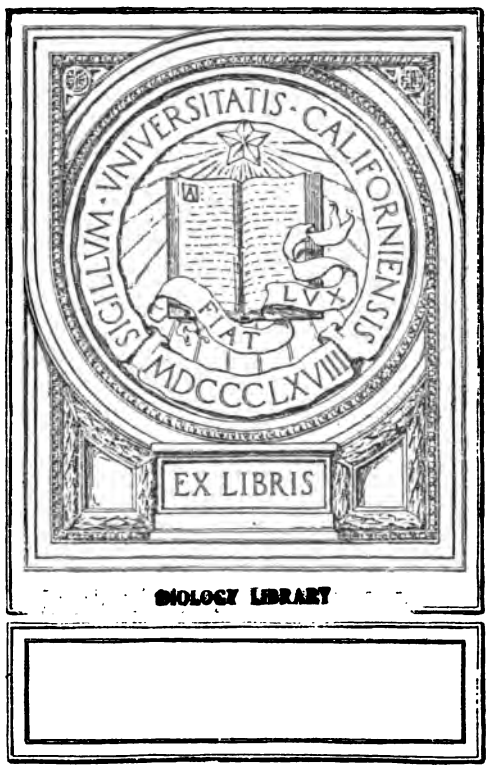

DAIRY BACTERIOLOGY

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BY

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TRANSLATED

FROM THE SECOND DANISH EDITION, WITH ADDITIONS AND REVISIONS

BY

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TRANSLATOR'S PREFACE

THE success of Professor Orla-Jensen's "Dairy Bacteriology," in the original as well as in the German, Dutch, and Finnish translations, has led me to hope that an English translation might find a welcome. The author has had the unique experience of an intimate knowledge of the highly-evolved dairy industries of two countries so widely different as Denmark and Switzerland, and, as the reader will find, he is keenly interested in the valuable work which has been carried out in English-speaking countries, particularly in America.

The translation has been based on the second Danish edition of 1916, which is the standard text-book on its subject for Danish students of dairying. As Professor Orla-Jensen has spared himself no pains in correcting and adding to the text in order to bring it thoroughly up-to-date, the present edition may be regarded as an entirely new one. Certain portions of purely local interest have been omitted, though the number of omissions on this account have been very few; the scientific character of the work as a whole renders it of international interest.

At the present time, when the question of pure milk is beginning to attract the attention which it deserves in the United Kingdom, it is hoped that the work may offer some useful suggestions to those engaged on the difficult problems which the whole question involves. In spite of the modesty of the author's claims I consider that his book should also convey something to those who can read between the lines, and on this account I venture to hope that not only students, but also dairymen and all others who have to deal with milk and dairy products, whether from the medical, veterinary or analytical side, will find something of interest in its pages.

In conclusion, I wish to express my thanks to Mr. D. R. Wood, Public Analyst for the County of Somerset, for kindly reading the MS. and offering useful hints.

PAUL S. ARUP.

Liverpool.

AUTHOR'S PREFACE

In order to avoid the inclusion of superfluous matter in this work on Dairy Bacteriology, I have, on the one hand, omitted from the teaching of dairy practice everything which is not exactly of bacteriological interest, and, on the other hand, from the bacteriological side everything which is not of interest in dairy practice. It is, therefore, assumed that the reader will have obtained from other sources a knowledge of dairy practice, and that through the study of this book he will obtain some guidance in the bacteriological technique.

This work is not a treatise, but only a text-book, for which reason one must not expect to find in it mention of every single microorganism to which is ascribed the power of affecting milk or dairy products in one way or another. I have purposely scrutinised the literature on the subject as closely as possible, and through my own investigations I have sought to form an opinion as to the actual conditions. The book is in all details built up on my own experiences, culled from twenty-five years of research work, and I, therefore, feel that I can confidently recommend it to my pupils.

ORLA-JENSEN.

Copenhagen.

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DAIRY BACTERIOLOGY

PART I

GENERAL

Chapter I

Microorganisms and Fermentations

By **microorganisms** or *microbes*, we understand all organisms which are too small to be seen by the naked eye; they remained unknown until it had become possible to construct strong magnifying glasses or the combination of lenses which make up the *microscope*. Bacteria were first observed by the Dutch optician *Leeuwenhoek* in 1675, but no real knowledge of their nature was gained until the latter third of the last century, when *Pasteur* carried out his classical researches. Bacteriology is thus a comparatively new science; nevertheless it has already revolutionised both medical science and the technology of the fermentation industries, among which may be included the dairying industry.

As far as the fermentation industries are concerned, only three groups of microorganisms come into consideration, *Bacteria*, *Yeasts* and *Moulds*. Bacteria and yeasts proper are *unicellular*, while moulds are generally *multicellular*. Unicellular organisms may be united in chains, but the individual cells of the chain show no differentiation as regards structure or function, excepting such modifications as may be due to differences in age or nutrition. In the moulds, on the other hand, it is possible to distinguish between two groups of cells; first, the *mycelium*, which is concerned with the nutrition of the organism, forming a tangled network in the nutrient medium, and second, the fine thread-like shoots which bear the reproductive organs and generally project out of the medium so that the spores may be carried away by air currents (see Fig. 8).

The size of microorganism is given in micromillimetres, or *microns*, designated by the Greek letter μ, being one-thousandth part of a millimetre. Bacterial cells generally measure from 0·5 to 2 μ in thickness. Some idea of their minuteness may be formed on consideration of the fact that the space of a cubic millimetre will hold one thousand million bacteria of average size. From Figs. 1, 2 and 3 it will be seen that the cells of yeasts and moulds are from five to ten times as large as those of the bacteria.

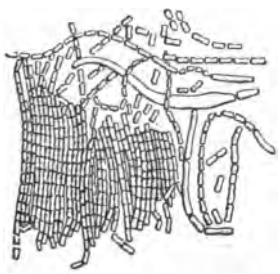


FIG. 1.—Acetic Acid Bacteria (*Bacterium aceti*). (After *Hansen.*)

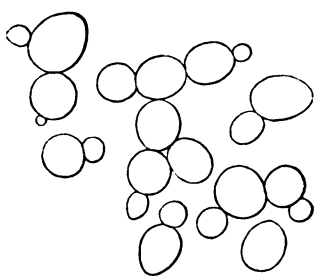


FIG. 2.—Brewers' Yeast (*Saccharomyces cerevisiæ*). (After *Hansen.*)

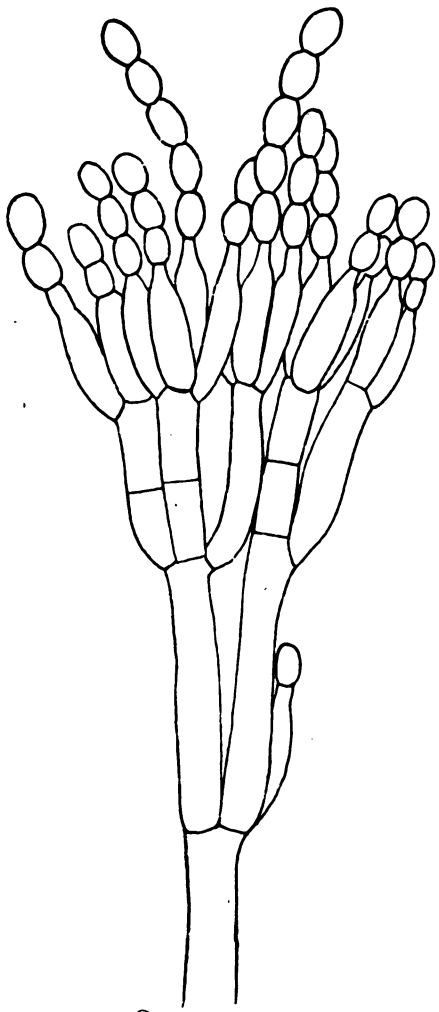


FIG. 3.—*Penicillium glaucum.*

All magnified 1,000 times (× 1,000).

GROWTH AND REPRODUCTION

Under normal conditions a bacterial cell will soon grow to its maximum size, often becoming more or less elongated in form, when a cross partition appears dividing it transversely into two

separate daughter cells of equal size, which in their turn grow longer and divide, the process being repeated indefinitely. This mode of reproduction is known as *fission*. Yeast cells, on the other hand, reproduce by *budding*, or gemmation; small round outgrowths appear and continue to develop until they attain the size of the mother cell (see Fig. 2).

In the moulds we generally find growing points as in the higher plants; only the outer cells are capable of reproduction, which mostly takes place by fission and only rarely by budding.

Spore Formation.—As the higher plants form seeds, so many of the microorganisms form spores, the function of which is to preserve the species under adverse conditions. Spores are accordingly more resistant to the influence of desiccation, poisons and high temperatures than the ordinary cells. Two distinct

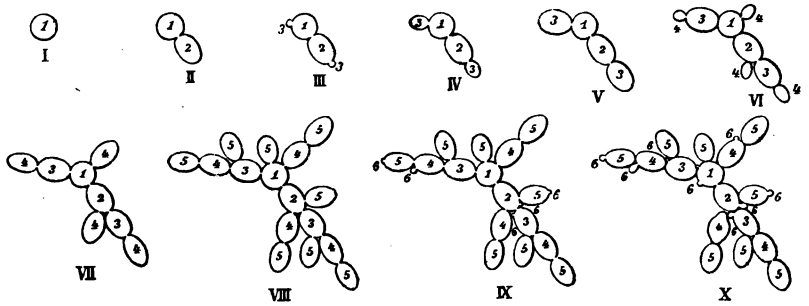


FIG. 4.—Growing Top Yeast. (After *Mitscherlich.*) I. At 7 p.m. II. Next morning, 8 a.m. III. 9 a.m. IV. 10.15 a.m. V. Noon. VI. 3.30 p.m. VII. 8 p.m. VIII. 9 p.m. IX. 10 p.m. X. 11 p.m.

types are found: *endogenous* and *exogenous* spores. It is comparatively seldom that the cell becomes completely changed into an *Arthrospore*, absorbing reserve food material freely and thickening its wall[1]. Endogenous spores are formed inside a cell while exogenous spores arise as constrictions on the end of a cell. Bacteria only form spores of the first mentioned type, only one spore appearing in each cell. If the spore causes the cell to bulge out locally, drumstick (*Plectridium*) or club-shaped (*Clostridium*) formations arise according as the spore lies at the end or in the middle of the cell. Germination in a direction approximately at right angles to the length of the cell is said to be lateral, and germination from the end polar. The yeasts likewise form endospores only, but several spores, up to ten in number, may be

[1] In this connection it may be mentioned that, according to *Preisz'* investigations ("Centralblatt f. Bakt.," 1 Abt., 1918, Bd. LXXXII., p. 321), the spore proper (the refractile body) is always only condensed reserve food material; outside this may be demonstrated a mass of protoplasm from which growth proceeds.

formed in a cell. As a rule, two, three or four spores are found together (Fig. 7), and they generally grow by budding like the ordinary yeast cells.

When moulds form endospores (*e.g.*, the various *Mucors*) this takes place in specially shaped cells known as *sporangia*, which may contain several hundred spores (Figs. 8A and 8B). As a rule, however, the moulds form exogenous spores ; these are known as *Conidia* when formed as constrictions on specialised spore bearing members (*Conidiophores*) (see Figs. 3 and 9), and as *Oidia* when formed on the ordinary branches of the mycelium (Fig. 10). Considered as spores, the oidia are less characteristic than the conidia ; the *Chlamydospores*, however, are exceptional, being unusually thick walled oidia, which may be formed anywhere in the mycelium (Fig. 11). In a chain of conidia, the outer members are generally the oldest, and the inner ones the youngest.

STRUCTURE AND CHEMICAL COMPOSITION OF CELLS

The main constituent of the cell is a viscous jelly-like transparent solution of proteins, known as *protoplasm, which is the living substance of the cell.* It is an extremely complex mixture of unstable compounds, which are largely built up of combinations

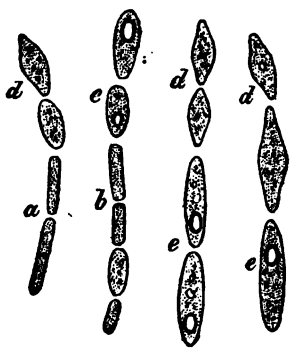


FIG. 5.—Clostridia. (After *Prazmowski.*) Spore Formation in the Common Butyric Acid Bacteria.

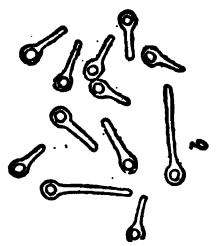


FIG. 6.—Plectridia. (After *Migula.*) Spore Formation in the Tetanus Bacterium.

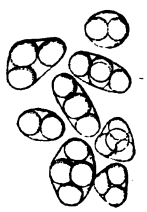


FIG. 7.—Spore Formation in Wine Yeast. (After *Hansen.*)

All × 1,000.

of a considerable number of different amino acids. The feeble acid and basic properties of these acids are also characteristic of the proteins, a circumstance which enables the latter to combine loosely with a vast number of other substances, thus fulfilling a condition necessary for the vital processes. Oil drops and other reserve foodstuffs are often found in the protoplasm, and as a rule

there are one or more spaces containing cell sap (*vacuoles*). Young and vigorous cells are nearly filled with protoplasm; old or starved cells have only a thin layer on the cell wall. All cells possess a *nucleus* which, although similar to the rest of the protoplasm in composition, is of a still more complex nature. When reproduction takes place, the nucleus is divided between the two new cells.

The protoplasm is bounded by a *cell wall* which grows thicker as the cell ages. It is composed not of cellulose, as in the case of

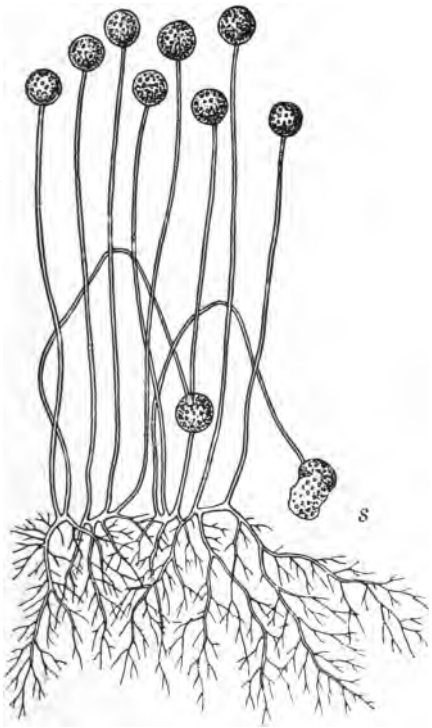


FIG. 8A.—*Mucor Mucedo.* (After *Kerner.*)

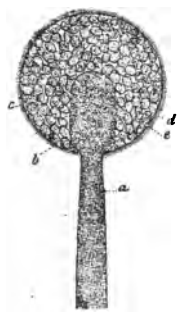


FIG. 8B.—Transverse Section of a Single Sporangium. (After *Brefeld.*)

the higher plants, but of an allied carbohydrate which has been named hemicellulose; it becomes coated with a nitrogenous substance identical with or very similar to chitin, the chief constituent of the shells or exoskeletons of the crustacea and the insects. Mucin, a substance which forms a sticky mass with water, may also be present. As these substances are typical of many of the lower forms of animal life, it may be surmised that the bacteria form a sort of link between animal and plant life, and this theory derives some support from a consideration of their mode of life. The cell wall may sometimes swell up considerably; in cases where

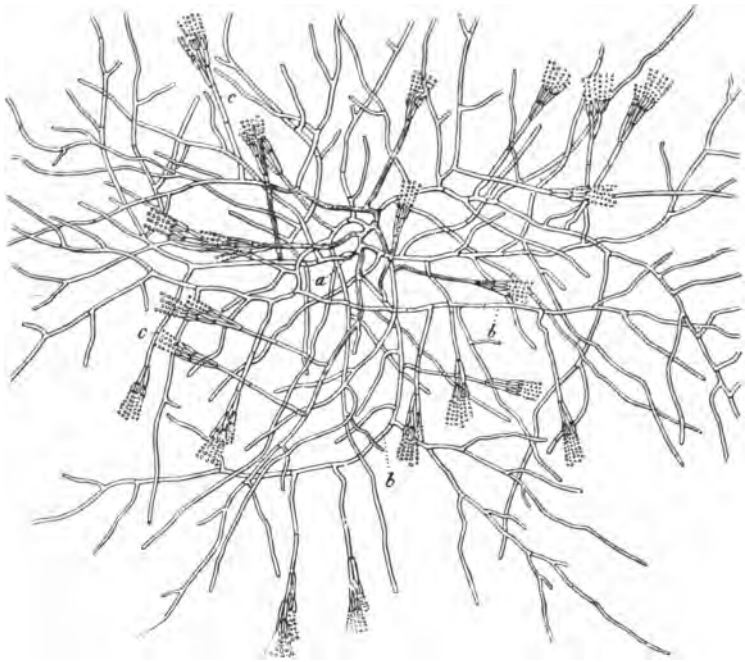


FIG. 9.—*Penicillium glaucum.* (After *Brefeld.*)

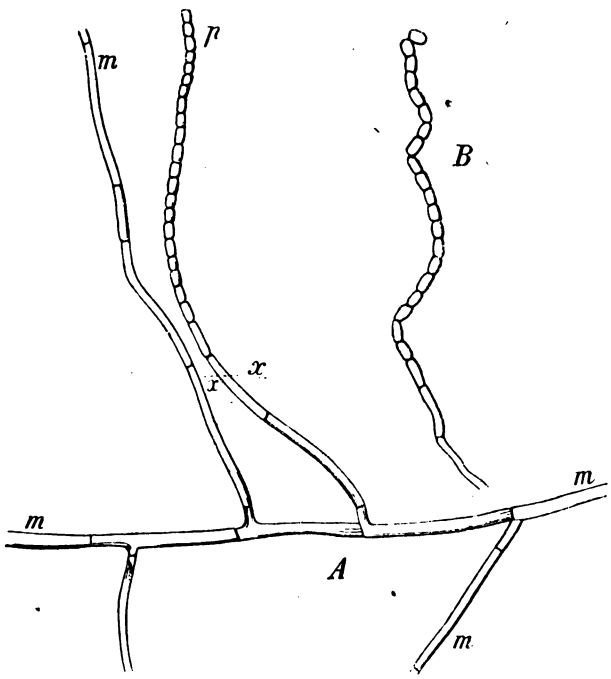


FIG. 10.—*Oidium lactis.* (After *de Bary.*)

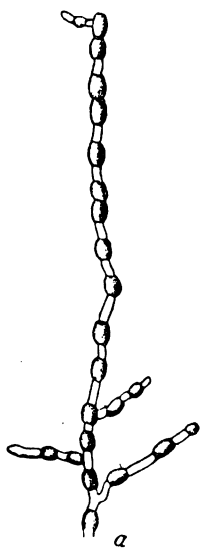


FIG. 11.—*Mucor racemosus* with Chlamydospores. (After *Brefeld.*)

it retains its sharp contour, it is known as a capsule. Numbers of cells may thus form large jelly-like masses, known as zoogloœa Occasionally the cell walls will become assimilated with the surrounding liquid, which then becomes slimy or ropy throughout (see Figs. 13, 14 and 15).

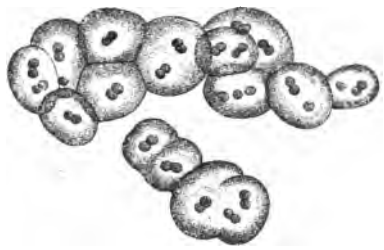


FIG. 12.—Zoogloœa of "*Leuconostoc*," a Coccus which forms slimy lumps in Cane Sugar Solutions. (After *Zopf.*) × 1,200.

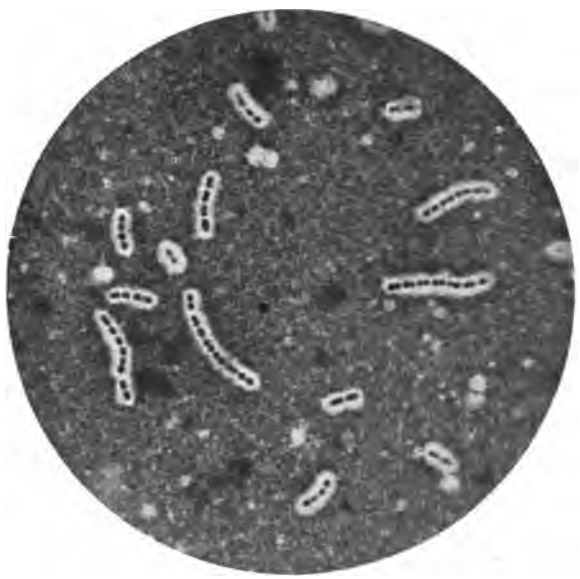


FIG. 13.—The Bacterium of "Long Milk." Capsule Stage. Milk not yet slimy. × 1,000.

NUTRITION

The following twelve elements enter into the composition of all organisms :—Oxygen, Hydrogen, Carbon, Nitrogen, Sulphur, Phosphorus, Chlorine, Sodium, Potassium, Calcium, Magnesium and Iron. It follows that substances composed of these elements are necessary for the normal growth of microorganisms. Some of them, *e.g.*, sulphur, calcium and iron, are only used in such minute

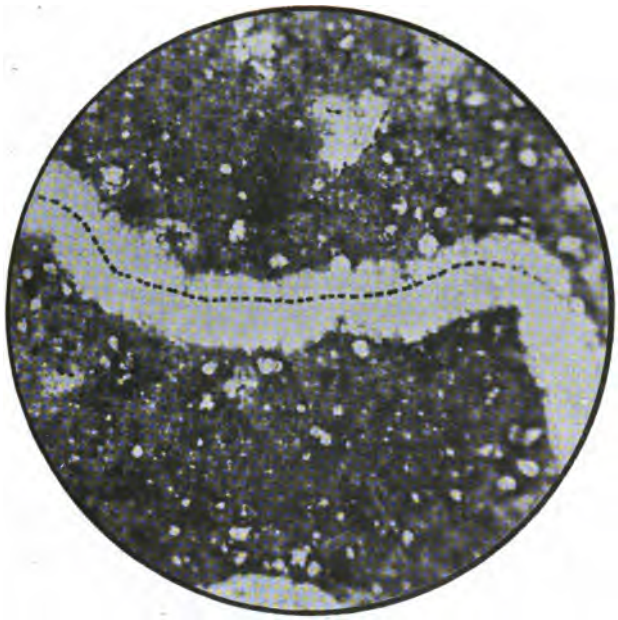


FIG. 14.—The Bacterium of "Long Milk." Slimy Stage. × 1,000.

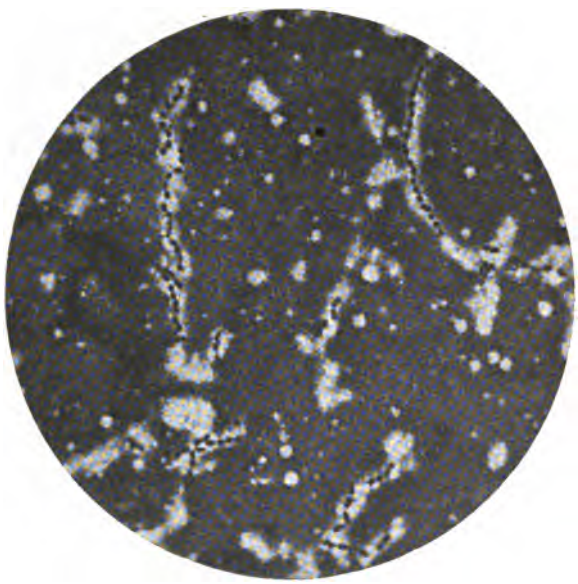


FIG. 15.—The Bacterium of "Long Milk." Milk less slimy, but beginning to go thick owing to acid formation. × 1,000.

quantities that it is unnecessary to add them specially to the nutrient media if ordinary tap water is used. Chlorine and

sodium are only required in appreciable amounts by organisms normally living in salt liquids, *i.e.*, marine and many pathogenic organisms. Like all other organisms, the microorganisms are able to derive the necessary sulphur, phosphorus and metallic elements from inorganic salts. These salts are found in the required proportions in plant ashes, and formerly it was the practice to add them to nutrient media in this form. If microorganisms are cultivated in milk or the extracts of meat or plants they will generally be well supplied with the requisite inorganic nutriment.

Nitrogen.—Microorganisms may be divided into two main groups according to their ability or inability to assimilate all the nitrogen they require from inorganic sources. A few species belonging to the former group can assimilate nitrogen from the air, and thus incidentally improve the soil for plant growth. The great majority of these, however, require their nitrogen in the form of ammonia or nitrates ; they are represented by the typical water bacteria, acetic acid bacteria, and many yeasts and moulds. Belonging to the group which cannot grow in the absence of proteins or the immediate decomposition products of proteins are many of the putrefactive bacteria and the true lactic acid bacteria.

Carbon.—Microorganisms fall into two groups with respect to carbon assimilation. A few species of soil bacteria which oxidise ammonia to nitrates resemble the higher plants in being able to utilise atmospheric carbon dioxide as their sole source of carbon. Unlike the higher plants, however, they are best able to carry out this process in darkness—no bacteria tolerate direct sunlight. Most microorganisms require organic sources of carbon. Some putrefactive bacteria can obtain all the carbon they need from proteins, thus presenting a broad analogy to the carnivorous animals. As a rule, however, microorganisms require special sources of carbon such as carbohydrates, alcohols and organic acids, and often show marked preferences for certain substances ; thus some species will only grow in presence of certain sugars, a circumstance which is put to advantage in distinguishing closely related species from one another.

Oxygen.—Oxygen and Hydrogen are assimilated from most of the nutrient substances and from water. Water must be regarded as the most important of the nutrient substances ; it constitutes about four-fifths of the total substance of the microorganism, besides which it is absolutely essential to life as a solvent and distributing medium for all the other nutrient substances. Like animal and plants, most microorganisms are capable of assimilating oxygen direct from the atmosphere. The analogy is only super-

ficial, for nearly all microorganisms can live without atmospheric oxygen for shorter or longer periods, provided that other sources of energy are available, and towards some species oxygen acts as a poison. Organisms which cannot live without atmospheric oxygen are defined as *aerobic*, and those which can do without it as *anaerobic*. The latter group is subdivided into facultative and obligate anaerobes, which are respectively helped or hindered in their growth by the presence of atmospheric oxygen. As a rule

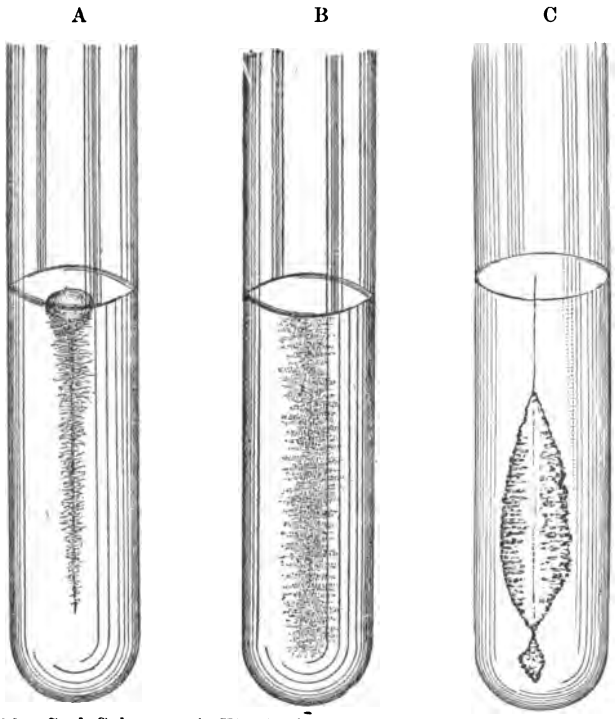


FIG. 16.—Stab Cultures. A. The Anthrax Bacterium (*aerobic*). B. The Swine Erysipelas Bacterium (*facultative anaerobic*). (After *Migula.*) C. The Tetanus Bacillus (*obligate anaerobe*). (After *Ball.*)

aerobic organisms will, sooner or later, form a film on the surface of the culture solution or spread over the surface of solid media, only penetrating slightly below the surface. Facultative anaerobes will grow equally well at all depths or on the surface, while obligate anaerobes only thrive below a certain depth. These relations are illustrated by the accompanying figures. The true lactic acid bacteria generally tolerate air, but thrive best in its absence; in stab cultures, therefore, they will not spread over the surface, but penetrate the medium evenly from all points.

FERMENTATION PROCESSES

The assimilated nutrient substances are employed, partly in building up the cells during growth and reproduction, and partly as sources of energy for these and other vital processes. All substances are not equally suited for both purposes, and distinction may be made between the nutrient substances proper and those which merely function as sources of energy. As long as active reproduction is taking place, a considerable proportion of the latter will be pressed into service as cell-building material, later on to be decomposed into simpler products, when the energy thus liberated will be utilised by the microorganisms. That portion of the period occupied by the reproductive process during which no appreciable amounts of decomposition products are separated is known as the *incubation period*. The decomposition products, which bear a certain general comparison with the substances contained in the urine of animals, are known as fermentation products, and fermentations are to be regarded as decompositions brought about by microorganisms. Exceptions occur when the organic substances undergo complete oxidation to carbon dioxide and water; such changes are looked on as respiration processes analogous to those observed among the higher organisms. *Fermentation thus implies only a partial decomposition through the agency of microorganisms.* It is evident that such a process entails a certain loss of efficiency, and consequently a larger consumption of material. Complete decomposition is only effected by aerobic microorganisms, more particularly by the moulds which, as previously mentioned, are able to form aerial shoots. Moulds only function as fermentation organisms when excluded from access to air, under which circumstances many of them produce alcohol, like the yeasts. It will now be understood that the most typical of the fermentation organisms must be facultative or obligate anaerobes. Fermentations are also known which are simple oxidations, such as the acetic acid fermentation. Processes of this nature require a plentiful supply of air, and can, therefore, only be carried out by aerobic organisms.

Fermentations and fermentation organisms are often named after their most characteristic products, *e.g.*, alcohol and butyric acid fermentations, lactic acid or butyric acid bacteria. Sometimes the name is derived from the substance attacked, *e.g.*, cellulose fermentation or cellulose bacteria. The former system is the more logical. *Soil formation* is the natural bacterial decomposition of plant remains into products, which, as a rule, are not stinking. *Putrefaction* is the decomposition of animal remains into products which are usually stinking. As

animal tissues consist for the most part of proteins, the term putrefaction has become synonymous with the bacterial decomposition of proteins. Formerly, when nothing was understood of the real nature of the different fermentations, they were generally looked on as chemical changes which apparently originated spontaneously, and which might improve a natural product. In cases where the natural product was spoilt instead of being improved the process was known as putrefaction. As alcohol fermentation is the most widely-known example, it is often supposed that the evolution of gas is characteristic of fermentations in general; but in its modern sense the term fermentation includes a number of processes, such as the lactic acid and acetic acid fermentations, in which no gas is produced.

ENZYMES

The manifold chemical changes due to microorganisms are carried out through the agency of certain special substances, known as enzymes. These are bodies of unknown composition, possibly more complex than the proteins, which may be separated more or less easily from the protoplasm, and which are able in small amounts to induce certain chemical changes in relatively large amounts of material. Enzyme action may be **extracellular or intracellular,** according as the enzyme acts outside (**exoenzyme**) or inside (**endoenzyme**) the cell in which it has been formed. To the former class belong the digestive enzymes of animals; as the function of these is to prepare the food for assimilation, they must necessarily act outside the assimilating cells. Many microorganisms form enzymes which are analogous to these. On the other hand, the typical fermentation enzymes decompose or oxidise the assimilated food material inside the cell, and must therefore act inside the cell, so that the energy liberated in the process may be directly available to the cell.

Distinction was formerly made between *unorganised ferments*, *i.e.*, enzymes such as are contained in the digestive juices, and *organised ferments*, by which were understood the microorganisms. It was not until 1897, when *Buchner* succeeded in separating from yeast the enzyme which brings about alcoholic fermentation (zymase), that it became clear that all fermentations are ultimately due to enzyme action.

The activity of enzymes increases with the temperature, but above certain limits the enzymes, like the proteins, become denatured and lose their special properties. The *optimum* for most enzymes lies between 35° and 65° C. In aqueous solution they are generally rendered completely inactive at temperatures

between 65° and 85° C. In the dry state, some enzymes will stand temperatures of about 150° C.

Enzymes resemble living organisms in being destroyed by heat; on the other hand they are unaffected by many substances which act as poisons towards living protoplasm, such as toluene, ether, chloroform, ethereal oils, phenol, salicylic acid and benzoic acid. Some such substance, usually toluene, may therefore be added to an enzyme solution to keep it sterile; boric acid is added to preparations of rennet. Most enzymes act best in feebly acid, all but neutral, solutions; pepsin is exceptional in acting in presence of the appreciable proportions of acid contained in the gastric juice.

As a rule, enzymes are named after the substances on which they act, thus *maltase* acting on maltose, *lactase* acting on lactose, *urease* on urea, etc. In some cases, however, previous usage has established other names. *Diastase* or, better, *Amylase* (*amylum* = starch) converts starch into dextrin and maltose; it occurs plentifully in malt, and plays an important part in the brewing and distilling industries; during the mashing process it converts the starch into fermentable products. It also occurs in the saliva and the pancreatic juice of mammals; as it only makes its appearance some time after birth, newly-born animals are unable to assimilate starchy foods. *Invertase* hydrolyses saccharose, *i.e.*, cane or beet sugar, into dextrose (d. glucose) and lævulose (d. fructose), a mixture which is found in honey and many fruits. Invertase, maltase, lactase and amylase are the chief **carbohydrate splitting enzymes**. Enzymes which hydrolyse fats into glycerol and fatty acids are known as **lipases,** and those which split proteins as **proteolytic enzymes.** Proteins may be hydrolysed by stages, each stage yielding a simpler product, thus: proteins to metaproteins to proteoses to peptones to polypeptides to amino acids. The first decomposition product of casein is *paracasein*, which, according to *Hammarsten*, differs from natural casein in being precipitated by the small amounts of calcium salts found in solution in normal cow's milk[1]. A microorganism which secretes proteolytic enzymes will always coagulate milk, and then gradually redissolve, *i.e.*, peptonise, the precipitated paracasein; unless the organism in question also belongs to the acid-producing group, the process of peptonisation will tend to produce an alkaline reaction in the milk.

Peptonising organisms generally liquefy gelatine more or

[1] The coagulation of casein by rennet appears to be analogous to the coagulation of other proteins by heat. See *Orla Jensen*, "Kemiske Undersögelser over Maelkens Koagulering og Koaglets Oplöselighed i Saltvand." Det kgl. danske Videnskabernes Selskabs Oversigter, 1914, No. 4. Chemische Untersuchungen über die Gerinnung der Milch und über die Löslichkeit des Ger. in Salzwasser. Zeitschr. f. Physiol. Chem., 1914, Bd. XCIII., p. 283.

less readily, being usually referred to as *liquefying organisms*. The proteolytic enzymes of the gastric juice, *chymosin* (rennet) and *pepsin*, only carry the cleavage of proteins as far as the peptone stage; the *trypsin* of the pancreas effects complete hydrolysis to amino acids. The *erepsin* of the intestinal juice is particularly thorough in its action, but only attacks proteins which have already been partially broken down by other enzymes. Casein is exceptional in being directly attacked by erepsin[1].

Oxidising and reducing enzymes are known as **oxidases and reductases**; they are of prime importance in connection with the breathing of animals and the reduction of carbohydrates to fats. *Storch's* well-known reaction, by which it is possible to ascertain whether milk has been heated to over 80° C. or not, depends on the presence in milk of an oxidase which is destroyed at 80° C., and can transfer the loosely bound oxygen of hydrogen peroxide to paraphenylene diamine or certain other colourless substances giving coloured products. Paraphenylene diamine gives a violet or, in the presence of casein, a blue colour. Many vegetable products, such as potatoes, fruits or fungi, contain oxidases as well as substances which yield coloured products on oxidation; hence the darkening in colour which takes place when they are cut into pieces and left exposed to air; if the material has been boiled, the oxidase will have been destroyed, and no darkening occurs. Reductases, on the other hand, are generally recognised by taking advantage of the fact that many substances, such as methylene blue, are decolorised on reduction. *Catalase* occupies a position intermediate between oxidases and reductases; it decomposes hydrogen peroxide, but the oxygen so liberated will not act on paraphenylene diamine or similar oxidisable substances. Catalase only occurs in small quantities in milk, but plentifully in blood; as constituents of the blood always pass into milk, which is drawn from diseased udders, an abnormally copious evolution of oxygen in the catalase test (see p. 159) is to be regarded as a bad sign.

Certain poisons, easily destroyed by heat, known as **toxins**, are closely related to the enzymes. They are found in a few plants, in poisonous reptiles and other animals, and are frequently secreted by microorganisms. The various toxins are generally the active agents in diseases due to pathogenic organisms. Healthy living tissues are highly resistant towards enzyme action, and are provided with special poisons, bactericidal substances, which repel

[1] It follows that microorganisms which secrete no other enzymes than erepsin attack casein, but do not liquefy gelatine. Conversely, the author has found that micrococci which liquefy alkaline, but not neutral or acid gelatine, do not peptonise casein.

bacterial invasion unless they have previously been weakened by the action of toxins. To counteract this, the higher organisms secrete substances, known as anti-toxins, which play an important part in recovery from disease and the subsequent more or less permanent *period of immunity* from the disease in question.

VARIABILITY

Microorganisms are classified together when they resemble one another in structure and habits of living and growth. Many microorganisms, however, are very liable to vary in appearance and in their biological characteristics, so that we may often have to deal with dissimilar forms which nevertheless belong to the same species. As we have really no sure guide enabling us to distinguish between essential and non-essential characteristics, it may often be difficult to determine whether we are dealing with *different species or only variants of the same species.* Undoubtedly, the biological properties, such as those which relate to nutrition, growth and energy, are more important in this respect than outward form, and, again, the means by which energy is produced are of more importance than the particular raw materials from which the energy is derived. Though it may be granted that an organism which derives its energy from an alcoholic fermentation instead of a complete oxidation to carbon dioxide and water would hardly be classified among the higher organisms, yet it is often observed that closely related organisms are able to utilise different nutrient materials. If a lactic acid organism undergoes variation, it does not follow that it will start producing alcohol or butyric acid instead of lactic acid, but it may easily lose its power to ferment a polysaccharide, such as milk sugar, which requires a special enzyme, lactase, to convert it into fermentable material. In other words, *the intracellular enzymes are much more characteristic of a given microorganism than the extracellular enzymes.* It may be assumed that the chief products of a fermentation process will always be the same under similar conditions, but the by-products may vary considerably according to the condition of the organism. The ability to produce certain substances which affect the taste, smell or colour of the medium is especially variable. The nature of the cell wall may also vary, so that an organism may appear sometimes with and sometimes without a capsule, or the cell wall may disintegrate into a mucilaginous mass. Bacteria are particularly liable to undergo this form of degeneration—especially the lactic acid organisms—generally as the result of over-nutrition; an analogous instance is seen in fatty degeneration in animals.

Temperature is an important factor in determining the form of

microorganisms ; on cultivation at temperatures approaching the maximum, acetic acid bacteria may be induced to undergo a striking transformation. In the case of acid-producing bacteria, the high concentrations of acid produced in the medium will also tend to cause the appearance of abnormal forms, known as *involution forms*, and in old cultures of lactic acid bacteria strange elongated, swollen or even branched cells may often be observed. Prolonged cultivation under adverse conditions is the best method of producing new varieties ; thus *Emil Christian Hansen* induced yeasts permanently to lose their capacity of forming spores by

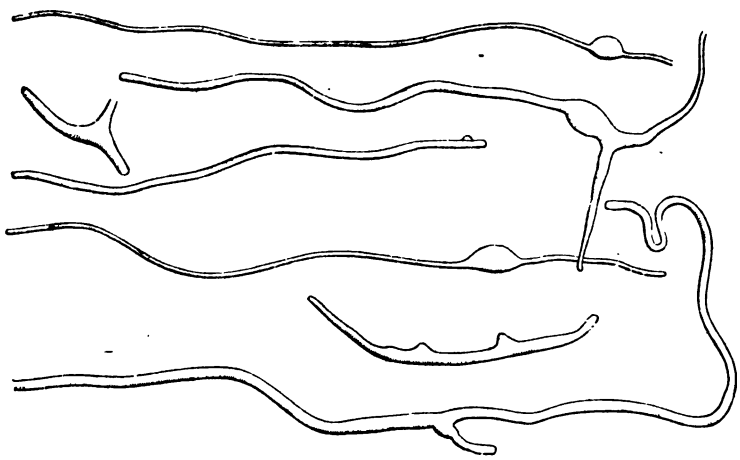


FIG. 17.—Involution Forms of *Bacterium aceti* produced by cultivation at 39° to 41° C. (After *Hansen.*) × 1,000.

cultivating for several generations at temperatures above the maximum for sporulation.

