasts that will produce a lactic-acid and a mild alcoholic fermentation in milk will make kefir, although to obtain the most desirable flavor certain organisms may be essential.

Hammarsten (34) shows in the following table (Table 1) the changes brought about in cow's milk by this fermentation:

TABLE 1.—Chemical analysis of kefir

Item	2 days old	4 days old	6 days old	Item	2 days old	4 days old	6 days old
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Casein.....	2.570	2.586	2.564	Fat.....	3.619	3.630	3.626
Lactalbunin.....	.425	.405	.390	Ash.....	.641	.624	.630
Peptones.....	.071	.089	.120	Lactic acid.....	.665	.832	.900
Lactose.....	3.700	2.238	1.670	Alcohol.....	.230	.810	1.100

It will be observed that the changes were confined almost entirely to the lactose and its by-products. The casein remained unchanged, and the increase in the peptones was insignificant. The lactalbumin decreased slightly. The casein of kefir is, according to this chemist, not especially soluble, but may be more easily digestible because of its finely divided condition. The lactose diminished appreciably, and there was a corresponding augmentation of alcohol and lactic acid. A certain part of the lactose is consumed in the formation of carbon-dioxide gas not included in this analysis.

The following directions are given for making kefir when the grains are available: The dry grains are softened by soaking in warm water, which should be changed several times. When the grains rise to the surface and become white and gelatinous they are ready for use. One part of these grains is used to three parts of milk

which has been thoroughly heated to destroy the bacteria already present. The bottles in which the milk and grains are placed should not be stoppered but should be protected from the dust by cloths, inverted cups, or plugs of cotton. They are held at or near 14° to 16° C. (57° to 60° F.) and stirred or shaken frequently. After 8 to 10 hours the milk is strained through cloth and put into tightly stoppered bottles at the same temperature as before. The bottles should be shaken every few hours to prevent the formation of lumps of precipitated casein. The kefir is ready for use at the end of 24 hours; if held longer than this it is advisable to keep it on ice to check the fermentation. The temperature at which the milk is fermented is important in controlling the relative amounts of alcohol and lactic acid. At higher temperatures the percentage of alcohol is increased, while as the temperature is lowered the alcoholic fermentation is diminished and the quantity of lactic acid formed is greater. After the fermentation is once started the grains may be discarded and new kefir made by adding one part of the fermented milk to three or four parts of fresh milk. In order to remove the grains the kefir should be strained through cheesecloth, and after thorough washing to remove the curd, the grains may be dried by exposure to the sun on pieces of blotting paper. In this condition they are said to retain their vitality for several years, although many of the yeasts in the outer part of the grain are killed by the desiccation. It may be necessary to break up the grains with the fingers. When in the wet stage they should not be larger than a walnut.

The routine manner of preparing kefir does not promote the growth of the grains to any great extent. When it is desired to increase the quantity of grains, a milk of low fat content, 2 or 3 per cent, is used, and the product is held at 15° C. for 48 hours. The grains are then washed and transferred to fresh milk every 48 hours until the desired growth is obtained.

Kefir grains are not easily obtained in this country, but a good imitation of kefir can be made by carrying on simultaneously in sealed bottles an alcoholic and a lactic fermentation. Better results can be obtained by inducing the alcoholic fermentation in buttermilk. In this way it is possible to avoid much of the trouble from the formation of lumps of curd. If buttermilk is made for this purpose from whole or skim milk, careful attention should be given to the time of curdling and the breaking up of the curd. This is essential to a smooth, creamy kefir. Ordinary bread yeast may be used for the alcoholic fermentation, but as this yeast does not ferment lactose it is necessary to add cane sugar to the milk. If buttermilk is used in making kefir, the following steps should be taken:

Buttermilk may be obtained from a dealer or prepared as directed on page 7. The yeast is prepared by adding a half teaspoonful of sugar to a 6-ounce or 8-ounce bottle of boiled and cooled water. Half a yeast cake is added to this sugar solution and set in a warm place overnight. This will give an active culture of the yeast and obviate the necessity for adding the yeast cake directly to the milk. This yeast culture should be ready at the time the buttermilk is received or, if made at home, at the time it is curdled.

One to one and a half per cent of sugar is added to the buttermilk. On the quantity of sugar added to the buttermilk will depend

the extent of the alcoholic fermentation. Theoretically about one-half of fermented sugar may be converted into alcohol; that is, milk to which 1 per cent of cane sugar has been added may contain after the fermentation 0.5 per cent of alcohol. The quantity of sugar added should be governed by the amount of carbon dioxide it is desired to have in the finished product. This should be sufficient to make the kefir distinctly effervescent and impart to it the peculiar, sharp taste of charged water, but should not be developed enough to blow the fluid out of the bottles when the stoppers are removed. Experience shows that 1 to 1.5 per cent of sugar will give the proper amount of gas. This may be approximated by adding sugar in the proportion of 2 even teaspoonfuls of sugar to each pint of milk. When the buttermilk and the yeast culture are ready, the sugar is dissolved in the buttermilk.