METHODS OF CULTURE

It is not possible to compound a universal nutrient medium, for some substances may be essential for the well-being of certain organisms and poisonous to others. While certain water bacteria will only thrive in very dilute solutions of organic nutrient matter, yeasts and moulds thrive best in wort and fruit juice, milk bacteria in milk, and pathogenic bacteria in meat broth or blood serum. Such natural media are of variable and partly unknown composition, and, therefore, do not lend themselves so well to the study of fermentations from the chemical point of view. For this purpose artificial media containing as far as possible only substances of known composition in definite proportions are the best ; for bacteria which require proteins, a preparation of peptonised fibrin known as Witte peptone is used ; unfortunately this involves the

introduction of a substance of which the composition is not quite constant; only the best brands, prepared under standard conditions, should be used. Most milk bacteria thrive in the following solution :—Tap water, 1 litre; sodium chloride, 2 grams; dipotassium phosphate, 2 grams; magnesium sulphate, 1 gram; dextrose, 20 grams; peptone, 20 grams.

This solution has an alkaline reaction, and phosphoric acid should be added until it only just turns litmus paper blue. For the cultivation of water bacteria and most pathogenic organisms, the medium should be distinctly alkaline; for yeasts and moulds it should be acid. The media are distributed in flasks or test tubes closed with plugs of non-absorbent cotton wool and sterilised by heating in an *autoclave* for a quarter of an hour at 110° to 120° C.

The *Freudenreich* flask is the most convenient vessel to use, as the contents are not so easily dried up or infected as in test tubes. If the nutrient solution is to be used for the investigation of acid

FIG. 18.—*Freudenreich* Flask.

FIG. 19.—*Petruschky* Flask.

formation, then 10 c.c. are introduced into each test tube, and the inoculated solution is titrated when the maximum acidity is certain to have been reached, say, after fourteen days at 30° C. The amount of acid produced by lactic acid bacteria increases with the amount of nitrogenous nutrient material in the medium; these organisms thrive better on casein peptone [1] than on *Witte's* peptone, but most of the rod-shaped lactic acid bacteria thrive best on an extract of autolysed yeast [2].

In order that the cultures may be kept at definite temperatures the bacteriological laboratory must be equipped with several *incubators* heated by gas or electricity, and, if necessary, cooled by water circulation, in such a way that the temperature is automatically kept constant.

In order to examine microorganisms, to obtain them as pure cultures or to determine their numbers, they must first be isolated. This may be accomplished by *Koch's* method of plating, which consists in distributing a definite small quantity of the liquid containing the organisms in a solid transparent medium which

[1], [2] See footnotes at end of this Chapter.

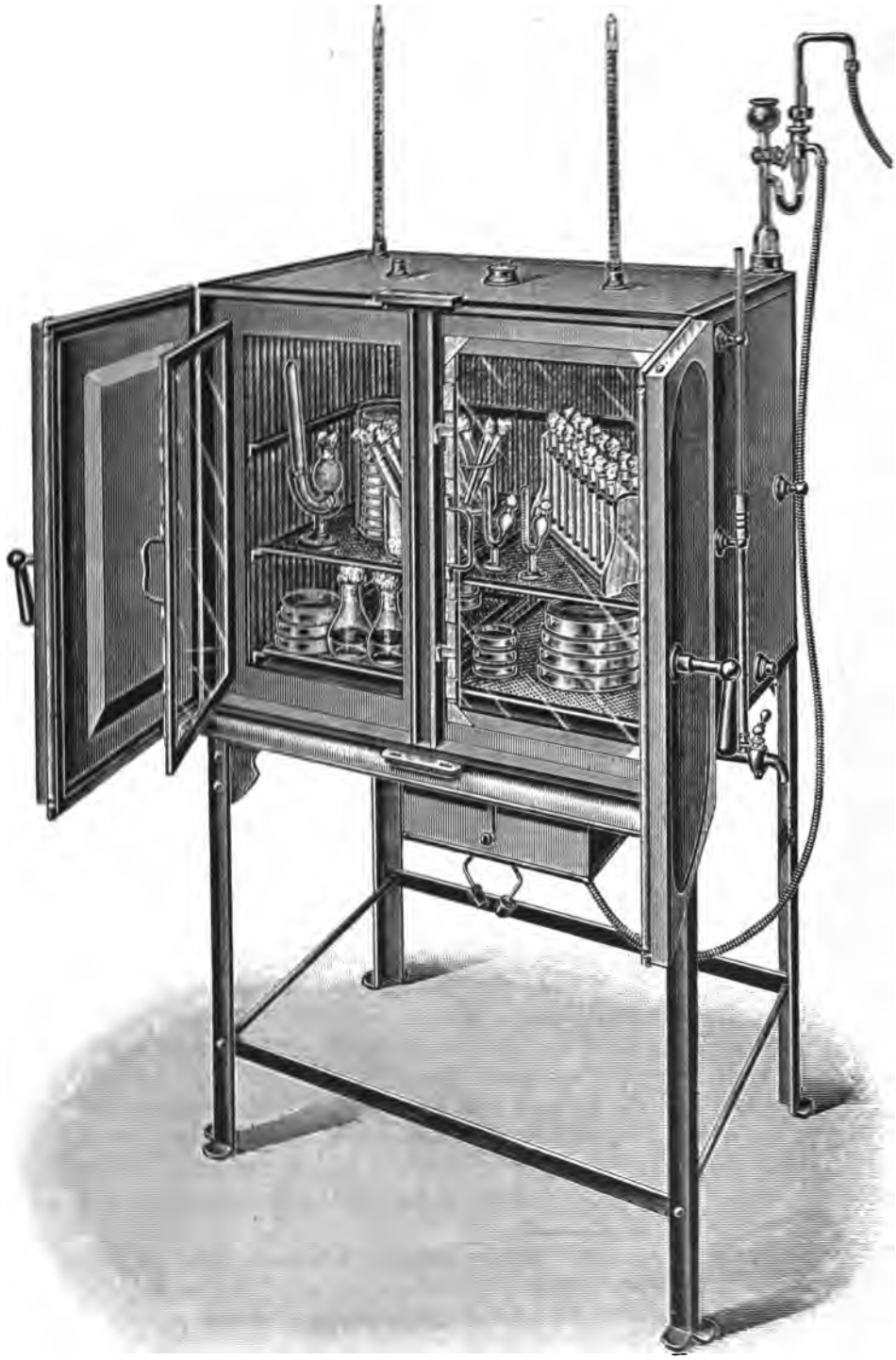


FIG. 20. Incubator with Petri Dishes, Plugged Test Tubes, Apparatus for Measurement of Gas Production, etc.

has previously been melted, and then allowing this to set in a thin layer. The isolated germs, fixed in position, will after some time multiply into colonies visible to the naked eye. From the number of colonies, the number of organisms per cubic centimetre of the original liquid can be calculated. Care must be taken that the temperature of the melted medium is not high enough to cause injury to the organisms. For milk bacteria the best media are whey or the solution recommended above, with the addition of 12 per cent. of gelatine or 1½ per cent. of agar. Gelatine may be regarded as a protein, whereas agar, a product obtained from certain seaweeds, is composed of carbohydrates known as pectins. Gelatine is to be preferred, as it can be made to yield clearer media than agar, but as it melts slightly over 20° C. or lower if it has been heated too frequently or at too high a temperature, it must be replaced by agar when dealing with organisms which only thrive at higher temperatures. If litmus or chalk be added, acid-producing organisms may at once be recognised. Solid media are usually allowed to set in *Petri* dishes, flat dishes with lids, which have been sterilised dry at 150° to 170° C. Organisms which do not grow in presence of oxygen are best cultivated in *Burri's* tubes, which are closed at one end by a rubber stopper and at the other by a plug of cotton wool; on removing the stopper, the agar column may be shaken out and examined for colonies. Some obligate anaerobes form characteristic surface colonies; to obtain these, the Petri dish must be kept in an atmosphere of hydrogen or in a vacuum. The oxygen which gradually leaks in may be absorbed by alkaline pyrogallol solution. The simplest plan is to use a vacuum desiccator, which is tilted during exhaustion in such a way that the chemicals (5 grams of pyrogallol and 50 c.c. of 10 per cent. caustic potash) in the sulphuric acid container are only mixed when the desiccator is stood straight after evacuation.

FIG. 21.—Desiccator for Anaerobic Cultivation.

As a rule, the liquids to be examined contain so many germs that they must be diluted with sterilised water before plating, in order to avoid overgrowth. The number of colonies to be aimed at is 40 to 200 per plate, and it will generally be necessary to make

several dilutions to achieve the desired result. A number of 100 c.c. Freudenreich flasks sterilised in the autoclave with 50 c.c. of water should be prepared in readiness, and also some dry sterilised plugged pipettes, graduated in quarters of a cubic centimetre. When making counts in milk, the following dilutions may be made :—

Dilution 1. 1 c.c. milk in 49 c.c. water, of which 0·5 c.c. in Petri dish No. 1.

„ 2. 0·5 c.c. of Dilution 1 in 49·5 c.c. water, of which 0·5 c.c. in Petri dish No. 2.

„ 3. 0·5 c.c. of Dilution 2 in 49·5 c.c. water, of which 0·5 c.c. in Petri dish No. 3.

The number of organisms per cubic centimetre is obtained by multiplying the number of colonies on plates 1, 2 or 3 by 100, 10,000 or 1,000,000, respectively. Butter is measured by means of a rounded platinum spoon, holding exactly 0·25 c.c. The spoon and the knife used for scraping off the surface of the butter must be sterilised by passing through a Bunsen flame before use. If the water used for dilution is warmed to about 40° C., the butter will readily melt from the spoon and become distributed on shaking. Cheese gives more trouble, as it must be weighed and comminuted; about 1 gram of cheese may be accurately weighed into a Freudenreich flask containing 50 c.c. of water and ground up by repeatedly pressing it out in the water by means of a flamed glass rod. An alternative method, resembling that for determining the soluble nitrogenous matter, is to grind 10 grams of the cheese with sterile water at 40° to 50° C. in a sterile mortar, pouring the emulsion into a sterilised 250-c.c. measuring flask and making up to the mark with sterile water. This method is to be preferred as a more representative sample is obtained, a matter of some importance as the organisms are usually very unevenly distributed in cheese. Further, the cheese is more easily ground up in the mortar than in the flask, while the number of organisms introduced by infection from the air will be negligible compared with the large number present in the cheese.

In all cases uniform working methods should be adopted in order that the results may be comparable; the colonies should be counted after incubating for a definite time, say seven days at 20° C. The counting is facilitated by placing the plate on a squared glass plate resting on a black surface.

For use when travelling, *Petruschky* flasks (Fig. 19), sterilised with the proper amount of nutrient gelatine, are more convenient than Petri dishes; they are generally used in *water examinations*, which are best carried out on the spot, as an increase in the

number of microorganisms generally takes place during transit. Bacterial multiplication may, however, be avoided to a considerable extent if the samples are packed in ice. For counts of water samples the peptone gelatine recommended above may be used, modified as follows: the sugar is omitted, and the neutralised medium is treated with 15 c.c. of 10 per cent. soda solution per litre. In these, as in all bacterial counts, it is impossible to get all the germs present to grow on the same medium; as many typical water bacteria, for example, the thread-forming and sulphur organisms, will not grow on gelatine at all, the counts must not be regarded as representing the actual numbers of organisms present, though they furnish useful indications as to the relative purity of samples if the same working conditions are adhered to throughout. It must also be remembered that long chains of bacteria or large pieces of mould mycelia only yield single colonies. The method is thus subject to considerable error, a fact which may easily be demonstrated by comparing the results with those obtained by direct microscopical counts. According to *Barthel's* investigations, 2 to 200 times as many organisms are found by direct counts as by plating according to *Skar's* method [1]. Pasteurised milk cannot be examined by the direct method as there are no means of distinguishing by microscopic inspection between living and dead bacteria [2].

Furthermore, one cannot be certain that any particular colony may not have arisen from several different species which may have adhered together or become intertwined in the original liquid. If, therefore, a *pure culture* is desired, it will be necessary to sow plates from a well-isolated colony, and only when the resulting colonies are found to consist exclusively of the desired organism is it possible to be sure that the individual colonies are pure. It is still safer to make a single cell the starting point, a method which is described under the next heading.

Pure cultures are best preserved in Freudenreich flasks on nutrient agar. As a rule *stab cultures* are made by piercing the medium with a platinum wire on the point of which a trace of the culture has been picked up. *Streak cultures* are made of organisms which require free access to air; the agar is allowed to solidify in a slanting position so as to expose a large surface, and the infected wire is drawn lightly across the surface. In order to protect stab cultures of anaerobic bacteria from the air, the cotton wool plug is flamed and pushed down the test tube till it

[1] "Milchwirtschaftliches Zentralblatt," 1912, p. 455.

[2] *H. W. Conn* (U.S. Public Health Report, 1915, No. 295) gives some valuable information concerning the limits of accuracy in the bacteriological analysis of milk.

nearly reaches the agar surface. A plug of absorbent cotton wool soaked in alkaline pyrogallol is then inserted a short distance above the first plug, and the tube is closed by a rubber stopper or a cotton wool plug soaked in melted paraffin wax (see Fig. 22). As soon as the cultures have made growth they must be kept at a low temperature or they will quickly die. The acid-producing bacteria keep better, the smaller the amount of sugar contained in the agar medium. Lactic acid bacteria may be preserved alive for years if not more than 0·25 per cent. of dextrose is employed, but it is safer to sow into fresh media every month. If it is desired to keep lactic acid bacteria in perfect condition with respect to their action on milk, they should always be cultivated in milk, and reinoculated into fresh sterilised milk as frequently as possible.

FIG. 22. — *Stribolt's* Method for the Cultivation of Anaerobic Bacteria. (After *Salomonsen*.)

Inoculation is carried out by means of a platinum wire sealed into a glass rod or fixed into a screwed aluminium holder; the end of the wire may be shaped as desired; when dealing with liquids it is looped. The wire is always flamed immediately before use.

In order to demonstrate the presence of certain organisms which may be greatly outnumbered by other species, the method of plating fails; it will be necessary to use the *enrichment method* of inoculating a small quantity of the material into a medium which favours the growth of the organism in question to the disadvantage of others; the cumulative effect of several reinoculations into the same medium will often be the production of a pure culture of the desired organism, or at any rate a culture in which the organism can easily be recognised. Such methods are employed, among others, for demonstrating the presence of pathogenic organisms.

METHODS OF EXAMINATION

Of all the methods in use for the determination of the species of microorganisms, microscopic examination is one of the most important, for until the shape and size of the cells have been noted it will not even be possible to say definitely whether one is dealing with a yeast or a bacterium. The more highly developed the organism, the completer will be the information to be gained by microscopic examination, especially if the various stages of

development are studied. In this way the moulds may readily be identified. Greater difficulties are encountered in dealing with the two other groups, which are much simpler in morphological structure; in these groups cultural and especially biochemical characteristics are more important as means of identification. It has already been mentioned that the appearance of a stab culture will reveal the character of the organism with respect to atmospheric oxygen, and as to whether it produces proteolytic enzymes or coloured products. Sometimes even the shape and general macroscopic appearance of the colony may be so characteristic as to afford in itself a means of identification. By varying the composition of the nutrient medium characteristic preferences may be discovered—for example, an investigation may be made to determine which sugars are fermented with the production of acid or acid and gas. Satisfactory results, however, can only be

FIG. 23.—*Jörgensen's* Moist Chamber.

got by finding out what fermentation products are formed under certain conditions.

For detailed descriptions of the microscope, works on microscopy or optics may be consulted. It need only be mentioned here that high magnification is achieved as the product of the magnifications due to the two systems of lenses, the eye-piece (ocular) and the objective. The object to be examined is placed on a glass slide and covered with a square or round cover slip of thin glass. For high magnifications, a drop of cedar-wood oil of the same refractive index as glass is placed between the cover slip and the special immersion objective, an arrangement which entails less loss of light than when air intervenes, and which is essential when magnifications of 1,000 diameters are required.

In order to determine whether an organism is motile or non-motile, it is observed in a hanging drop adhering to the under side of a cover slip placed over a hollow slide; in this way the growth and development of the organism may also be observed. In examining moulds, which require a plentiful supply of oxygen, the cover slip is separated from the slide by a glass ring, and the whole arrangement, known as a *moist chamber*, is sealed together

with vaseline ; a drop of water is placed on the slide to keep the air in the chamber moist, and to prevent evaporation of the drop on the cover slip. The moist chamber is also employed in making *single cell cultures* as follows : a miniature gelatine plate is made on a special cover glass marked with numbered squares, and with the aid of the microscope the most isolated cells are sought out and their positions noted with respect to the markings on the cover glass (see Fig. 23). The colonies which grow in these positions may then be sown separately into suitable media. The first yeast culture of undoubted purity was prepared in this way by *Emil Christian Hansen*, an achievement of far-reaching importance in the brewing industry. The method is not adapted

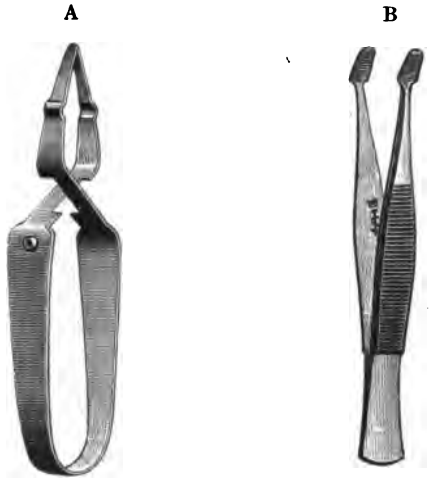


FIG. 24.—A. *Cornet's* Forceps. B. *Kühne's* Forceps.

for the preparation of pure cultures of the bacteria, as these are so much smaller than the yeasts.

Bacteria may be seen more clearly if they have been *stained* after fixation on the cover slip. Fixation is accomplished by picking up the cover slip by the edges, between the thumb and first finger, and twice drawing it slowly through a Bunsen flame ; as long as the fingers are not burnt, the preparations will not be overheated ; the bacteria are killed, and on this account stain better. If the culture medium is whole milk, the preparation should be freed from fat by immersion in chloroform before staining. The cover glass is immersed for a few minutes in the staining solution in a small dish or watch glass, washed with pure water, laid on the slide and dried on the surface by means of filter paper ; it should be handled by means of the *Kühne's* forceps (Fig. 24). The stain may also be dropped on to the cover slip held clamped by means of the Cornet forceps (Fig. 24). The

stains most used are alcoholic solutions of *methylene blue* or *fuchsine*. The former, which must not be diluted too much with water, is specially suited for preparations from milk, as it does not stain the casein strongly ; if fuchsine is used, a red patch may be formed in which it is impossible to distinguish the bacteria. As fuchsine stains very deeply, it is used in dilute solution ; its staining capacity is increased by the addition of phenol (*carbol fuchsine*). Tubercle bacteria, which are coated with wax, and intracellular spores, which are very resistant to staining, may be stained by warming with carbol fuchsine, and when thus stained they retain the colour so persistently that mineral acids fail to remove it. Methylene blue is also suited better than fuchsine for staining broth cultures. Acid cultures should, however, first be neutralised, unless the methylene blue solution has been treated with a little alkali.

Gram's method of staining is especially useful in the identification of bacteria ; it depends on the fact that microorganisms after treatment with gentian violet are stained a violet black by a solution of iodine in potassium iodide, and retain this stain more or less completely on treatment with absolute alcohol [1]. Bacteria which retain the stain are known as *Gram-positive*, and those which lose it as *Gram-negative*. Most yeasts and moulds and all true lactic acid bacteria are Gram-positive, while most of the harmful milk bacteria are Gram-negative. The Gram method lends itself well to the examination of lactic acid cultures and sour milk preparations in general, as the casein is completely Gram-negative.

Instead of staining, *Burri's Indian ink method* may be adopted ; a drop of the liquid to be examined is placed on a cover slip, mixed with a little sterile *liquid Indian ink*, allowed to dry, mounted in water, and examined. If the liquid is acid it should be neutralised, as the colloidal Indian ink is coagulated by acid. Treated in this way, the organisms show up white on a black background. Permanent preparations may be made by mounting in Canada balsam instead of water.

[1] *V. Jensen* ("Hospitalstidende," 1912, No. 20) recommends a 0·5 per cent. solution of methyl violet in water, and a solution of 1 gram of iodine and 2 grams of potassium iodide in 100 c.c. of water.

Notes to page 17.

[1] 100 gm. sugar free casein (precipitated by acid) were digested for a week at blood heat with a litre of water containing 4·6 per cent. HCl and 2 gm. pepsin. The resulting solution contained, after neutralisation, sterilisation and filtration, about 1 per cent. N and 1·2 per cent. NaCl.

[2] At 50° C. the digestion is completed in 24 hours. The sugar disappears at the same time. The highly acid solution contains about 2 per cent. N.

Chapter II

Bacteria

MANY bacteria differ from the yeasts and moulds in being capable of independent motion, which is accomplished by means of *fine threads, known as flagellæ*, growing from the protoplasm. The *monotricha* have a single flagellum growing from one end, and the *lofotricha* a tuft of flagellæ similarly situated; the *peritricha* have flagellæ all round the cell. Motility may be restricted to certain stages of development, chiefly the period of active growth; on the other hand, a large number of bacteria appear to be non-motile at all stages, and on this account it may be presumed that they are without flagellæ; unfortunately these extremely fine thread-like structures can only be rendered visible by means of complicated methods of staining which sometimes fail. There is, however, no doubt that the disposition of the flagellæ affords

FIG. 25.—*Bacterium pyocyaneum, monotrich.* (After *Migula.*) × 1,000.

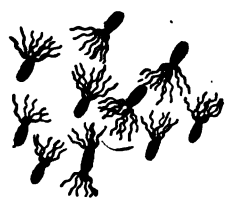


FIG. 26.—*Bacterium syncyaneum, lofotrich.* (After *Migula.*) × 1,000.

the most important of the morphological methods of distinction, and one on which the classification of the bacteria should primarily be based. Thus the monotricha and the lofotricha require comparatively simple nutrient material, being chiefly water bacteria, while the peritrich bacteria are typical fermentation organisms [1].

According to their shape the bacteria are divided into *sphere, rod* or *screw* forms. As the first-mentioned have no axis of length, division is possible in three dimensions, and, as a matter of fact, cases are known where division takes place in either one, two or

[1] *Orla Jensen*, "Hovedlinierne i det naturlige Bacteriesystem." Videnskabernes Selskabs Oversigter, 1908, No. 5. Die Hauptlinien des Natürlichen Bakteriensystems. Verlag von *Gustav Fischer*, Jena, 1909.

three dimensions, resulting in the **Streptococcus, Micrococcus** and **Sarcina** forms respectively, provided that the cocci remain united after cell division has taken place. Streptococci may be compared with strings of beads, Micrococci form *tetrads, i.e.*, squares composed of four spheres, and Sarcinæ form bundles of cells.

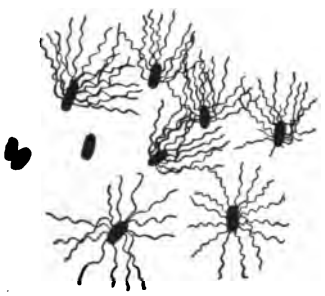


FIG. 27.—*Bacterium typhosum, peritrich.* (After *Migula.*) × 1,000.

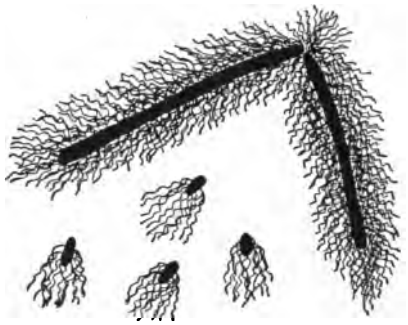


FIG. 28.—*Bacterium vulgare, peritrich.* (After *Migula.*) × 1,000.

Gocci which adhere together in pairs are known as **Diplococci,** and those forming irregular aggregates resembling clusters of grapes are named **Staphylococci;** these names, however, have no systematic significance. Cocci which divide in more than one direction do not as a rule stretch before division, but form two hemispheres immediately after division. Many streptococci

FIG. 29.—Various Cocci. (After *Flügge.*) × 1,000.

behave similarly and resemble chains of spheroidal links, flattened as though compressed along the direction of growth. Other streptococci become distinctly elongated, forming oval or even rod-shaped links; an example is seen in *Streptococcus lactis*, the commonest of the lactic acid bacteria. With the exception of a few sarcinæ, the cocci have never been found to form spores.

The rod-shaped bacteria are divided into the genera **Bacterium** and **Bacillus.** Originally the term bacillus indicated long rods,

later, according to *Migula*, motile rods, while now it generally denotes spore-forming rods.

The screw-shaped bacteria, which always have polar flagellæ, are classified into the genera **Vibrio** and **Spirillum**. The vibrios are monotrich and form single curves, being comma-shaped; the spirilla are lofotrich and more or less screw-shaped. Certain spirilla are said to be able to form spores.

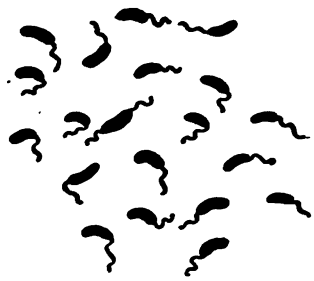


FIG. 30.—*Vibrio cholerœ*. (After *Migula*.)

FIG. 31.—Various Screw-shaped Bacteria. (After *Flügge*.) × 1,000.

The above remarks may be condensed into the following tabular classification into families and genera :—

Family 1.—**Coccaceæ, or Sphere Forms.**

Genus *Streptococcus*, dividing in one direction.
Genus *Micrococcus*, dividing in two directions.
Genus *Sarcina*, dividing in three directions.

Family 2.—**Bacteriaceæ, or Rod Forms.**

Genus *Bacterium*, without spores.
Genus *Bacillus*, with spores.

Family 3.—**Spirillaceæ, or Screw Forms.**

Genus *Vibrio*, monotrich.
Genus *Spirillum*, lofotrich.

As bacteria may lose the power to form spores, the distinction between the *bacterium* and the *bacillus* groups is not sharp. The distinctions between the micrococci and the sarcinæ, and between the vibrios and other monotrich rod forms, are still more ill-defined. On the whole, the above system of classification, due to *Lehmann* and *Neumann*, is unsatisfactory, as it groups together organisms which have but little in common and separates others which should be grouped together. It is only mentioned here as being that which is most commonly used. As far as the true lactic acid bacteria are concerned, I propose to use generic terms of more essential significance.

Together with the bacteria may be classified the **Sulphur Bacteria,** the **Thread Bacteria** and the **Ray Fungi.** Sulphur bacteria are always present where hydrogen sulphide is produced, for example in rotting seaweed, where they transform the poisonous gas into sulphates necessary for plant growth. Colourless and red species are known in forms of almost endless variety. The thread bacteria, like the sulphur bacteria, are typical water organisms; they are found attached to solid objects, and their outer cell walls form sheaths in which may be accumulated large amounts of iron. They may cause stoppages in water pipes, and contribute towards the formation of bog-ore. The ray fungi, or **Actinomycetes,** may be regarded as being intermediate between the bacteria and the moulds, especially the Mycomycetæ (Mucorinæ); on the one hand their cells are as slender as those of the bacteria, on the other hand they ramify and form oidia by constriction. They are of very common occurrence in soil, contributing to its characteristic smell. They may develop in butter, in which they produce a similar smell [1]. One variety, *Actinomyces bovis* (Fig. 32), is the cause of actinomycosis in cattle. The organisms of diphtheria, and especially those of tuberculosis, are closely related to this group.

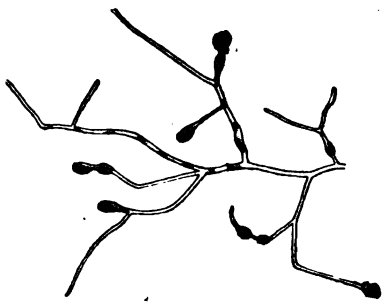


FIG. 32.—*Actinomyces bovis.* (After *Boström.*) × 1,000.

The Spirilla are also typical water bacteria, being difficult to cultivate artificially. As representing the vibrios, only the well-known cholera vibrio need be mentioned (Fig. 30). In dairy

[1] *Orla Jensen,* "Centralblatt für Bacteriologie," 2 Abt., 1902, Bd. VIII., p. 250.

practice we have to deal almost exclusively with cocci and rod-shaped organisms; special attention will therefore be paid to these groups in this work.

THE LACTIC ACID FERMENTATION

This is the most important fermentation in dairy practice. In the *true lactic acid fermentation*, lactic acid is practically the sole product arising from the fermented sugar. The chief by-products are carbonic and acetic acids. The production of carbonic acid

FIG. 33.—*Thermobacterium bulgaricum.* Grown in sterile milk. Stained with methylene blue. The grains are round and dark blue. × 1,000.

in recognisable amounts is a sign that the lactic acid bacteria are in full vigour; acetic acid, on the other hand, only appears in appreciable quantities in stale cultures, or when the bacteria are given a plentiful supply of air, either by aerating the liquid during fermentation or by allowing it to expose a large surface to the air. *Pseudo lactic acid fermentations* are characterised by the formation of considerable quantities of volatile acids, gases and other by-products, such as succinic acid and alcohol. In view of the fact that in dairy practice only the true lactic acid fermentation can be turned to economic advantage, while the pseudo lactic acid fermentations generally cause trouble, the terms "true" and

"pseudo" may be taken as corresponding with the concepts of useful and harmful lactic acid bacteria.

Lactic acid was first studied systematically by the Swedish chemist *Scheele* in 1782 [1]. It is a syrupy liquid, entirely soluble in water, alcohol and ether. The most convenient method of preparing it pure is to evaporate sour whey to a syrup, and to extract this with ether. If chalk is stirred into the fermenting liquid from time to time the acid will be neutralised, and thus prevented from weakening the bacteria, which will then be able to ferment the whole of the sugar. Lactic acid may be prepared on

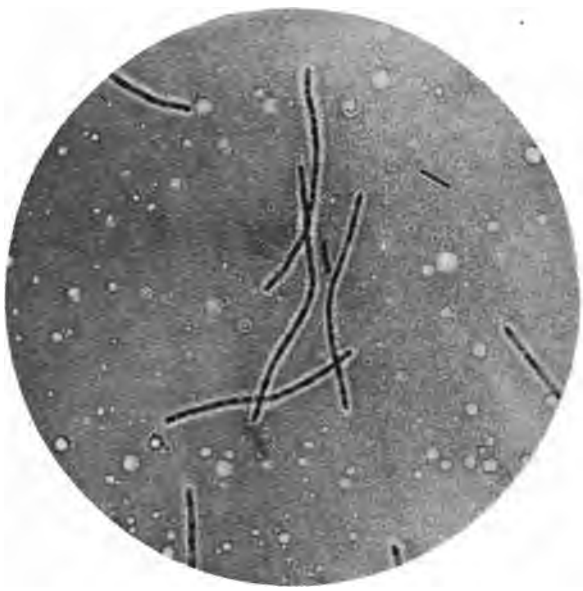


FIG. 34.—*Thermobacterium bulgaricum.* Grown in milk pasteurised by heating previously to 80° C. for half an hour. Stained with methylene blue. The grains are long-drawn and red. Capsule clearly shown × 1,000.

a large scale from a 10 to 20 per cent. solution of maltose, made by saccharifying starch with extract of malt containing active diastase. The solution is treated with chalk, sterilised and fermented by a pure culture for a week or two at 50° C., the culture being kept pure at this temperature. As calcium lactate is formed, the solution gradually sets to a pasty mass of crystals, which is pressed and decomposed with sulphuric acid; after filtering off the calcium sulphate the liquid is evaporated, preferably *in vacuo*, till it contains 40 to 80 per cent. of lactic acid. The yield is about 75 per cent. Lactic acid and its salts are largely used as mordants in the dyeing and tanning industries. Before the War, Germany

[1] "Kgl. Vetenskaps Academiens nya Handlingar," Bd. III., p. 120.

produced 1,000 tons annually. Polarimetric observation reveals three forms of lactic acid, dextro and lævorotatory, and inactive. The last-mentioned is a mixture of the two optically active acids in equal proportions, and differs from these in the solubility of its salts as well as in its optical properties [1]. In order to identify a lactic acid bacterium, it is necessary to know what form of lactic acid it produces under certain conditions.

In 1857, *Pasteur* discovered that the lactic acid fermentation was due to the action of certain bacteria [2], and soon after the discovery of the method of making gelatine plate cultures, *Hueppe*, in 1884, succeeded in isolating one of these bacteria [3]. This organism, which was named *Bacillus acidi lactici*, is not one of the true lactic acid bacteria, but belongs to the aerogenes group, which will be dealt with later. The organism which plays the principal part in the self-souring of milk had already been correctly described by *Lister*, in 1878, as an oval diplococcus, and given the name *Bacterium lactis* [4]. A similar form was isolated by *Grotenfeld* in 1879, and named *Streptococcus acidi lactici* [5]. The importance of this organism was, however, only recognised by *Leichmann* in 1894 [6], and by *Gunther* and *Thierfelder* [7] in 1895, who demonstrated that it produced dextro lactic acid in milk. *Leichmann* named it very appropriately the bacterium of sour milk, *Bacterium lactis acidi*. The large number of lactic acid bacteria now known really only resemble one another in not forming spores, and therefore being comparatively easily destroyed by heat.

The True Lactic Acid Bacteria. — These bacteria ferment carbohydrates and higher alcohols to lactic acid; they only grow in presence of proteins or complexes of amino acids, and not in presence of ammonium salts or single amino acids. They are Gram-positive, non-motile, non-sporing rod or sphere forms. It will only be possible here to mention the more important characteristics by which nearly related forms are best distinguished. The following is a brief summary of the results of the author's recent researches on this subject [8]:—

[1] The lactic acids are best identified by conversion into zinc salts. The active zinc lactates rotate the plane of polarised light in senses opposite to those due to the free acids, and they crystallise with two molecules of water, corresponding to 12·9 per cent. H_2O, which is not driven off below 140° C. The inactive zinc lactate is much less soluble, and crystallises with three molecules of water (18·2 per cent. H_2O), which is more readily driven off on heating.

[2] "Comptes rendues," tome 45, p. 913.

[3] "Mitt. Kais. Ges.-Amt.," Bd. II., p. 309.

[4] "Trans. Path. Soc. of London," vol. 29, p. 425.

[5] "Fortschr. Medizin," Bd. VII., p. 121.

[6] "Milchzeitung," Bd. XXIII., p. 523.

[7] "Archiv. für Hyg.," Bd. XXV., p. 164.

[8] "The Lactic Acid Bacteria." Monograph published in English by the Danish Academy of Sciences, Copenhagen, 1919.

(A) **No catalase formation, nitrate reduction nor surface growth.**

(a) *Forming only traces of by-products in addition to lactic acid.*

Rod forms .. Genera *Thermobacterium* and *Streptobacterium.*

Sphere forms .. Genus *Streptococcus.*

(b) *Generally forming appreciable amounts of gas and other by-products in addition to lactic acid.*

Rod forms .. *Betabacterium.*

Sphere forms .. *Betacoccus.*

(B) **Usually forming catalase, reducing nitrates and showing surface growth.**

Rod forms .. *Microbacterium.*

Sphere forms .. *Tetracoccus*[1].

Group B has been included because it forms a limiting group to the lactic acid bacteria on the one side, just as the Coli and Aerogenes group do on the other, though none of these can be reckoned as true lactic acid bacteria. The last-mentioned group includes group A, and also *Bacterium bifidum*, which, on account of its branched form and obligate anaerobic character, occupies a unique position.

Group A.—Although we may well suppose that the genera *Streptobacterium* and *Streptococcus* and the genera *Betabacterium* and *Betacoccus* are respectively more closely related to one another than the various rod forms to one another, and the various sphere forms to one another, we will, however, seeing that the rod forms as a whole are stronger acid producers than the sphere forms, keep up the old tradition and treat the rod and sphere forms separately.

(1) **Rod Forms** (named *Lactobacilli* by *Beijerinck*).—Short or long, straight or curved rods which may grow out into long threads. Sometimes they contain granules which stain more readily with methylene blue than the rest of the protoplasm. They may occur in pairs or chains of varying lengths and regularity of form. To this group belong the most typical of the lactic acid bacteria, which produce, and are able to stand, greater quantities of lactic acid than the other lactic acid organisms. Some of them may form as much as 3 per cent. of lactic acid in milk. The members of the *Thermobacterium* genus are long rods, which thrive at 40° to 50° C. or even higher temperatures, but not below 22° C.; they will generally obtain predominance in milk kept above 40° C. They form lævo or inactive lactic acid, and with one exception, *Tbm.*

[1] Common term for acid-forming Micrococci and Sarcinæ.

cereale (*Bacillus Delbrücki*), they attack casein to a considerable extent, and thus come to play an important part in the ripening of strongly scalded cheeses such as Emmental or Gruyère. To this genus belong the strongest acid producers, *e.g.*, *Tbm. helveticum* (Bacterium casei ϵ), which may produce over 2·7 per cent. of inactive lactic acid, and which plays an important part in the ripening of Emmental cheese. *Tbm. bulgaricum* (*Bacillus bulgaricus*) forms up to 1·7 per cent. of lævo acid, and *Tbm. jugurt* forms as much inactive acid as *Tbm. helveticum*, and grows in peculiar feathery-shaped colonies. These two last-mentioned organisms occur in Bulgarian sour milk.

Organisms belonging to the *Streptobacterium* genus are short or long chains of short or long rods, which as a rule have a maximum

FIG. 35.—*Thermobacterium helveticum* from Emmental Cheese. (After *Freudenreich.*) × 1,000.

FIG. 36.—*Streptobacterium casei* from Danish Dairy Cheese. × 1,000.

of 35° to 40° C.; they gradually come to predominate in dairy products which are kept at temperatures below 35° C., and are therefore frequently found in cheese. They form inactive or dextro acid. *Sbm. casei* (*Bacterium casei* α) hydrolyses casein, while *Sbm. plantarum* does not do so.

The *Betabacteria* almost always form inactive acid, and when in a freshly isolated state, perceptible amounts of by-products; they have no action on casein, and as a rule do not grow well in milk. As examples may be named *Bbm. breve* and *longum* (*Bacterium casei* γ and δ respectively). The former ferments arabinose strongly, and frequently also xylose; it has a maximum temperature of 38° C. The latter never ferments arabinose, but frequently ferments xylose and raffinose; its maximum temperature is 45° C. *Bbm. caucasicum* is the chief constituent of Kefir

grains ; it forms appreciable amounts of acid together with yeast, and its optimum temperature lies below 30° C.

(2) **Sphere Forms.**—*The Streptococci* are related to the *Streptobacteria*, the points of similarity having been mentioned above. They usually grow out into long chains when cultivated in broth, but in milk and on solid media they may present varied appearances. They grow well in milk, and always form dextro lactic acid, though very seldom more than ½ to ¾ per cent., *i.e.*, not much more than is required to coagulate milk. They are better able to grow on the surface of solid media than the rod forms, being less strictly anaerobic in character, but they do not form spreading

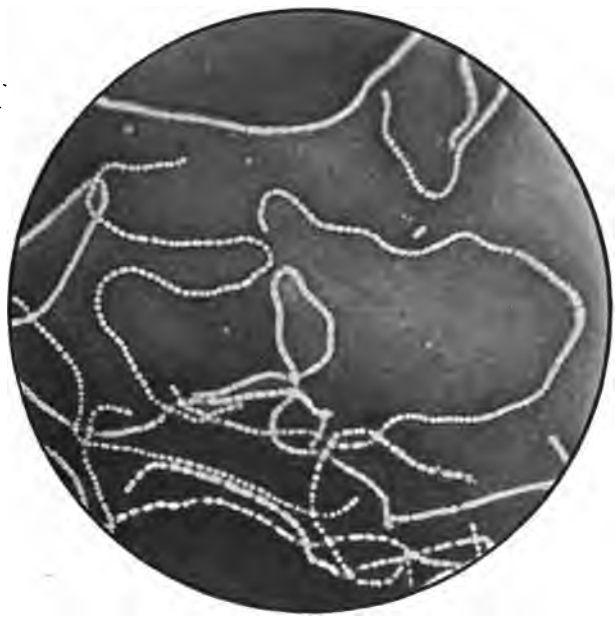


FIG. 37.—*Streptococcus cremoris.* (*Streptococcus lacticus.*)

colonies. With the exception mentioned below, the *Streptococci* show very little tendency to hydrolyse casein, and completely lose the power to do so if not cultivated in milk. Their optimum temperature is as a rule 30° C., and many strains do not grow above 37° C. *Sc. thermophilus*, however, grows best at 40° C. ; it is irregular in shape, and is easily isolated from milk which has been kept at a fairly high temperature. The greatest interest attaches to *Sc. cremoris*, which is used for ripening cream in butter making ; it forms long chains in milk and grows best at 25° to 30° C., but not at blood heat. Slime-producing strains are represented by the " bacterium of long milk," and *Sc. Hollandicus.* The former of these was first described by *Gerda Troili-Petersson* under the name

of *Bact. lactis longi*[1], and the latter by *Weigmann*[2]; this organism was to be found in the ropy whey formerly used in the manufacture of Dutch (Edam) cheese. *Sc. mastitidis* (*Sc. agalactiæ*), which produces notable quantities of lactic acid in milk, is the cause of mastitis or inflammation of the udder in cows. It may be recognised by the orange colouring matter which it produces after some time in agar or broth with casein peptone and soluble starch. Unlike *Sc. cremoris*, it ferments saccharose, maltose and dextrin. *Sc. pyogenes* is a general term for a number of pathogenic streptococci which do not coagulate milk; they cause boils and many other similar diseases in animals and human beings.

Belonging to this group are also the common bacteria of sour milk, *Sc. lactis* (*Bact. lactis acidi, Leichmann*), which always obtain

FIG. 38.—*Streptococcus cremoris* (*Storch's* No. 18). × 1,000.

FIG. 39.—*Streptococcus thermophilus.*

predominance in milk which is kept at ordinary room temperature, appearing mostly as diplococci in milk. It ferments dextrin, but not saccharose. *Sc. fœcium* is also a typical diplococcus form, which grows even at 50° C., and is very common in the manure of mammals. Other related organisms appear both as diplococci, and as longer chains in the same culture. They are distinguished according to their power to ferment a number of different substances such as glycerine, sorbitol, maltose, dextrin and salicin, and generally also pentoses and saccharose; their maximum temperature is about 45° C. As an example may be mentioned *Sc. liquefaciens*[3], which liquefies gelatine and produces a bitter taste in cheese.

[1] "Zeitschr. für Hyg. und Infectionskrankheiten," 1899, Bd. XXXII., p. 361.

[2] "Milchzeitung," 1899, Bd. XVIII., p. 18.

[3] *Freudenreich* originally named this organism *Micrococcus casei amari* ("Landwirt. Jahrbuch der Schweiz," 1894, p. 136).

The *Betacocci* have been so named by the author because they are generally found in sugar and other beets, swedes and mangold wurzels, especially when these are in a state of decomposition. In countries where such roots are largely used as cattle fodder, the Betacocci are of very common occurrence

FIG. 40.—*Streptococcus lactis.*

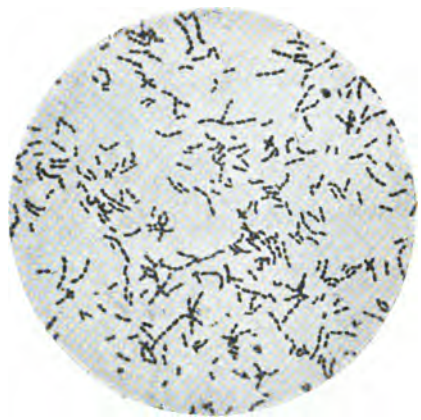


FIG. 41.—*Betacoccus* from thin juice from Nakskov Sugar Factory.

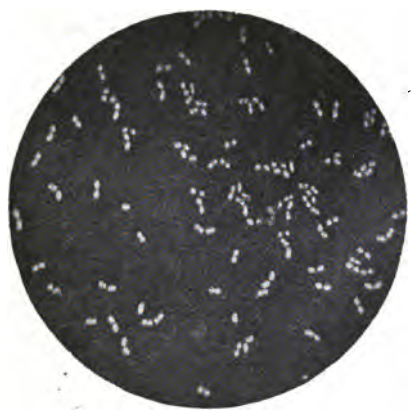


FIG. 42.—*Streptococcus liquefaciens (Freudenreich's Micrococcus casei amari).*

FIG. 43.—*Streptococcus liquefaciens (Escherich's Streptococcus coli gracilis).*

in milk and in the cheese made therefrom. The Betacocci occur as diplococci or short chains, which are not to be distinguished microscopically from the streptococci which have been dealt with above. They generally form lævo lactic acid, gas and other by-products, and render saccharose broth more or less slimy. The slime formation may best be observed in

stab cultures in saccharose gelatine. Some species liquefy this medium after some time, though they do not decompose casein. The Leuconostocs (Fig. 12, p. 7), which may give a deal of trouble in beet sugar manufacture, are Betacocci. They grow at temperatures as low as 5° C., and some species thrive better at room temperature than at 30° C. The sour cabbage bacterium, *Sc. brassicæ*, also belongs to this group.

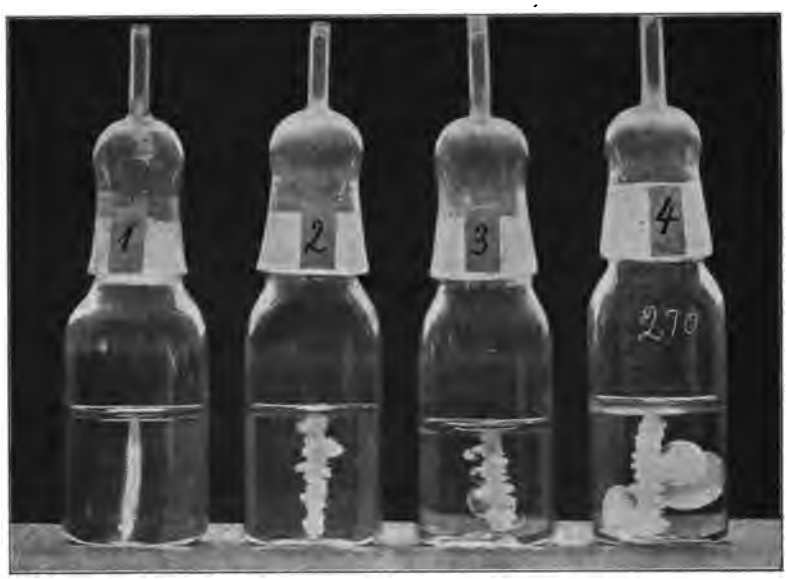


FIG. 44.—Various Betacocci in Stab Cultures in Cane-sugar Gelatine.

Group B.—The bacteria belonging to this group are not true lactic acid bacteria, differing from the forms hitherto described in forming catalase, reducing nitrates and generally growing well on the surface of solid media.

The microbacteria are very small rods, usually only 0·3 to 0·4 μ thick, which stand fairly high temperatures, and are therefore to be found in pasteurised milk. They form lactic acid, and some of them (*Bacillus acidophilus*) are common intestinal organisms. To these bacteria are related a group of small rod bacteria which liquefy gelatine, but only produce traces of acid.

The tetracocci include the acid-producing forms of the *Micrococci* and *Sarcinæ*. Division into these two groups is not feasible, and confusion may even occur with the Streptococcus group, as many of the Tetracocci appear as diplococci ; they may, however, readily be distinguished by their power to decompose hydrogen peroxide, for, as mentioned above, they differ from the true lactic acid bacteria in producing catalase. They produce less lactic acid

than the Streptococci, while they form notable amounts of acetic acid besides. They are more aerobic in character than the Streptococci, forming as a rule large surface colonies which are often coloured yellow, orange or pink. Most of them liquefy gelatine, though generally slowly, and they will therefore also coagulate milk, though the quantity of acid formed is seldom sufficient to accomplish this. Several of the liquefying species probably play some part in the ripening of certain cheeses, for example *Tetracoccus liquefaciens*[1], which forms white colonies and produces dextro lactic acid. The Tetracocci are found in great numbers in cow dung and earth, whence they find their way into dust; they are well able to stand desiccation and common salt, and many of them will also stand heating to over 70° C. They are common skin bacteria, some giving rise to pustules and others to inflammations, which are generally not of a dangerous character. According to *Beijerinck*, certain lactic-acid-producing sarcinæ which occur in soil, but are of no importance in dairy practice, produce large amounts of carbon dioxide and hydrogen.

The Pseudo Lactic Acid Bacteria.—These are motile or non-motile, Gram-negative short rods with rounded ends, which seldom form chains or threads of any length. They do not require organic nitrogen, and in stab cultures show profuse surface growth. As a rule they do not liquefy gelatine, and are mainly intestinal and excremental bacteria. The gas which they produce from sugars consists of carbon dioxide and hydrogen in widely varying proportions; according to certain American investigators, the composition of the gas formed affords a basis for the classification of these organisms[2].

The aerogenes bacteria are non-motile rods which produce large amounts of gas, being able to convert most of the sugar present into gas, especially if the acid which is produced is neutralised. The gas may contain up to three times as much carbon dioxide as hydrogen, while aerogenes forms are known which may even produce carbon dioxide alone. In dairy bacteriology two types may specially be distinguished. The one forms much slime which gives rise to outstanding colonies with the shiny appearance of porcelain; appreciable amounts of alcohol are formed, but not acid enough to coagulate milk. If the acid is neutralised by chalk as fast as it is formed, the milk will gradually be converted into a thick slime. To this type belong certain pathogenic bacteria, such

[1] The author ("Landwirt. Jahrbuch der Schweiz," 1904, pp. 349 and 369) has described this organism under the name *Micrococcus casei liquefaciens*.

[2] *L. A. Rogers, W. Mansfield Clark, Brooke J. Davis* and *Alice C. Evans* ("Journ. of Infectious Diseases," vols. 14, 15 and 17).

as *Bacterium pneumoniæ*, and some bacteria which, according to *Gillebeau*, cause inflammation of the udder. The second type, to which belongs *Bacterium lactis aerogenes*, produces less slime, and its colonies on gelatine are often but little larger than those of the lactic acid streptococci. They generally coagulate milk by the production of succinic acid and lævo lactic acid. The aerogenes bacteria can convert the citric acid of the milk into acetic and carbonic acids. On corn and flour aerogenes bacteria are found which produce a yellow colouring matter. *Bacterium cloacæ* is a liquefying aerogenes bacterium. Motile forms related to this organism may be regarded as a link with the Proteus bacteria. In cheese such forms are often found, but these form spores in one end of the cell, and must therefore be designated as aerobic Plectridia.

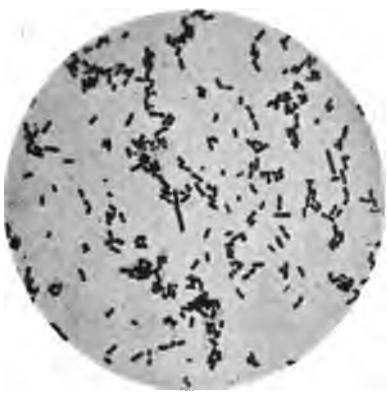


FIG. 45.—The Coli Bacterium which spoilt the Milk and Butter at Duelund Dairy in 1888. (After *C. O. Jensen.*) × 1,000.

The coli bacteria are as a rule peritrich, though often they are only slightly motile. They differ from the aerogenes bacteria in producing about equal quantities of carbon dioxide and hydrogen, and also in their power to decompose casein. In the latter respect they show a certain resemblance to the true lactic acid bacteria, but they carry the decomposition further than the amino acid stage, producing evil-smelling bodies, a circumstance which links them with the putrefactive bacteria. The kind of lactic acid formed by the coli bacteria is influenced to some extent by the particular sugar fermented, and also by the nature of the nitrogenous nutrient matter present. They commonly form large amounts of succinic and acetic acids. Their surface colonies may resemble those of the aerogenes bacteria in exceptional cases, but as a general rule, they spread out in thin, skinny layers. Numerous species of coli bacteria are known, many being pathogenic, giving rise to intestinal and other diseases such as typhoid fever and dysentery. *Bacterium typhosum* (Fig. 27) distinguishes itself by being very actively motile, while the dysentery bacteria are non-motile. None of these form gas. The commonest cases of meat poisoning are also due to certain coli bacteria, *Bacterium enteriditis*. The very dangerous coli bacteria which are dealt with here can easily be distinguished from the ordinary coli bacteria by the fact

that they do not ferment lactose to any appreciable extent. Even though they may make milk slightly acid to begin with, they always turn it alkaline at last.

THE PROPIONIC ACID FERMENTATION

The second fermentation process to be described is that in which sugar or calcium lactate is converted into propionic, acetic and carbonic acids. This fermentation is of particular interest in dairy practice in connection with the formation of cavities or "eyes" in cheese. The author's investigations on the ripening of Emmental cheese have shown that the above-mentioned products mostly appear at the stage at which the eyes are formed [1];

FIG. 46.—*Bacterium acidi propionici* (*a*). × 1,000.

FIG. 47.—*Bacterium acidi propionici* (*b*). Grown at 39° C. × 1,000.

and *Clark* has shown that normal eyes contain nothing but carbon dioxide [2]. The presence of the organisms in question can only be demonstrated after several successive inoculations into peptone broth containing calcium lactate instead of sugar. If such a medium be innoculated with almost any kind of cheese, active propionic acid fermentation will be found to have set in after systematic cultivation for a week or two.

Bacterium acidi propionici was first isolated by *Freudenreich* and the author [3]. These bacteria are non-sporing, non-motile and Gram-positive. Several species are known which differ considerably in appearance. The species (*a*), which is mainly instrumental

[1] "Studier over de flygtige Syrer i Ost." Doctoral thesis, published by *J. Gjellerup*, Copenhagen.

[2] U.S. Dept. of Agriculture, Bureau of Animal Industry, Bull. 151, 1912.

[3] "Über die im Emmenthalerkäse stattfindende Propionsauregärung" ("Landwirt. Jahrbuch der Schweiz," 1906, p. 320.)

in forming eyes in Emmental cheese, resembles the streptococci, but it does not coagulate milk. To this group belong various colour-producing species which may form red and brown spots in Emmental cheese [1]. Other strains (*b*) are stout irregular rods, some of which become either branched or club-shaped, and stain so unevenly that, like the diphtheria bacteria, they present a segmented appearance. These species produce sufficient acid (not lactic acid) from milk sugar to coagulate milk. The propionic acid bacteria do not decompose casein. Some of them are anaerobic, but may be accustomed to aerobic conditions; when this occurs, their fermentative powers are gradually weakened. The aerobic varieties form outstanding colonies in stab cultures, and are on the whole as prone to slime production as the aerogenes type. The propionic acid bacteria grow at temperatures between 15° and 40° C. According to *Burri* [2], they occur in appreciable numbers in cow dung, and probably they find their way into milk from this source.

THE BUTYRIC ACID FERMENTATION

In this process carbohydrates or lactates are converted into a number of different products, among which butyric acid, carbon dioxide and hydrogen have attracted particular notice. In addition to these, notable amounts of lactic, acetic, propionic and formic acids are formed, and sometimes also various alcohols, so that the process is an extremely complicated one.

The first butyric acid bacterium was described by *Pasteur* in 1861, a discovery which for the first time revealed the existence of obligate anaerobic organisms; the butyric acid bacteria will under no circumstances grow in presence of atmospheric oxygen. They are fairly large rods which form spores, assuming either the Clostridium or Plectridium forms (Figs. 5 and 6); the spores exhibit polar germination. In the butyric acid bacteria it is often possible to demonstrate reserve food material which stains blue or violet with iodine; this is most noticeable just before sporing if grown on starchy material such as potato slices. The young bacteria are generally Gram-positive. The true butyric acid bacteria can subsist on inorganic nitrogen, and in conjunction with aerobic bacteria they can assimilate atmospheric nitrogen[3]. They do not attack proteins; in this respect they differ from a

[1] *Thöni* and *Allemann*, "Centralblatt für Bacteriologie," 2 Abt., 1910, Bd. XXV., p. 8. According to the author's investigations, stab cultures of white varieties of the propionic acid bacteria are often red below the surface of the medium.

[2] "Landwirtschaftliches Jahrbuch der Schweiz," 1912, p. 481.

[3] *Winogradsky*, "Archives des Sciences biologiques," 1895, tome 3, p. 295, and *Bredemann*, "Zentralblatt f. Bact.," 2 Abt., 1909, Bd. XXIII., p. 385.

number of nearly related obligate anaerobic bacteria to be described in connection with the putrefactive process. As all the non-sporing bacteria in a liquid can be killed by heating for a few minutes at 90° C., the sporing organisms may readily be isolated by this means. If the liquid is rich in butyric acid bacteria, a small quantity may be inoculated after heating, into a tube containing a deep layer of sugar agar, but if only a few of these organisms are present their number must be increased by adding the heated liquid to sterile milk which all but fills a bottle securely closed by a spring stopper; the fermentation should not be allowed to proceed too far before the bottle is opened, or the pressure of the accumulated gases may easily cause a burst. The butyric acid bacteria ferment most sugars (not mannitol), and are said to be able to ferment starch. Butyric acid is the chief product formed in milk. They occur principally in soil, manure and flour.

According to *Grassberger* and *Schattenfroh*[1], distinction is to be made between the motile and the non-motile forms, *Bacillus butyricus mobilis* and *B. b. immobilis*. The former is peritrich, and readily forms spores which are killed after only three minutes' boiling; it does not ferment calcium lactate. It is responsible for the large amounts of butyric acid found in certain sour milk cheeses[2]. The latter is somewhat larger and forms spores less readily, but on the other hand the spores are decidedly more resistant to heat, for they will survive one and a half hours' boiling. According to *Barthel*[3], it is far more common in milk than the motile form, which accounts for its regular occurrence in the human and animal intestines. As a gas-producing organism, it may give rise to the formation of cavities in cheese. It may sometimes be pathogenic, and is supposed by some to be merely a degenerated variety of the organism of the cattle disease, blackleg or quarter evil (*Bacillus Chauvœi*), which is normally peritrich and attacks proteins. The butyric acid bacteria grow well at 16° to 40° C.

While the butyric acid bacteria do not attack cellulose, a number of closely related Plectridium forms carry out a cellulose fermentation which is of prime importance in the digestive process of herbivora and in soil formation. Other allied forms ferment the pectins which cement together the vegetable cells, and come to play an important part in the retting of flax and hemp.

[1] "Archiv. f. Hygieine," XXXVII., XLII. and XLVIII.

[2] *Freudenreich* and *Orla-Jensen*, "Landwirtschaftliches Jahrbuch der Schweiz," 1905, p. 312.

[3] "Obligat anaerobe Bakterien in Milch und Molkereiprodukten" ("Centralblatt f. Bacteriologie," 2 Abt., 1910, Bd. XXVI., p. 1).

THE PUTREFACTIVE PROCESS

The preceding sections have been devoted to certain carbohydrate fermentations. We now pass on to the fermentations of the proteins, which, according to our previous definition, come under the heading of putrefaction. In this process two distinct phases may be recognised: first, the protein hydrolysis or peptonisation, due exclusively to the proteolytic enzymes, in which the proteins are split up into soluble amino acids, and second, the decomposition of these acids by other enzymes (amidases, oxidases and reductases), with the formation of ammonia and various evil-smelling substances. While the final products of carbohydrate fermentation are always acid, those of protein fermentation are always alkaline. As the action of proteolytic enzymes and also the growth of the organisms which secrete them are inhibited by small quantities of free acid, it is easy to understand why the presence of carbohydrates in appreciable amounts will prevent putrefaction; it is only when the carbohydrates and their acid products have been destroyed or neutralised that the process of putrefaction will obtain a proper start. As all bacteria which secrete proteolytic enzymes may take part in a putrefactive process, we have to deal with a large number of types. We will first deal with the aerobic putrefactive bacteria, and then with those which are obligate anaerobes, for the former initiate the process and, consuming the available atmospheric oxygen, they prepare the ground for the latter group, which then carry the decomposition further; it is especially during this second phase that the evil-smelling products, so characteristic of putrefaction, are formed.

A. The Aerobic Putrefactive Bacteria. 1. **The Fluorescent Bacteria.**—These are non-sporing motile rods with polar flagellæ, producing a fluorescent green colouring matter (insoluble in chloroform) on neutral or alkaline media. The commonest species are monotrich and Gram-negative. They do not ferment lactose, and some are liquefying, others non-liquefying. Several, especially the non-liquefying species, are denitrifying organisms, *i.e.*, they reduce nitrates to free nitrogen, and thus rob plants of their nitrogenous nutrient matter. They are very widely distributed in soil and water, and can as a rule grow at temperatures only slightly above 0° C. A species which is particularly active in liquefying gelatine, *Bacterium fluorescens liquefaciens*, hydrolyses fats, and may therefore play an important part in turning butter rancid. *Bacterium pyocyaneum* (Fig. 25) is a nearly related form which also liquefies gelatine and hydrolyses fats, but it grows so slowly at ordinary temperatures that it does

not spoil butter under normal conditions. In addition to a fluorescence, this organism produces a blue colour (which is soluble in chloroform and is turned red by acids). It is often found as one of the active organisms in inflammations, when it imparts a blue or green colour to the pus. A similar but Gram-positive, lofotrich and non-liquefying species is the bacterium of blue milk, *Bacterium syncyaneum* (Fig. 26), which in addition to a fluorescence produces a grey colour which is turned blue by acids; it can therefore only colour milk blue in the presence of lactic acid bacteria. A peptonising rod form which colours neutral milk blue was isolated by *Carl Lind*, working in the author's laboratory. In all cases the blue coloration starts on the surface of the milk. The last-mentioned organism is perhaps identical with the water bacterium, *B. cyaneofuscus*, isolated by *Beijerinck* [1], which produces blue spots in cheese.

The group most nearly allied to the fluorescent bacteria are the marine phosphorescent bacteria, which cause dead fish to become luminous. Being marine organisms, they require a nutrient medium containing sodium chloride.

2. **Peptonising Cocci.**—The liquefying micrococci and sarcina forms, especially those which do not produce acid, generally take part in putrefactive processes. Several of the colour-producing varieties thrive well in the outer paste which becomes the rind of many cheeses.

3. **Coli Bacteria.**—As mentioned above, these are intestinal organisms which find their way into milk from the excreta. Being acid-producers, they will generally retard rather than promote putrefaction, as carbohydrates are present in sufficient quantity both in the intestine and in milk. In the absence of carbohydrates, however, they act as typical putrefactive bacteria, decomposing amino acids.

4. **The Proteus Bacteria.**—As their name implies, these rod forms may assume many different shapes and sizes, sometimes growing out into long threads which may form a tangled network. They form no spores, and do not show any uniformity in their behaviour towards Gram's stain; as a rule they are actively motile, being plentifully supplied with flagellæ all over the cell. The proteus bacteria mostly ferment sugars, especially dextrose, with the production of succinic and acetic acids and various gases, and they generally liquefy gelatine. A non-liquefying form, *Bacterium Zopfii*, whose colonies ramify so freely on gelatine that it might easily be mistaken for a mould, is frequently found in milk. The most typical of the aerobic putrefactive bacteria is

[1] "Botanische Zeitung," 1891, Bd. XLIX., p. 704.

Bacterium vulgare (Fig. 28), which coagulates and peptonises milk ; it can grow at low temperatures. Many closely related forms are pathogenic. The ptomaine poisons produced in meat are generally due to certain proteus and coli bacteria. The proteus group includes several colour-producing bacteria, such as the *Bacterium synxantum* of yellow milk and *Bacterium erythrogenes* of red milk. The latter, however, differs from the other proteus bacteria in being non-motile. *Bacterium prodigiosum*, which produces a fine red colour, especially when grown on starchy media, is far commoner of occurrence than the two last-mentioned organisms ; this

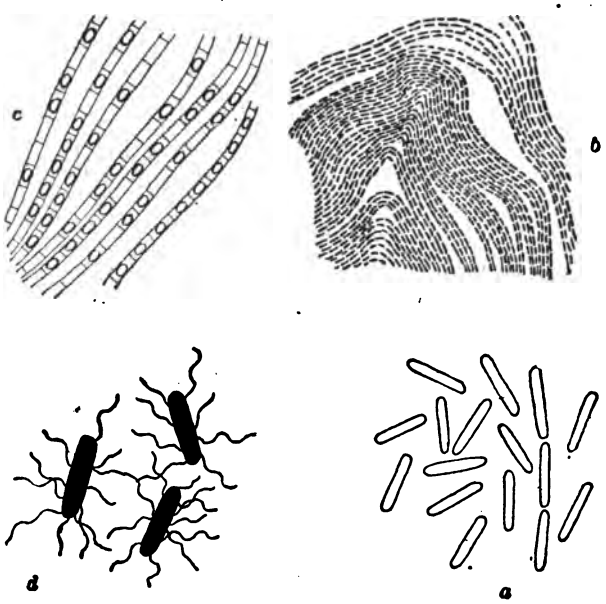


FIG. 48.—*Bacillus subtilis*. (After *Migula*.) *a*. Active rods before spore formation. *d*. Stained to show the flagellæ. *c*. Rods with spores. All these × 1,000. *b*. A piece of film. × 100.

phenomenon was formerly taken for the appearance of blood on the Host. As the red colouring matter is not soluble in water, the bacterium cannot colour milk red, but can only form red spots on the cream layer. It is Gram-negative, and most varieties coagulate milk quickly by forming both acid and rennet. The author has shown that it hydrolyses fat as actively as *Bacterium fluorescens liquefaciens*.

5. **The Hay and Potato Bacteria.**—These organisms are distinguished by their ability to form spores which are more resistant to heat than those formed by the butyric acid bacteria. They are only killed after three to six hours' boiling, and a few species which do not grow at ordinary temperatures form spores capable of

resisting twenty hours' boiling. The spores germinate laterally. The organisms of this group are typical soil bacteria, finding their way into the soil from hay, potatoes and other feeding stuffs, in which they were first found. With the dust, and from the excrements of the stable, they find their way into milk, rendering it very difficult to sterilise. Being obligate aerobes, they generally form films on the surface of the culture solution and carry the oxidation of carbohydrates almost to completion. They contain diastase, which enables them to hydrolyse starch; if bread contains spores of these bacteria, which have not been killed in baking, it becomes slimy after a few days. They peptonise actively, are Gram-positive and peritrich. The two best-known species, *Bacillus subtilis* and *Bacillus mesentericus*, are best distinguished from one another by cultivation on potato slices, on which the former produces a smooth film and the latter a wrinkled film. *Bacillus mycoides* is most frequently found in incompletely sterilised milk; it forms stellate colonies like those of *B. Zopfii*, in stab cultures, before the gelatine is melted. *Bacillus anthracis* (Fig. 16A), grows similarly, but is non-motile. Most of the hay and potato bacilli are thermophile, that is they are able to grow at high temperatures, many of them growing well at 50° C., and some even at 60° C. Other species actually prefer temperatures of 50° to 70° C., and do not grow at ordinary temperatures; these are often non-motile, and anaerobic rather than aerobic. When masses of hay or other vegetable matter become warm, this is in the first place due to the oxidation or respiration of the vegetable matter itself, but when the temperature reaches 50° C. the vital processes of the plant remains cease, and further production of heat is due to the action of the thermophile bacteria until their maximum temperature is reached. Further rise in temperature and spontaneous ignition may take place owing to purely chemical oxidation, *i.e.*, combustion.

B. Anaerobic Putrefactive Bacteria.—All the organisms of this group are peritrich spore-forming rods which closely resemble the butyric acid bacteria, from which, however, they differ in being able to attack proteins. The most important member is *Bacillus putrificus* which contributes more than any other organism to the development of the putrefactive odour; it is able to grow in solutions of pure proteins. The *Plectridium fœtidum*, isolated by *Weigmann* [1] from cheese, which produces in milk a smell like that of Limburg cheese, is, according to *Barthel* [2], identical with *Bacillus putrificus*. The author [3] has

[1] "Centralblatt f. Bakt.," 2 Abt., 1896, Bd. II., p. 150.
[2] "Centralblatt f. Bakt.," 2 Abt., 1910, Bd. XXVI., p. 1.
[3] "Centralblatt f. Bakt.," 2 Abt., 1904, Bd. XIII., p. 754.

examined a similar Plectridium form which was isolated from cheese, but which differs from the last-mentioned organism in being able to ferment calcium lactate and not thriving in the absence of special sources of carbon. Further, it produces in milk formic, butyric and valerianic acids. Several poison-producing pathogenic forms also belong to this group, *e.g.*, *Bacillus botulinus*, which forms poisons in sausages, and *Bacillus tetani*, the organism of tetanus or lockjaw (Figs. 6 and 16c).

The subject of putrefactive fermentation has been dealt with at some length for two reasons: first, the putrefactive organisms are the most harmful with which we have to deal in dairy practice, and second, the ripening of cheese is a process of protein decomposition which takes a special course thanks to the acid which is always formed at the outset.

The few milk bacteria which are not connected with the fermentation processes already described will be dealt with in Part II.

Chapter III

Yeasts and Moulds

UNLIKE the bacteria, the yeasts and moulds prefer acid nutrient media, their natural habitat being soft, juicy fruits. They also thrive in sour milk and dairy products, in which they can produce a variety of changes owing to the number of enzymes which they secrete. They generally oxidise lactic acid, and thus render the medium better adapted for the nutrition of bacteria; it is only with the co-operation of yeasts and moulds that putrefactive fermentation can become established in sour milk. Yeasts and moulds play an important part in the ripening of many sour milk and soft rennet cheeses. As most moulds hydrolyse fats, they contribute largely towards the development of rancidity in butter. Most yeasts and moulds can grow at comparatively low temperatures. While bacteria only develop in media containing at least 20 to 30 per cent. of water, moulds require only 14 per cent., and will therefore grow on comparatively dry feeding stuffs, whence they find their way with the stable dust into milk.

A. YEASTS

The best-known property of these organisms is the power to ferment sugars with the production of alcohol and carbon dioxide. They find technical application in the manufacture of beer, wine and spirits, while pressed yeast is used in baking to raise the dough. The sporing and the non-sporing forms are known as *Saccharomycetes* and *Torulæ*, respectively. The non-sporing *Mycodermæ* occupy a special position; like the moulds, they only produce alcohol quite exceptionally, but they are able to oxidise this substance.

1. **Saccharomycetes.**—These are the most typical of the alcohol-producing yeasts (Figs. 2, 4 and 7). As the form of the cells is not very characteristic, and subject to considerable variation, the various species are best distinguished by the scheme of *Emil Christian Hansen*, which is based on the investigation of the spore formation and observation of the time which elapses before spores are formed under certain conditions. Moist plaster of Paris blocks are particularly well adapted for this purpose. The pure cultures used in breweries are generally round or oval yeasts, which

develop comparatively few spores in each cell; certain wild yeasts, which spoil beer, grow as elongated cells and form large numbers of spores in each cell. Yeasts which collect at the bottom of the liquid towards the end of the fermentation are known as bottom yeasts; those which rise to the surface are known as top yeasts. Bottom yeasts are employed in slow fermentation at low temperatures, in the making of light wines and beers of the lager type. Top yeasts, on the other hand, cause more vigorous fermentations, and are used in the making of heavier wines and beers and spirits. Bakers' yeast is usually a top yeast. The yeasts used in the production of alcoholic beverages ferment maltose and sucrose, but not lactose; the saccharomycetes met with in dairy practice ferment lactose and sucrose, but not maltose.

2. **The Torulæ.**—These are smaller than the saccharomycetes and play only a subordinate part in the alcoholic fermentation industries. On the other hand, they are of far more frequent occurrence in dairy products, while the formation of alcohol in Kefir and similar beverages is chiefly due to the action of certain torulæ which ferment lactose either by themselves or in symbiosis with certain lactic acid bacteria. A lactose-fermenting species, *Torula amara*, which lives in sycamore leaves, will, according to *Harrison*, turn milk bitter in a few hours. The torulæ which do not ferment sugar are still more widely distributed; according to the author's investigations, they develop freely in butter which is kept for some time. Certain species which form characteristic stellate colonies in stab cultures hydrolyse the fat more or less vigorously, provided that the butter is sufficiently acid. A certain torula which hydrolyses fat will colour butter red; the growth of this organism is inhibited by common salt.

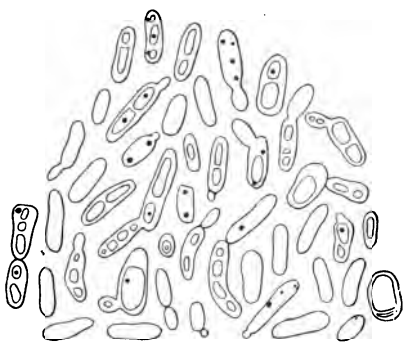


FIG. 49.—*Mycoderma cerevisiæ.* (After *Holm.*)

3. **Mycodermæ.**—While most yeasts form a film on the surface of the liquid after the fermentation is over, the mycodermæ form a dull-looking film at the very outset. As a rule they are elongated cells containing a few bright granules. In the making of Emmental cheese, a home-made rennet is generally employed which, if properly made, should be a practically pure culture of *Thermobacterium helveticum* covered by a mycoderma film which excludes

air, and thus secures anaerobic conditions favourable to the growth of the lactic acid bacterium. This is an instructive example of the way in which an organism may act beneficially by indirect means. The mycoderma in question ferments dextrose but not disaccharides.

B. MOULDS

It will not be necessary to enter into the classification of the moulds, as only a few species are met with in dairy practice; the yeast-like forms, *Monilia*, *Cladosporium* and *Oidium lactis*, and a few *Penicillium* species.

1. **Monilia and Cladosporium.**—Both of these groups reproduce by budding, so that in young cultures they cannot be distinguished from yeasts; it is only at a later stage that the cells become elongated and form a true mycelium which grows upwards into the air. The Monilia group further resembles the yeasts in being able to bring about a true alcoholic fermentation. A species which becomes black in old cultures, *Monilia nigra*, will, according to *Burri* and *Staub* [1], form deeply penetrating black spots on the rind of hard cheeses. On the other hand, the blackening of sour milk cheeses and soft cheeses is due to *Cladosporium herbarum*, a mould which is fairly prevalent in dairies [2]. A nearly related form, *Cladosporium butyri*, which is first white, then green, brown and finally black, has been found by the author [3] to play an important part in the development of rancidity in butter. Like several of the mycodermæ, it produces an ethereal fruity odour in milk, but does not bring about alcoholic fermentation. The above-mentioned Monilia and Cladosporium species liquefy gelatine, and are able to grow in presence of large amounts of salt, so that their growth in cheese is not inhibited by salting.

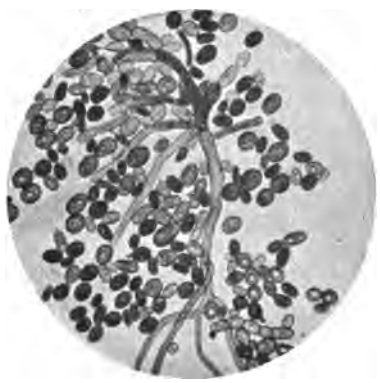


FIG. 50.—*Monilia nigra*. (After *Burri* and *Staub*.)

2. **Oidium lactis.**—This white mould is hardly ever absent from any milk. It thrives better in cream than in skim milk, and

[1] "Landwirtschaftliches Jahrbuch der Schweiz," 1909, p. 487.

[2] This mould was first observed by *Herz* (Milchzeitung, 1885). *Adametz* has described several black moulds in his book, "Ueber die Ursachen und Erreger der abnormalen Reifungsvorgänge beim Käse" (Bremen, 1893).

[3] "Landwirtschaftliches Jahrbuch der Schweiz," 1901, p. 367.

better in sour than in fresh milk. It is always this mould which forms the velvety layer on sour milk which has been kept too long. On sugar gelatine it only shows luxuriant development of aerial threads in places where lactic acid bacteria are also growing, which shows that it thrives best in presence of lactic acid. It liquefies gelatine very slowly. As already mentioned, it has no specialised conidiophores. It is mostly found on the surface of many soft cheeses, and contributes towards the development of rancidity in butter. Several allied forms are known, some of which, according to *Weigmann*, will produce a smell of turnips and other feeding stuffs in milk. A red form, *Oidium aurantiacum*,

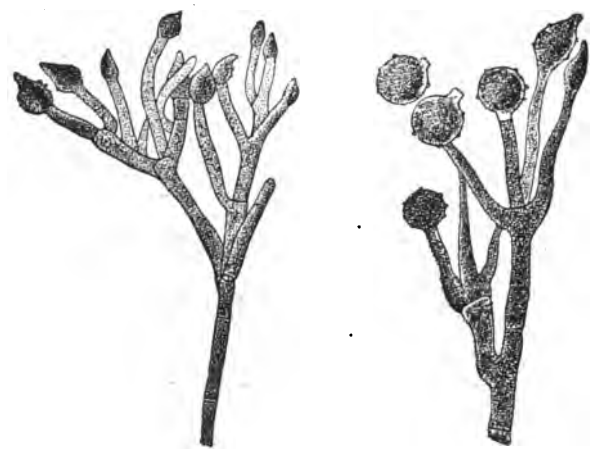


FIG. 51.—Two varieties of *Penicillium brevicaule*.

will, according to *Adametz*, produce orange or red spots on the surface of cheese.

3. **The Penicillia.**—These are represented by numerous species, difficult to distinguish from one another. Their distinguishing feature is a sheaf of conical cells, growing out from the upper end of the conidiophore, from which the conidia (usually green) are formed by constriction. The name of this group of moulds is derived from *Penicillium*, a brush, owing to the characteristic tassel or brush-like appearance presented by the arrangement of the spores. The commonest species is *Penicillium glaucum* (Figs. 3 and 9), which liquefies gelatine and produces the well-known mouldy or musty smell. A nearly related form, which thrives particularly well on apples, produces an ethereal fruity odour. In Roquefort, Gorgonzola and Stilton cheeses, a special species, *Penicillium roqueforti*, is found, which forms shorter conidiophores and somewhat larger conidia. On sugar gelatine its colonies develop an uneven and often fairly wide white rim;

they have no smell, and do not liquefy gelatine. On a fatty medium this mould produces the characteristic smell and taste of Roquefort cheese. Among the many similar forms which are known, only those can be employed which form a perfectly colourless mycelium. If they colour the nutrient medium yellow or brown, or their conidia become brown instead of green after a short time, they will colour the Roquefort cheese brown. According to *Staub*, a mould of this kind, *Penicillium casei*, will produce on the surface of cheese yellowish brown spots which gradually become reddish brown, and coalesce so that the whole of the rind becomes coloured. On Camembert and other French soft cheeses, are generally found *Penicillium camembertii*, which forms pale green or greyish green conidia, and *Penicillium candidum*, which forms conidia up to half a centimetre long, being perfectly white even in the ripe state. Both these moulds liquefy gelatine slowly. While all the species mentioned above form conidia which are perfectly smooth and spherical when ripe, *Penicillium brevicaule* forms irregular warty conidia (Fig. 51); this mould is usually yellow or brown and grows on manure; according to *Weigmann* and *Wolff*, it produces a smell of turnips or onions in milk and dairy products [1]. According to *Thom*, varieties of this mould are often found on Camembert cheese.

[1] "Centralblatt f. Bakt.," 2 Abt., 1909, Bd. XXII., p. 657.

PART II

Chapter I

Cleaning and the Procurement of Milk

CLEANING

Systematic Cleanliness is the golden rule in dairy practice :— more than this, it includes all that is of prime importance in Hygiene ; and it brings every one into actual contact with the science of Bacteriology. Unfortunately it is only too obvious that few people realise the true significance of cleanliness. For example, in dusting a room, many think that they are serving the interests of cleanliness by simply whirling up the dust with a dry cloth from places where it is most conspicuous only to let it settle in a thin layer all over the room after some time ; again, many will wash a number of floors with the same pail of water, which may at last become as black as ink, or wipe the inside of cooking utensils with swabs which may be in a most disgusting condition. It must also be said that most people consider the daily brushing of their teeth with a tooth brush which is never cleaned as the acme of cleanliness.

As the great majority of the defects of milk and dairy products arise through lack of cleanliness, a few remarks concerning the *cleaning of the surfaces with which the milk comes into contact* will not be out of place. If the cleaning is to be effective, the dirt must not merely be spread over a larger area, but it must be completely removed, and not only must the microorganisms present be killed, but care must be taken that no fresh organisms are introduced. The main object in cleaning is to get rid of the microorganisms, and as these are usually embedded in the dirt, the first step is to secure the removal of the dirt ; in doing this, the great majority of the microorganisms will be removed as well, while the few which remain will be prevented from multiplying owing to lack of nutrition. Moreover, even sterile dirt will always be undesirable as it renders the surface of the vessel rough, and even if odourless at the start, it will acquire an unpleasant taste and smell on being submitted to the processes necessary for the killing of the micro-organisms. In order to remove the dirt, which in our case consists of the constituents of milk, it must be dissolved or at least loosened ; thus fat will be loosened by the use of hot water. The

water should, however, not be used too warm to begin with, or the proteins will be rendered insoluble. It is well-known that cheese cloth quickly becomes stiff and useless if not rinsed in cold water before washing in boiling water. By the use of soda or lime, the casein is dissolved and the fat is emulsified, *i.e.*, reduced to a fine state of division. These chemicals also act as poisons towards bacteria, *i.e.*, *disinfectants*. They are most effective if used together, but unfortunately tinned vessels do not stand this treatment well. Soda is to be preferred to lime for the cleaning of such vessels, for though it is somewhat more energetic in its action on tin, it removes fat more readily. In order to prevent the tinning on pasteurisers from being attacked, it is best to soak the crust which has formed in a cold solution of soda, whereupon it will easily be removed on scrubbing with clean warm water. The plates of centrifuges may be cleaned in the same way. With lime, the fat forms insoluble lime soaps which makes the tin dull and rough. On the other hand, lime is to be preferred for wood work, partly because if rubbed in as milk of lime it will remain where it is for some time, so that its disinfecting action will be prolonged, and partly because the lime soaps fill up the pores and render the surface of the wood smoother and firmer. The cleaning must be finished with several rinsings with plenty of water, or some of the chemicals and dissolved dirt will remain behind. The last rinsing should be with hot (sterile) water to ensure rapid drying. Wood easily frays with too much heating, but good wood will stand liberal quantities of pure warm water and even steam; churns are best cleaned by simply rinsing repeatedly with water at 90° C. Tinned or other metal vessels should if possible be boiled out or steamed as a final treatment, thus ensuring an extra sterilisation and rapid drying. This last point is a very important one, for in spite of all reasonable care absolute cleanliness and sterility are seldom achieved, but if only the vessels are dried as soon as possible, no new vegetation will be allowed to develop in them. Wherever possible, make the best use of direct sunlight, which both dries and sterilises. Wind and through draughts are also useful provided that they bring no dust. All that has been said regarding pails, apparatus and piping applies equally well to the cloths and scrubbing brushes used in cleaning; these must be thoroughly cleaned and finally scalded with boiling water and dried to prevent them becoming slimy; they may conveniently be dried in the boiler room. Every dairyman should clearly understand that cloths and brushes may do more harm than good if not perfectly clean. It is well known that cleaning cannot be effective if the vessels have inaccessible corners or rough surfaces; frayed woodwork or rusty pails should therefore not be tolerated.