The yeast culture is added to the buttermilk in the proportion of 1 teaspoonful to 1 quart of buttermilk.

The buttermilk is mixed thoroughly and bottled. The bottles should be very strong, as sufficient gas pressure is sometimes generated to break ordinary bottles. The heavy bottles used for ginger ale or other carbonated drinks answer this purpose very well. They should be carefully cleaned and boiled or steamed before being filled and then stoppered tightly. The stoppers should be wired or tied securely in place.

The product is put in a cool place to ferment. If the fermentation is too active the kefir will have a yeasty taste, and the curd is likely to become lumpy and filled with large gas bubbles. A temperature of 18° to 21° C. (65° to 70° F.) will be found satisfactory for kefir which is to be used on the third or fourth day. The floor of a cool cellar is a convenient place to ferment kefir made in the home. The bottles should be shaken as often as may be necessary to keep the curd in a finely divided condition. The finished product should be smooth and creamy, effervesce rapidly when poured from the bottle, and have the pleasant, acid taste of buttermilk, with the added sharpness caused by the gas and the trace of alcohol. Kefir 2 or 3 days old may have a yeasty taste, but if it has been properly made this will disappear as the fermentation of the sugar nears completion; made under these conditions, it should be used when 3 to 5 days old, but if put on ice it may be held for a week or even longer.

KUMISS

The missionary monks and other wanderers who first penetrated the undulating, treeless plains of European Russia and central and southwestern Asia brought back descriptions of a fermented drink which in the light of more recent investigations is easily recognized as kumiss. These vast prairies are inhabited by tribes of nomads who live in tents or squalid huts in the winter and wander during the summer, seeking pasture for their horses, their herds of cattle, or flocks of sheep. They are all horsemen, and by a process of selection in which they have probably played only a passive part they have developed an exceptionally hardy race of horses. The mares give much more than the ordinary quantity of milk, which constitutes almost the entire food of the people during the summer. This is never used in the fresh condition but is fermented to make kumiss.

Unlike kefir, there is no dried ferment, seeds, or grains with which the fermentation of the mare's milk is started. When it becomes necessary to establish the fermentation anew, it is the practice of the natives to add to milk some fermenting or decaying matter, such as a piece of flesh, tendon, or vegetable matter. Whatever the material used to supply the essential organisms, it is evident that the milk is so cared for that a combination of an acid and an alcoholic fermentation is favored and the necessary bacteria and yeast are soon established. No doubt the change in the milk is produced under different circumstances by different combinations of bacteria and yeast, and there are usually present various contaminating organisms which are detrimental or at least are not essential to the production of the kumiss. Native kumiss makers lay great stress on the quality of the milk, the breed of the mares, and the condition of the pastures; but it is probable that their troubles ascribed to variations in these conditions are more likely attributable to imperfectly controlled bacteriological factors.

There was at one time much interest in kumiss as a therapeutic agent in the treatment of tuberculosis, and sanatoriums were established in Russia where invalids could get this treatment. It is probable that the benefits, real or imaginary, derived from it came from the general methods, which correspond somewhat to present practices, rather than from the action of kumiss.

Mare's milk is lower in nutritive value than cow's milk, as Table 2, taken from Richmond's Dairy Chemistry (77), shows:

TABLE 2.—Average composition of cow's milk and mare's milk

Milk from—	Water	Fat	Sugar	Casein	Albumin	Ash
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Cow.....	87.10	3.90	4.75	3.00	0.40	0.75
Mare.....	90.06	1.09	6.65	1.89		.31

The composition of kumiss varies somewhat with the age, the rapidity of the fermentation, and the nature and extent of contamination with extraneous organisms. The analysis in Table 3 is taken from Richmond's Dairy Chemistry (77, p. 241):

TABLE 3.—Composition of kumiss made from mare's milk

Item	1 day old	8 days old	22 days old	Item	1 day old	8 days old	22 days old
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Water.....	91.43	92.12	92.07	Albumin.....	0.25	0.27	0.24
Alcohol.....	2.67	2.93	2.93	Albumose.....	.98	.76	.77
Lactic acid.....	.77	1.03	1.27	Fat.....	1.16	1.12	1.30
Sugar.....	1.63	.50	.22	Ash.....	.35	.35	.35
Casein.....	.77	.85	.83				

It will be observed that this fermentation produces no changes that could be expected to increase appreciably the digestibility of the nitrogenous part of the milk except the possible advantage of a finely divided curd. Mare's milk differs from cow's milk in giving

with rennet a softer, more friable curd, but it is not certain that this property would increase the value of kumiss.