While clean metal ware may be sterilised by simply prolonging the steaming sufficiently, wood work presents a more difficult problem, and should the dairy be troubled with an infection of harmful organisms, the usual cleaning should be supplemented with a washing with 1 to 2 per cent. formaldehyde made by diluting commercial formalin with twenty to forty times its bulk of water; it is advisable to protect the hands from the action of this chemical, which is best distributed by means of a garden syringe. In dealing with churns or combined churns and workers, the disinfecting action may be intensified by keeping them well closed for several hours after washing, as the formaldehyde vapour has a disinfecting action. A 2 per cent. solution of ammonium bifluoride is also a very effective disinfectant. The vessels must be rinsed and dried before using again. The growth of moulds is best prevented by avoiding excessive dampness; even in cellars used for storing cheese, the humidity need not exceed 90 per cent. If the walls of the cellar have become mouldy (*Cladosporium herbarum* often appears only as very small black spots) they must immediately be limed. Two per cent. of copper sulphate may be added to the lime with advantage. In investigating the cause of any defect in cheese, it should not be forgotten that the cutting and stirring appliances and the cheese cloths may be infected, and that the wooden thermometer case may harbour bacteria.

In dealing with the subject of cleaning, it may be well to give a more explicit definition of what is understood by infection. By an infection in the broadest sense of the term, we understand any admixture which may minimise the value of the product in question. If the foreign substance is sterile, the resulting condition is a purely chemical matter. If the foreign matter is not sterile, we shall be dealing with what is generally known as an *infection* (opposite *disinfection*), and this is a purely biological matter. The latter trouble will generally be more serious in its consequences than the former, as the effects of the biological action increase on keeping. Milk especially is subject to rapid alteration, not only because it is an ideal nutrient medium for many microorganisms, but also because, unlike most other dairy products, it is a liquid. A solid medium can only be spoilt gradually by an infection from without spreading inwards, and then only if the medium is not too dry to allow of the growth of microorganisms.

THE PROCUREMENT OF MILK

The most troublesome sources of milk infection are the udder and teats of the cow, and the vessels with which the milk comes

into contact; compared with these, contamination from the air usually plays quite a minor part.

An important influence on the quality of the milk is often ascribed to the **feeding,** and it is well known that certain fat-soluble colouring and odiferous matters contained in the fodder may pass into the milk, and also that the composition of the milk fat is largely influenced by the nature of the feed. At times the natural acidity and the sensitiveness to the action of rennet may be slightly influenced, but otherwise the chemical composition of the milk is not sensibly affected by the factor in question [1]. It should, however, not be forgotten that the feeding is the most important factor in determining the consistency of the dung, and the thinner the dung the dirtier the cows. Moreover, the feed will directly or indirectly determine the nature of the bacteria predominating in the dung, and thus the nature and number of the bacteria in the milk. It will therefore be understood that the most important effect of the feed on the quality of the milk is of a bacteriological nature, and this can partly be eliminated by keeping the cows clean; it is, however, difficult to do this properly if they are suffering from diarrhœa, and it is therefore of prime importance to avoid all risk of digestive trouble. Sudden changes in the feeding are especially to be avoided; in spring, the first green fodder should be given in the stable, gradually decreasing the proportion of dry fodder. If diarrhœa occurs in winter, the proportion of beet must be decreased and the ration made up with hay, while care must also be taken that the drinking water is not too cold. Should the weather be cold and rainy in summer, the cows should be kept in the shed at night, or supplied with covers. Beet and turnip tops very often cause trouble, though this is rather of a bacteriological than of a chemical nature. If the tops are not given in large amounts, they will do no harm provided that they have been harvested properly, but if they have lain in the field and become wet and soiled with earth, they will develop undesir-

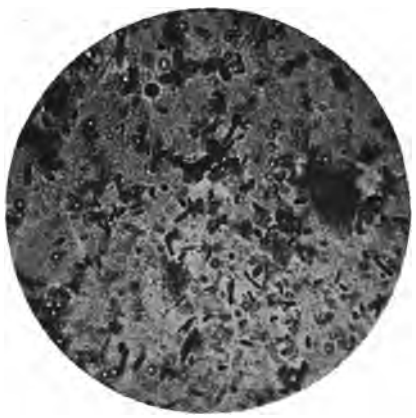


FIG. 52.—Rotting Swede containing numerous Pectin-fermenting Plectridia.

[1] *Orla-Jensen,* "Landwirtschaftliches Jahrbuch der Schweiz," 1905.

able fermentations. Diffusion slices [1] are generally supposed to exert a particularly undesirable influence on milk in connection with cheese making ; but as a matter of fact, they are harmless in a properly soured condition ; on the other hand, if they are given in spring or summer when they have begun to putrefy, they will be just as dangerous as any other putrefying roots ; the author has found that such material is the chief source of butyric acid and aerogenes bacteria in milk.

It is obvious that cleanliness will be promoted by cutting the hair on the udders and hindquarters of the cows as short as possible. **The condition of the ground or the covering of the stable floor** has a most important bearing on cleanliness. The most favourable conditions are on the pastures in dry weather. In rainy weather the cows may be badly soiled with mud which, as has been mentioned above, is a fruitful source of sporing bacteria ; milk obtained under these conditions is on this account difficult to sterilise. In damp meadows or during a prolonged spell of wet weather, microorganisms will grow abundantly on the surfaces of plants. The floor of the cowshed must be well covered with fresh straw, but it is better for the cows to rest on a clean cement floor than on decaying straw or husks which give rise to dust rich in microorganisms. Peat, especially the long fibred variety, is good, but it must not be allowed to remain until it becomes sloppy. If the shed is not planned so that the cows cannot lie down in their dung, someone must be at hand to remove or cover up the dung at once. There should be a sharp fall in the floor where the dung is deposited. If once the udders have become badly soiled, it will be impossible to obtain the milk in a decent condition. Rubbing the udder with a cloth, which will very quickly be dirtied, is of little avail ; proper cleaning of the udders will be very difficult of accomplishment on large farms. Prevention will be found to be far easier than cure, and if this matter were only given the attention which it justly deserves, the farmer would be rendering a most valuable service to the cause of clean milk.

It should go without saying that everyone who touches food should have **clean hands,** but it is of little avail that the milkers wash their hands only to soil them again immediately they commence their work. Of course, something will be gained if the hands are thoroughly washed after the milking of each cow, provided that they are not soiled again by touching the bottom rim of the pail or the cobwebs on the beams, or by slapping the dirty flanks of the cow to make it give room for the milking. Clean hands and clean clothes are without doubt much to be

[1] A by-product from beet sugar manufacture, which is used as fodder in countries where this industry has been developed.

desired, but in the first place we must insist on clean cows and clean sheds ; when these points have been gained the rest will no doubt follow as a matter of course. Dry milking is naturally more hygienic than wet milking, but as experience has shown, it is extremely difficult to carry out in practice ; it is facilitated by smearing the teats with a little vaseline or fatty material. This practice, which was first proposed by *Gillebeau* of Berne, has the additional advantage that the dirt is taken up by the greasy matter ; should a drop of the grease fall into the milk it will not mix readily with the latter, and it will be removed on straining through cotton wool. The hope that cleaner milk would be obtained by the use

FIG. 53.—Milking Room at Fauerholm.

of **milking machines** has hitherto been disappointed. These machines, with their numerous corners, cavities and rubber tubes, are so difficult to clean and sterilise that they require far more intelligent and conscientious attention than they are likely to receive at the hands of the average milker.

As already mentioned, the cleaned *Pails* should receive a final rinsing with hot sterile water, and this is especially necessary if the farm has not a **good water supply.** It has often been maintained that bad drinking water for the cows will give rise to bad milk ; this, however, only applies in cases where the cows may have contracted some disease through the water ; the direct infections caused by rinsing the pails with bad water will have far

more serious consequences. To avoid the introduction of dirt into the milk in filling or emptying the pails, cans or churns during weighing or measuring, care must be taken that these vessels do not become soiled during transport, and especially that they do not become plastered with mud round the bottom rim where they will be gripped on emptying. The cans should be covered with tarpaulin during transport, and they should not be stood on the ground but on clean planks.

The possibility of infection being carried by **Flies** is by no means to be overlooked as microorganisms may be brought from any place where the flies may have lodged. In cowsheds the danger is best avoided by removing the dung as often as possible and by hanging up, just below the ceiling, wide shallow enamelled dishes containing skim milk to which four tablespoonfuls of formalin have been added per litre (or quart).

To ensure **fresh air** during milking, the cowshed must be well ventilated beforehand, and as many doors as possible be kept open during the milking; in this way, the additional advantage of a good light will be secured. Cleaning operations of any kind or feeding should be avoided just before or during the milking, in order that the air may be as free from dust as possible. Naturally, it would be an advantage if the hindquarters of the cows could be brushed down just before milking [1]; but the evil effects of the dust raised in the process are only to be avoided if a special milking room is available. Fig. 53 illustrates the room set aside for this purpose at *Fauerholm*, near Frederiksborg, whence the city of Copenhagen receives its highest grade milk, which is known as "Ismaelk" (ice milk), as it is received into pails specially designed by *Busck*, which are provided with jacketed bottoms containing a freezing mixture.

The milk is (or should be) strained on the farm, in order to remove the coarsest of the dirt particles. By means of *Ulander's* filter, in which the filtering medium is a layer of cotton wool, most of the finer dirt particles may also be removed. Although the renewal of the cotton wool each time it becomes choked may entail some expense, this filter is strongly to be recommended, as the benefit to be derived from the immediate removal of the dirt is incomparably greater than that derived from the subsequent cleaning which the milk may receive on arrival at the dairy, when the soluble dirt and the bacteria contained therein will have become distributed throughout the milk owing to the shaking of the cans during transport. The best practice of all is to prevent the dirt from ever entering the milk by tying a straining cloth over

[1] In the procurement of the American "certified milk" the cows are often vacuum cleaned before milking.

the pail, as is done when using *Gurler's* pail (Fig. 54); the cloth must of course be changed frequently in order to avoid the dirt particles thereon being broken up and washed through by the impact of the milk from the udder.

We need not here enter into the discussion of other refinements calculated to further the production of clean milk, regardless of cost, which have been adopted in some other countries, for the public catered for in such cases is of necessity strictly limited; the points which do immediately concern the general public are the necessity for improved cowsheds and for more efficient and intelligent labour for the tending of the cows and the cooling of the milk. As, however, these matters also involve extra expense, the only hope of progress lies in the institution of a system of *payment according to quality*.

The following figures illustrate the points discussed above :—

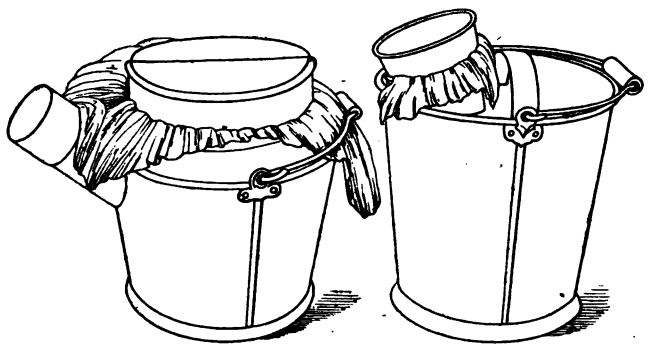


FIG. 54.—*Gurler's* and *Stadtmüller's* Milk Pails. (After *Conn.*)

Cow dung contains over 1,000 million organisms per gram.

Straw and earth contain up to twenty million organisms per gram.

According to *Barthel*, the air in a well-kept cowshed contains on an average, 300,000 organisms per cubic metre during the dinner hour, and over a million during the feeding of the cattle.

According to *Harrison*, 20,000 organisms fall into the milking pail per minute if the manure is removed and straw laid during the milking, but only 1,000 if this work is done one hour before the milking.

However clean the cows and the shed may be kept, it is impossible to obtain absolutely sterile milk as the udder, even when healthy, always contains some bacteria which find their way in through the opening in the teats[1]. There will always be a fairly

[1] A few investigators are of the opinion that bacteria also find their way into the udder through the blood.

large number of bacteria in the milk duct, and if high grade milk is desired, the very first portion of the fore milk from each teat should always be excluded. Thus the author found the following results on milking from a washed udder and teats into sterile bottles :—

In the fore milk from the four quarters, 16,000 organisms per cubic centimetre.

In the middle milk from the four quarters, 480 organisms per cubic centimetre.

In the strippings milk from the four quarters, 360 organisms per cubic centimetre.

The number of bacteria present in the udders varies ; if the teats are regularly washed and disinfected, and protected from dirt between the milkings, *e.g.*, by enclosing them in a waterproof bag, the bacteria in the milk may be reduced to ten per cubic centimetre. The American "*certified milk*" which contains less than 10,000 organisms per cubic centimetre, keeps extremely well ; instances have been observed of this milk keeping quite good at 0° C. for more than a month, and only containing 1,000 organisms per cubic centimetre after a week's keeping.

According to *Burri*, milk fresh from the cow, obtained under ordinary circumstances, contains 3,000 to 86,000, and on an average, 21,000 organisms per cubic centimetre.

Chapter II

The Normal and Abnormal Microflora of Milk

A. THE NORMAL FLORA

MILK fresh from healthy clean cows does not contain many organisms beyond a few from the air, and those which normally occur in and on the udder and teats. These organisms are nearly all micrococci and sarcina forms, most of which are without action on milk, while a few both acidify and peptonise it. According to *Arthur Wolff* [1], certain alkali producing short rod forms (*Bacterium lactis innocuum*) rank next to the micrococci in point of numbers, in fresh milk. In appearance, these organisms and their colonies closely resemble the aerogenes bacteria, but they do not ferment sugars, and they render milk feebly alkaline instead of acid without causing any other change. According to *Burri* and *Hohl*, *Streptococcus liquefaciens* is occasionally found as a pure culture in the udders of healthy cows [2].

Milk which has been less carefully handled contains, in addition to the above, coli, aerogenes, proteus and hay bacteria, ray fungi, moulds, yeasts, fluorescent bacteria and sometimes also butyric acid bacteria. The coli, aerogenes, proteus and butyric acid bacteria generally come from the dung, the fluorescent bacteria from the water used for rinsing the pails, and the others chiefly from the bedding and the stable dust. If the cattle are on pasture, the fluorescent and sporing bacteria may come from the ground. Curiously enough, the typical streptococci are seldom found in milk fresh from the cow. According to *Barthel's* investigations [3], they occur in cow dung with which they are distributed over the fields, being therefore found on all cultivated plants. From the latter they find their way back to the cow, and milk

[1] Inaugural Dissertation, Zurich, 1908. *Bacterium lactis innocuum* is probably identical with the organism known in the literature as *Bacterium alcaligenes*.

[2] "Schweitzerische Milchzeitung," 1916, Nos. 3 to 8.

[3] "Landbruks-Akademiens Handlinger och Tidsskrift," 1905, p. 403. It will, however, be necessary to revise these investigations since we have now learnt better to differentiate between the various species of lactic acid bacteria. Thus a large proportion of the lactic acid bacteria of the plants are not streptococci in the narrower sense, but betacocci.

may thus be infected with streptococci *not only* direct from the manure, but also from the bedding and the dust from the fodder. The milk pails are equally important as sources of infection, for they will as a rule be impregnated with lactic acid bacteria. These bacteria may also be introduced by flies.

The bacteriological state of milk will be determined at the outset according to the degree of care with which it has been handled, and the nature of the bedding and the feed. If, however, milk is kept for any length of time, the *temperature* at which it is kept will play an all-important part as it is the factor which determines which groups of microorganisms shall develop in preference to other groups. Not only the various groups of bacteria, but also the milk itself must take part in the struggle for existence, for milk contains *bactericidal substances* which, however, are gradually weakened in their action, presumably by the bacteria themselves; only at low temperatures, at which bacterial development is inhibited to a considerable extent, can these substances retain their activity for any length of time, and at these temperatures it is found that the number of organisms decreases at first instead of increasing. In the following table are given the *numbers of organisms* after twenty-four and forty-eight hours in the same milk kept in sterile flasks *at different temperatures*. The milk originally contained 84,000 organisms per cubic centimetre, of which 2,000 were liquefying.

	0° C.	12° C.	20° C.	30° C.	38° C.	45° C.
	After twenty-four hours keeping.					
Gelatine—						
Total .	52,000	8,200,000	163,000,000	380,000,000	17,400,000	12,000,000
Liquefying	18,000	1,600,000	6,000,000	200,000	0	0
Agar total .	—	—	—	460,000,000	20,000,000	100,000,000
	After forty-eight hours keeping.					
Gelatine—						
Total .	252,000	27,000,000	350,000,000	380,000,000	3,000,000	1,200,000
Liquefying	60,000	1,800,000	2,000,000	0	0	0
Agar total .	—	—	—	500,000,000	60,000,000	222,000,000

* The milk was clotted.

It will be seen that at 0° C. the number of microorganisms was lowered in twenty-four hours from 84,000 to 52,000 owing to the action of the bactericidal constituents of the milk. At the same time the number of liquefying bacteria had increased, and the

examination after forty-eight hours showed the total number to have increased. Chilling to 0° C. will therefore not prevent bacterial development indefinitely. At temperatures above 10° C., bacterial multiplication is rapid; thus in the present example, after twenty-four hours at 12° C., the number of organisms had increased one hundred fold, and at 30° C., five thousand fold. Thirty degrees to thirty-five degrees is the optimum for most milk bacteria; already at 38° C., many of them cease to grow and, moreover, the inhibitory effect of the acid which is formed increases with the temperature, as is shown in the table, particularly in respect to the liquefying organisms. In order to make bacterial counts of samples kept at the higher temperatures, it was necessary to employ agar, preferably in *Burri's* tubes (see p. 19) at 40° C., as organisms would be present which would not grow at ordinary temperatures. A comparison between gelatine and agar plates from milk kept for forty-eight hours at 38° C. showed that the original flora was being suppressed, while a new flora consisting entirely of rod-shaped lactic acid bacteria was making its appearance. At 45° C., a temperature unfavourable for the development of the common milk bacteria, this change proceeds much more rapidly. The lactic acid rods which develop at this temperature are mostly thermobacteria producing lævo lactic acid. The results of investigations of the flora of milk at different temperatures, by *Conn* and *Esten* [1], *Arthur Wolff*, *Luxwolda* [2] and the author, are as follows:—

1. Under 5° C. the fluorescent bacteria predominate.

2. Between 5° C. and 10° C., in addition to fluorescent bacteria, proteus bacteria, micrococci, alkali producing rods, and, as *Beijerinck* has shown, also some rods which produce an aroma of fruit.

3. Between 10° C. and 15° C. betacocci, streptococci, and some species of aerogenes bacteria in addition to the above.

4. Between 15° C. and 30° C. the streptococci, especially *Sc. lactis*, predominate.

5. Between 30° C. and 40° C. coli and aerogenes bacteria and lactic acid forming rods in addition to streptococci.

6. Above 40° C. lactic acid forming rod bacteria and Saccharomycetes, which ferment lactose, predominate. The streptococci which are found are now chiefly *Sc. thermophilus* and *Sc. fœcium*.

It is well known that the lower the temperature to which milk is cooled the better it keeps; in this connection, some results obtained by *Kjaergaard Jensen* may be quoted; they represent the bacterial counts of the same milk kept for eighteen

[1] Ann. Rep. Storr's Exp. Station, 1904.
[2] "Centralblatt f. Bakt.," 2 Abt., 1911, Bd. XXXI., p. 129.

hours at the temperatures which are most general in actual practice.

	Number of bacteria per cc.
Immediately after milking	1,480
After standing eighteen hours at 9° C. ..	2,100
,, ,, 12° C. ..	5,600
,, ,, 15° C. ..	156,000
,, ,, 18° C. ..	550,000
,, ,, 21° C. ..	6,750,000

Naturally, the cleaner the milk the greater will be the benefit derived from cooling. As already pointed out, it is not advisable to keep milk longer than twenty-four hours, even if cooled to 0° C.; if it is to be kept longer it must be frozen in order to avoid the risk of the development of fluorescent and other water bacteria which produce an unpleasant taste; at somewhat higher temperatures certain toxic proteus bacteria will also develop. For these reasons cooled milk or cream which has stood for any length of time are to be regarded with suspicion, even if apparently unchanged. As a rule milk does not become coagulated when kept at temperatures below 10° C., but above this temperature coagulation takes place in the course of a few days owing to the action of rennet and acid forming bacteria. At 20° C. milk quickly becomes coagulated, obviously owing to the formation of acid; at this temperature the streptococci, especially *Sc. lactis*, develop so freely that after a time they will come to constitute about 90 per cent. of the bacterial flora. The presence of large amounts of lactic acid inhibits the growth of other milk bacteria, for which reason the sour milk thus produced is harmless as an article of food. As already mentioned, at higher temperatures the streptococci give place to rod bacteria which produce higher concentrations of lactic acid. As regards the gas forming lactic acid bacteria, a few of these grow even below 10° C., but as typical intestinal bacteria they have as a rule a high optimum temperature and will most readily obtain predominance at 38° to 40° C.; this temperature is somewhat high for the streptococci, and the slowly growing lactic acid rod bacteria will only come to exercise an inhibitory effect on the other forms at a later stage. For similar reasons the temperature mentioned is also the most favourable for the development of the anaerobic sporing bacteria which form butyric acid. On the other hand, the aerobic sporing hay and potato bacilli, which often constitute the main flora of pasteurised milk, are practically inert in raw milk; their spores do not germinate at the ordinary temperature, and at higher temperatures the growth of their vegetative cells is inhibited

by the lactic acid formed by other organisms; as a group, they are extremely sensitive to acid. There are, however, varieties of *Bacillus mycoides* which can become accustomed to fairly large amounts of acid and can even be induced to produce acid themselves.

As the aerobic organisms grow best near the surface of the milk, and the anaerobic near the bottom, the pseudo lactic acid bacteria will be more plentifully represented in the cream, and the true lactic acid bacteria will predominate near the bottom of the vessel[1]. Hence the spontaneous curdling of milk by souring always starts from the bottom. While several of the pseudo lactic acid bacteria produce lævo lactic acid, the common lactic acid streptococci produce the dextro acid only; the result of the combined action of the two groups is a mixture of inactive and dextro acids. After some time, these organisms are supplanted by lactic acid forming rods, not only at higher temperatures (*thermobacteria*), but also after keeping at lower temperatures (*strepto-* and *betabacteria*); as many of these rods form inactive acid, the so-called fermentation acid will consist chiefly of this variety.

In the **spontaneous souring of milk** at the ordinary temperature, three stages may be distinguished. At the outset the original flora of the milk develops rapidly; as this is poor in true lactic acid bacteria, the milk will only acquire a slightly unpleasant smell and taste which may be described as *stale*. Only by degrees do the streptococci gain the upper hand, whereupon the milk becomes coagulated, and the acid which is formed masks the first taints. Finally comes the third stage in which the lactic acid rods predominate and in which the concentration of the acid may rise from 0·6 per cent. to well over 1 per cent. Before this happens however, a plentiful development of yeasts and moulds (especially Torulæ and *Oidium lactis*) will have taken place, and these consume the acid or neutralise it by producing ammonia or other basic products of protein hydrolysis; in the latter case their action is similar to that of chalk excepting that they destroy most of the acid instead of conserving it. Through the joint action of all these organisms the milk sugar will be fermented and the lactic acid destroyed, so that the way is prepared for the putrefactive organisms. As a rule, however, many weeks must elapse before this state of affairs is brought about. The shallower the layer of milk, the quicker will the acid disappear, provided, of course, that the milk does not dry up in the meantime.

[1] When whipped cream, *i.e.*, cream containing numerous air bubbles, is kept at the ordinary temperature, coli and aerogenes bacteria will obtain predominance in it.

B. THE ABNORMAL FLORA

The changes brought about by the normal flora in milk cannot be looked on as defects unless they appear at too early a stage. Thus the fact that milk turns sour on standing is in itself not a defect, but if the milk arrives at the dairy in a sour condition it is certainly defective. Defects of milk we understand to be not the normal but the abnormal changes. *Defects of milk* may be spoken of as primary if present from the outset, or secondary if they appear at a later stage in the history of the sample. In the former case they may simply be due to changes to which the milk is naturally subject at different stages of the period of lactation, or they may originate from the fodder or diseases of the cows; in the latter case they will be due to the action of microorganisms which find their way into the milk either during the milking or at a later stage.

Primary Milk Defects.—It is well known that just after calving or towards the end of the period of lactation the milk is abnormal in composition and coagulates badly with rennet. While colostrum has a strongly acid reaction, milk obtained towards the end of the lactation period is rather neutral than acid. Owing to the rapid growth of the fœtus, much potash and especially phosphoric acid are used up instead of passed into the milk. On the other hand, the percentage of sodium chloride often increases to such an extent that the milk tastes salt or bitter salt. It may be pointed out that so long as the cow is not in calf, the milk may be suitable for cheese making during two to three years[1].

The feed may have an undesirable effect on the milk by imparting to it an abnormal taste and smell, the best known examples being a taste of onions through feeding with onions, a turnip-like taste through giving liberal amounts of turnips and swedes, mustard and rape seed cake containing mustard, and a bitter taste from lupins (unboiled) or large amounts of vetches. Furthermore, poisonous substances may be derived from certain plants which, however, are generally avoided by the cows when on pasture. Greater danger attaches to such poisons as iodine, arsenic and mercury compounds which may be given as medicines and thus passed into the milk. For this reason no milk should be sent from any farm where the cows are given medicine of any kind, without the sanction of a veterinary surgeon. Similarly, disinfectants such as carbolic acid may pass through the blood into the milk, or they may be absorbed direct from the air, in which case the milk will also be unsuitable as food. Finally the

[1] *Orla Jensen*, "Landwirtschaftliches Jahrbuch der Schweiz," 1905, p. 542.

milk may be poisonous on account of toxins which have passed into it as the result of fevers or serious digestive troubles.

An indirect influence may of course be exercised by *diseases of the udder*, among which *inflammation of the udder or mastitis* and *tuberculous udder* are the most important. The alterations in the composition of the milk are bacteriological as well as chemical. In the first place the reaction of the milk is altered; frequently it becomes alkaline rather than neutral, due to the inflammation produced by *Streptococcus mastitidis*, though as a rule it eventually becomes acid as this organism produces appreciable amounts of lactic acid. The milk acquires a bitter, salt or other unpleasant taste, and the percentage of lactose, the most constant factor in the milk as long as the udder remains healthy, falls off appreciably. Flakes and lumps of pus and casein will be seen in the milk, and the colour changes. With streptococcic mastitis the milk becomes yellow, and with tuberculous udder bluish. Occasionally the milk may be coloured red by blood, and generally speaking, increasing quantities of the constituents of the blood pass into the milk while the normal constituents of the milk fall off. At last a watery secretion containing pus is obtained which can no longer be described as milk.

Inflammation of the Udder.—The most dangerous form of this disease is that caused by *Streptococcus mastitidis*. It is very infectious, being easily transmitted from one cow to another, so that for this reason alone it is desirable that the milker's hands should be washed after milking each cow. Inflammation of the udder is also occasioned by *Bacterium pyogenes* and certain coli and aerogenes bacteria and micrococci. *B. pyogenes* is a very small rod form which may produce a very unpleasant smell in the milk. A similar form, *Bacterium minimum mammæ*, which decomposes casein, but does not liquefy gelatine, and which produces small amounts of lactic acid, has been found repeatedly by *Gorini* [1] in the udders of cows which have not been milked properly. Under these conditions, micrococci, possibly the same as those which are normally found in and on the udder, may gain predominance. This may also happen if the udder is in an unhealthy state owing to chills. The micrococci generally only produce light catarrhs. *Bacterium pyocyaneum* may also be found in inflamed udders.

As most of the bacteria mentioned here may give rise to stomach and intestinal diseases, milk from inflamed udders must be regarded as dangerous.

Udder and other Tuberculosis.—Owing to the prevalence of

[1] "Revue générale du Lait," 1907, Vol. VI., No. 24.

bovine tuberculosis in general (in Denmark 30 to 50 per cent. of the cows are affected in one form or another), it is no wonder that tuberculosis of the udder is often met with. Generally speaking, large herds seem to be affected the most. As udder tuberculosis makes rapid progress, the cows from which nursery milk is obtained should be examined by a veterinary surgeon at least once a fortnight. Owing to the dangerous nature of this disease, the Danish law orders the slaughtering of cows with tuberculous udders. Tuberculosis of the udder is, however, not the only form of the disease which may involve the infection of the milk with tubercle bacteria. This may also very well happen in cases of tuberculosis of the uterus and kidney or the intestine, and even tuberculosis of the lungs may be dangerous in this respect as the animals swallow most of the slime which they bring up, with the result that the bacteria pass into the manure. *Every form of open tuberculosis* must therefore be regarded as a source of danger as far as the milk is concerned. As tubercle bacteria do not grow at temperatures much below blood heat, they will not multiply in milk or milk products under normal conditions, but as they are not killed by small amounts of lactic acid they can live in buttermilk. In the separating of milk the majority of the tubercle bacteria are removed with the separator slime, though appreciable numbers pass into the cream while only very few remain in the separated milk. Raw milk is on this account less dangerous than raw cream or butter. Tubercle bacteria can live in butter for a much longer period than it is usually kept nowadays. In cheese making the great majority of the bacteria as well as fat globules are precipitated with the curd, for which reason milk and especially fresh cheese may be far more dangerous than whey. According to *Harrison*[1], the hard cheeses may contain virulent tubercle bacteria even after keeping for two months. The latest researches have established that the organisms of human and bovine tuberculosis are different varieties, the latter being less dangerous to adults than was formerly supposed though dangerous to children. Tuberculosis is usually contracted by adults through inhaling the dried saliva of consumptive persons. On the other hand, bovine tuberculosis is very dangerous to calves and pigs, for which reason a law was passed in Denmark at the suggestion of *Professor B. Bang*, ordering all dairies to heat separated milk and buttermilk to 80° C. before returning it to the farmers, who use it chiefly for feeding pigs. It is regarded as a matter of the greatest importance that pathogenic germs are excluded from Danish butter in a similar manner.

[1] "Landwirtschaftliches Jahrbuch der Schweiz," 1900, p. 317.

Other Diseases of the Cow.—Just as milk from cows suffering from tuberculosis of the uterus and intestine may easily become infected with tubercle bacteria, so milk from cows affected by other diseases of the sexual and digestive organs may become infected with the corresponding organisms. Experience has shown that the milk should not be used for cheese making until ten days after calving, the reason being that not only is the chemical composition abnormal, but also that during this period the milk is especially subject to infection from the uterus, and to taints from the disinfectants often used during calving. The small rod form, *Bacillus abortus*, which causes abortion, may occasionally be found in milk[1]. The consequences of ordinary diarrhœa have already been mentioned. Chronic diarrhœa, when the motions contain blood or are otherwise abnormal, is far more dangerous, for in such cases we are dealing with an infectious disease and the organisms in question (according to *C. O. Jensen*, often coli bacteria of the swine fever group) may cause similar intestinal trouble in human beings, especially in children. Mortality among calves will always be a danger signal for human beings. Finally there will always be a danger of infection through the milk in cases of anthrax and all skin diseases such as foot and mouth disease, cowpox, etc., more especially if the udder is affected, and possibly also in cases of udder actinomycosis, acute lung diseases and rabies. The chemical composition of the milk is affected through several of these diseases.

Secondary Milk Defects.—*Milk may also be infected with pathogenic organisms from human beings*, and any one suffering from an infectious disease or living in the same house with sufferers should be forbidden to handle or sell milk. It is generally accepted that tuberculosis, diphtheria, scarlatina, cholera and typhoid fever may be transmitted by milk. Epidemics of typhoid, cholera, scarlet fever and diphtheria have undoubtedly been caused repeatedly through milk infections. Although *Bacterium typhosum* does not ferment lactose, it grows freely in milk, and it is also stated to be able to live for some time in butter and cheese ; like the organisms of the other diseases mentioned above with the exception of that of anthrax, it is killed by pasteurisation at a low temperature.

[1] *Schroeder* and *Cotton*, U.S. Dept. of Agric., Bur. of Animal Industry, 28th Annual Report, 1911 ; *Alice C. Evans*, "Journal of the Washington Academy of Sciences," Vol. V., No. 4, 1915, and "Journal of Infectious Diseases," Vol. XVIII., No. 5, 1916. According to the latter paper, *Bacillus abortus* and other similar forms seem to be fairly common udder bacteria, to be found in over 20 per cent. of the milk fresh from the cows. It appears to be chiefly a fat-splitting variety (*var. lipolyticus*), which is killed after warming only to 52° C. for thirty minutes.

While many of the above mentioned defects, however dangerous they may be, will not be readily detected and indeed are only to be demonstrated with great difficulty, the following defects will hardly escape observation, and it is accordingly these which are commonly alluded to in speaking of milk defects. We will begin with those which are visible and then pass on to defects of taste and smell.

Fermenting and Gassy Milk.—Milk may be described as fermenting if it shows copious evolution of gas even before coagulation sets in, and gassy if this only occurs after keeping for some time at a high temperature (see the fermenting test). Both defects are due to coli and aerogenes bacteria (more seldom to yeast), and the difference between them is only determined by the degree of infection. Fermentation may arise through direct infection from fermenting fodder or when the cows are suffering from violent diarrhœa or aerogenes mastitis. Both fermenting and gassy milk should be avoided in cheese making. Fermenting cream may give trouble in churning.

Prematurely Coagulating and Cheesy Milk.—Badly-cooled milk may coagulate when only a few hours old ; as the milk will not be appreciably sour in such cases the phenomenon must be ascribed to an action resembling that of rennet ; it is always due to exceptionally active development of peptonising lactic acid bacteria (*Streptococcus liquefaciens* or Tetracocci), which will probably have started to secrete coagulating enzymes in the udder. The well-known phenomenon of milk being specially liable to curdle in *thundery weather* does not seem to be ascribable to any other reason than the high temperature which usually precedes a thunderstorm. Milk which has been coagulated by rennet is generally described as cheesy ; it can easily be distinguished from sour milk by the separation of clear whey and the contraction of the coagulum. If gas-producing enzymes are present as well, the curd will become flaky or lumpy owing to the disturbance of the milk by rising bubbles. The same effect may be produced in practice in presence of coagulating bacteria alone, if the milk is stirred or shaken.

Slimy and Ropy Milk.—Milk may become slimy on standing, and sometimes the effect is so pronounced that the milk may be pulled out into threads a metre long. The change is most marked at 18° to 20° C. At higher temperatures the slimy organisms may be suppressed by the lactic acid, but even in pure cultures they form more slime at the temperatures mentioned. According to *Gillebeau*, one of the commonest slime-producing organisms is *Micrococcus Freudenreichii*, a large coccus[1] 2 μ thick, which

[1] "Landwirtschaftliches Jahrbuch der Schweiz," 1891, p. 135, and 1902, p. 342.

liquefies gelatine and produces an appreciable effect in milk within five hours. Like several allied forms, it may occur in bad water. If a stable once becomes infected with these organisms, nothing short of a very thorough disinfection will eliminate them. The author has isolated a non-liquefying micrococcus, which, being an obligate aerobe, only makes the surface of the milk slimy; in pure cultures, the sliminess is preserved for many weeks; the milk becomes faintly alkaline, but is not peptonised. It turns the surface of agar brownish black. *Storm* has isolated a motile rod which similarly only turns the surface of the milk slimy; it coagulates and peptonises the milk. As already mentioned, the slime is derived from the outer portion of the cell membrane. While the organisms mentioned can form slime from the protein of the milk, the lactic acid bacteria which produce slime require sugar in order to do so, but, on the other hand, they make the milk slimy throughout. As has been mentioned, the most important slime-producing lactic acid bacteria are certain varieties of *Sc. cremoris*.

As far as the Swedish "long milk" or the Dutch "long whey" are concerned, sliminess is a desirable characteristic, but otherwise it is highly undesirable, for slimy cream gives a bad yield of butter, and slimy whey is difficult to press out of cheese, collecting under the rind, and, as *Burri* has shown, causes the cheese to crack at a later stage[1]. For this reason slimy bacteria are no longer used in making Dutch cheese, as other lactic acid bacteria have been found to possess the same advantages without the disadvantage in question. Further, slimy organisms easily lose their characteristic property, especially if grown at higher temperatures; conversely, the common *Streptococcus cremoris* is inclined to make milk slimy if cultivated at lower temperatures for any length of time. Other lactic acid bacteria, pure (certain thermobacteria), as well as pseudo (certain aerogenes bacteria), may cause sliminess.

Coloured Milk.—In the old-fashioned process of allowing milk to stand in order to let the cream rise, coloured spots often appeared on the cream, or the milk would gradually become blue or red throughout. As these defects have not been met with in practice since the introduction of the centrifuge, they need not detain us here. They are due to the colour-producing organisms already mentioned (see pp. 44, 45 and 46).

Milk with an Unclean Sour Taste.—There is no defect more commonly met with in dairies than this one, as it is in no way due to foreign or rare milk organisms. It is simply the stale stage prematurely reached owing to insufficient cooling.

[1] "Centralblatt f. Bakteriologie," 2 Abt., 1904, Bd. XII., p. 192.

Milk with Stable or Grass Taste.—Formerly it was supposed that a stable smell was exclusively due to air absorbed in the stable. The taste, however, usually becomes worse after the milk has left the stable, and it has been shown to be due to the bacteria which produce the smell in the stable, *i.e.*, the intestinal bacteria. A heavy infection with manure will not only give the milk a taste of manure by direct means, but will introduce a number of bacteria which will continue the decomposition of the specific constituents of the manure and, naturally enough, do not neglect the constituents of the milk itself. In the same way, milk may acquire an unduly strong aroma of grass in spring. In the usual course some principles of colour and taste will always pass into the milk from the young grass, but the strong odour of herbs, which is appreciated by some people, will only arise on infection with the liquid secretions of grass.

Milk with a Taste of Turnips.—As already mentioned, the milk acquires this taste when the feed includes too great a proportion of the pungent principles characteristic of the Cruciferæ, turnips and swedes being especially dangerous if given in a rotting condition or too cold, so as to cause digestive troubles. Under these conditions other roots may, of course, also have an undesirable effect[1]. The taste-producing constituents of the roots need not necessarily pass into the udder, but may also be introduced into the milk with the manure, and, still worse, they will then be accompanied by the microorganisms which vegetate on the roots, and which, therefore, are equipped with those enzymes which are capable of hydrolysing such substances as the glucosides of mustard oil. According to *Weigmann*, the active organisms in such cases are chiefly coli bacteria, *Penicillium brevicaule* and certain species of oidium. *C. O. Jensen* found in the course of his well-known researches at *Duelund* dairy, which led to the pasteurisation of cream for butter making[2], that a coli bacterium living in water could produce in the milk a very unpleasant turnip-like taste even when the cows had not been fed on roots at all. *Bacterium fluorescens liquefaciens* also produces a taste of turnips, and this defect is accordingly often met with in milk which has been kept for any length of time at a low temperature. *Weigmann* has isolated a non-liquefying fluorescing bacterium, *Bacterium carotæ*, which produces a strong smell of carrots in all nutrient media. The author has cultivated this

[1] According to *Johannes Rolle*, "Zeitschrift f. Untersuchung d. Nahrung u. Genussmittel," 1915, Bd. XXX., p. 361, feeding with liberal amounts of beets may cause betain to pass into the milk. As this substance is a base, it will delay the coagulation of milk by acid.

[2] *C. O. Jensen* and *Lunde*, "Forsögslaboratoriets," 22 Beretning, 1891.

organism for years, the smell of the cultures remaining as strong as ever.

Milk with a Soapy Taste.—As is well known, this taste arises when milk is neutralised with alkalies, and is therefore produced by bacteria which chiefly produce ammonia. As the neutralisation of the milk is counteracted by acid production, and the ammonia-forming bacteria grow better at low temperatures than the acid-forming bacteria, it will readily be understood that the defect in question is most noticeable in cooled milk. *Bacterium sapolacticum*, isolated by *Eichholz*, can grow even at 5° C.; it is a non-liquefying fluorescent organism.

Bitter Taste in Milk.—This taste nearly always occurs in milk which has not been completely pasteurised, being produced by the peptonising sporing bacteria. Bitter substances are nearly always formed in the first stages of protein hydrolysis. The defect may also be due to the cow, to certain feeding stuffs, yeasts (*Torula amara*) and peptonising cocci, especially *Streptococcus liquefaciens*. According to *Weigmann*, *Bacterium Zopfii*, *Bacterium lactis innocuum* and aerogenes bacteria may turn milk bitter.

Metallic and Tallowy Taste in Milk.—Badly-tinned vessels will always impart a metallic taste to milk which is acid or fairly warm. In Denmark the pasteurised separated milk is sent back warm from the co-operative dairies to the farms, and in such cases it is difficult to prevent the cans from being attacked to a somewhat greater extent than usual, and thus giving the milk a metallic taste. The metallic taste must not be confused with *the tallowy taste* which may arise when the cream layer is exposed to direct sunlight, or through the action of certain microorganisms. As the metallic taste which is caused by copper is not to be distinguished from the tallowy taste, it may be presumed that copper salts promote by purely catalytic means the oxidation processes owing to which the tallowy taste arises. According to *Rosengren* [1], it is only milk or cream which is or has been heated which gets a foreign taste from the copper. The same holds good for butter made from the cream. According to *Storch*, certain lactic acid bacteria may cause a tallowy taste. Most liquefying rod bacteria (the hay bacillus, etc.), may produce a sickly, tallowy taste at first, but the more rapidly they peptonise the milk the sooner will the taste become bitter. The author has found that some aerobic non-sporing rod bacteria occasionally occurring in water are generally the cause of the tallowy taste which may arise in milk on long standing [2].

[1] "Landbrugsforsögsmeddelelse," No. 197, 1920.

[2] As an instructive example it may be mentioned that a dairy which

Bacteria are known which form hydrogen sulphide from sulphur, and therefore also from vulcanised rubber. Milk which has passed through rubber hose pipes, such as in milking machines, may therefore, on warming to a relatively high temperature, acquire a smell of rotten eggs.

supplied Copenhagen with pasteurised cream which tasted good on arrival, but which became tallowy before it reached the consumers, eliminated this defect by rinsing the transport cans with boiled water. The rod bacteria mentioned above were found both in the cream and the water from this dairy.

Chapter III

The Preservation of Milk and its Treatment for Direct Consumption

PRESERVATION

THE general methods used for the preservation of foods are constant cooling, short heating, concentration, and the addition of bactericidal substances. All four methods or combinations thereof are applied to milk.

Cooling is most used because it is the cheapest method and because the milk suffers no chemical change in the process. The principle of the method has been discussed above, and it is well-known that the sooner and the more thoroughly the milk is cooled the better will be the result. Even in cold weather the milk cannot be cooled quickly enough by simply allowing it to give up its heat to the air. It is absolutely necessary to stand the pails in cold water which is frequently changed ; the level of the water must be higher than that of the milk in the pail. The water container should be fitted with a grid or the bottom should be fluted so that the water may circulate freely under the pails. The same object may also be achieved by standing the pails on a flat surface if their lower rims are provided with holes. The water inlet pipe should reach the bottom while the overflow should be at the top. On large farms it is best to have a tank above the cowshed, which is filled just before milking, and from which the water slowly flows through wooden troughs situated on the coolest side of the shed ; the cooling may thus be started during the straining process. The pails are first placed in the end of the trough nearest to the outlet and are moved by degrees towards the inlet end, whence they are removed from the trough. The troughs must be covered over by a lean-to roof sufficiently high to allow a man to stand under it. The used cooling water may with advantage be used for drinking water for the cows. When the milk has been cooled to the temperature of the water, it may be further cooled by standing the pails in ice water or by passing it over a cooler through which a mixture of four parts of ice in small lumps and one part of salt is circulated. In summer time the cooler may be moved out into the fields, though if this is done the

consumption of ice will be unreasonably large. The accompanying illustration shows the arrangement of the cooling apparatus at Fauerholm dairy which supplies Copenhagen with milk. Here the milk is cooled to 3° C. By allowing the milk warm from the cow to flow over a cooler or a special aerating apparatus, some of the unpleasant animal odour is eliminated, but at the same time the milk loses carbon dioxide and takes up oxygen, with the result that the development of the aerobic putrefactive bacteria will be favoured at the expense of the true lactic acid bacteria. As

FIG. 55.—Cooling and Straining of Milk at Fauerholm. In the foreground Ice is being put into the bottom of *Busck's* Milk Pail.

aeration will also expose the milk to air infection, it may often do more harm than good on the whole. This will be the case when the cows are in the stable, but here it will be easy to secure rapid and thorough cooling by other means. It is a different matter when the cows are on pasture where the air will be pure, and more than three hours may elapse before the milk arrives at the farm to be cooled; in this case immediate cooling on an aerating appliance will do a great deal of good. *Weigmann*[1] has found that pasture milk contains on an average, when retailed, six times as many bacteria as stable milk obtained under the same conditions

[1] "Centralblatt f. Bakteriologie," 2 Abt., Bd. XLV., p. 105.

of cleanliness, a fact which is undoubtedly to be attributed to the delay in the cooling of the former. Mid-day milk can only be satisfactorily preserved until the following morning by the use of ice ; as this further adds to the cost of production incurred by milking three times a day instead of twice, it is a question whether the extra 6 to 7 per cent. of milk and milk fat which, according to *Fleischmann* [1], is gained in this way, is not too dearly paid for, considering the present cost of labour. There is no doubt that the bad state in which the mid-day milk arrives at the dairies can only influence the dairy products for the worse.

Heating.—As already explained, cooling only serves to preserve milk for a comparatively short time, for it would entail too great an expense to maintain the low temperatures necessary to prevent all bacterial development. Milk cannot be kept for any length of time without the aid of heat. The temperature and time of heating necessary to sterilise milk depend very largely on its quality, and on the size of the bottles or tins which are used. With tins, water filled autoclaves are best as the heat is transferred more rapidly and uniformly by this method. By heating for one hour at 105° C., or for a quarter of an hour at 115° to 120° C., milk will usually be rendered sterile, and may then be kept for years, especially if care be taken to close the bottle immediately after the autoclave is opened, and while the milk is still boiling. The bottles will be practically free from air, and as the most resistant spores require an abundant supply of oxygen in order that they may germinate, the keeping properties of the milk will be greatly enhanced. In order to prevent the fat globules from collecting as a solid lump in the neck of the bottle, the milk should first be *homogenised*. As sterilised milk is brown in colour and considerably altered in chemical composition, it cannot be recommended for children. The steriliser invented by *Jonas Nielsen* is less destructive in its action, the milk being momentarily heated to 130° to 135° C. by being pumped through a system of small thin pipes heated by steam. Burning and the change in taste which this causes, are completely avoided if only the milk is kept during the time of heating at a pressure sufficiently high to prevent it from boiling. According to the author's investigations, even milk to which have been added very resistant bacterial spores, already becomes sterile in forty seconds at 135° C., and in fifty seconds at 130°, but only after ninety seconds at 125° C. It naturally follows that the milk must be cooled very rapidly after this drastic heating in order that it may not become brown ; this is accomplished by means of a system of piping similar to that which is used for the

[1] "Das Molkereiwesen," Brunswick, 1875, p. 81.

heating process, but which is surrounded by cooling water instead of by steam. If the milk sterilised in this way is filled into sterilised cans, avoiding infection from the air (*Jonas Nielsen* has patented special appliances for this purpose), it may be sent any distance. On passing through *Jonas Nielsen's* steriliser the milk undergoes a partial homogenisation. Milk and cream intended for export should, in order to meet all eventualities, be sent as cold as possible, and in this connection it may be mentioned that the results of cooling are in general far more noticeable in heated than in raw milk (provided only that the milk vessel has not been

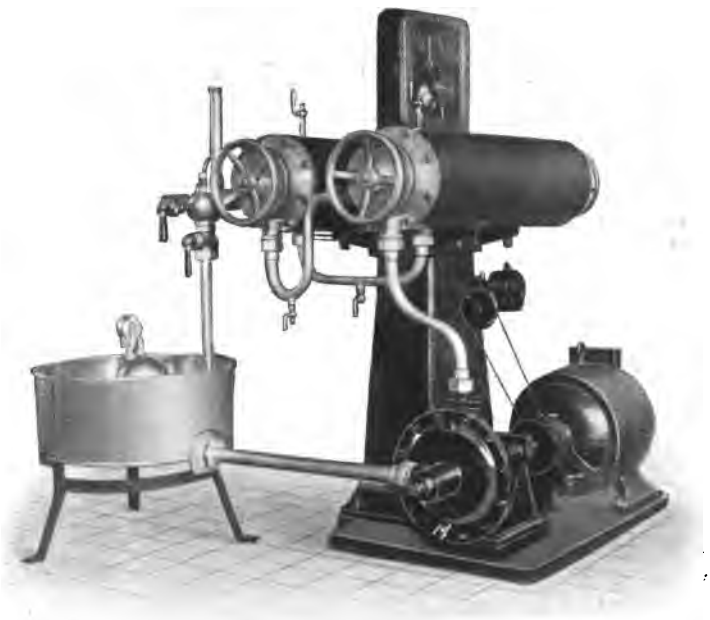


FIG. 56.—*Jonas Nielsen's* Steriliser.

contaminated or rinsed with unboiled water), for the bacteria which grow best at low temperatures are the first to be destroyed on heating while the most resistant spores only germinate at high temperatures. In the production of sterilised cream (export cream) homogenisation also plays a very important part, as cream with a certain fat percentage appears to be richer the more finely divided the fat globules. Cream, however, like condensed milk, cannot be homogenised at quite so high a pressure (at the most 150 atmospheres) as ordinary milk which may be treated right up to 250 atmospheres.

If the object is only to free the milk from pathogenic germs, and to increase its keeping powers to some extent, complete *sterilisa-*

tion is not necessary and a less drastic treatment, *i.e.*, *pasteurisation*, will suffice. The easiest method consists in heating the milk for a short time, two minutes at the most, in a continuous pasteuriser. It has been shown [1] that the keeping powers of milk may be improved by heating even to 70° to 75° C. in this way. According to American researches [2] a far better result is obtained at 80° C., and as this temperature is necessary to ensure the destruction of the tubercle bacteria, it has been fixed as the minimum by the Danish pasteurisation law. Very little is gained by increasing the temperature to 85° C., and the milk or cream easily acquires a " cooked " flavour if it cannot be cooled rapidly enough. On the other hand, skim milk should be heated to 90° to 95° C. if, as is the practice in Denmark, the milk is to be returned from the co-operative dairy to the farms in the unrinsed cans; in this way the remains of milk in the cans will also be pasteurised.

From the bacteriological point of view, approximately the same results may be achieved by prolonged heating at lower temperatures as by short heating at higher temperatures; in America milk is often pasteurised for half an hour at 60° to 70° C. The author has found that this method of treatment has a less destructive effect on the proteins [3], and is therefore to be recommended for the treatment of milk for infants or for cheese making, where it is of importance to preserve the natural properties of the milk. The following table shows that on heating to over 70° C., albumin coagulation occurs fairly rapidly, while the milk loses its power of coagulation with rennet [4].

Time heated in minutes.	Percentage of albumin coagulated.				Time to coagulate with rennet according to Schaffer in minutes.				
	70° C.	75° C.	80° C.	90° C.	Unheated.	70° C.	75° C.	80° C.	90° C.
Momentarily	—	15	39	71	13	—	14	17	26
5	13	49	88	100	15	16	19	28	28
15	18	62	91	100	15	17	22	60	60
30	30	83	100	100	15	19	32	57	57
60	37	93	100	100	14	20	34	46	46

[1] " Forsögslaboratoriets 22 Beretning," 1891.

[2] *Harding* and *Rogers*, New York Experimental Station, Bulletin 172, 1899.

[3] " Landwirtschaftliches Jahrbuch der Schweiz," 1905.

[4] It must be added that the extent of the change suffered varies greatly with different samples of milk. In the experiments recorded in the table the same samples were used throughout for the same times of heating, but different samples for different times of heating; a maximum effect was soon reached, which was first exceeded when the milk was heated to a temperature sufficiently high to turn it brown.

The first visible effect of heating is the gradual destruction of the cream line, and as the public usually judge the richness of the milk according to the thickness of the cream layer which separates out, this matter is not without significance in practice. The cream line is already affected by heating to 63° C. for some time [1], and as it happens that heating for half an hour at this low temperature is critical for most milk bacteria [2], especially the colon bacteria, this method of treatment, which we will refer to as *low temperature pasteurisation* (*the American holder process*), would seem to be the best. Moreover, the bacteria which cause foot and mouth disease, diphtheria, cholera, dysentery, typhoid and other intestinal diseases, are killed just as surely by low temperature pasteurisation as by short heating to 80° to 85° C. Different opinions have been held with regard to tubercle bacteria, but according to *Barthel* and *Stemström's* researches [3] there can no longer be any doubt that these organisms will under normal conditions be killed even by only heating to 60° C. for ten to twenty minutes.

Whichever method of pasteurisation may be adopted, it is of the greatest importance to prevent the formation of skin and froth, as these act as insulators, preventing the transference of heat. This point was first demonstrated by *Theobald Smith* [4]. Thus, when pasteurising at low temperatures in large vessels, the milk must be in constant though not violent motion. Another condition is that the milk must not contain so much acid or rennet that the heating will precipitate the casein which may thus come to enclose and protect the bacteria. Pasteurisation can by no means be trusted in implicitly to cover all defects, for the result depends largely on the state of the raw milk. Fresh and cleanly handled milk may be practically sterilised on pasteurisation, though the milk flowing from the pasteurisers in some dairies frequently contains several thousand bacteria per cubic centimetre, and the subsequent passage over the coolers will, of course, not improve matters in this respect. The expression of the efficiency of pasteurisation as the percentage of bacteria killed on the number originally present is somewhat misleading; using the processes described above, the percentage is usually over ninety-nine with bad milk, but it often falls as low as ninety with good milk, although the actual number of organisms is always many times greater in pasteurised bad milk than in pasteurised good milk.

[1] *Weigmann*, "Mitt. des Deutschen Milchwirtschaftliches Verieins," 1914, Bd. 31; *Burri*, "Schweitzerische Milchzeitung," 1915, Nos. 42 and 43.

[2] *Ayers* and *Johnson*, "Journ. of Agric Res.," 1915, Vol. III., No. 5.

[3] "Meddelande," Nos. 117 and 118, från Centralanstalten för försöksväsandet pa jordbruks området, 1915.

[4] "Journ. of Experimental Medicine," New York, 1899, vol. 4.

It is obvious that the sooner the individual particles of the milk can be heated, the shorter will be the time occupied by the process of heating, and by spraying the milk into the pasteurising vessel, as is done in *Oscar Lobeck's Biorisator*, an effect is attained by momentary heating to 75° C, which is as efficient in killing the bacteria and as lenient towards the milk itself as that attained by heating to 63° C for half an hour. *Biorisation* may thus be regarded as a process intermediate in character between *high* and *low temperature pasteurisation*. Unfortunately the atomiser becomes choked so easily that the apparatus cannot be recommended in its present form. On investigating the Biorisation process [1], the author obtained the following remarkable results :—Not only the milk treated at 70° C., but also that treated at 80°, 85° and 90° C., kept worse than that treated at 75° C. Milk treated at 70° C. rapidly became sour, while that treated at 80° C. became putrid, mainly owing to the action of hay and potato bacilli. *Tholstrup Pedersen* [2] repeated these tests, heating the milk as quickly as possible in a boiling water bath. As this method of heating is less rapid than biorisation, the best results were obtained at a somewhat lower temperature, *i.e.*, 70° C. The fact that hay bacilli and other heat-resisting organisms grow quicker in milk heated to higher temperatures can only be explained by the supposition that the bactericidal constituents of the milk are killed at temperatures above 70° C. ; *Tholstrup Pedersen* has adduced other evidence in favour of this supposition. From this it follows that the bacterial count of milk immediately after pasteurisation is not a perfectly reliable gauge of its keeping power, as the keeping power depends just as much on the bactericidal properties of the milk as on the count (and of course also as on the nature of the organisms present). If the milk is kept at 10° C. or lower temperatures, these properties are more noticeable in lightly pasteurised milk in which the surviving organisms have become weakened by heating than in raw milk, an appreciable diminution in the number of organisms taking place during the first twenty-four hours.

If milk is pasteurised at a high temperature (85° to 95° C.) or boiled (as in *Soxhlet's* apparatus for pasteurising nursery milk), only the spores survive, and milk or milk foods (*e.g.*, chocolate) treated in this manner will therefore not become sour on standing, but will develop putrefactive or butyric acid fermentations. In most cases *Bacillus mycoides* and other aerobic sporing bacteria will be found. The milk soon acquires an unpleasant, sickly taste, a particularly dangerous feature being that poisonous sub-

[1] "Maelkeritidende," 1915, p. 483.
[2] "Maelkeritidende," 1916, p. 231.

stances may be formed before any apparent change has set in. Immediate cooling to under 14° C., or better still to under 10° C., delays these changes considerably. As many of the sporing bacilli can grow and even thrive well at high temperatures, the slow cooling of milk pasteurised by the methods under consideration may have disastrous results; *Tholstrup Pedersen* has shown [1] that milk treated thus is affected most rapidly at 70° to 60° C. At these temperatures non-motile, aerobic rods will appear after only three hours' standing; after four hours the milk is so changed that it will no longer stand boiling, and after six to eight hours it coagulates owing to the action of fermentation acid and bacterial rennet. At 60° to 50° C., the bacteria in question do not grow so rapidly, but in their place anaerobic plectridium forms develop freely. It is only under 50° C. that the common hay and potato bacilli appear, while at 40° to 30° C. the true butyric acid bacteria appear as well. The above outline gives an idea of the possible bacterial developments in milk pasteurised at high temperatures and allowed to cool spontaneously. It has an important bearing on the treatment of skim milk in the Danish co-operative dairies; as was mentioned above, the skim milk is sent back warm from the pasteuriser to the farms; it is contained in cans holding up to 50 litres, and after three to four hours its temperature may be above 50° C., and in summer sometimes above 60° C.[2] Formerly it was thought that the holding of milk at high temperatures over long periods enhanced the effect of pasteurisation, but now it is known that while this treatment certainly tends to destroy the normal flora of the milk, especially the lactic acid bacteria, it also encourages the growth of another and far more dangerous group of organisms. For this reason skim milk should be thoroughly cooled in the dairy; there is no reason for heating it to temperatures above 80° to 85° C., the limit set by the Danish pasteurisation law. If this procedure is adopted, the cans must be cleaned before receiving the cooled milk; the extra trouble thus involved is amply paid for by the saving in coal which may be effected by the use of the regenerative system of cooling; moreover, the quality of the separated milk will be improved both as regards taste and keeping powers, while the tinning on the cans will last longer. It should be pointed out that the cleaning of the cans by the dairy by no means relieves the consumers from the obligations in this connection. The harmful bacteria may also be suppressed by souring the milk, but

[1] "Maelkeritidende," 1915, p. 817, and 1916, p. 35.

[2] These investigations also point to the necessity for caution in hay-box cookery, the food being kept for many hours at temperatures favourable to the development of thermophilic bacteria.

as the resulting product cannot always replace unsoured milk, there is no need to go to this trouble unless facilities are lacking for cooling the milk to under 14° C.[1] From the results of these investigations it may also be understood why milk or cream which has been homogenised at 60° to 70° C., and which commonly has to stand for some time before it can be sterilised, may acquire a bad taste. The homogenisation should rather be accomplished at 80° to 90° C., after which the milk should be sterilised at once.

While high temperature pasteurisation alters the relative proportions between the numbers of the good and the harmful bacteria to the advantage of the latter group, the opposite effect may be produced by low temperature pasteurisation. *Ayers* and *Johnson*[2] have shown that the lactic acid bacteria have, as a group, greater heat-resisting powers than was formerly supposed, and that a larger relative proportion of these organisms is to be found in milk which has been warmed to 63° C. for half an hour than in the raw milk. This would appear to be an additional recommendation for low temperature pasteurisation. On the other hand, it must be mentioned that the lactic acid bacteria which survive this process sour the milk slowly at ordinary temperatures, and that there is yet a possibility that the milk treated in this way may suffer undesirable changes before it becomes sour. If the raw milk is particularly good, and therefore poor in true lactic acid bacteria, there is a possibility that it may eventually become just as harmful after low temperature pasteurisation as after heating to higher temperatures. In ordinary milk the true lactic acid bacteria are only killed off entirely by heating to 77° to 82° C. for half an hour. In agreement with this observation, *Tholstrup Pedersen*[3] found that milk which had been heated to 80° C. still contained many lactic acid bacteria, but that milk heated to 85° C. contained none. The author has found that the lactic acid bacteria growing at high temperatures, *i.e.*, the thermobacteria, and in addition the microbacteria, *Streptococcus thermophilus*, *Streptococcus fœcium*, *Streptococcus glycerinaceus*, and a few micrococci are the lactic acid bacteria which best survive heating. The heat-resisting powers of the thermobacteria have been taken

[1] The foregoing remarks, as well as those on the same subject on p. 70, are of particular interest in connection with the Danish co-operative dairy industry, in which separated milk is obtained as a by-product from butter making and sent to the farms, where it is extensively used for feeding pigs. The matter is, however, of general interest as an example of the great importance of cooling after pasteurisation. In the large dairies of the United Kingdom the separated milk is usually treated as recommended above before sending to biscuit and other factories.—*Translator.*

[2] U.S. Dept. of Agric. Bureau of Anim. Industry, Bull. 161, 1913; "Journ. of Agric. Research," 1914, vol. 2, No. 4.

[3] "Maelkeritidende," 1915, p. 85.

advantage of in the Swiss cheese making industry for many centuries. Pure cultures are obtained in the acid vats by filling up with warm "*Schotte*" (boiled clarified whey) so that the temperature rises to about 60° C. The thermobacteria are of no great interest in connection with pasteurised milk, as they develop very slowly in it unless kept at temperatures over 35° C.

According to *Weigmann's* [1] investigations on low temperature pasteurisation, practically the whole of the bacterial reduction takes place during the first ten minutes, the remaining 1 or ½ per cent. is halved during the second ten minutes, while practically nothing happens during the last ten minutes. It is, however, necessary to continue for the last ten minutes if the milk is pasteurised in bottles, as it is considerably colder near the bottom than at the top. *Ayers* and *Johnson* have even continued heating for six hours without having observed any further bacterial reduction. It follows that the few bacteria which are not killed during the first ten to twenty minutes cannot be got rid of without raising the temperature, so that there is nothing to be gained by lengthening the time beyond the prescribed thirty minutes. According to the author's investigations, the advantages of prolonged low temperature pasteurisation would be illusory if only for the reason that prolonged heating (*e.g.*, for five hours), even to as low a temperature as 60° C., causes considerable chemical changes in the milk, and, what is still worse, the development of the thermophilic putrefactive bacteria will be favoured by keeping the milk for so long a time between 60° and 70° C.

The concentration of milk consists either in condensing *in vacuo* or complete evaporation to dried milk.

Distinction is made between **sweetened and unsweetened condensed milk,** the latter also being called *evaporated milk.* Both are as a rule sold in hermetically-sealed tins, but the sweetened milk may also be shipped in cans or barrels. As the condensation is carried out at 50° to 60° C., the multiplication of bacteria is by no means excluded during this process, and the milk should therefore previously be freed from as many germs as possible by heating to the neighbourhood of 100° C.

Fourteen to sixteen per cent. of cane sugar is added to the milk which is to be sweetened, and the product is evaporated to a third of its bulk, cooled quickly and stirred meanwhile so as to prevent

[1] *Weigmann, Wolff, Trensch* and *Steffen* have confirmed *Ayers'* and *Johnson's* results, and also shown that the proportion of lactic acid to other bacteria is higher in stable milk than in pasture milk after low temperature pasteurisation, because the former is richer in lactic acid bacteria than the latter ("Centralblatt f. Bakteriologie," 2 Abt., 1916, Bd. XLV., p. 63).

the formation of large crystals of the difficultly soluble milk sugar, and finally filled into steamed (preferably sterile) vessels. Milk which has been treated in this manner is by no means sterile, but the high sugar content inhibits the development of microorganisms, as in jam. Quite a number of orange and white micrococci can, however, always be found in the milk, and occasionally also yeast, red as well as white Torulæ, of which latter a few ferment saccharose even in these high concentrations, and, therefore, can make the tins bulge out [1]. If the milk has not been thoroughly aerated after it has been in the vacuum pans, it is only in the top end of the tins that alcohol is formed. In this end moulds may develop in addition to yeasts, but their vitality is cut short as soon as all the oxygen present in the tin has been used up. This stage, however, need not necessarily mark the cessation of the harmful effect of the moulds, as the proteolytic enzymes contained in the moulds, after having digested the mycelium itself, may continue to act on the surrounding medium; *Rogers*, *Dahlberg* and *Evans* have thus shown [2] that certain reddish brown lumps which are now and again found in old condensed milk, and the origin of which has hitherto been a mystery because no cells were found therein, are formed by *Aspergillus repens* in the way described above.

As the unsweetened condensed milk is not so viscous, it must be homogenised to prevent the separation of cream; it must be sterilised after having been tinned, in order that it may keep. As highly concentrated milk coagulates to a gelatinous mass at the high temperatures used in sterilisation, the unsweetened milk cannot be evaporated so far as that which has been sweetened; the author was the first to show [3] that the soluble calcium salts present in the milk are the cause of this phenomenon, their concentration naturally increasing with that of the milk; if acid has

[1] *Hammer* has named this yeast *Torula lactis condensi* (Iowa Agric. Exp. Station, 1919, Bull. 54, pp. 211—220. According to the author's researches, the micrococci as well as the yeasts come in the majority of cases from the sugar which is used, it is therefore very necessary that the sugar should be heated to a sufficiently high temperature after having been dissolved. The large amount of cane sugar in condensed milk inhibits the development of the ordinary cane-sugar-fermenting Saccharomycetes; only *Zygosaccharomycetes* (*i.e.*, certain sexually differentiated Saccharomycetes) will be able to grow in the concentration of cane sugar in question, but these are, so far as the author is aware, never found in milk products.

[2] "Journal of Dairy Science," 1920, vol. 3, p. 122.

[3] *Hoppe Seyler's* "Zeitschrift f. physiol. Chemie," 1914, Bd. 93, pp. 299, 300. In this work it is shown that the coagulation of faintly acid milk on boiling, which is a well-known phenomenon in cookery, is not directly due to the slight amount of acid, but to the soluble lime salts formed by it. The conditions causing the coagulation of normal (not acid and not condensed) milk on sterilisation at 130° to 140° C. are more complicated, as in this case a proteolytic decomposition also takes place. For further information regarding these conditions, see the author's work in "Landwirtschaftliches Jahrbuch der Schweiz," 1905, p. 235.

been formed in the milk the concentration of soluble calcium salts is further increased. The worst conditions occur if rennet-forming bacteria are present in the milk in addition to acid-forming bacteria, as the amount of calcium necessary to precipitate the paracasein is only half that necessary to precipitate the casein. It is therefore obvious that only milk of the very best quality can be used for condensing, and such milk is of course also the easiest to sterilise with satisfactory results. According to *Hunziker's* investigations[1], the bacteria which are found in incompletely sterilised evaporated milk are not so often hay and potato bacilli as a slender peptonising, non-acid-forming, non-sporing rod bacterium. This organism presumably belongs to the group of microbacteria which the author points out as being able to stand comparatively high temperatures for non-sporing bacteria. If the tins of evaporated milk bulge out, this is always due to anaerobic spore formers, *i.e.*, butyric acid bacilli as well as anaerobic putrefactive bacteria. In leaky tins in which the milk has been infected after sterilisation one may, of course, find lactic acid bacteria as well as many other stray organisms. In order that the bad tins may be picked out, the manufacturers should always keep the condensed milk for a short time before sending it out. Fermentation in sweetened milk will always show itself in the course of about a week at 20° to 25° C., while the "incubation" time for sterilised unsweetened milk often lasts two to three weeks, even at a temperature of 30° C.

Milk is dried on drums *in vacuo* (*Eckenberg's* method), on drums heated to 140° C. (*Hatmaker's* method), or by finely dividing it and causing it to meet a current of air at 120° C., whereby it is instantaneously converted into powder (spray process). Of these methods, *Hatmaker's* is the easiest and cheapest, but the powder which is produced is difficultly soluble even if alkali has been added. The dried milk made by the spray process is far better, being completely soluble when freshly prepared. This is due to the fact that no denaturisation of the proteins takes place, as the current of warm air is cooled right down to 60° C. immediately on meeting the sprayed milk, owing to heat being absorbed in the process of evaporation. All the methods can of course be combined with an initial condensation of the milk. Only dried milk made by *Hatmaker's* process is sterile. The dried milk made by the other processes can of course be made more or less free from germs by pasteurising the milk more or less thoroughly at the

[1] *Otto F. Hunziker*, "Condensed Milk and Milk Powder," Illinois, 1920. This work, which is based on wide experience, is strongly recommended to any one wishing to obtain information on these special branches of dairy practice. See also *C. Porcher*, "Le Lait Desséché," Lyon, 1912. *Porcher* strongly recommends dried milk for infant feeding.

outset. Only skimmed milk is adapted for drying, as the milk fat is quickly oxidised owing to the large surface presented by the powder, and thus acquires a highly unpleasant taste. For this reason condensation must still be regarded as the best method for preserving whole milk, at any rate when it is a question of preserving it for a long time.

Preservatives.—If it is only a question of preserving the sample for analysis, comparatively large amounts of antiseptics may be used, *e.g.*, copper sulphate, potassium dichromate or formaldehyde (0·1 per cent.), but all such substances must be excluded if the milk is to be used as food for animals or human beings. According to *Lazarus* [1], the following can be added per litre of milk without the taste being affected: 3 grams sodium carbonate or bicarbonate, 1 to 2 grams boric acid, 0·75 gram salicylic acid. Soda naturally delays the souring, but not always the coagulation, as the growth of the rennet-forming bacteria is promoted thereby; it has also a favourable action on the development of the pathogenic bacteria. Boric acid is without effect in the above proportions, while the salicylic acid inhibits the growth of bacteria to some extent [2].

Behring has recommended the addition of a slight amount of formaldehyde to milk, 1 to 10,000 parts, to destroy the tubercle bacteria. This is certainly the highest dilution which can have any bactericidal action whatever.

The only substance which can come into consideration in the present case is hydrogen peroxide, which is decomposed by the catalase of the milk into water and oxygen, the latter having an antiseptic action in the nascent state. The Danish engineer *Budde* has shown that the decomposition takes place most rapidly at 45° to 50° C., and as several species of bacteria are killed only by heating to these temperatures, the so-called *Buddisation* consists in treating the milk for several hours (stirring at first) at 52° C. with 0·35 part per thousand of hydrogen peroxide; the bacteria are thus subjected to the simultaneous action of poison and heat. Commercial 3 per cent. hydrogen peroxide cannot be used on account of the poisonous impurities which it contains, and also the fact that it would dilute the milk with 1 per cent. of water; pure hydrogen peroxide must be used, which renders the method too costly, especially as all that is achieved is pasteurisation and not sterilisation. *Barthel* has shown that milk treated by Budde's process no longer gives *Storch's* reaction [3]. In order to sterilise milk by means of hydrogen peroxide, 1 to 2 parts per thousand must be

[1] "Zeitschrift f. Hygieine," VIII., p. 207.

[2] According to *Proks*, 2 per cent. of benzoic acid has no greater antiseptic action than 1 per cent. of salicylic acid.

[3] "Nordisk Maelkeritidende," 1903, No. 5.

used, in which case the milk acquires a very disagreeable taste. Moreover, the milk is appreciably affected by the milk enzyme galactase (see under the ripening of cheese) in the course of a few weeks, all of which goes to show that milk cannot be preserved for any length of time without having recourse to strong heating[1]. By adding substances containing catalase, such as liver extract (*Hepin*), blood serum or yeast extract, the excess of hydrogen peroxide may be removed immediately before the milk is used; this process was used on a large scale for the production of milk for infant feeding (*Perhydrase milk*) on the estate of *Prince Ludwig of Bavaria*. According to the author's researches, *ozone* has no bactericidal action on milk, and only gives it a highly disagreeable taste.

In this connection, mention may be made of the suggestion to use *the ultraviolet rays* of the mercury arc lamp for sterilising milk. As is known from *Finsen's* researches, these rays have a bactericidal action, and water has been sterilised on a large scale in this way. Milk, however, is somewhat impervious to the rays and, moreover, it generally contains highly resistant spores; it is therefore doubtful whether the method will ever come to have any practical significance, notwithstanding the number of appliances which have already been devised for carrying it out. *Powerful alternating currents* are said to have been applied with greater success.

TREATMENT OF MILK FOR TOWN SUPPLIES

As the general methods usually available for producing good milk have already been discussed, the subject of the *proper treatment of milk which is to be retailed in towns* may be dealt with quite briefly. The difficulties to be faced are often considerable, for as the milk has often to be sent long distances by railway, it may not reach the consumer until it is six to thirty hours old, and even then it may have to stand for a long time under unfavourable conditions before it can be used up. Only milk which has been cleanly handled from the outset can be expected to keep good under these conditions. On arrival at the large dairies, the milk is first graded, smelt and tasted, and frequently sampled for further examination. After weighing or measuring, the milk is *freed from particles of dirt* by filtering or centrifuging. This treatment, at a stage when the bacteria have long since been distributed throughout the milk, can only be justified on æsthetic grounds; it can have no influence on the keeping power of the milk. Some of the bacteria will naturally be removed, but, on the other hand, large clumps of bacteria are broken up with the result

[1] As hydrogen peroxide itself has a solvent action on proteins, it will rather promote the action of the proteolytic enzymes than inhibit it.

that plate counts show apparently more bacteria than before cleaning. The best results are got with the so-called *cleaning separator* (Fig. 57), which first whirls out the coarser and heavier particles in the form of slime, and then removes the finer particles (but also a number of fat globules if the milk is cold), by pressing the milk through a filter cloth which is stretched out in the form of a cylindrical bag. As the illustration shows, the bowl of the centrifuge is comparatively large in diameter and the filtration cylinder is supported by a truncated cone through the bottom of which the milk enters. *Laval* has constructed a cleaning centrifuge with plates which acts only by separating the slime

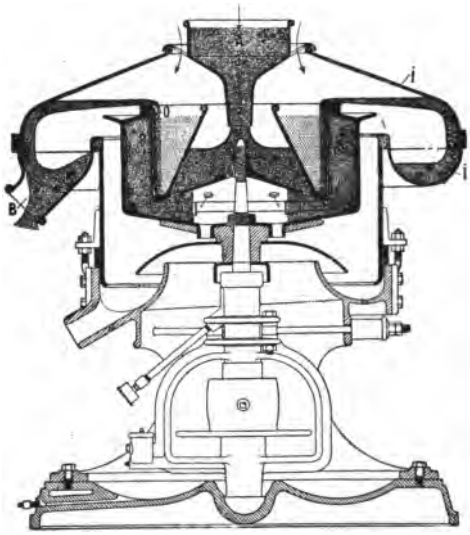


FIG. 57.—*Heine's* Cleaning Separator.

(Fig. 58). The milk enters under the bottom plate into the large slime chamber, and then passes in between the plates to the outer side of the outlet pipe along which it is discharged from the top.

After cleaning the *milk is cooled* to a few degrees above 0° C. on surface coolers through which brine is circulated from the refrigerator, after which it is run into well-cleaned cans or bottles and kept cold. In summer it is necessary to use ice on the carts from which the milk is retailed. Obviously, the necks of the bottles must be free from cracks, holes or crevices in which dirt may collect. The bottles are best closed by aluminium caps, pressed on by machinery so that they can only be removed by breaking them. Stoppers which can be removed and replaced at will must have a proper seal; they have the advantage that they

serve to protect the milk from flies and dust after the bottle has been opened and the milk partly used, and they allow of the shaking up of the milk in order to distribute any cream which may have separated. The best stopper of this description is that shown in Fig. 59. It consists of a fairly stout porcelain stopper which is held firm by being simply clamped against the under side of the collar on the bottle neck; it is easily removed when the bottle is opened, and is therefore easy to clean; the joint is made by a paraffined paper ring which should constantly be renewed;

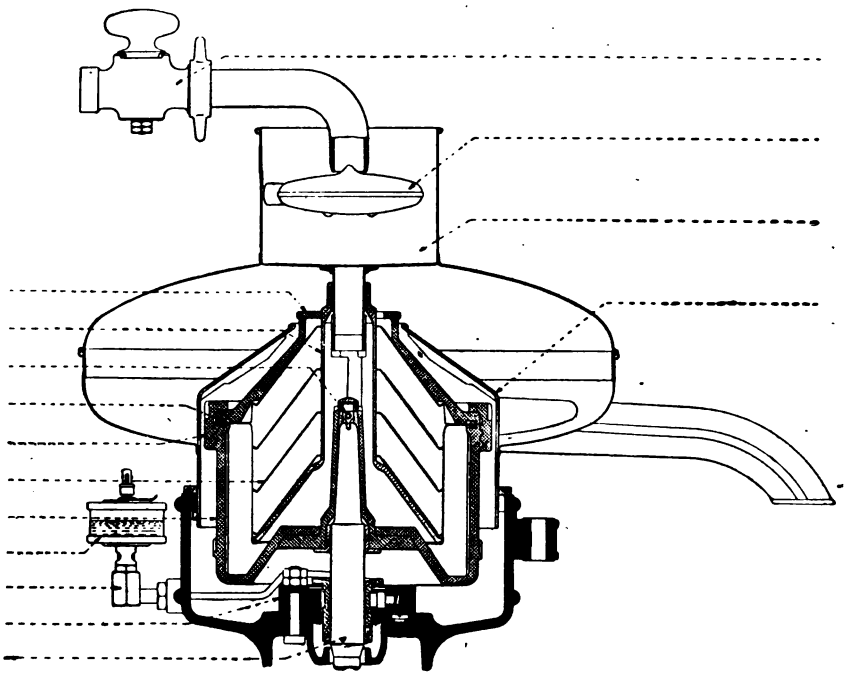


FIG. 58.—*Laval's* Cleaning Separator.

rubber rings are not so good, as they are not always free from smell. (Another method which is largely used on grounds of economy is to close the bottle by a paraffined cardboard disc which fits tightly inside a groove in the neck of the bottle; this method is less satisfactory than those described above, for after the disc has been removed and replaced, it easily becomes soaked through with the milk, and the internal groove in the neck of the bottle is objectionable on grounds of cleanliness.—*Translator*.)

Milk is often *pasteurised*, the better to overcome the difficulties which beset the problem of keeping it good until it can be retailed. For the reasons stated above, this should be accomplished by the low temperature process or by short heating (flashing) to 70° to

75° C.[1] Low temperature pasteurisation (holder process) is best carried out in closed bottles completely immersed in water, as is done in the Danish beer pasteurising apparatus; treated in this way, the milk does not lose its carbon dioxide, while the bottles are pasteurised as well. As the milk does not give off its loosely combined carbon dioxide at the temperatures employed, no appreciable pressure is developed in the bottles. If the milk is pasteurised in bulk and then bottled, the bottles must be sterilised beforehand. *Ayers* and *Johnson*[2] propose to bottle the milk warm in a warm room, and to let it stand for ten minutes before cooling; as the temperature will only fall about 5° C. at the most during this period, the organisms which have gained access to the milk in the bottle during filling will generally be destroyed, and the milk will keep as well as if it had been pasteurised in the bottle.

FIG. 59.—Hygienic Stopper.

Milk which has been pasteurised rationally according to the methods suggested above still gives a positive *Storch's* reaction, and is consequently not recognised as pasteurised according to the Danish law, the definition being reserved for the milk which has been strongly heated so as to give a negative test. In any legislation on this subject, it is highly desirable that distinction should be made between high and low temperature pasteurised milk. In the case of skim milk or buttermilk, however, the *Storch* test may quite well be employed as the criterion. Milk which is to be retailed to the public should either be raw, in which case it should be derived from herds kept under strict veterinary supervision, or it should be pasteurised by the low temperature process; in addition to control by inspection of the recording thermometer charts in the dairy, the

[1] *Tholstrup Pedersen* proposes simply to slime centrifuge the milk after it has been warmed to 70° C. This proposal is worthy of consideration, especially as *Prescott* and *Breed* ("Journal of Infectious Diseases," 1910, vol. 7) have shown that the white blood corpuscles, which carry much dirt and many bacteria with them, are more readily separated from warm milk. Unfortunately the cream line is affected by this treatment, as was shown in the trials made with the first *Laval* cleaning centrifuge ("Deutsche milchwirtschaftliche Zeitung," 1912, No. 12). Milk is, therefore, now cleaned by centrifuging at 10° to 12° C. instead of at 70° C.