Kumiss is made and offered for sale in this country, but as this is usually made from cow's milk, it is, more correctly, kefir.

YOGURT

In passing to a consideration of the fermented milks used by the people of the countries bordering on the eastern end of the Mediterranean, a preparation quite distinct from that of the Caucasus and the Russian steppes is found. Kefir and kumiss are limpid, mildly acid, and distinctly alcoholic; but the yogurt, yahourth, or jugurt of the Turks, the kissélo mléko of the Balkan people, the mazun of Armenia, the gióddu of Sardinia, the dadhi of India, and the leben or leben raib of Egypt, are all quick-curdled milks, decidedly acid, and with very little or no alcohol. The method of preparation is also very different. Goat's, buffalo's, or cow's milk may be used. This is usually boiled and sometimes is reduced by evaporation to one-half its original volume. In the latter case it is not used as a drink, but is eaten, frequently with the addition of bread, dates, or other food.

A portion of the previously fermented milk is used to ferment the fresh milk. Unlike kefir, it has no seeds through which the fermentation can be transmitted, but the essential organism is sometimes preserved by drying the fermented milk and reducing the dry material to a powder. This constitutes the podkwassa, or maya. The organism giving these milks their distinctive character is evidently identical in them all, or, more properly speaking, may be any one of the several varieties of a distinct and closely related group. On account of its peculiarities, some of which are exceptional and striking, and the importance attached to it by discussions both in the scientific and the popular press, a brief résumé of its characteristics is given:

This bacterium was probably first observed by Kern (47), who incorrectly designated it as *Dispora caucasicum*. His description, however, is so limited that it is impossible to attach the name he proposes to any particular organism. Later Beyerinck (7), described under the name *Bacterium caucasicum*, and Freudenreich (28), as *Bacillus caucasicus*, organisms isolated from kefir which agree in their essential features with those obtained from yogurt. Rist and Khoury (78) isolated from Egyptian leben two bacilli to which they gave the names *Strepto-bacillus lebens* and *Bacillus lebens*. Grigorooff (32) and Cohendy (15) isolated similar organisms from Bulgarian fermented milk. These various bacteria are undoubtedly nearly or quite identical, and all are included under the name *Lactobacillus bulgaricus*, now generally adopted. Work by Hastings (35) and by Heinemann and Hefferan (39) indicates that this bacterium is not peculiar to the eastern fermented milks, but is widely distributed, having been isolated from milk, soil, saliva, feces, and various soured foods. White and Avery (91) believe that this bacterium is the representative of a group of closely related bacteria which they divide into two types on the basis of their activity in milk and the nature of the lactic acid formed. The action of this organism on milk distinguishes it from all other known bacteria. At the optimum temperature milk is curdled in a few hours with a rather soft curd,

frequently somewhat slimy, which ordinarily does not separate from the whey even on long standing. In 24 hours the milk may show acidity equivalent to nearly 2 per cent of lactic acid, and on standing several days this may become about 3 per cent. The most active of the ordinary lactic-acid bacteria seldom exceed 1 per cent lactic acid. The more active type of *Bacterium caucasicum* forms the inactive lactic acid, whereas the levorotatory acid is produced by the type forming acid more slowly. Small amounts of other organic acids and traces of alcohol are formed.

This bacterium is evidently the essential organism of yogurt, matzoon, ceiddu, leben, and similar fermented milks. Other bacteria are always present, some of them habitually and others only occasionally. Some of these may have an influence on the flavor, whereas others are inert. It is probable that there is none, with the exception of *Bacterium caucasicum*, that can not be replaced by other species without appreciably affecting the results. Doubtless slightly different varieties of fermented milk have developed in various localities owing to different combinations of bacteria or of bacteria and yeasts. The Egyptian leben is reported to contain alcohol, but not in quantities sufficient to produce an effervescence such as is observed in kefir or kumiss. One of the ordinary lactic-acid bacteria seems to be always present with the *B. caucasicum*, and it is probable that if it is not essential it is of some assistance in starting the lactic fermentation and, especially if the temperature is low, in suppressing contamination before the *B. caucasicum* has time to develop sufficient acid to check extraneous bacteria.

Hastings and Hammer (36) could not detect evidences of proteolytic enzymes by the usual tests, but found in old-milk cultures a distinct peptonization of the casein which was not traceable to the action of the acid. This change is so slow and so small that it can not be considered as having any influence on the digestibility of the milk. Otherwise the only changes in the milk constituents are in the conversion of the sugar to lactic acid and very small amounts of volatile acids and traces of alcohol.