[2] U.S. Dept. of Agric., Bureau of Animal Industry, Bull. 240, 1915.

efficiency of the process should be checked first and foremost by ascertaining whether the number of microorganisms is satisfactorily low, which will be the case if it shows a long time for reduction (at least nine hours) in the reductase test (see p. 166). In this way a double purpose will be served, for the test will only show satisfactorily with *freshly pasteurised milk*, and this is exactly what is required, for, unless pasteurised milk is fresh, it will be a source of danger, and should be regarded in the same light as an adulteration, as it purports to be better than raw milk, whereas in reality it is worse. As low temperature pasteurised milk, like that pasteurised at a high temperature, cannot be relied on to sour spontaneously, it should not be sold as raw milk, but should be plainly marked with the date of pasteurisation.

The high temperature pasteurised milk which is at present sold in Copenhagen is often as rich in bacteria as raw milk, and always shows copious evolution of gas in the fermenting test on account of its comparatively high content of butyric acid and pseudo lactic acid bacteria. The latter probably find their way into the milk on the open coolers and from unsterilised bottles. The advantages offered by such milk are exceedingly doubtful, and any one who fears the risk of infection had far better buy raw milk and boil it.

According to the author's repeated investigations, the ice milk supplied to Copenhagen, which was mentioned above (sold from carts at about 9 a.m.), contains on an average 60,000 bacteria per cubic centimetre, and the ordinary milk from large dairies, from several hundred thousand to about two millions. Small dairies generally sell far inferior milk, the counts sometimes running as high as one hundred million organisms per cubic centimetre. Better conditions can hardly be expected without further legislation. The author suggested at the International Dairy Congress at Stockholm[1] that no hard and fast rule should be laid down once and for all as to the maximum number of bacteria and the minimum percentage of fat in different grades of retail milk, but that the dairies should be required to make some guarantee which should be plainly stated on the label in such a way that it could not be misunderstood. Competition would then bring about a gradual improvement in the quality of the milk, and the authorities would only have to see that the dairies did not promise more than they fulfilled. In this way the smaller dairies which can guarantee nothing, would be suppressed, and the trade would pass entirely into the hands of the large and well-regulated firms[2].

[1] "Maelkeritidende," 1911, p. 731.

[2] The bacteriological condition of the milk retailed in the large towns of the United Kingdom leaves quite as much to be desired. It is to be

The author has proposed the following regulations for the milk supply of Copenhagen[1]:—(1) The milk trade of Copenhagen should be concentrated in the hands of a few authorised firms possessing the proper equipment and managed by competent experts, such dairies to be under municipal control in all details. (2) The prices paid for milk by the dairies to the producers, as well as the prices paid by the consumers to the dairies, should depend on the hygienic qualities of the milk, its cleanness, keeping qualities and fat content. (3) All persons having anything to do with the milk up to bottling should be under medical supervision, and the herds from which the milk is derived should be under effective veterinary supervision. (4) At the dairies, samples from each farmer should be taken at least once a week to be tested by the reductase and fermenting test, and analysed for fat; these tests should be carried out by impartial persons appointed by the municipality in conjunction with a representative of the dairy. (5) According to the results of the reductase and fermenting test, the milk should be graded into four classes (see p. 170), the standard price to be that of second grade milk with 3·3 per cent. of fat (the average fat percentage in Copenhagen milk). First grade milk to command a higher price, and third and fourth grade milk lower prices, a certain bonus or deduction being calculated for each tenth per cent. of fat over or under the average. (6) First and second grade milk, sold for direct consumption, should be pasteurised by the low temperature process; third and fourth grade milk, which should only be sold for cooking and baking, should be pasteurised at a high temperature. (7) After pasteurisation all milk should be cooled to about 4° C., and kept on ice or in refrigerators during transit or in the shops. It should be plainly marked with the name of the dairy, its grade and fat percentage, as well as the date of pasteurisation; it should not be

feared that any attempts to effect improvement of milk by stimulating competition, as suggested by Professor Orla Jensen, would prove abortive, owing to the absence of any real competition in many parts of the country, and that the grading of milk by legalised standards will prove the only remedy for the unsatisfactory state of affairs. The enhanced market value of milk only gives emphasis to the necessity for cleaner handling and scientific treatment, and it is most unsatisfactory from the point of view of the public that the enormous increase in price has not been accompanied by any improvement in quality. The best example of what may be achieved under climatic conditions often worse than those of the United Kingdom is to be seen in the American Bacteriological Standards for Milk (U.S. Public Health Reports, Reprint No. 192). Here the regulation of the market value of milk according to its bacteriological condition receives the attention which it deserves. Standards for certified special milk are often as low as 10,000 bacteria per cubic centimetre, and in many of the large towns the standards for raw milk are 500,000 bacteria per cubic centimetre, or even less.—*Translator.*

[1] "Ugeskrift for Laeger," No. 16, 1919.

sold later than two days after pasteurisation, while first grade milk which is to be used as nursery milk should not be allowed to stand for more than one day after pasteurisation. (8) While first and second grade milk should be sold exclusively in bottles, third and fourth grade milk should only be sold in cans.

No question concerning milk, and it may even be said no question concerning the public welfare, is of greater importance than that of the **Nutrition of Infants,** and it will naturally claim our attention here. As *Bunge* was the first to show, the milks of different animals are richer in proteins and calcium phosphate the quicker the animals for which they are intended form flesh and bone. To illustrate this point, the author has collated the following table of average analyses of different milks :—

	Woman	Ass.	Mare.	Cow.	Goat.	Sheep.	Sow.	Bitch.	Rabbit.
Water . .	88·3	90·3	90·6	87·7	86·8	78·9	82·2	80·1	69·5
Proteins . .	1·6	1·8	2·0	3·4	3·7	6·2	6·9	7·3	15·5
Lactose . .	6·4	6·2	5·8	4·8	4·6	5·0	2·2	2·8	2·0
Fat . . .	3·4	1·3	1·2	3·4	4·1	8·9	7·7	8·5	10·5
Ash . . .	0·3	0·4	0·4	0·7	0·8	1·0	1·0	1·3	2·5
K_2O . .	0·08	0·08	0·10	0·18	0·15	0·20	0·19	0·14	0·25
Na_2O . .	0·03	0·03	0·02	0·04	0·06	0·08	0·08	0·08	0·19
CaO . .	0·04	0·11	0·12	0·17	0·19	0·24	0·45	0·45	0·89
MgO . .	0·006	0·013	0·012	0·017	0·02	0·02	0·016	0·02	0·05
P_2O_5 . .	0·04	0·14	0·13	0·20	0·29	0·34	0·34	0·51	0·99
Cl. . . .	0·05	0·03	0·03	0·10	0·10	0·13	0·07	0·16	0·13
Number of days taken to double the weight.	180	—	60	47	22	15	14	9	6

The table shows that the milk of the ass and the mare only differ from human milk in containing somewhat less fat and a little more calcium and phosphoric acid ; as they both resemble human milk in being alkaline towards litmus, and contain at least as much casein as albumin, they form the best substitute in cases where infants cannot be breast-fed. Unfortunately it is nearly always necessary to have recourse to cow's milk, as the only kind of milk available in large quantities. In order to make cow's milk resemble human milk, water, sugar, and, if possible, also cream, should be added to it. Cane sugar is often used as being the cheapest, but as it favours the development of butyric acid bacteria in the intestine at the expense of the true lactic acid bacteria[1], milk sugar or malt extract is to be preferred. As the child grows older, it becomes possible to depart more and more from the composition of the mother's milk, and the additions of sugar and

[1] *Paul Sittler,* "Die wichtigsten Bakterietype der Darmflora beim Säugling," Wurtzburg, 1909, p. 60.

water to cow's milk may be gradually decreased. As the infant begins to secrete larger amounts of diastase, oat gruel or other starchy liquids may be substituted for sugar. Infectious germs are destroyed by boiling the milk in a saucepan or a *Soxhlet's* apparatus. The albumin, which is none too plentiful to begin with, is thus coagulated, while the casein is altered in such a manner that the milk coagulates more slowly and less completely with rennet. The change does not, as was previously supposed, solely consist in the precipitation of certain calcium salts which are a necessary factor in coagulation—when the milk comes into contact with the acid gastric juice the effect will be neutralised—but, according to the author's investigations[1], a real denaturing of the casein takes place, which must mean that it becomes less digestible, for the coagulation of casein by rennet is (see p. 13)

FIG. 60.—*Orla-Jensen's* Household Pasteurising Apparatus.

only a sign that the first stage in digestion has been completed. The fact that raw cow's milk is coagulated into lumps immediately on entering the stomach is regarded by the author as a practical provision against the passage of large amounts of undigested casein through the stomach. Furthermore, the milk acquires a cooked flavour, and its natural enzymes, bactericidal constituents and antitoxins are destroyed. Even though opinions may be divided regarding the practical bearing of all these changes in connection with infant nutrition, it is certain at least that many children cannot stand boiled milk, for which reason it is advisable to employ low temperature pasteurisation in freeing infants' milk from pathogenic germs. Both in order to be on the safe side as regards tubercle bacteria and to avoid the too rapid separation of cream—an undesirable feature in infants' milk—the lowest tem-

[1] "Landwirtschaftliches Jahrbuch der Schweiz," 1905, p. 241.

peratures should not be employed; it will only be necessary to see that the milk is not heated above 70° C. The author has designed a household pasteuriser which consists simply of a water bath large enough to retain a temperature of not under 65° C. for half an hour after it has been heated to 70° C. and the source of heat has been removed. Hot and cold water may also be mixed in the bath so as to obtain a temperature of 78° C., the filled bottles then being placed in it for an hour; already, after five minutes, the milk will be at 67° C., and the water at the same temperature. The bottles must be fully immersed; as they are closed during warming, the formation of foam or skin is avoided. As with *Soxhlet's* bottles, the stopper may be replaced by a rubber teat. The pasteurised milk must of course be kept cold [1].

Recent investigations have shown that milk contains a fat soluble as well as two water-soluble **vitamines,** which substances are absolutely indispensable for the normal growth of young animals. The fat-soluble and one of the water-soluble vitamines are only destroyed by prolonged boiling or at temperatures over 100° C. The second water-soluble vitamine, on the other hand, already begins to be destroyed at a comparatively low temperature, a fact which more than any other warns us against heating infants' milk more than absolutely necessary. The lack of this last-mentioned vitamine causes scurvy, or, particularly in infants, *Barlow's* disease [2]. By adding lemon or orange juice to the milk, the missing vitamine will be replaced.

The fat-soluble vitamine is only sparingly distributed in nature (besides in milk fat, it is only found in fairly large amounts in cod liver oil), for which reason milk fat and butter cannot be completely replaced by other fats.

It is well known that milk contains a sufficiency of all the substances necessary for the nutrition of the infant, with the exception of iron [3]. At birth, the child is provided with a reserve

[1] The household pasteurising apparatus is sold by *Apoteker Delholm,* Vaisenhusapoteket, Copenhagen.

[2] In contradistinction to *Barlow's* disease, rachitis is due to lack of lime. According to *P. Röhmann* ("Die Chemie in Einzeldarstellunge," Berlin, 1916), the three following causes may be operative:—(1) Primary lack of lime, owing to the mother's milk immediately after birth not containing enough lime; later on it will be sufficiently rich in lime, but then it is customary to change over to farinaceous foods, which are very poor in lime. (2) Secondary, lack of lime due to bad assimilation of lime salts, caused by disturbances in the secretion of bile, or in the action of the intestinal glands, so that the lime is kept back as salts of the fatty acids. (3) Disturbances in the cells which deposit the lime in the bones; these may be of a local nature, or due to the thymus gland, the secretions of which have an influence on this process.

[3] A litre of milk, direct from the cow, contains, on an average, only half a milligram of iron. If the milk contains appreciably more iron, it will be in the form of inorganic salts from iron vessels with which the milk has

of iron in the liver, but when, after six to nine months, this has been used up, food containing iron must be given. Spinach and egg-yolk (beaten up in thin semolina gruel or buttermilk soup) are the best media for introducing iron, and the large lecithin content of egg-yolk no doubt also contributes to the growth and welfare of the child, lecithin being an important constituent of nerve and brain.

come into contact. Normal human milk contains twice to three times as much iron as is present in cow's milk. On centrifuging, most of the natural iron, as well as of the lecithin—the latter is probably combined with the proteins—passes into the cream. Again, on churning, the bulk of these constituents remains in the buttermilk, so that clear filtered butter fat is free from lecithin. It will be seen from this that buttermilk, besides being richer in fat than separated milk, is also richer in lecithin and organically combined iron, both substances of very great physiological importance; it follows that separated milk in which fat has been emulsified cannot replace unseparated milk. If it is found that buttermilk is more easily digested than ordinary milk, the reason is that its casein is already so finely divided that it can no longer form large lumps in the stomach.

Chapter IV

The Applications of the Lactic Acid Fermentation in the Dairy Industry

In Northern and Central Europe "long" and "thick" milks are prepared in the household as articles of diet; in Southern Europe and the neighbouring regions of Asia and Africa, other forms of sour milk are prepared which generally also develop a more or less vigorous alcoholic fermentation. These milk products, which are all credited with great dietetic value, appear under different names in each country without necessarily being different in nature. Thus, the author found the same organisms in the Sardinian *Gioddu* as in the Bulgarian *Yoghurt*, while Kefir grains have been found to vary in composition to such an extent that all that they could be said to have in common was their peculiar structure. All these products are of considerable bacteriological interest, as in many cases they contain different organisms which mutually benefit one another, forming genuine illustrations of *symbiosis*. They may be classed in two groups according to the completeness of the symbiosis. To the first group belong **Mazun** (Armenia) and **Yoghurt** (Bulgaria), and to the second group, **Leben** (Egypt), **Kumys** (Russian Steppes) and **Kefir** (Caucasus).

The products of the first group contain both true lactic acid bacteria and lactose-fermenting Saccharomycetes. The latter contribute to the aroma, but otherwise play a minor part. The principal organisms are rapidly growing lactic acid streptococci and strongly acidifying rod forms. The former are less anaerobic than the latter and prepare the way for them. The rod form of Mazun produces dextro lactic acid, while that of Yoghurt, *Thermobacterium bulgaricum*, produces the lævo acid. In addition to the last-mentioned organism, another rod form may occur in Yoghurt; this forms feathery colonies in agar tubes and produces inactive lactic acid; the author has named it *Thermobacterium Jugurt*. Good **Yoghurt**[1] may be prepared by boiling or pasteurising good milk, cooling to 50° C., inoculating with the proper organisms (the yeast may be dispensed with), bottling and keeping at 40° C. At higher temperatures the rods will predominate, while at lower temperatures these will be displaced by

[1] This name is derived from the Turkish "*Jugurt.*"

streptococci. As soon as the milk has coagulated, it is cooled in running water and placed on ice; the production of acid must be stopped at the right moment, otherwise the Yoghurt will become too sour. Sometimes the milk is concentrated to half its original bulk before inoculation, in which case the product will be more nutritious, but less refreshing than that prepared from unconcentrated milk. It will then correspond in composition to Yoghurt made from Buffalo milk, which is considered particularly choice. According to *Metschnikoff*, the *Yoghurt rod bacteria inhibit the growth of the intestinal putrefactive organisms* to a greater extent than do the other lactic acid bacteria, and thus prevent digestive troubles, rheumatism, calcareous growths, and, generally speaking, prolong life. Centenarians are said to be more numerous in Bulgaria than in other countries, and considering the claims which were made on its behalf, it is no wonder that Yoghurt at one time came into demand in all civilised countries. People ate not only Yoghurt, but Yoghurt tabloids, which frequently contained no living lactic acid bacteria, but always *Bacillus mycoides* or other sporing organisms. To what extent *Thermobacterium bulgaricum* can become acclimatised to the conditions of the alimentary canal of man and animals is extremely doubtful[1]. The author has never succeeded in finding this organism in the fæces of adults, even after large daily doses of Yoghurt. Neither was it possible to find this easily recognisable rod form in the fæces of an infant constantly fed on milk which had been inoculated with a few drops of Yoghurt[2]. However, by inoculating a little of this fæces into milk which was kept at 45° C., the Yoghurt rod was obtained as a pure culture. A more rational procedure would be to introduce into the intestine those lactic

FIG. 61. — *Bacterium bifidum*, distinctly branched. From fæces of bottle-fed infant. × 1,000.

[1] Thus *Hull* and *Rettger* found that *Thermobacterium bulgaricum* could not be acclimatised in the alimentary canal of the white rat ("Centralblatt f. Bakteriologie," 1 Abt., 1914, Bd. LXXV., p. 219).

[2] A slight addition of Yoghurt has an aperient action, and it is certainly more harmless than other aperients. The thermobacteria seem to be able to thrive in the stomach of young animals as long as they are fed on milk alone, and where the production of hydrochloric acid is lower than later on.

acid bacteria which are known to thrive there under normal conditions, *i.e.*, the obligate anaerobic rod form *Bacterium bifidum* (*Bacillus bifidus, Tissier*)[1], and various streptococci which the author finds to be related to *Streptococcus fœcium* and *glycerinaceus*. The common bacteria of sour milk, *Sc. lactis* and *Sc. cremoris*, do not thrive in the alimentary canal. If the typical intestinal lactic acid bacteria do not always obtain predominance spontaneously, it will be owing to a faulty diet. If care be taken that the diet contains a far greater proportion of carbohydrates (especially starch, maltose and lactose) than of proteins, then the lactic acid bacteria will always be able to gain the upper hand over the putrefactive bacteria. This result may be achieved by eating more fruit, farinaceous and milk foods than meat, eggs and ripe peas and beans. It should also be borne in mind that man is not purely herbivorous, and can only digest very small amounts of cellulose. The nutrient substances enclosed in voluminous greens are too difficult of access for our digestive system, and are to a great extent carried out into the large intestine, where, together with the cellulose, they set up various anaerobic fermentations. Already, in 1876, *Baumann* showed that intestinal putrefaction is just as pronounced on a pure vegetarian diet as on a meat diet; it is most successfully avoided by the rational selection of a mixed diet.

In the second group of sour milk products alcoholic fermentation plays a far more important part, although the lactose fermenting Saccharomycetes are generally replaced by Torulæ which are unable to ferment milk sugar directly, but only after it has been hydrolysed to dextrose and galactose by certain lactic acid bacteria. This is the case in **Leben**; according to *Rest and Khoury*[2], the lactic acid rod forms occurring in this product are only feeble acid producers growing at the ordinary temperature. *Olav Johan Olsen Sopp*[3] found a similar case of symbiosis in the Norwegian **Kaeldermaelk** (cellar milk), which is made from boiled milk by inoculating it with a special kind of ropy milk, and keeping it as cold as possible; on account of the large amounts of lactic acid (up to 2·5 per cent.) and alcohol (about 0·5 per cent.), which are eventually formed, this preparation keeps particularly well and is not even inclined to turn mouldy; it often forms a substitute for fresh milk when the cows are away from the farms on the mountain pastures, and was formerly very commonly used by the Norwegian peasantry. **Kumys** is made from mare's milk,

[1] "Recherches sur la flore intestinale normale et pathologique du nourisson," Paris, 1900.
[2] "Annales de l'Institut Pasteur," 1902, p. 65.
[3] "Centralblatt f. Bakteriologie," 2 Abt., 1912, Bd. XXXIII., p. 1.

which, on account of its high sugar content, is well adapted for alcoholic fermentation, being capable of developing up to 3 per cent. of alcohol. According to *Rubinsky's* investigations[1] a yeast which ferments milk sugar and a rod bacterium which produces over 1 per cent. of lactic acid are the organisms which play the most important part in the production of Kumys. In pure cultures the lactic acid rod does not grow under 23° C., but in conjunction with the yeast it grows at the ordinary room temperature. Kumys should, however, not be made at too low a temperature. **Kefir** presents the greatest interest, for the so-called Kefir grains, which resemble small cauliflowers (Fig. 62), are produced by the symbiotic growth of the active organisms. Sectional preparations (Fig. 63) show them to consist of a network of small or large rods, in which yeast cells are enclosed. The

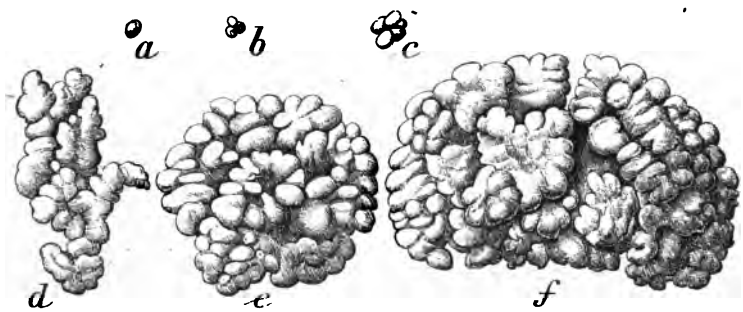


FIG. 62.—Kefir Grains, natural size. (After *Kern.*) Above, dried. Below, swollen.

rods are chiefly *Betabacterium caucasicum*, a lactic acid bacterium resembling that of Leben in its chief characteristics, while the yeast also resembles that of Leben in fermenting saccharose and maltose, but not lactose without the help of lactic acid bacteria. Kefir grains generally also contain strongly acidifying Streptobacteria. The Betabacteria are best found by inoculating into yeast water, which is the only medium in which they thrive well. In this symbiosis, the lactic acid bacteria appear to be more dependent on the yeast than conversely. In good Kefir, only rod bacteria and yeast will be found, and the former always collect round the latter, showing a mutual attraction. Kefir grains imported direct from the East exhibit vigorous growth, and have often been regarded as a miraculous remedy for many ills. Grains obtained from several European depots were of quite a different nature; all of these contained a torula which fermented lactose

[1] "Studien uber den Kumiss," Inaugural Dissertation, Leipzig, 1910, Verlag Gustav Fischer, Jena.

directly, and a few of them were found to contain some Gram-negative rods in addition to the usual Gram-positive rods. The Gram-negative rods must be classed with *Bacterium cloacæ* or rather with the Proteus species; they are peritrich and non-sporing; they ferment practically all sugars [1] with copious evolution of gas, production of alcohol and small amounts of acid (acetic and lactic). They coagulate milk, forming a slimy film which sticks fast to the bottom of the flask, and on shaking

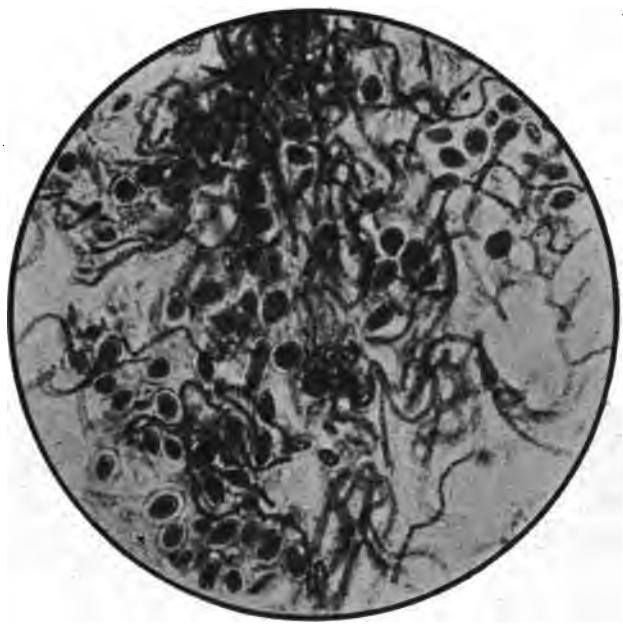


FIG. 63.—Section through a Kefir Grain. × 1,000.

clots together, enclosing other microorganisms and casein. These rods therefore doubtless play some part in the formation of the

[1] In addition to the common mono and disaccharides, they ferment raffinose and inulin, and, among the alcohols, glycerol and mannitol; but, like the true lactic acid bacteria, they do not ferment dulcitol. The aerogenes bacteria mentioned by *Kunze* in his exhaustive work on Kefir fermentation ("Centralblatt f. Bakteriologie," 2 Abt., 1909, Bd. XXIV., p. 112) are possibly identical with these. This investigator also describes certain butyric acid bacteria which are said to play an important part in Kefir fermentation, and, as a matter of fact, a butyric acid fermentation can always be induced by inoculating stale Kefir grains into sterilised milk. The butyric acid fermentation, however, only does harm, and it can only be regarded as a defect here as in all other milk products. In order to avoid this fermentation, the Kefir grains should be revived by inoculation into milk distributed in shallow layers. Kefir grains occasionally have certain Mycodermæ on the surface. The author was formerly inclined to regard these as organisms which normally contributed towards the growth of the grains, but now regards their presence as a defect.

grains; they confer on the Kefir its slimy consistency and bring about the peptonisation of the casein, besides contributing towards the production of alcohol. In Kefir made from these grains, various streptococci always appear, which contribute towards the production of acid, but for the most part they appear to be casual guests. According to *Freudenreich*, who was one of the first investigators of the Kefir fermentation[1], certain special strains of streptococci play a part in this process. The grains investigated by *Freudenreich* appear to occupy an intermediate position between the two varieties described above.

The preparation of Kefir from the dried grains of commerce requires a somewhat lengthy process of revival. The grains must first be soaked in lukewarm water and then in lukewarm milk which is constantly to be changed before it coagulates. This treatment must be continued till the grains produce sufficient gas to make them rise in the liquid, which may take a whole week. The grains which float are then added to milk in the proportion of 2 to 3 kilos per 10 litres. If there is not a large proportion of grains to milk, the bacteria of the milk itself may easily come to predominate. Under favourable conditions, the weight of the grains will increase by over 40 per cent. in fourteen days. The milk is kept at 20° to 25° C., and frequently stirred or shaken. When active fermentation has set in, the grains are strained off and may, after washing with clean water, be used for a fresh batch; the milk is bottled, closed up with spring stoppers and kept for one or two days at 15° to 20° C.; at higher temperatures the bottles may easily burst. The precipitated casein is distributed by careful shaking, and the resulting product is an effervescent, somewhat thick fluid containing about 1 to 1½ per cent. of acid, and about 0·25 per cent. of alcohol. On longer standing the taste becomes less pleasant owing to the progress of alcoholic fermentation; in the course of six days, the alcohol content may increase to over 1 per cent. Kefir is undoubtedly of greater dietetic value than Yoghurt, for the author has found that on the drinking of Kefir, the proportion of Gram-positive rod bacteria rapidly increases in the fæces, while the yeast is completely digested, the fæces showing only the empty cell walls. As extract of yeast counteracts certain illnesses, the healing properties of Kefir must be ascribed to the yeast. The author has found that yeast extract promotes the development of the lactic acid rods, but inhibits the development of streptococci, especially the pathogenic species. For people who have comparatively little hydrochloric acid in the stomach, Kefir, like

[1] "Landwirtschaftliches Jahrbuch der Schweiz," 1896, p. 1.

Yoghurt, acts favourably by simple reason of its large content of lactic acid.

As a third group of milk products may be mentioned **Sparkling Whey,** and **Whey Champagne,** in which alcoholic fermentation is very pronounced. In the Carpathians, sparkling whey made from sheep's milk (*Urda* and *Skuta*) is a favourite beverage, and in Chile whey champagne is made by the fermentation of whey with the addition of various saccharine and aromatic substances.

In addition to its applications in the preparation of these national milk products, the lactic acid fermentation plays an important part in butter making from soured cream and in the manufacture of margarine from soured skim milk. Its application in the manufacture of lactic acid has already been touched upon (see p. 31). The souring of cream will now occupy our special attention.

THE SOURING OF CREAM

Like most practical discoveries, the souring of cream for butter making owes its origin more or less to chance. In order to avoid churning every day, it became the custom on small farms to save the cream for several days and sometimes even for a whole week, the different batches being mixed together, and naturally the cream became sour. In many cases it was sour before mixing, as the milk was often kept in a warm place for the cream to rise. In the same way, the custom of churning sour cream ("gathered cream") naturally arose in the American creameries where fresh cream is not available. Originally the butter prepared from soured cream was extremely bad, but experience soon showed how the worst defects might be avoided, and it was found that under certain circumstances a particular aroma would be imparted to the butter, which could not be produced with unripened cream, and was appreciated by those who had acquired the taste for it. Further, the use of ripened cream results in an increased yield of butter as the fat globules coalesce more readily during churning when the casein has been precipitated. Finally, the butter made from ripened cream keeps better than that from unripened cream. For these reasons the practice of souring cream was continued when the dairies became so large that churning became a daily operation. Even in Central Europe, where butter was generally churned from fresh cream, it has become the custom to use soured, or at any rate slightly soured, cream. The only difference between "sweet" and "sour" butter will soon be that the former is usually not salted.

As the self-souring of cream is the oldest process, we may first deal briefly with the changes which it involves. An actual

example may be taken from *Conn and Esten's* investigations[1]. Although the cream was hand-skimmed, it did not show a high bacterial count to start with. It was allowed to stand at 20° to 21° C.

From the accompanying table it is seen that the count increases steadily during the first forty-eight hours, and even reaches to over 1,000 million bacteria per cubic centimetre, after which it decreases. At first all the different groups of bacteria increase in numbers, but later on the lactic acid bacteria gain the upper hand, and by degrees suppress the other organisms. The most persistent seem to be the pseudo lactic acid bacteria and the alkali-producing bacteria (*Bacterium lactis innocuum*), the cream having been particularly rich in the latter at the outset.

Time after skimming.	No. of bacteria per cubic centimetre of cream.	Percentages of the Bacterial Groups.				
		True lactic acid bacteria.	Pseudo lactic acid bacteria.	Alkali-forming bacteria.	Liquefying bacteria.	Other bacteria.
3 hours.	195,600	6·2	7·6	66·2	6·4	13·6
12 ,,	4,750,000	5·1	1·8	70·2	11·8	11·1
24 ,,	54,000,000	37·4	5·1	33·8	2·1	24·4
36 ,,	528,000,000	90·2	5·0	4·7	0·1	0
48 ,,	1,023,000,000	94·6	3·1	2·1	0·2	0·
60 ,,	994,000,000	96·1	3·0	0·9	0·1	0
72 ,,	687,000,000	95·4	2·3	1·8	0·1	0
84 ,,	420,000,000	96·3	1·1	2·0	0·6	0

Among the lactic acid bacteria, only the Streptococci multiply at first, but after the count (lactose gelatine plates) has reached the maximum, bacteria belonging to other groups gradually appear and begin to suppress those present. At the same time, yeast and *Oidium lactis* appear, as in milk. The period of the maximum count, which always synchronises with the thickening of the cream, is also the period of the purest lactic acid fermentation. If the cream becomes over-sour, the most beneficial of the lactic acid bacteria will disappear and other microorganisms will develop which may spoil the keeping qualities of the butter, but which may, on the other hand, impart to it a stronger aroma. It is difficult to fix any particular degree of acidity at which the souring should be interrupted, for, as we shall see later, the degree

[1] "The Ripening of Cream," Storr's Agricultural Experiment Station Report, 1900, p. 13.

of acidity is inversely proportional to the fat percentage. The consistency is a far better guide than the acidity, especially in view of the difficulty in measuring an exact amount of the thick cream, and in determining the end point in the titration. As soon as the cream has the proper consistency, the souring should be stopped by churning and washing out the bulk of the lactose, or, if churning cannot be proceeded with immediately, by thorough cooling.

In order to promote the souring, a **Lactic Acid Culture** or **Starter** may be added. In this way the first stage of the souring may be shortened, *i.e.*, the stage during which many different kinds of milk bacteria develop; although some of these may tend to improve the aroma of the butter, most of them will in all probability do harm. The final stage of the souring will also be shortened, especially if an over-sour starter, such as *buttermilk*, is used. Buttermilk is probably the oldest form of starter used, and if it originates from butter of good aroma, and has not been diluted with bad water, it will impart a good aroma to the product; as a rule, however, the development of harmful organisms will be encouraged and butter defects will be perpetuated by this method. A safer method is to *let extra good milk sour spontaneously*, using it as a starter when it has reached the right stage; this method became prevalent before the so-called pure cultures were put on the market. The introduction of these cultures was due to the initiative of *Storch*, who found that different species or strains of lactic acid bacteria produced very different odours or tastes in the soured products [1]. Among other lactic acid bacteria, *Storch* isolated a stout streptococcus, " No. 18 " (Fig. 38), which produced a particularly fine aroma. This particular strain was not used in actual practice to any extent, but already in the same year the firm *Blauenfeldt and Tvede* put cultures on the market, and others soon followed their example. All these cultures which are propagated in the best pasteurised milk, generally contain several different species of lactic acid bacteria, and very probably they owe their good qualities to this very fact, for a higher degree of acidity is obtained by the co-operation of several species than by the action of one species alone, while lactic acid bacteria in pure cultures are apt to degenerate or become slimy. As a rule, diplococci are met with among the stout streptococci (*Streptococcus cremoris*). *Boekhout* [2] and *Storch* [3] have described a non-souring or slightly souring streptococcus which appears to con-

[1] " Forsögslaboratoriets," 18 de Beretning, 1890.

[2] " Vereeniging tot Exploitatie eener Proefzuivelboerderij te Hoorn," Verslag over det jaar, 1917.

[3] Researches carried out some years before his death, still unpublished.

tribute towards the aroma in the souring of cream. The author[1] has isolated this bacterium from commercial starters, and finds it to be a typical milk organism, possibly a variety of *S. cremoris* which has lost the power to form acid, but on the other hand developed an increased power to produce aroma. Also *Hammer* and *Baily*[2] have shown that the acid-forming bacteria proper produce more volatile acids (which constitute part of the aromatic substances) in symbiosis with another bacterium. *Dry cultures* may be obtained as well as *liquid cultures*. The latter are dried on some neutral material such as lactose or starch, and products are obtained which are more permanent than the liquid preparations and better suited for sending long distances. On the other hand, dry cultures will not keep indefinitely, and it is not advisable to buy them unless they are stamped with the date of preparation or a date after which they must not be used. In any case they must be revived by propagating several times before they can be used, and they are, on account of the method of their preparation, much oftener infected with yeasts and moulds than the liquid cultures. Their use is therefore not to be recommended if liquid cultures can be obtained instead.

The full benefits of the use of pure cultures are only attained when all the other microorganisms in the cream have previously been killed by pasteurisation, and it was only after this practice had been introduced that commercial cultures came into extensive use. The pasteurisation of the cream and the subsequent use of good cultures have more than anything else contributed towards making Danish butter a uniform and well-keeping product. Pasteurising above 80° C. brings the additional advantage of killing the tubercle bacteria which pass into the cream in large numbers on separating; the resulting buttermilk will thus be rendered harmless to pigs and calves, and the butter harmless for human consumption. The legal enforcement of pasteurisation of cream at a temperature of at least 80° C. by the law passed, in Denmark in 1898, with the object of combating the results of bovine tuberculosis, must therefore be signalised as an important step forward in Danish dairy practice.

Although there can be no doubt that the pasteurisation of cream can only be a benefit, it is still maintained by many that the commercial cultures do not produce so strong an aroma in the butter as is obtained by spontaneous souring. Pasteurisation does away with the first stage of spontaneous souring, while the development of yeasts and yeast-like moulds is avoided during the

[1] "The Lactic Acid Bacteria," monograph published in English by the Danish Academy of Sciences, Copenhagen, 1919.

[2] Iowa Agricultural Experimental Station, 1919, Bulletin No. 55.

last stage by the use of commercial starters; naturally both of these factors have an influence on the aroma of the butter. The most aromatic butter, that from *Isigny*, is still made by the old-fashioned methods from very over-ripe cream in which aroma-producing yeasts have been found. At the same time, the aroma depends on the nature of the feed as well as on the action of micro-organisms; very probably it is the resultant of the combined influence of both factors. Thus it is well known that grass butter has a far more pronounced aroma than winter butter, and there can hardly be any doubt that the fine aroma of *Isigny* butter is largely attributable to the excellent Norman pastures. In this connection, however, it should be pointed out that the aromatic substances originating from the feed do not always pass unchanged into the milk (whether through the udder or by way of the manure); the most usual course of events is the secretion of complex substances, which only give rise to aromatic products on hydrolysis. Microorganisms which can effect these hydrolyses will thus be aroma-producing in milk or cream which contains the substances in question, but not necessarily in any milk or cream. Several *butter aroma bacteria* (mostly belonging to the Coli group) have already been isolated and recommended for use in conjunction with lactic acid bacteria in the ripening of cream, and the fact that none of them have attained to any practical significance is probably to be attributed to the circumstances just mentioned. At present we have only certain lactic acid bacteria, producing a faint aroma, which can invariably be used with advantage; the above-mentioned discovery of an organism which might be used in conjunction with these in order to increase the aroma may be of great practical value. Experience shows, however, that butter which has a pronounced aroma does not generally keep well, and it will always be more to the advantage of the producer to make sure of the keeping qualities than to concentrate attention on the aroma, which, after all, is a matter of local or even only temporary preference as far as the public is concerned, and this can be influenced to a certain extent by the large producers.

We will now pass on to the detailed discussion of the ripening of cream by modern methods. The first question of importance is the **fat percentage of the cream.** As the lactic acid bacteria do not act on the butter-fat, they must necessarily produce the characteristic butter aroma and acid from the other constituents of the milk. Seeing that the aroma is produced outside the fat globules, one might suppose that it would be washed out as easily as the lactic acid, but this is not the case. Thus *Isigny* butter has a stronger aroma than any other make, but its process of

manufacture involves a very thorough washing. The explanation is that butter-fat is able to absorb essential oils and other odiferous substances, both pleasant (a property which has been made use of in perfumery) and unpleasant, and the aroma of the cream will therefore pass into the fat globules. The greater the proportion of fat globules present, the less aroma will be available for each globule, and experience also shows that a high fat percentage is not conducive to the production of an aromatic butter. Unfortunately there is a tendency to work with richer cream in dairies where increasing quantities of milk have to be treated; thus in Denmark it was formerly considered inadvisable to churn cream containing more than 20 per cent. of fat, but of late the practice has generally been to increase the percentage to thirty or even more. Fortunately difficulties in churning prevent the raising of the latter limit to any extent.

As far as the **pasteurising of the cream** is concerned, it need only be mentioned that out of consideration for the keeping properties of the butter, the pasteurising temperature should be as high as possible. As the cooked flavour which milk acquires on heating does not originate in the fat, cream is not particularly sensitive to this treatment, and if the subsequent cooling be sufficiently rapid, no harm will be done by raising the temperature to 95° C. In this connection we must bear in mind the well-known fact that milk fat occurs naturally in different stages of *supercooling*, and that a partial alteration of this condition must take place before the butter can "come." As all the constituents of the fat melt completely on pasteurisation, freshly pasteurised cream is difficult to churn. The longer the cream has stood, the nearer will the fat globules be to the point of crystallisation, and the quicker will the butter come. In order to promote crystallisation, it is usual to cool the cream as completely as possible for a short time immediately after pasteurisation, and then to warm it up to the souring temperature[1]. The crystallisation of fat, however, takes time, and less is achieved by a cooling of short duration at a low temperature than by a more prolonged cooling at a somewhat higher temperature. *Prolonged cooling, lasting many hours,* is more practicable after the souring process than before, and under these circumstances the cream is only cooled to the souring

[1] *Cf.* the author's theory of churning ("Maelkeritidende," 1907, p. 943) *van Dam's* investigations ("Molkereizeitung," 1915, Nos. 25 and 26) clearly show the great importance of cooling the cream to a low temperature. At 16° C. all the fat was liquid even after twenty-one hours. At 11° C. one-half of the fat had solidified in the same time, and at 6° to 8° complete crystallisation had taken place in four hours. It is, therefore, recommended to cool the cream for some time to 6° C., especially in autumn.

temperature after pasteurisation[1]. On the usual type of surface cooler the cream is exposed to great risk of infection from the air, but, on the other hand, certain evil-smelling substances may be eliminated, while oxygen will be absorbed. Opinion is divided as to whether the latter is advantageous or not. *Aeration* tends to inhibit the development of lactic acid bacteria, but it may possibly promote the production of aroma; at any rate the amount of volatile acids is increased thereby[2]. It will be best to pour the starter into the cream tanks before the cream, so that the souring of the cream may commence immediately after the pasteurisation; the surviving microorganisms (chiefly bacterial spores) will then have no time to develop. It follows as a matter of course that the starter must be properly distributed throughout the whole bulk of the cream, and the cream should be stirred occasionally so that it may become uniformly sour.

As the **amount of starter** which is necessary to sour the cream in a given time depends on the **souring temperature,** these two factors may be discussed simultaneously. As the harmful bacteria which have resisted pasteurisation grow best at high temperatures, the cream should be soured at the lowest possible temperature. The author has found the optimum for vigorous cultures of *Streptococcus cremoris* to be 25° C., so that there is no reason why this temperature should be exceeded. On the other hand, the temperature should not be so low that the lactic acid bacteria do not thrive well, for then the full aroma will not be obtained. Without doubt, 18° to 20° C. is the best temperature, and if, as is sometimes the case in Denmark, a somewhat lower temperature of 15° C. to 17° is frequently employed, it is either because the dairies have not the equipment necessary to cool the cream to a low temperature after souring, or else for the reason that it is obviously more convenient to carry out the process in such a way that the cream will be as nearly as possible at the churning temperature when it is ripe. In order to secure a pure fermentation, and especially to obtain a good firm butter, *Rosengren* has recommended in Sweden, and *Weigmann* in Germany, to sour the cream at a still lower temperature, *i.e.*, at 8° to 12° C., and to add 10 to 20 per cent. of starter. It will be seen that many different methods are used in practice, and it is hardly possible to lay down any hard and fast rule except the following:—*The fresher and cleaner the milk, the higher should be the souring temperature, and the lower the percentage of starter used, and conversely,*

[1] See the author's article "Can a Cleaner Churning be attained by a more thorough Cooling of the Cream." ("Maelkeritidende," 1910, p. 801).

[2] *Kayser,* "Contribution à l'étude de la fermentation lactique," Doctoral thesis, Paris, 1894.

the older and dirtier the milk, the lower should be the temperature, and the higher the percentage of starter. If the souring is carried out at 18° to 20° C., the amount of starter should, nevertheless, not be below 4 to 5 per cent., for the cream should in any case be ripe before nine p.m., so that there will be time to cool it in order that it may stand at a low temperature overnight. The completer the cooling, the less will be the likelihood of oversouring, the smaller the loss on churning, and the firmer the butter. Cooling is best accomplished by the addition of crushed ice which is stirred into the cream. If ice is not available, it is necessary to use ripening tanks, furnished with cooling jackets or pipes, through which cold water can be circulated. *Ahlborn's* cream-ripening tank[1] is of a type which can specially be recommended; several imitations of it are made in Denmark, some of them being improvements on the original type. It is doubtful whether any advantage is to be gained by covering the ripening tank with a lid if it stands in a clean and dry room; the air between the cream surface and the lid will be saturated with moisture, thus affording ideal conditions for the development of moulds. For example, if two plates of milk are left to sour, one of them being covered with another plate, a vigorous growth of mould will be found on the surface of the covered milk after twenty-four hours, while none will be seen on the uncovered milk.

We may now discuss the **starter,** which influences the quality of the butter more than anything else. It is of great importance to keep the starter pure, cleaning and sterilising everything with which it comes into contact with the greatest care. Only perfectly fresh morning milk must be used in its preparation. By the systematic application of the reductase and fermenting test (see p. 166), it will be easy to pick out the farmers who supply the cleanest milk. By passing through a clean separator, the milk will be further purified, for, as was mentioned above, a large proportion of the bacteria pass into the cream, and particularly into the separator slime. As regards the pasteurisation of the starter milk, the chief point is to carry it out in such a manner as to secure the best conditions for the development of the lactic acid bacteria which are to be added to the milk. Formerly the lactic acid bacteria were supposed to thrive best in raw milk, as this contains dissolved albumin, and from this point of view, low temperature pasteurisation would be the best. On the other hand, there is a good deal to be said in favour of high temperature pasteurisation, which destroys the foreign bacteria more effectively and also destroys the bactericidal constituents of the milk, which

[1] "Maelkeritidende," 1910, p. 1094.

may inhibit the activity of the useful and the harmful organisms alike. In order to clear up this question, the author made a series of souring experiments, in which milk heated for half an hour to various temperatures was used. The experiments were all made with two grades of separated milk, one showing a low bacterial count (A), and the other being an average retail milk (B). The samples were inoculated with 1 per cent. of a culture of lactic acid bacteria in milk.

Pasteurising temperature.	Bacteria inoculated and temperature at which kept.	B. After 18 hours. Appearance.	B. After 18 hours. Acidity.	B. After 28 hours. Appearance.	B. After 28 hours. Acidity.	Bacteria inoculated and temperature at which kept.	A. After 18 hours. Appearance.	A. After 18 hours. Acidity.	B. After 18 hours. Appearance.	B. After 18 hours. Acidity.	B. After 28 hours. Appearance.	B. After 28 hours. Acidity.	Bacteria inoculated and temperature at which kept.	A. After 18 hours. Appearance.	A. After 18 hours. Acidity.	B. After 18 hours. Appearance.	B. After 18 hours. Acidity.	B. After 28 hours. Appearance.	B. After 28 hours. Acidity.
Raw.	None.	g_1	32	g_1	36	None.	g_1	34	g_1	38	g_1	38	None.	s_2	30	s_3	29	s_3	35
60°		f	7	f	9		f	8	f	14	,,	24		,,	27	,,	31	,,	36
65°		,,	7	,,	7		,,	7	,,	11	f	22		f	26	,,	32	,,	35
70°		,,	7	,,	7		,,	8	,,	10	,,	19		,,	13	g_1	21	,,	34
75°		,,	7	,,	8		,,	8	,,	6	,,	12		,,	11	f	18	c_1	31
80°		,,	7	,,	8		,,	7	,,	7	,,	7		,,	8	,,	8	f	8
85°		,,	7	,,	7		,,	7	,,	7	,,	7		,,	8	,,	8	,,	8
90°		,,	7	,,	7		,,	7	,,	7	,,	7		,,	8	,,	8	,,	14
95°		,,	7	,,	7		,,	7	,,	6	,,	7		,,	8	,,	8	,,	8
Sterile.	20°	,,	8	,,	8	30°	,,	8	,,	8	,,	8	40°	,,	8	,,	8	,,	8
Raw.	None.	f	13	c_1	14	*Strepto-*	g_2	34	g_3	38	g_3	40	*Strepto-*			s_2	28	c_2	35
60°		,,	11	f	12	*coccus*	g_1	25	g_1	27	g_1	36	*coccus*			,,	27	,,	33
65°		,,	7	,,	10	*cremoris.*	f	15	f	21	f	36	*thermo-*			,,	25	,,	30
70°		,,	9	,,	13		,,	11	,,	20	,,	33	*philus.*			f	19	s_3	24
75°		c_1	15	c_1	17		,,	10	,,	11	f	18				,,	19	s_1	24
80°		g_1	16	g_1 c_1	16		,,	10	,,	11	g_1	33				g_1	29	,,	34
85°		,,	15	g_1	17		,,	10	,,	11	,,	31				,,	29	g_1	33
90°		,,	14	,,	16		,,	12	,,	18	,,	28				g_1 c_1	30	g_1 c_1	33
95°		,,	14	,,	15		,,	15	,,	22	,,	33				g_1	33	,,	36
Sterile.	64°	f	8	f	8	30°	,,	20	,,	29	,,	31	40°			g_1	37	g_1	40
Raw.						*Strepto-*	g_1	34	g_3	38	g_2	40	*Thermo-*	s_3	34	s_1 c_1	39	s_1 c_1	60
60°						*coccus*	f	16	g_1 s_1	27	g_1 s_1	34	*bacte-*	,,	30	,,	33	s_1	43
65°						*lactis.*	,,	16	,,	26	,,	33	*rium*	f	26	,,	35	s_1 c_1	50
70°							,,	15	fg_1	29	g_1	35	*helve-*	,,	9	f	20	g_1 s_1	43
75°							,,	14	g_1	32	,,	37	*ticum.*	f g_1	21	s_1 c_1	36	c_1	63
80°							,,	17	,,	34	,,	39		f	12	f	16	g_2	44
85°							,,	17	,,	35	,,	39		,,	12	,,	26	g_1	30
90°							,,	14	,,	33	,,	36		,,	13	,,	16	g_1 s_1	36
95°							,,	14	,,	33	,,	37		,,	13	,,	16	g_2	21
Sterile.						30°	,,	19	,,	35	,,	38	40°	,,	22	,,	35	,,	60
Raw.						Vigorous	g_1	41	g_1	36	g_1	40	*Thermo-*	g_2	71	g_1 c_1	64	g_3	80
60°						starter.	,,	40	,,	34	,,	39	*bacte-*	f	15	g_1	51	g_1	64
65°							,,	41	,,	35	,,	41	*rium*	,,	16	g_1 c_1	41	,,	48
70°							,,	41	,,	35	,,	42	*bulga-*	,,	19	f	18	s_2	27
75°						A 30	,,	42	,,	34	,,	41	*ricum.*	,,	19	,,	20	g_1 s_1	28
80°							,,	44	,,	36	,,	42		,,	18	f_1 g_1	19	c_1	27
85°						B 30	,,	40	,,	35	,,	40		f g_1	19	g_1	20	,,	31
90°							,,	42	,,	32	,,	41		g_1	21	,,	21	g_1	30
95°							,,	43	,,	33	,,	41		,,	24	,,	36	,,	39
Sterile.							,,	43	,,	35	,,	42	40°	,,	63	,,	60	,,	70

f = Fluid. g = Gelatinous curdled. s = Spongy curdled. c = Cheesy (casein contracted and much clear whey). fg = Semi-solid.

See further under the "Fermentation Test."

The acidity is given in Soxhlet-Henkel degrees (see p. 161).

The original acidity of the milk was 7; the acidity of the sterilised milk was, however, 8.

The table first shows the behaviour of the uninoculated milk on standing; at 20° C. it was practically unaltered after twenty-eight hours, which is explained by the slow growth of the heat-resisting bacteria at the ordinary temperature, whereas at 40° C. it was affected fairly quickly. The sudden interruption of the increase in acidity on heating the milk above 75° C. is explained by the fact that the heat-resisting lactic acid bacteria will survive heating to 75° C., but not to 80° C.; microscopic examination confirmed this view, as streptococci were found in great numbers in the milk heated to 75°C., but none were found in that heated to 80° C. or more[1]. From the results already considered, we may draw the conclusion that *it is impossible to be sure of the exclusion of foreign lactic acid bacteria from the starter unless the milk has been heated to at least* 80° C. *Microscopic* examination also shows the milk pasteurised at 75° C. to contain most hay bacilli after standing, 75° C. being the lowest temperature at which the bactericidal substances of the milk are completely destroyed. These points are particularly well illustrated in the case of the milk which was kept at 64° C.; in this milk only those thermophile bacteria grew which were not in the slightest degree affected by the pasteurisation, but which, on the other hand, seem to be fairly sensitive towards the bactericidal substances in the milk. This explains the somewhat paradoxical result that under these conditions the milk which has been heated to 75° C. or over is that which is most rapidly affected.

The conditions are still more complicated in the inoculated milk. In the raw as well as in the low temperature pasteurised milk the lactic acid fermentation is inhibited by the bactericidal substances but encouraged by the lactic acid bacteria of the milk itself, and in the milk heated up to 70°, 75° and 80° C., a sharp struggle for existence takes place between the lactic acid and the hay bacteria, which often results favourably for the latter at the outset. Finally, it must be borne in mind that the higher the temperature to which the milk has been heated, the less oxygen will it contain. As the different species of lactic acid bacteria are differently affected by the conditions under consideration, no definite rule for pasteurisation can be laid down on this head. Thus it was found that *Streptococcus cremoris* and the nearly related *Sc. thermophilus* develop most slowly in the milk heated to 75° C. (and the latter also in the milk heated to 70° C.), while *Sc. lactis* shows a minimum capacity for souring (though not very pronounced) in the milk which has been pasteurised at low temperatures and retained its bactericidal constituents. The point of special interest is that the vigorous lactic acid culture (starter) is only slightly affected by the

[1] Living micrococci are, however, still found in milk pasteurised at 80° C.; they grow best at 20° C.

previous treatment of the milk (perhaps a maximum capacity for souring is found in that heated to 80° C.). On the other hand, the two thermobacteria which are distinctly anaerobic in character are greatly influenced by the previous treatment of the milk, as is seen from the acidity and also, in the case of *Thermobacterium bulgaricum* (of Yoghurt), by direct microscopic examination (*cf.* the illustrations, p. 30). Both these organisms grow best in raw milk, where the oxygen is quickly consumed by other organisms, and in sterile milk which is quite free from oxygen. *Thermobacterium helveticum* also shows a maximum of souring in the milk heated to 75° C. in which the bactericidal substances have been destroyed, but which still contains a little dissolved albumin. Of the lactic acid bacteria employed in these experiments, this was the only one which was influenced by the albumin, all the others growing better in milk which had been heated to 80° C. or above.

From what has been said we may draw the conclusion that no harm will be done by pasteurising the starter milk thoroughly. All that is necessary is to avoid discolouration or burning, which may cause difficulty in judging the aroma of the starter or impart a cooked flavour to the butter in cases where large amounts of starter are used. Experience shows that pasteurisation for one hour at 85° gives good results. The milk is heated in a water bath, stirring frequently, and it is cooled as quickly as possible to the souring temperature, stirring carefully, the pail or can being placed in running water. Aeration by pouring the milk from vessel to vessel is unnecessary; the risk of infection is only increased by exposure to the air, and especially by contact with more vessels than is strictly necessary. It is a great mistake to pass the pasteurised milk over a cream cooler. As in the case of cream, undesirable fermentations are best avoided by keeping the souring temperature low, and moreover, it is unsound as a matter of principle to accustom the bacteria to a sensibly higher temperature in the starter than that at which they are required to act in the cream. A temperature of 22° to 23° C. is quite satisfactory if only this is kept constant by placing the starter cans in a sufficiently large water-bath at the right temperature. *Boekhout's* and *Storch's* aroma bacteria, it may be noted, do not thrive well at higher temperatures. On the other hand, it is not advisable to lower the temperature further, as *Sc. cremoris* nearly always degenerates on propagation several times at temperatures below 22° C. As cream is always soured under this temperature, we find here a new and hitherto unknown reason why the use of buttermilk for this process is unsatisfactory in the long run. The water-bath may be made of wood or galvanised or tinned iron, and covered in in the same way as the culture apparatus, so that only

the tops of the cans project. The cans are covered with steam sterilised double covers which allow of air circulation. As nothing is gained by keeping the starter cold overnight, there is no reason to add more culture than is necessary to ripen the starter before the following morning; about ½ to 1 per cent. is sufficient. Immediately before use the starter is skimmed, as the top layer will be richest in foreign germs if infection has taken place[1]. The starter is given a more liquid consistency by stirring it vigorously, after which it is poured into the cream ripening tank. As the starter is more easily titrated than cream, its acidity should be checked from time to time. Experience has shown that the best results are obtained with an acidity of 90° to 100° (number of cubic centimetres of normal soda per litre, corresponding to 18 to 20 c.c. of decinormal soda per 20 c.c. of milk). This corresponds to 36 to 40 *Soxhlet-Henkel* degrees. If the starter becomes weak and shows a lower acidity although it has been kept at a temperature over 22° C. all the time, the culture used is weak, while if the acidity is too high the milk has probably been oversoured, and the most beneficial bacteria will not be in full vigour. The culture will thus often contain rod-shaped lactic acid bacteria, in which case it will be best to use a new culture.

What has been said regarding the starter also applies to the **culture** from which the starter is made, only in this case still greater care is necessary, for an infection in the starter need only degrade the quality of a single churning, while an infected culture may cause repeated trouble. Fortunately it is easier to keep the culture pure, and there is not the same difficulty in obtaining the small amounts of finest grade milk required for this purpose as may be experienced in obtaining sufficient quantities of milk in the condition in which it should be used for the starter. Herein lies the advantage of propagating the culture and the starter separately instead of simply inoculating the new starter milk with some of the old starter. It is a good plan to keep two or three cultures going independently of one another, inoculating daily into fresh milk, for by comparing the tastes of the different cultures the detection of defects in taste is much facilitated and as it would be a very exceptional misfortune if all the cultures became bad at once, at least one good culture will always be available if the precaution is taken of renewing any bad cultures as soon as possible. Several forms of culture making apparatus are on the market; it is important that means should be provided

[1] If the milk is stirred during the souring there is no benefit to be derived from this precaution. According to *Boström's* experiments at Alnarp dairy, the lactic acid bacteria thrive better if the milk is stirred occasionally. By this means local over- or under-souring is avoided.

for keeping the temperature of the milk as constant as possible during the souring period.

Before leaving the subject of cream ripening, it may be pointed out that the so-called **souring defects** need by no means be ascribed to infections of totally foreign groups of microorganisms such as pseudo lactic acid bacteria, yeasts and moulds; as often as not they may originate from the true lactic acid bacteria. Thus *Storch* once isolated a lactic acid bacterium which was able to produce a *tallowy* taste in cream and butter, and *C. O. Jensen* found, almost simultaneously, lactic acid bacteria, some of which gave the butter an *oily* taste, and others which imparted a *burnt* or *malty* taste [1]. The last-mentioned taste is often produced by strains of *Sc. lactis*. From the foregoing it will be seen that the species of lactic acid bacterium chosen for the culture is by no means a matter of indifference.

Buttermilk is obtained as a by-product from butter making; its good qualities have already been mentioned. It becomes particularly good when the cream is pasteurised and soured with a pure starter. As the most favourable stage of the lactic acid fermentation has always been reached before the churning, the buttermilk quickly deteriorates in taste and throws out large lumps of casein unless it is kept quite cold. It follows as a matter of course that water should not be added to buttermilk which is to be retailed in towns; under these circumstances all washing and rinsing of the butter should be done with ice-cooled buttermilk from a previous churning. Separated milk is not so suitable for this purpose, as it causes the buttermilk to curdle more quickly. The undiluted buttermilk will always have a lower acidity than the starter which has been used in souring the cream from which it is made, even if the cream and the starter milk have been soured in exactly the same way. *Tholstrup Pedersen* [2] has shown that the difference is largely due to the fact that the buttermilk loses its carbonic acid during churning; the sour starter will also show a lower acidity if it is shaken before titration.

The Souring of Separated Milk.—In the manufacture of margarine, separated milk is soured and churned into an emulsion with melted fat. The aroma of the soured milk is taken up by the fat in much the same way as occurs in butter making; the difference between the two cases lies in the fact that while the aroma is taken up by the fat globules in the cream during the ripening process, this occurs in margarine only during the churning, or emulsifying process, and the ensuing operations. As regards the actual souring of the separated milk, this subject has

[1] "Forsøgslaboratoriets," 22 de Beretning, 1891.
[2] "Maelkeritidende," 1916, p. 65.

been discussed at some length in dealing with the preparation of starters for the ripening of cream, and the process does not differ materially from that described under the souring of cream. *Blichfeldt*[1] has devised an appliance for the **continuous souring of separated milk,** consisting of a closed cylindrical vessel into which fresh separated milk is introduced, and from which soured skim milk is withdrawn simultaneously. The contents of the vessel are kept stirred, and by regulating the temperature and the rate of output, the acidity of the product may be kept constant. The apparatus is worked under sterile conditions as far as the avoidance of infection from outside is concerned, while the fresh separated milk must be efficiently pasteurised before use, which of course is also the case in the tank souring process. The continuous process has the advantage that by its means a large amount of milk may be treated in a relatively small space.

The Preservation of Stable Manure by addition of Whey.—This process has been proposed by *Barthel*[2], and will have economic value where whey is plentiful and peat is unobtainable. The lactic acid fixes the ammonia in a form easily available for the nutrition of plants (or the nitrifying bacteria). By using 50 to 100 litres of whey per 1,000 kilos of manure, at a cost of about 7*d*., the increased yield to be obtained from good soil will amount to the value of 6*s*. 8*d*. In this connection it may be mentioned that all dairy refuse, even the washing water, has great fertilising value in virtue of the nitrogenous matter which it contains. The washing water should therefore, wherever possible, be used for surface irrigation on the fields instead of, as is sometimes done, run into ditches where it will putrefy. In this case the milk sugar, or rather the lactic acid formed therefrom, is a drawback, but as a rule the latter will be neutralised by the large amounts of lime used in cleaning.

[1] English patent 4504, 1912. The patent covers continuous fermentations.

[2] *C. Barthel* and *Sigurd Rhodin*, Meddelande Nr. 57 från Centralanstalten för försöksvasanet på jordbruksonirådanet, 1912.

Chapter V

The Normal and Abnormal Microflora of Butter

THE NORMAL FLORA

FRESH **butter from unpasteurised cream** will naturally contain the same microorganisms as milk, and the bacterial changes which take place on keeping will be the same as those which occur in milk kept at the same temperature. Thus at low temperatures water bacteria will tend to predominate, while at the ordinary temperature the fresh butter will soon become sour owing to the rapid development of lactic acid bacteria, and, in particular, *streptococci*. Later on, lactic acid rod bacteria, yeasts and moulds appear. As the moulds, which hydrolyse the fat, only appear on the *surface*, the keeping qualities of the butter will be greatly enhanced by packing it in large casks instead of in small flat slabs. Such small pieces will, unless frozen, be subject in the course of a few days to the same changes, originating on the surface and gradually working towards the centre, as occur in a mouldy soft cheese. **Butter from pasteurised ripened cream** will have a much simpler flora to begin with, as *Streptococcus cremoris*, used in the ripening process, will generally be the principal organism present. This organism, however, does not seem to be capable of living long in butter, and is gradually replaced by yeast, and generally also by lactic acid rod bacteria. Moulds, which are an unavoidable infection from the air, gradually appear on the surface. As the yeasts and bacteria only develop in the small drops of water which constitute only about one-sixth of the weight of the butter, it is obvious that butter will never show such high bacterial counts as milk and cheese. The number of microorganisms in butter will depend on the amount of nutrient matter and antiseptic substances present in the water droplets, *i.e.*, on the *washing*, *working* and *addition of salt, boric acid or other preservatives* [1]. Properly treated butter seldom contains

[1] The preserving action of salt is more pronounced the lower the percentage of water in the butter. Thus in butter containing 2 per cent. of salt, the aqueous portion will contain 12·5 per cent. of salt if the water percentage is 16, but 20 per cent. if the water percentage is 10. The development of microorganisms is only completely inhibited when the

more than a few million bacteria per cubic centimetre. In butter from unripened cream, the bacterial count increases during the first few days [1], after which it decreases. In butter from ripened cream the count is highest when the butter is freshly made (ten to twenty million lactic acid bacteria per cubic centimetre), and decreases steadily in the course of a few weeks to a few hundred thousand, and sometimes even to as low a figure as a few thousand, the reason being that the original lactic acid bacteria die off at a quicker rate than their successors develop, for the available nutrient matter, especially lactose, is continually falling off. In cases where fat hydrolysis is taking place, this source of carbon will be replaced by glycerine, and the lactic acid bacteria will then again fare better.

On keeping for any length of time, butter acquires a stronger flavour, and it becomes rancid quicker than other fatty materials on account of the large amounts of water and nutrient substances which it contains. Before the introduction of margarine, compound and other substitutes, it was a common custom in Central Europe to preserve the butter fat by melting and separating off the other constituents ; the pure butter fat kept good if preserved in well closed stone jars in a cool place.

Duclaux [2] was the first to study **the changes which take place in butter on keeping.** He found that direct sunlight promoted the action of atmospheric oxygen, *i.e.*, its function was similar to that of the oxidases, causing oxygen to combine with the constituents of the butter-fat, especially the olein. *Duclaux* also found that butter was spoiled by various moulds to which he ascribed an action similar to that of sunlight in oxidising the fat. The author's investigations [3] show that there is an important difference between the action of sunlight and that of micro-organisms. While *fats are principally oxidised under the action of sunlight*, the iodine value being reduced, *they are hydrolysed by the microorganisms into fatty acids and glycerine*, the acid value being increased. Oxidation causes changes in taste which are much more undesirable than those caused by hydrolysis ; if butter is

concentration of salt reaches 25 per cent., *e.g.*, with 13 per cent. of water and 3·3 per cent. of salt (tinned butter). If permissible by law, it is specially recommended to add 0·75 per cent. of benzoic acid, or 2 per cent. of sodium benzoate, or a mixture of 0·5 per cent. of benzoic acid and 0·5 per cent. of sodium benzoate. As benzoic acid is a physiological product (it is transformed to hippuric acid), it may be regarded as one of the less objectionable preservatives.

[1] The highest count ever obtained by the author from butter made from unripened cream was 59 millions per cubic centimetre.

[2] "Le lait," Paris, 1894.

[3] "Studien über das Ranzigwerden der Butter" ("Centralblatt f. Bakt.," 2 Abt., 1902, Bd. VIII., p. 11). References to earlier literature given here.

exposed to strong sunlight for only one hour, its surface becomes bleached and perfectly uneatable. The taste thus produced bears the closest resemblance to that of bad tallow, and is generally described as "*tallowy*." *The rancid taste proper* is only produced by the microorganisms, and is due to the lower members of the fatty acid series, *i.e.*, the volatile fatty acids, as well as certain esters which have a fruity odour, such as ethyl and amyl acetate. The glycerine, which is usually completely transformed by the microorganisms, is the starting substance in the formation of the esters as well as of the alcohols which may be formed. As most fat hydrolysing organisms require air for their development, *the exclusion of air* is the most important precaution necessary to protect the butter from the changes under discussion. For this reason, butter intended for use in warm climates is packed in hermetically sealed tins. *Heat*, like sunlight, promotes the oxidation of the fat. According to *Ritsert*[1], *carbonic acid*, even in darkness, will impart a tallowy taste to butter; this point is not without bearing on the ripening of cheese, as considerable quantities of carbonic acid are often produced in cheese.

The most important fat hydrolysing microorganisms are *Bacterium fluorescens liquefaciens*, *Bacterium prodigiosum*, *Oidium lactis*, *Penicillium glaucum* and *Cladosporium butyri*. As they do not form spores, they are all easily destroyed on heating; of the two bacteria, *B. fluorescens liquefaciens* is, as mentioned above, very widely distributed in water, for which reason there is a danger in letting butter come into contact with water or ice. In many cases the advantages gained by pasteurisation are nullified when we introduce bacteria with the washing water, which have a worse effect on the keeping qualities of the butter than those which were destroyed in pasteurising. It should be made a principle to pasteurise (in a regenerative apparatus) all the water used for rinsing the cream cans, ripening tanks and churns, and for washing the butter. Moulds may come from the starter (or from the milk if the cream has not been pasteurised), from the air or from the packing material. By treating the casks and parchment paper for twenty-four hours or longer with concentrated brine, nutrient substances are extracted and the mould spores are considerably weakened. It is better to treat the inside of the casks with melted paraffin wax immediately before use; they will then be sterilised and rendered airtight. If this is done, parchment paper may be dispensed with, and the wood will not soak up brine and increase the tare at the expense of the nett weight[2]. Although *Oidium lactis* and *Penicillium glaucum*

[1] Inaugural dissertation, Berne, 1890.
[2] *Rogers*, U.S. Dept. of Agric., Bureau of Anim. Ind., 1906, Bull. No. 89.

are the most active in hydrolysing fat, they do not spoil the butter to the same extent as the above-mentioned bacteria. Not only do the moulds hydrolyse the fat, but they also consume part of the free fatty acids, especially the lower members of the series; the acids produced by the bacteria, however, are allowed to accumulate to such an extent that the fat hydrolysing bacteria, which, curiously enough, are fairly sensitive to acid, are destroyed. This explains the fact that very rancid butter may occasionally be sterile some distance from the surface. The formation of the esters which are so characteristic of rancid butter is due to *Penicillium glaucum* and especially to *Cladosporium butyri*. The former, however, only forms esters in symbiosis with *Oidium lactis*, which mould also promotes ester formation by *Cladosporium butyri*. Certain mycodermæ which do not themselves hydrolyse fat may also promote the formation of esters. According to the investigations of *H. C. Jacobsen*[1], exactly the same microorganisms are responsible for rancidity in margarine.

All the above-mentioned organisms grow better and quicker in unsalted butter than in salted butter. The water bacteria are particularly sensitive to salt, and by packing the butter in large casks the moulds, which require air, will obtain the smallest possible surface for attack; Danish butter and similarly prepared butters therefore contain, as a rule, only lactic acid bacteria and yeast. Among the yeasts, however, there are certain torulæ which are by no means innocuous and which may, especially in symbiosis with lactic acid bacteria, hydrolyse butter-fat to varying extents. The reason why the lactic acid bacteria promote hydrolysis in such cases is that the yeasts in question thrive best in presence of a little lactic acid[2]. While the lactic acid bacteria, generally speaking, promote the growth of yeasts and moulds, they inhibit the action of the fat hydrolysing bacteria to a considerable extent, so that it is difficult on the whole to say whether the souring of cream will as a general rule contribute towards the preservation of butter. According to the observations of *Rogers* and *Gray*[3], butter from pasteurised sweet cream keeps better than that from pasteurised sour cream, and the addition of a little lactic acid to the pasteurised cream has the same undesirable effect as souring with a starter. In all circumstances it is essential that the starter used should contain only pure cultures of good lactic acid bacteria, and no yeasts or moulds. The safest method of preventing yeasts and moulds as well as other harmful organisms from developing to any extent in

[1] "Folia Microbiologica," 1918, V. 2.
[2] *Orla Jensen*, "Maelkeritidende," 1910, p. 965.
[3] Experimental Station Record, 1909, No. 5.

[redacted]

The original defects in taste are due to original milk defects (and therefore also possibly to the feed), to milk defects arising at a later stage, *i.e.*, secondary defects, faulty ripening and impure salt. What has been said under the heading of milk regarding **stable, grass, turnipy** and **bitter tastes,** applies also to butter. When butter has a strong taste of grass it often contains numerous small gas bubbles, which show the defect to be due to gas-producing organisms, *i.e.*, intestinal bacteria. Yeasts which ferment lactose may produce gas bubbles and give to the butter a peculiar **yeast-like taste.** Defects of this nature are generally to be avoided by pasteurising the cream. On the other hand, pasteurisation will not prevent a **metallic taste** (which may arise in butter through washing with ferruginous water), and secondary defects due to *faulty souring*. A **cooked taste** is not so often due to heating the cream to too high a temperature as to heating it for too long, as may occur if the cooling after pasteurisation is too slow; the taste often arises through the milk having too high an acidity, which causes the proteins to separate and burn on the pasteuriser. A **burnt taste** may also arise in this way; as already mentioned, a **burnt, oily** or **tallowy taste** may also be due to faulty souring, while the effect of sunlight or copper salts in producing tallowiness has also been alluded to. If the salt used contains appreciable amounts of magnesium salts, it may give a bitter taste.

Although for the sake of uniformity distinction has been made between original and secondary defects, it must be admitted that the line of demarcation between the two is by no means sharp. Thus a defect such as *unclean taste*, which is produced by various putrefactive bacteria (proteus, coli bacteria, etc.), may develop sooner or later in the history of the butter. The term original defects will be applied to such defects as come out immediately or during the first few days after the butter has been made, and then disappear either partially or completely; by secondary defects will be understood those which develop gradually and become worse as time goes on.

Secondary defects are of course counteracted by any conditions tending to increase the keeping powers of the butter, *i.e.*, good raw material, efficient pasteurising, pure starter, proper churning, thorough washing with good water, proper working and salting, clean air, sterile and airtight packing, and the most thorough cooling possible. Defects in appearance include **mouldy spots** which are generally accompanied by a mouldy smell; it must be mentioned that many kinds of moulds may grow on butter besides those which generally cause rancidity, and although most of these organisms may be able to hydrolyse fat, moulds are known which do not do so. *Oidium lactis* cannot as a rule be seen in butter

with the naked eye, but most of the other moulds form *green, brown or black spots.* As has already been mentioned, butter from sweet cream may be coloured *red* by certain torulæ, which in symbiosis with lactic acid bacteria hydrolyse fat and also give an oily taste in butter. *Actinomyces chromogena* turns butter brown and gives it an unpleasant **earthy smell.** Among the secondary defects of taste, the **sour cheesy** taste deserves special consideration. The acid is chiefly due to lactic acid rod bacteria, so that the defect is particularly likely to arise when the butter has not been properly freed from buttermilk or when it contains lumps of casein in which these cheese bacteria may initiate a cheese-ripening process. The defect, however, only attains its worst form when a symbiosis with yeast gives rise to fat hydrolysis [1]. Certain yeasts may produce a **fishy or train-oil taste.** In marshy districts or where the land is occasionally inundated by sea or brackish water, this defect may appear in fresh butter, and is then due to the grass or small crabs which are found in great numbers in the grass. Certain bacteria are also said to be able to produce a fishy taste by forming trimethylamine from lecithin [2]. Some of the defects which may appear in butter after keeping for any length of time are of a purely chemical nature, like the oxidation process discussed above, and their appearance may be accelerated by iron and possibly other salts. It is therefore of importance that the salt used in butter should be chemically pure [3]. It has been said that dairy salt may contain fat hydrolysing bacteria [4]. Fresh pure salt is of course sterile, but when kept in the dairy, numerous organisms (thousands per gram) may collect on its surface, and *Weigmann* has therefore proposed to dry the salt in an air oven at 100° C. before use.

As butter defects which are apparently of the same nature may arise in different ways, and conversely, as is often seen to be the case in butter grading, the same defect may pass under different names, it is hardly possible in the present state of our knowledge to go into further detail as regards the secondary defects. As, moreover, most of the defects sooner or later pass into the stage

[1] The author was the first to show that the ability of certain yeasts to hydrolyse fat is promoted in the presence of lactic acid bacteria: "Bakteriologische Studien über die dänische Butter" ("Centralblatt f. Bakt.," 2 Abt., 1911, Bd. XXIX., p. 610). This was later confirmed by *Sandelin:* "Die Hefen der Butter," Helsingfors, 1919.

[2] Thus, *Cusick* ("Journal of Dairy Science," 1920, Vol. III., p. 194) is of the opinion that this defect can be produced by *Bact. ichthyosmius*, a motile Gram-negative rod showing dirty white surface growth, peptonising milk while producing slight amounts of acid, and producing gas from cane sugar, but not from lactose.

[3] *Rogers, Berg* and *Potteiger*, U.S. Dept. of Agric., Bureau of Anim. Ind., 1913, Bull. 162.

[4] *Wolff*, "Milchzeitung," 1914, p. 545.

known as *rancidity*, it is probable that they are only forerunners of this principal defect.

The dairies can easily control the keeping qualities of the butter which they produce, and thus of any possible defects, by making a practice of keeping samples of the freshly worked product in small jars at about 8° to 14° C., and tasting these after a week and a fortnight.

Chapter VI

The Ripening Processes of the Different Cheeses

THE *methods of preservation*, involving the use of preservatives or bactericidal substances, include the use of harmless acids such as acetic or, better, lactic acid. The latter is not added but produced by allowing the material to be preserved to set up a lactic acid fermentation. This method is applied to the preservation of beet slices, white cabbage (*sauerkraut*) and other sugar containing fodders and foodstuffs which contain too much water to be dried without the aid of artificial heat. In such cases it is only necessary to exclude air as completely as possible so that the acid-consuming moulds are kept down. The same process may also be applied to milk. If the yeasts and moulds are destroyed by pasteurising in bottles, and the milk is inoculated with a vigorous culture of lactic acid bacteria, it will remain unchanged after the souring process has been completed ; a similar principle is applied in the making of cheese, which is always based on a vigorous development of lactic acid bacteria. Under normal conditions the acid which is produced will always inhibit the growth of other bacteria, and in the closely pressed mass of cheese no moulds will develop, owing to the absence of air. If, as in the case of the hard cheeses, an additional protection is afforded in the shape of a firm cheese rind, all risk of infection is excluded, and a permanent product is obtained.

The origin of cheese making was without doubt a desire to preserve the valuable constituents of milk in a permanent and easily marketable form. The primitive process, therefore, only involved the drying and salting of the curd, a process which is still employed in several places in the East. Subsequently it was discovered that the curd would also keep without drying, and that with suitable treatment it would acquire other valuable properties into the bargain. The art of cheese making thus became not only one of mere conservation, but also the production of a *palatable food*, and in the case of the soft cheeses it may well be said that attention has been concentrated on the latter point.

The curd is separated by the action of either *rennet or lactic acid* ; we may commence with an examination of the mechanism of the two processes.

The active principle in **rennet** is a *proteolytic enzyme Chymosin*, the action of which does not cease with the coagulation of the milk and the contraction of the casein, but continues in the cheese with the formation of soluble proteins. The author's researches have established that cheese rennet exerts a powerful solvent action on the proteins of milk, and that this action is promoted by the addition of small amounts of acid [1]. American researches [2] have shown that the ripening of cheese is accelerated by increasing the amount of rennet used. As pepsin is also present in rennet, it was formerly supposed that the solvent action on the proteins was exclusively due to this enzyme. Careful investigation, however, has conclusively shown that chymosin itself is a proteolytic enzyme which can act in the presence of smaller amounts of acid than pepsin [3]. The addition of pepsin to the milk used for cheese making appears to have no influence on the ripening process. Trypsin, on the other hand, has a decided influence on cheese, but may easily cause a bitter taste [4]. In this connection, mention may be made of **galactase,** a proteolytic enzyme which, according to researches by *Babcock* and *Russel* [5], is a normal constituent of milk, and which just after its discovery in 1897 was assumed to play a most important part in the ripening of cheese. Subsequently it transpired that galactase does not play any notable part in the ripening of soft cheeses, in which, however, it is particularly plentiful [6]; and the fact that this enzyme is not indispensable to the ripening of hard cheeses is amply demonstrated by the fact that good cheese has been produced on a large scale from milk which has been heated to over 80° C., at which temperature the enzyme is destroyed.

As regards the **action of lactic acid,** this is of interest not only in the making of sour milk cheeses, but also in the making of rennet cheeses. As is well-known, casein occurs in milk as a calcium salt, a dicalcium caseinate (the calcium compounds of the proteins usually form milky solutions in water), and when casein is precipitated by acid it is not due to transformation into paracasein as occurs with rennet, but simply to the abstraction of lime by the acid. At the same time a little lactoglobulin is precipitated. The greater the percentage of casein in the milk, the more acid

[1] "Landwirtschaftliches Jahrbuch der Schweiz," 1904, p. 404, and 1907, p. 97.
[2] Seventeenth Report of Wisconsin Agricultural Experiment Station, Madison, 1900.
[3] *Petry*, "Wiener Klinische Wochenschrift," 1906, p. 143.
[4] *Orla Jensen*, "Nyt Tidskrift for Fysik og Kemi," 1897, p. 92, and "Landwirtschaftliches Jahrbuch der Schweiz," 1901, p. 197.
[5] Wisconsin Agric. Expt. Station, Bull. 14, 15 and 19.
[6] *Orla Jensen*, "Centralblatt f. Bakt.," 2 Abt., 1900, Bd. VI., p. 793.

will be required for complete coagulation. The higher the temperature, the easier the coagulation. Thus, at 18°, 30°, 40° and 100° C., 0·6, 0·5, 0·25 and 0·1 per cent. respectively of lactic acid will generally be required, corresponding to 80, 72·5, 53·5 and 27·5 c.c. of decinormal caustic soda per 100 c.c. respectively. For this reason, milk is often warmed when the casein is to be precipitated. If a temperature of 70° C. is exceeded, appreciable amounts of albumin are also precipitated ; larger yields of cheese are therefore obtained by using milk pasteurised at high temperatures. On heating to over 90° C., all the albumin and globulin are precipitated ; the more completely this takes place the more easily will the milk coagulate, for all solid particles, including fat globules, serve to stiffen the coagulum. The same milk will therefore coagulate at a lower acidity when pasteurised at a high temperature than when pasteurised at a low temperature. Acid coagulum differs from rennet coagulum in not contracting so much, so that it does not separate such large quantities of whey when allowed to stand undisturbed. This affords a means of distinguishing between the two types of coagulation. In the case of a bacterium which coagulates milk, a titration will at once decide whether a quantity of acid sufficient to be entirely responsible for the coagulation has been produced ; if not, then the bacterium must also have produced a coagulating enzyme like rennet. The coagulating power of rennet is increased by the addition of acid ; the reason for this is not only that the acid promotes the action of the enzyme, but also that it forms soluble calcium salts which facilitate the precipitation of the paracasein. The coagulation of pasteurised milk by rennet may thus be promoted by the addition of a fair amount of acid, *e.g.*, in the form of buttermilk [1], and also by the addition of calcium chloride (100 c.c. of a 40 to 50 per cent. solution to 100 litres of milk). The contraction of the rennet coagulum is also promoted to a certain degree by the addition of acid, the whey separating most readily at a concentration of about $\frac{1}{4}$ per cent. of lactic acid, when all the casein is converted into the mono-calcium salt ; for this reason, the cheese dries better if prepared from slightly acid milk than if prepared from fresh milk. If the milk is so sour that there is a suspicion that the resulting cheese will be too dry, all that is necessary is to dilute it with water before adding the rennet ; in this way, the concentration of both the free acid and the soluble calcium salts will be decreased. Conversely if a higher acidity is desired, the

[1] In the making of Cheddar cheese from pasteurised milk, *Sammis* and *Bruhn* (Bureau of Anim. Ind., 1913, Bull. 165) recommend besides the addition of starter milk containing $\frac{1}{4}$ per cent. of acid the use of 1 part of normal hydrochloric acid per 100 parts of milk.

milk may be allowed to ripen at a temperature favourable to the development of good lactic acid bacteria (15° to 20° C.), or an appropriate amount of buttermilk, starter or other lactic acid cultures may be added. The longer the time occupied in making the cheese, the sourer will be the curd. The temperature of the curd when placed in the cheese press determines the species of lactic acid bacteria which shall obtain predominance. If scalding is omitted, and the curd is cooled by kneading after the whey has been run off, the bacterial flora will be quite different from that which results when heat is applied and the curd is taken direct from the warm whey. By scalding and carefully cutting the curd, the separation of the whey will be facilitated; a means is thus afforded of shortening the curd forming process, and of regulating the degree of acidity of the cheese.