In making yogurt in this country better results are obtained by using with the *B. caucasicum* a culture of an ordinary lactic-acid organism such as is used in making buttermilk. *B. caucasicum* growing alone in milk forms usually a rather slimy, tenacious curd which can not be broken up into the smooth, creamy condition essential to a good buttermilk. If this organism is grown in combination with the ordinary lactic-acid organism, a more friable curd is obtained, and the sliminess is not so evident. The two organisms can be carried in mixed culture only with great difficulty, as the high acid soon suppresses the ordinary form. The most satisfactory results can be obtained by making buttermilk in the ordinary way and churning it with an equal quantity of milk curdled with the yogurt organism. This procedure gives the desirable texture of buttermilk and a distinctive flavor.

If a culture can be obtained, yogurt can be made in the home. With a reasonably active dry or fluid culture the following procedure should be satisfactory:

One-half pint of milk is heated in a double boiler and held at the boiling point for one-half hour. This milk is cooled to about 100° F.

(about blood heat). At this temperature the container will feel warm, but not hot, to the touch. A considerable quantity of the culture is added to this milk. If it is in the form of tablets three or four should be used.

The milk is transferred to a bottle or fruit jar or, better still, to a vacuum-insulated bottle, which has been rinsed with boiling water and kept overnight in a warm place. Good results may be obtained by placing the bottle or jar containing the milk in a dish of water warmed to about 100° F. The most favorable temperature for the fermentation is at or a little below blood heat. At a little higher temperature the organism grows faster, but the curd is likely to separate from the whey as a tough mass. At a lower temperature the growth may be so slow that other bacteria gain the ascendancy.

By the following morning a thick, somewhat stringy curd with a sharp, acid taste should have developed. One teaspoonful of this curdled milk is added to from 1 pint to 1 quart of milk which has been heated in a double boiler, held at the boiling point for one-half hour, and then cooled. This milk is held as before, and the curd which has formed is broken up by being shaken vigorously in a fruit jar.

This process may be repeated as long as the curdled milk has a smooth, acid curd free from undesirable flavors and particularly the yeasty flavor and odor characteristic of bread dough. The so-called *Lactobacillus bulgaricus*, under favorable circumstances, will suppress other bacteria by its vigorous acid formation, but yeasts are favored by the acid condition of the milk and sooner or later make their appearance. Every precaution should be taken to protect the milk from exposure to the air and to sterilize all utensils with boiling water. When evidences of yeast contamination appear it is best to start with a fresh culture.

Yogurt may be made more palatable by adding to two parts of the yogurt one part of cold water, or, better still, cold-charged water, which can be bought in siphons at drug stores. Sugar and lemon juice or other fruit flavor, or chocolate sirup, may also be used for this purpose. The sugar should be added in the form of a sirup, as granulated sugar dissolves very slowly in the cold yogurt.

In making yogurt on a large scale the process is not essentially different except that it is advisable to carry a small culture, about 1 quart, to inoculate the milk to be made into buttermilk. Every precaution should be taken to maintain the purity of the culture. It is advisable to carry duplicate cultures independently so that a good one will always be available.

Expensive outfits for making fermented milks are on the market, but although they may be convenient they are by no means essential. For the smaller dairy the following procedure will probably be found satisfactory:

A small culture is propagated from day to day as indicated in the directions given above. In a similar way a culture of the ordinary sour-milk organism is carried. This may be obtained from many of the commercial laboratories.

The milk to be fermented should be thoroughly pasteurized. If a small quantity—5 to 10 gallons, for instance—is to be made, it may be done by holding a can of milk in a tub or vat of water heated

by a steam hose. If a larger quantity is made, one of the starter cans used in creameries will be found convenient. These are essentially cylindrical vats with mechanical stirrers and a jacket which can be filled with steam for heating or water for cooling. The milk should be held at a temperature of at least 180° F. for not less than 30 minutes.

The milk is cooled to about 100° F. One-half of the milk is drawn off and inoculated with a culture of the sour-milk organism. The remaining half is inoculated with the bulgaricus culture obtained from daily propagation. The amount to be added will depend upon the quantity of milk to be fermented, the time at which it is desired to have it curdled, and the temperature maintained during the fermentation. This can best be determined by experience. On pint should be sufficient for any amount between 10 and 20 gallons.

The milk inoculated with the sour-milk organism may be held at ordinary room temperature. Precautions must be taken to hold that part inoculated with the bulgaricus culture at a temperature of 90° to 100° F. for several hours. If the milk is in cans it may be set in a tub of warm water. A large volume of milk in a warm room will maintain the proper temperature.

If it is impossible to hold the milk at the desired temperature, the amount of culture inoculation should be increased.

When the milk has curdled, which should be in 10 or 12 hours, the two lots should be poured together and thoroughly mixed by churning or stirring. It may then be bottled and put on ice to check the acid formation.

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