As nearly half the natural acidity of milk is due to the casein, it follows that the whey must have a much lower acidity than the milk from which it was made. In order to control the process of souring during cheese making, *the whey may be titrated* at different stages. Although the indications thus obtained will be of some value, they are far from accurate, and should be supplemented by a careful examination of the consistency of the curd. On coagulation, with careful treatment of the milk, most of the bacteria become enclosed in the curd and consequently lactic acid fermentation will take place far more rapidly within the curd than in the whey[1]. In the curd, however, most of the acid produced will be neutralised by the lime of the casein and the phosphates; accordingly it will sometimes be found that in spite of a vigorous lactic acid fermentation the acidity of the whey may remain unaltered during the process, or even decrease as happens in the making of Emmental cheese, for owing to the fact that the whey is scalded at a fairly high temperature, the loss of carbonic acid more than counterbalances the gain in lactic acid taken up by the whey from the curd. If the bulk of the whey is removed at an early stage, the remaining whey will be more acid than usual at the end of the process as the lactic acid which has diffused out of the curd will have been diluted to a less extent. *When the casein loses its lime* it becomes, as *van Slyke* and *Hart* were the first to show[2], *fairly readily soluble in a 5 per cent. solution of sodium chloride*, especially at 50° to 55° C., but this property is lost in the presence of an excess of acid. The author has shown[3] that this

[1] *Orla Jensen*, "Uber die im Emmenthalerkäse stattfindende Milchsäuregärung." "Landwirt. Jahrbuch der Schweiz," 1906, p. 287.
[2] New York Agric. Exp. Station, Bull. No. 261, 1905.
[3] "Zeitschrift f. physiologische Chemie," 1914, Bd. XCIII., p. 283.

is due to the easy solubility of monocalcium caseinate and paracaseinate, while free casein and paracasein are practically insoluble in salt solution. As cheese is always salted, these observations have an important bearing on cheese making; it will now be understood why *the acidity of the curd and the way in which it is salted come to have such an important effect on the consistency of the cheese*. When a slightly acid curd is salted or placed in brine, it will swell as if tending to dissolve, becoming elastic and semi-transparent. On the other hand, on excessive acidification or slow dry-salting, the curd will retain its original crispness for a long time. Monocalcium caseinate, though soluble in dilute salt solution, is insoluble in strong brine, for which reason the brine used in the cheese making dairies should contain at least 25 per cent. of salt; as lactic acid is constantly diffusing into it from the cheeses, it should be neutralised and filtered from time to time, as is done in Holland. According to *Rosengren*, 1 to 1·2 per cent. of salt in cheese can be considered normal; 2 per cent. or more makes the cheese dry, and inhibits the fermentation processes.

The action of acid has been dealt with at some length not only because the degree of acidity influences the consistency of the cheese, but also because it has a determining influence on the **course taken by the ripening process** which, owing to the empirical methods of treatment in vogue, determines more than anything else the nature of the resulting cheese. As the maximum acidity attained by the cheese depends first and foremost on the amount of whey which it contains, the classification into more or less sour cheeses accords fairly well with the division into *hard* and *soft cheeses*. In the former the production of acid will practically be limited to the amounts required to neutralise the lime, and the cheese will therefore ripen uniformly throughout. On the other hand, the relatively large amounts of acid present in the soft cheeses will only be neutralised after a considerable lapse of time, and as a rule the process is only completed by the aid of the ammonia which is formed on the surface. It follows that in such cheeses the ripening process will start at the surface and work inwards by degrees; for this reason the ripening may be accelerated by giving the cheeses a large surface relative to their bulk. The foregoing points are illustrated by the accompanying table which gives the percentages of soluble proteins and their decomposition products in different cheeses. Sol. N. stands for soluble nitrogen, *i.e.*. the nitrogen of the soluble proteins, plus that of the protein decomposition products present, Dec. N. for the nitrogen of the protein decomposition products, or amino acids which are not

precipitated by phosphotungstic acid[1]. Am. N. stands for ammonia nitrogen.

				Per cent. of total Nitrogen.			Per cent. of soluble Nitrogen.	
				Sol. N.	Dec. N.	Am. N.	Dec. N.	Am. N.
Hard Cheeses.	1	Emmental cheese, five months old.	Interior.	35·82	17·36	—	48·47	—
			Exterior.	29·22	12·57	—	43·02	—
	2	Emmental cheese twelve months old, ripe.	Interior.	33·15	17·35	2·37	52·34	7·15
	1	Edam cheese four months old, ripe.	Interior.	26·90	3·00	0·60	11·15	2·23
	1	Prize Danish dairy cheese from pasteurised milk, three months old.	Interior.	35·5	9·7	0·22	27·6	0·6
	2	Prize Danish dairy cheese from pasteurised milk, six months old.	Interior.	34·9	9·0	0·17	25·8	0·5
	1	Swiss skim milk cheese, eight months old, ripe.	Interior.	41·51	7·90	6·40	19·03	15·40
			Exterior.	35·90	7·40	5·20	20·61	14·50
	2	Swiss skim-milk cheese, sixteen months old, over-ripe.	Interior.	43·54	6·66	6·85	15·29	15·73
			Exterior.	53·59	9·11	5·37	17·00	10·02
	1	Roquefort cheese, ripe.	The whole bulk.	52·50	23·64	4·99	45·03	9·51
Soft Cheeses.	1	Brie cheese, not quite ripe.	Interior.	47·10	7·58	5·14	16·10	10·91
			Exterior.	53·50	21·33	12·37	39·87	23·12
	1	Camembert cheese, ripe.	Interior.	95·52	8·71	8·71	9·12	9·12
	1	Limburger cheese, six weeks old.	Interior.	24·82	5·27	4·37	21·23	17·60
			Exterior.	55·10	12·58	4·51	22·83	7·85
		Limburger cheese, ripe.	Interior.	99·82	4·33	11·97	4·52	11·99
Sour Milk Cheeses.	1	Schabzeiger.	The whole bulk.	37·35	16·58	5·80	44·39	15·53
	1	Norwegian Gammelost (old cheese), ripe.	Interior.	51·35	31·99	4·23	62·33	8·23
			Exterior.	69·47	38·57	7·42	55·52	10·68

The chief feature of the ripening process is the conversion of insoluble proteins into soluble substances. The table shows that

[1] For cheese analysis, see "Zeitschr. f. Nahrungs und Genussmittel," 1906, Bd. XII., p. 193.

in the hard cheeses usually only one-third of the casein becomes converted into water soluble proteins, whereas in the soft cheeses nearly all the casein undergoes conversion. This explains the apparent richness in fat of the soft cheeses, for when anything gives the sensation of melting in the mouth distinction is as a rule not to be made between melting proper, as in the case of butter, and a solvent action. Further examination of the soluble substances in hard cheeses shows that a larger proportion of these have been converted into amino acids than in the soft cheeses; these conditions may be summed up by saying that in hard cheeses *the ripening is less extensive but more thorough*, while in the soft cheeses it is extensive but not so thorough. This definition, however, cannot be regarded as a rigid one, for when the hard cheeses are not much older than the soft ones (see, for example, Edam cheese), the proportion of Dec. N. to Sol. N. is not large, and if the soft cheeses do only contain small amounts of amino acids, this is because these are quickly broken down into ammonia on the surface[1]. Now as ammonia readily combines with the lime free casein to form soluble salts, the high proportion of Sol. N. in the soft cheeses is partly attributable to the thoroughness of the ripening process. The most important factor in the ripening of the rennet cheeses, besides the various microorganisms, is the rennet. It is the rennet which produces the perceptibly soluble proteins and the microorganisms carry the degradation further. Further, as the action of the most important of the enzymes of the cheese ripening bacteria is inhibited, while that of rennet is promoted by the presence of acid, it will easily be understood why the practically neutral hard cheeses contain larger proportions of amino acids than the soft cheeses.

In the ripening of cheese, not only is the casein converted into easily digestible and palatable products, but the fat and the lactose also undergo changes. As might be expected, the fat is hydrolysed most rapidly in cheeses like Roquefort which are permeated with moulds. Rapid fat hydrolysis also takes place in cheeses made from separated milk, for here the fat globules are very minute and thus expose a large surface for attack. On the other hand, fat hydrolysis proceeds with extreme slowness in the common hard rich cheeses. As, however, butyric, caproic and capric acids have a very persistent taste, they contribute very largely to the aroma of the cheese even though they may only be present in small amounts. The reason why the choicer cheeses must pass through a long period of ripening in order to attain their characteristic piquant taste to the full, is that the processes

[1] *Orla Jensen*, "Centralblatt f. Bakt.," 2 Abt., 1900, Bd. VI., p. 773.

of fat hydrolysis and ammonia formation to which the production of the sharp taste (not to be confused with the salt taste) is due proceed with extreme slowness in these cheeses. Both of these processes seem also in the case of hard cheeses to start at the surface and gradually penetrate inwards.

While the fat is usually slowly decomposed in cheese, the **lactose** is the first constituent to undergo change. In hard cheeses it usually disappears in a few days and in soft cheeses in a week or two. Normally it is completely converted into lactic acid, which is neutralised by the lime and other bases present. Calcium lactate is not necessarily the end product, for it is quite frequently more or less completely converted into propionic acid by the fermentation process described on p. 41, whereby the normal "eyes" or cavities are produced in cheese. The butyric acid fermentation of calcium lactate however, must be looked on as a disease of cheese, for the evolution of gas will be too vigorous, while unpalatable or even poisonous substances may be produced. The author's researches on the volatile acids of cheese, summarised in the accompanying table, show that normally no more butyric acid is found in the ordinary rennet cheeses than will originate from fat hydrolysis.

After these general remarks, we may pass on to consider the microorganisms which play the chief part in the ripening of the different kinds of cheese. **The flora of the hard rennet cheeses** will be dealt with first. *Duclaux*, who was the first to investigate this field, found in cheese various sporing rod bacteria which he named *Tyrothrix*, *i.e.*, "cheese threads." He found both aerobic and anaerobic forms, *i.e.*, what now would be called hay and potato bacilli and anaerobic putrefactive bacteria. As the aerobic forms were able to decompose and dissolve casein, and the anaerobic forms produced the odour characteristic of cheese, *Duclaux* and all other contemporary investigators were agreed that the ripening of cheese was due to the combined action of these bacteria[1]. The constant success of *Duclaux* in isolating *Tyrothrix* bacteria from cheese was due to the fact that he employed an enrichment method which particularly favoured the growth of these bacteria. He introduced a small piece of cheese into broth in which the lactic acid bacteria did not thrive, and it was therefore only necessary to have a few *Tyrothrix* spores present from the outset in order that a *Tyrothrix* film should form on the surface of the liquid after some time, after which the anaerobic sporing bacteria, protected from the air, could grow rapidly.

The careful researches carried out by *Freudenreich* over a period

[1] *Duclaux*, "Le Lait," Paris, 1894, pp. 213—258.

of many years [1] have established that the *Tyrothrix* bacteria are comparatively rare in cheese, that normally they cannot develop in cheese even if introduced in large numbers, owing to their sensitiveness to acid, and finally that if conditions favourable to their growth are secured by using pasteurised milk and omitting the addition of lactic acid bacteria, they produce a disgusting taste of putrefaction in the cheese. The bacteria living in the

Kind of Cheese.		Found in 1,000 grams Cheese.										
		As cc. normal.		In grams.								
				Produced by fat hydrolysis.		Produced by the splitting up of the Casein (paracasein) and Lactose (lactic acid).						
		Total volatile acids.	Total ammonia.	Caproic acid.	Butyric acid.	Valerianic acid.	Butyric acid.	Propionic acid.	Acetic acid.	Formic acid.	Total volatile acids.	Total ammonia.
Emmental cheese.	Interior.	88·0	75·0	0·116	0·176	—	—	4·218	1·680	—	6·190	1·275
	Exterior.	75·0	55·0	0·928	1·232	—	—	2·812	0·900	—	5·872	0·935
Edam cheese.	Interior.	15·6	15·0	—	—	—	—	0·224	0·678	0·057	0·959	0·255
Swiss skim-milk cheese.	Interior.	81·6	267·5	0·986	1·496	—	—	2·405	1·200	0·138	6·225	4·548
	Exterior.	100·0	207·5	1·682	2·552	—	—	2·775	1·080	0·046	8·135	3·528
Roquefort cheese.	Whole bulk.	38·0	115·0	0·928	1·672	—	—	—	0·540	0·092	3·232	1·955
Camembert cheese.	Interior.	6·6	175·0	0·081	0·246	—	—	—	0·069	0·082	0·478	2·975
Brie cheese.	Interior.	11·3	95·0	0·139	0·572	—	—	—	0·204	0·008	0·923	1·615
	Exterior.	8·7	217·5	0·128	0·466	—	—	—	0·120	0·013	0·727	3·698
Limburg cheese.	Interior.	111·0	200·5	0·058	0·440	1·581	—	5·180	1·140	0·046	8·445	3·409
	Exterior.	104·5	220·0	0·232	1·003	1·550	—	4·529	0·822	0·046	8·182	3·740
Glarner Schabzeiger.	Whole bulk.	258·2	215·0	1·195	1·848	—	4·452	9·102	3·198	—	19·795	8·655

interior portions of the cheese are nearly exclusively of the lactic acid group, and it must consequently be these which are principally responsible for the protein hydrolysis which takes place. *Freudenreich*, who devoted special attention to **Emmental** cheese, found chiefly the rod-shaped species, and succeeded in showing that these were able to attack casein if only the lactic acid which they produced was neutralised by chalk as completely as it is neutralised in the hard cheese. This forms the basis of a

[1] The first of *Freudenreich's* papers on this subject appeared in "Annales de Micrographie," 1889, p. 257, while the subsequent ones are nearly all published in "Landwirtschaftliches Jahrbuch der Schweiz," 1891 to 1906.

correct understanding of the ripening processes which take place in these cheeses.

According to the author's researches, the proteolytic enzyme of the *lactic acid rod bacteria* must be regarded as an endoenzyme, as it is not liberated by the living cell, and its action recalls that of erepsin, for it produces amino acids from the casein without forming albumoses and peptones as intermediate products [1]. The large amounts of amino acids which are found in many hard cheeses are principally due to the action of this *endoerepsin* [2]. The bacteria themselves take no part in the process, for most of them will be dead before the bulk of the amino acids have been produced [3]; they do not thrive without sugar, and as all the lactose in hard cheeses will have been fermented in the course of a day or two, the ripening bacteria will already have reached their maximum (over 100,000,000 per gram) by then, after which they gradually fall off. Dead cells which are not exposed to desiccation generally digest themselves more or less completely owing to the action of the intracellular enzymes which thus set themselves free, so that they become able to exert their digestive action on the surrounding medium; this is what happens in cheese where the endoenzymes act under favourable conditions, not involving too great dilution. The author has demonstrated the presence of such enzymes in both hard and soft cheeses [4]. The fact that the ripening of hard cheeses depends on enzyme action pure and simple, and is not directly dependent on the action of living bacteria, is also demonstrated by the ripening of these cheeses at temperatures far below the minimum for cheese bacteria [5].

Of the various rod-shaped lactic acid bacteria isolated by *Freudenreich* from Emmental cheese, one particular species, *Thermobacterium helveticum*, seems to be indispensable for the production of the typical sweetish taste, and it is extremely interesting to see how the methods of manufacture, arrived at by practical experience, favour the development of this bacterium throughout the process. As the organism occurs in the fourth stomach of the calf, it will develop freely when the stomach is extracted in a warm place with "Schotte" (see p. 50). This is the usual practice in the Swiss dairies, the ripening bacteria thus

[1] "Centralblatt f. Bakt.," 2 Abt., 1900, Bd. VI., p. 840, and 1904, Bd. XIII., p. 521.

[2] *Freudenreich* and *Orla Jensen*, "Landwirt. Jahrbuch der Schweiz," 1899, p. 167.

[3] *Orla Jensen*, "Landwirt. Jahrbuch der Schweiz," 1906, p. 303.

[4] "Studien über die Enzymen im Käse," "Centralblatt f. Bakt.," 2 Abt., 1900, Bd. VI., p. 734.

[5] *Babcock* and *Russel*, Eighteenth Annual Report of the Wisconsin Agricultural Experiment Station, 1901, p. 136.

being introduced with the "*natural rennet*"[1]. As the stomachs, especially those of bad quality, also contain many harmful bacteria, it is advantageous to inoculate the rennet at once with a mixed culture of *Thermobacterium helveticum* and the mycoderma mentioned on p. 50[2], or to secure favourable conditions for the lactic acid bacteria simply by adding lactic acid (*e.g.*, some boiled acid Schotte) to the rennet. It is safer to use the pure *factory made rennet*, together with pure cultures of *Thermobacterium helveticum*, in sterile milk[3]. In order to secure the development of this organism to the exclusion of other bacteria, the cheese must be made warm and kept warm in the presses. This is achieved by scalding at a comparatively high temperature, taking the curd out of the warm whey in a lump, and above all by making the cheeses so large that they will retain their heat for a long time. Cheeses made in this way maintain a temperature of 50° to 35° C. during the first twenty-four hours, and under these conditions *Thermobacterium helveticum* is certain to obtain predominance.

At the same time *a lactic acid streptococcus* (*Sc. thermophilus*) having a high optimum temperature grows luxuriantly[4]. As this organism does not attack casein, it hardly plays any important part in the ripening of the cheese beyond the souring process, and possibly assisting in the production of favourable conditions for the growth of the lactic acid rod bacteria, in much the same way as occurs in Yoghurt and similar products.

While the drastic scalding weakens the gas-producing bacteria, the high temperature of the cheese while in the press favours the growth of the pseudo lactic acid bacteria, and if the milk was dirty there will already be a very vigorous development of blow holes. (Such a cheese (*Presslis*) develops a large number of pinholes.) Coli and aerogenes bacteria, as well as butyric acid bacteria, may also develop at a later stage, and it is therefore advisable to keep the cheese as cold as possible after it has left the press, and until all the lactose has been fermented. Any evolution of gas will do far more harm in the compact mass of Emmental cheese than in most other cheeses from which the gas can partially escape through the narrow fissures which mark off the original particles of curd. For this very reason the *normal cavities* or "**eyes**" are able to attain to a much larger size in

[1] *Freudenreich* and *Orla Jensen*, "Centralblatt f. Bakt.," Abt. 2, 1897, Bd. III., p. 545.

[2] *J. Thöni*, "Bacteriologische Studien über Labmägen und Lab." ("Landwirt. Jahrbuch der Schweiz," 1906, p. 181.)

[3] *Rosengreen* and *Haglund*, "Meddelande No. 101 fran Centralanstalten för försöksväsandet pa jordbruksomradandet," Stockholm, 1914.

[4] *Orla Jensen*, "Über die in Emmenthalerkäse stattfindende Milchsäuregärung." "Landwirt. Jahrbuch der Schweiz," 1906, p. 437.

Emmental cheese than in other makes. The eyes should only begin to form when the cheese has ripened to such a degree that it is sufficiently plastic to allow of their rounding off; if they form too soon, they become irregular in shape, and if the mass is made too dry, it will never become sufficiently plastic; in this state the cheese is known as "*Glásler*," having elongated cavities or clefts instead of proper eyes. In order to promote the formation of normal eyes, the cheese is brought into a room at 18° to 22° C. when it is two weeks old. Here the ripening process is accelerated and the propionic acid bacteria will gradually develop so that in the course of four to six weeks the eyes will have been fully formed. The cheeses are then brought into a cold place again. As the propionic acid bacteria are very sensitive to sodium chloride, it is possible to regulate the formation of eyes by the addition of more or less salt[1]; the chief reason for the adoption of the somewhat troublesome method of dry salting in the case of Emmental cheese, instead of methods by which the cheese receives its full amount of salt at an earlier stage, is doubtless that the cheeses would, in the latter case, only develop small eyes or none at all, as often occurs with small cheeses, which naturally tend to become salted too quickly. If the cheese has not been made sufficiently dry, or contains too many propionic acid bacteria at the outset, the development of the normal eyes will be excessive, and this is a defect which may be just as objectionable as the blowing at an earlier stage, which has been described above. On the other hand, if neither the ripening process nor the development of the propionic acid bacteria have progressed sufficiently in the "warm cellar," it may happen that the eyes will suddenly begin to form at a still later stage; an *after fermentation* of this nature will always tend to give a variable product. The presence of unusually large numbers of butyric acid bacteria will also give rise to an abnormal eye formation which, it may be noted, will be of the worst possible type[2]. In such cases a very energetic butyric fermentation sets in when the cheeses are from ten to fourteen days old, and under these circumstances they will not stand exposure to the temperature of the warm cellar.

Closely connected with the ripening process is the formation of drops of liquid or "**Tears**" in the eyes, a process which often first starts when the Emmental cheese is eight months old. The conditions determining the collection of this liquid are, first, that it shall not be too viscous, and second, that the pores of the cheese shall not be too fine. In the ripening of the cheese an appreciable proportion of the dissolved proteins are converted into amino

[1] *Orla Jensen*, "Landwirt. Jahrbuch der Schweiz," 1906, p. 437.
[2] *J. Thöni*, "Landwirt. Jahrbuch der Schweiz," 1906, p. 157.

acids, whereby the viscosity of the liquid is decreased, and as increasing amounts of the curd become soluble the pores in the cheese will become larger. The salt which at an earlier stage tended to cause the cheese to swell now has the opposite effect, but as the rind prevents contraction of the mass as a whole, the pores must become still larger. The salting and drying of the cheese finally bring about the precipitation of the sparingly soluble amino acids as white crusts, and these so-called "**salt stones**" may be distributed throughout the whole mass.

The reason why the ripening of Emmental cheese has been discussed so thoroughly is that this cheese has been studied more thoroughly than any other. In this respect, the **large-holed Swedish manor farm cheese,** which resembles the Danish-made Swiss cheese, comes next. According to *Gerda Troili-Peterson's* researches[1], the ripening of this cheese depends on *peptonising tetracocci* in addition to the lactic acid rod bacteria, and the formation of eyes on certain glycerine-fermenting aerogenes bacteria as well as the propionic acid bacteria. According to *Gorini*[2], peptonising tetracocci also play an important part in the ripening of **Parmesan cheese,** in the making of which the curd is scalded at a high temperature; this cheese differs from Emmental in being prepared without the addition of strongly acidifying lactic acid rod bacteria, a dough made from chopped calves' stomachs being used instead of the acid "natural rennet" described above. The peptonising cocci, just mentioned, generally develop freely in the fresh curd, for, unlike the *tyrothrix* bacteria, they can grow in the presence of acid. They are, however, quickly suppressed if too much acid is present, and will therefore be most prominent in cheeses which sour slowly or in cheeses made from milk which has been ripened at a temperature below 15° C., as they grow better than the true lactic acid bacteria under this temperature. The proteolytic enzyme secreted by the peptonising tetracocci is less sensitive to acid than the erepsin of the lactic acid rod bacteria, and its mode of action is intermediate between that of the latter and that of rennet. It would therefore appear to be best defined as an "*exotrypsin.*" The best investigated of these cocci is *Tetracoccus liquefaciens*, which produces the characteristic taste of **Tilsit cheese** or **Russian Steppe cheese,** and there is no doubt that it plays an important part in the

[1] "Centralblatt f. Bakt.," 2 Abt., 1909, Bd. XXIV., p. 343.

[2] Among the numerous papers by this investigator, special mention may be made of "Recherches sur les coccus producteurs d'acide et de préssures du fromage" ("Revue générale du lait," 1910, vol. 8, p. 337). The true significance of the action of the peptonising micrococci in the ripening of cheese was first pointed out by *Weigmann*, 1896 ("Centralblatt f. Bakt.," 2 Abt., Bd. II., p. 151).

ripening of these cheeses. Similarly, it is probable that it assists in the ripening of **Gouda cheese,** which is made on the smaller Dutch farms from perfectly fresh milk without the addition of lactic acid bacteria; under such conditions these organisms will have ample opportunity for development. *Boekhout* and *de Vries* have shown that the peptonising bacteria do not assist to any appreciable extent in the ripening of **Edam Dutch cheese,** which is nowadays generally made with a lactic acid starter [1], and they have not succeeded in demonstrating other specific ripening bacteria [2]. As Edam cheese is on the whole poor in typical products of bacterial action (amino acids and volatile acids), it would appear that the bacteria here play only a subordinate part, and that the principal changes are due to the rennet, as is indicated by *van Dam's* researches [3]. "Salt stone" is occasionally found in Edam cheese, but in this case it consists of calcium lactate and phosphate. Again, in **Cheddar cheese,** the peptonising bacteria do not bring about any changes of importance; here also a great deal of the action is due to the rennet, and the main feature in the making of this cheese is the production of a curd having a fairly high acidity at the outset, so that when the curd is pressed against a hot iron it can be drawn out into long silky threads (the hot-iron test). American investigators have found the flora in Cheddar cheese to consist almost exclusively of the true lactic acid bacteria, chiefly streptococci. The bacterial count reaches its maximum immediately after the cheese has been made and then decreases steadily, the rate of decrease being quicker the higher the temperature at which the cheese is kept [4]. By allowing the curd to become strongly acid through the agency of different streptococci, the author has succeeded in producing cheeses resembling Cheddar in texture and taste, so that there can be no doubt that in this case such bacteria actually accomplish more than the mere production of acid [5]. The streptococci cannot, however, be responsible for the large amounts of amino acids found in ripe Cheddar, so that here also we have evidence of the action of lactic acid rod bacteria. As a matter of fact, the author has never met with any kind of cheese in which lactic acid rod bacteria (strepto-

[1] "Revue générale du lait," 1910, vol. 6, p. 1.

[2] "Centralblatt f. Bakt.," 2 Abt., 1905, Bd. XV., p. 323, and 1906, Bd. XVII., p. 149. These authors have described a diplococcus-like rod bacterium which converts lactic acid into acetic acid, carbon dioxide and hydrogen, and which is said to have some significance in the formation of eyes in Edam cheese. It will stand 4½ per cent. of salt, and its optimum temperature is 21° C. (*ibid.*, 2 Abt., 1918, p. 130).

[3] "Centralblatt f. Bakt.," 2 Abt., 1910, Bd. XXVI., p. 189.

[4] *Harrison*, "Centralblatt f. Bakt.," 2 Abt., 1904, Bd. XI., p. 637. Subsequently *Harding* and *Prucha* made a thorough study of the flora of Cheddar cheese (New York Agric. Exp. Station, Bull. No. 8, 1908).

[5] "Centralanstalten's 97 Meddelse."

bacteria) could not be demonstrated in large numbers, a natural result of the ability of these organisms to overgrow all other bacteria under circumstances favourable to themselves. Thus, they also obtain predominance by slow degrees in the **Danish dairy cheese,** although this cheese, like Cheddar, is freely inoculated with lactic acid-producing streptococci by the addition of buttermilk.

Barthel ascribes to the streptococci a more important part in the ripening of cheese than has hitherto been done. He has shown that certain strains form, at ordinary temperatures, quite appreciable amounts of Sol. N. (see table, p. 143). The author has also found, with fair regularity, streptococci, especially strains of *Sc. cremoris*, which are conspicuously able to split casein into soluble products; as these bacteria gradually lose this power when cultivated on artificial media, it may be surmised that streptococci which do not hydrolyse casein to any appreciable extent may acquire this property when cultivated in milk and cheese. It must also be pointed out that many lactic acid bacteria grow better in milk to which rennet has been added than in ordinary milk; that is, their action is promoted by the rennet just as, conversely, the action of the rennet is promoted by the lactic acid.

In addition to the action of certain lactic acid streptococci which hitherto have not been further investigated, the chief factors in the ripening of the hard rennet curd cheeses are seen to be *the rennet, the exotrypsin of the peptonising tetracocci and the endoerepsin of the lactic acid rod bacteria.* The relative importance of each of these factors varies considerably in different cheeses, and determines the characteristic properties of each variety. Further differences arise owing to the fact that the above-mentioned groups of lactic acid bacteria include many different species, an illustration of the production of special characteristics owing to the action of a particular species of organism being seen in the case of Emmental cheese. The table on page 143 has been drawn up to illustrate the action of the several factors in ripening; the amounts of soluble nitrogen (Sol. N.), nitrogen of decomposition products (Dec. N.), and ammonia nitrogen (Am. N.) formed in milk after two months are given (*cf.* table, p. 133). All the cultures were treated with chalk and shaken regularly so that the lactic acid produced was neutralised.

Taking a wider view of the ripening process, as including not only the decomposition of the casein, we must also consider the propionic acid bacteria as a ripening factor. *Weigmann* is of the opinion that the aromas of the various kinds of cheese are principally due to the action of bacteria other than the lactic acid

organisms[1], but as yet he has not been able to adduce any evidence as to the correctness of his theory. *Rodella's*[2] contention that *the specific-taste-producing bacteria* are *obligate anaerobic sporing organisms* can hardly be supported, for it has never been proved that these bacteria reproduce themselves in rennet curd cheese[3], and when on rare occasion they have been found in large numbers, a harmful action has also been observed. The interior of a hard cheese should have a clean taste and smell, but *putrefactive processes in the rind* cannot be avoided unless special precautions are taken, and if the cheese becomes very old the interior may also

Milk with Chalk.	Percentage of Total Nitrogen.		
	Sol. N.	Dec. N.	Am. N.
Streptococcus lactis	2·51	2·02	0·23
Rennet	11·75	0·00	0·00
Streptococcus lactis and rennet . .	60·56	5·32	0·36
Tetracoccus liquefaciens . . .	75·70	12·12	1·89
Thermobacterium helveticum . .	36·12	34·60	3·91

become affected. An ordinary dairy cheese, not too poor in fat, may even be made to acquire quite a piquant flavour in the course of three to four months, if only certain changes are promoted in the outer layer, as is done in the case of the "smeared" soft cheeses. This method was at one time successfully applied by Fru *Hanne Nielsen*. Certain hard cheeses, *e.g.*, Tilsit cheese, have a tendency to undergo similar changes spontaneously, and may therefore be regarded as intermediate between the hard and the smeared soft cheeses. On the other hand, those hard cheeses which become permeated with moulds form a link between the hard cheeses and the soft mouldy cheeses; they will therefore be dealt with before passing on to the soft cheeses.

Cheeses like **Stilton, Gorgonzola** and **Roquefort,** which are permeated with moulds, resemble the other hard cheeses in their mode of ripening, *i.e.*, the process does not originate on the surface and work inwards. The surface is kept as clean as possible, and the cheeses are not made flat; on the contrary, they are shaped so as to expose a relatively small surface. In Stilton and Gorgonzola the moulds only develop slowly, as they owe their presence to

[1] "Mykologie der Milch," Leipzig, 1911, p. 223.
[2] "Centralblatt f. Bakt.," 2 Abt., 1903, Bd. X., pp. 499, 753; also 1906, Bd. XVI., p. 52.
[3] *Burri* and *Kursteiner*, "Landwirt. Jahrbuch der Schweiz," 1909, p. 442.

casual infection and not to artificial inoculation (at any rate, up till recently they were not purposely introduced); the initial stages of the ripening processes in these cheeses, therefore, follow much the same course as in the typical hard cheeses. On the other hand, Roquefort is inoculated with pure cultures of the required mould, *Penicillium roqueforti*, which means that a new factor comes into play at the outset, with the result that the ripening of this cheese, although accomplished at a low temperature (6° to 8° C.), only requires as many weeks for its completion as the ripening of the above-mentioned cheeses require months. The piquant taste and smell of this type of cheese are principally due to fat hydrolysis, and it is therefore possible to produce a similar aroma by inoculating the corresponding moulds into butter. These organisms also affect the casein to a considerable extent, as is shown in the table on p. 133. Roquefort, in common with the Norwegian Gammelost (the latter an acid curd cheese which is permeated with mould), contains more amino acids than any other cheese. The development of the moulds is promoted by making the fresh cheese strongly acid, for which reason the curd is not submitted to a prolonged working, the treatment resembling that applied to the soft cheeses, while during the early stages of the ripening the cheese is kept at 18° to 20° C. in order to promote lactic acid fermentation. As air is necessary for the growth of the moulds, the cheeses must be stabbed immediately after salting; before doing this the surface of the cheese must be cleaned carefully to avoid the transference of the organisms growing thereon to the interior by means of the needle; some of these organisms may colour the cheese red or turn it bitter. This is of particular importance if the cheeses are stabbed at later stages. They are stored in a comparatively dry room and placed on edge so that the holes shall not be closed up again. If it is desired to produce a typical Gorgonzola cheese in which certain bacterial fermentations will have taken place before the mould has commenced its action, the stabbing should not be commenced before the cheese is two months old. The bluish-green veins of Gorgonzola owe their origin to the practice of interlaying the fresh curd with acid curd which is twelve hours old and which has become strongly infected with *Oidium lactis* and various species of *Penicillium* on the surface. According to the results of the author's investigations with butter[1], the above-mentioned mixture of moulds seems to be particularly well adapted to produce the desired taste and appearance of Gorgonzola. In the case of Stilton, the moulds simply penetrate through fissures in the

[1] "Centralblatt f. Bakt.," 2 Abt., 1902, Bd. VIII., p. 369.

surface. The species of *Penicillium* found in Stilton and Gorgonzola appear to be mainly *Penicillium roqueforti*. This circumstance is explained by *Thom* and *Currie*[1] by the fact that *P. roqueforti* can thrive with less oxygen than the other Penicillia, and therefore obtains predominance in the interior of the cheese, even though the cheese has not been inoculated with it. In the making of Roquefort cheese the veins are produced where desired by dusting with mouldy bread, of which about 0·1 per cent. of the weight of the cheese is used; the mould grows not only in these places, but also in the holes made by the needle; it is able to overgrow the cheese bacteria owing to the low temperature (8° C.) at which the cheese is ripened. The bread used for the cultivation of the mould is best made from a dough containing equal parts of rye, barley and wheat flour, and a little lactic or acetic acid, in which acid fermentation is induced by the addition of some sour dough. The bread is very thoroughly baked, which, in conjunction with the action of the acid which has been added, destroys bacterial spores which otherwise would develop when the bread is set aside for the development of the moulds. It is then cut into slices and dipped into sterile water containing ½ per cent. of acetic acid, into which a culture of the mould has been stirred. The bread slices are placed close together on sterile shelves in a damp room and covered with sterile filter paper. As the change in temperature caused by the growth of the mould, when this sets in, is considerable, the initial temperature should be kept down to 9° to 10° C. When in the course of three to four weeks the bread has become thoroughly mouldy, the crusts are removed and the slices are dried for about ten days at 30° to 32° C., after which they are ready to be ground and sifted. The yield is 45 per cent. of the original weight of the bread.

Even if the work is carried out in rooms which are as sterile as possible, it is difficult to avoid infection with foreign moulds. The author has accordingly worked out a method[2] in which the strain of *P. roqueforti* which is used is little by little accustomed to stand comparatively large amounts of formaldehyde. Formalin can be added to the water in which the slices are dipped, whereby the development of the foreign moulds which cannot stand formalin is inhibited. A mould powder made in this way is sold by *Messrs. Blauenfeldt and Tvede*, of *Copenhagen*.

As already mentioned, the ripening process of the **soft rennet curd cheeses** starts at the surface and works inwards. At the same time, a ripening action due to the rennet takes place throughout the whole mass, even though it may not be very obvious. While the amounts

[1] "Journal of Biol. Chem.," 1913, vol. 15, pp. 249 and 259.
[2] "Maelkeritidende," 1919, p. 277.

of acid present in the interior of soft cheeses inhibit the action of the ripening factors hitherto considered, they promote the proteolytic action of the rennet and conserve its enzyme in an active state for a much longer time than is the case in the hard cheeses [1]. As will have been gathered from the preceding remarks, the soft cheeses may be divided into two groups: (*a*) The **"smeared" cheeses;** in these the moulds are suppressed and prevented from forming aerial hyphæ by daily smearing the cheeses either with a wet cloth or by hand, keeping them moist and protecting them as much as possible from the air by packing them closely together. (*b*) The **mouldy cheeses;** in these the growth of moulds is favoured by touching the surfaces of the cheeses as little as possible, keeping them dry and providing for free access of air.

The copious production of ammonia on the surface of the best-known of the smeared cheeses, **Limburger** and **Romadour,** has been found by the author [2] to be due to a *symbiosis* of peptonising tetracocci and *Bacterium casei limburgensis.* The latter is a non-motile, very irregular short rod which does not ferment lactose; it forms a film on media containing calcium lactate, and oxidises the lactic acid to acetic and carbonic acids; it does not attack casein, but forms traces of ammonia in milk, from the amino acids, which has a slight solvent action on the casein. This organism is therefore to be classed with the bacteria which produce a soapy taste in milk. Its most characteristic property is its ability to carry further the decomposition of the products of protein hydrolysis which have been produced by other micro-organisms; this is most strikingly illustrated by inoculating it into milk together with *Tetracoccus liquefaciens.*

Milk without Chalk.	Percentage of Total N.		
	Sol. N.	Dec. N.	Am. N.
Bacterium casei limburgensis . .	0·60	2·36	1·30
Tetracoccus liquefaciens . . .	61·61	8·73	1·43
Bact. casei limburgensis and Tetracoccus liquefaciens	67·59	32·18	14·99

The smeared crust of Limburger cheese consists, according to the unpublished researches of *Freudenreich,* chiefly of *Bacterium casei limburgensis* together with smaller numbers of peptonising organisms (chiefly *Tetracoccus liquefaciens,* a small spore-forming

[1] *Orla Jensen,* "Centralblatt f. Bakt.," 2 Abt., 1900, Bd. VI., p. 795.
[2] "Studier over de flygtige Syrer i Ost, etc.", Doctoral thesis, 1904, p. 74.

rod bacterium, and yeast); in view also of the results set out in the table on p. 146, there can hardly be any doubt as to the cause of the thorough-going decomposition processes which take place in this cheese. A similar flora will also develop on the rind of hard cheeses if they are kept damp, and it will be understood how the ripening of these cheeses may be modified so as to conform with the type generally associated with Limburger cheese. The ammonia formed on the surface gradually diffuses into the interior, and not only does it neutralise the lactic acid, thereby rendering possible the activity of the bacterial enzymes, but it also converts the casein into the readily soluble ammonium caseinate. The ripening of the soft smeared cheeses involves further complexities. In the ripe state these cheeses contain appreciable amounts of valerianic acid, an acid which is only produced by the above-mentioned bacteria in very small amounts, while on the strongly-smelling surface there are formed various typical products of putrefaction, such as hydrogen sulphide and indole. It is obvious that under normal conditions organisms other than those mentioned above participate in the process—*Weigmann* mentions *Plectridium fœtidum*—but whether their activity is to be regarded as at all desirable may be an open question; it is quite possible that Limburger cheese might have a wider market if it contained no products of putrefaction. According to *Laxa*[1], *Oidium lactis* participates in the ripening of certain Bohemian cheeses, which resemble Limburger cheese (*Harrach* and *Knoppist*); these cheeses therefore form a link with the mouldy cheeses.

The moulds play a part in the mouldy cheeses similar to that of the peptonising bacteria in the smeared cheeses. As a type we may take **Camembert,** which, thanks to the researches of *Roger*, *Mazé*[2], and *Thom*[3], is one of the most thoroughly investigated cheeses. The moulds which develop in this cheese are *Oidium lactis* (according to *Mazé*, *Oidium camemberti*, other oidium species, and a mycoderma), *Penicillium camemberti*, and *P. candidum*. The moulds of the Oidium group grow the quickest, while the Penicillium group first begins to develop after five to six days, when the surface has become a little drier. The chief difficulty lies in maintaining the proper humidity. If the air is too damp, the surface of the cheese becomes too slimy, as in the case of the smeared cheeses, and bacteria, yeasts, oidium, and even certain mucors, gain the upper hand; if too dry, on the other hand, the Penicillia grow too freely, causing the rind to shrivel; all the green Penicillia which develop (not excepting *P. roqueforti*) produce

[1] "Centralblatt f. Bakt.," 2 Abt., 1899, Bd. V., p. 755.
[2] "Annales de l'Institut Pasteur," 1910.
[3] U.S. Dept. of Agric., Bureau of Animal Industry, Bull. 115, 1909.

an undesirable taste. The moulds should not completely overgrow the surface, neither should they form too many conidia. The drying is regulated by providing for a suitable draught and by laying the cheeses on straw mats; after three weeks, when the cheeses have softened at the corners, they are transferred to another room and placed direct on the shelves, or sometimes on mats infected with bacteria. The moulds are now displaced by rod bacteria resembling *Bacterium casei limburgensis*, which appear as red and brown spots between the mouldy ridges, and the ammoniacal fermentation which starts under these spots gradually penetrates to the centre, resulting in the production of ammonium caseinate and other soluble proteins. The quicker the cheese ripens, the quicker will it become perfectly liquid and putrefying. A good saleable cheese should, therefore, be made firm and ripened at a low temperature; during the first few days, when the cheeses are salted and develop acid fermentation, they should be kept at 18° to 20° C.; the drying room should be kept at 13° to 15° C., and the ripening room, to which the cheeses are finally transferred for the chief fermentation to take place, at 10° to 12° C., or even lower. Frequently the process is completed in the boxes in which the cheeses are packed for transport. Cheeses never get the proper taste if they are put in the boxes too early. As soon as the interior of the cheese has become neutral in reaction the lactic acid bacteria will be able to act as in hard cheeses, but they act slowly and have no time to produce any noteworthy change before consumption. While *Oidium lactis* and the necessary bacteria will establish themselves of their own accord, there is some difficulty in establishing the desired species of Penicillium in places where the manufacture of Camembert is to be started. *Thom* recommends their cultivation on dry, sterilised rusks, wetted with a suspension of the conidia in water. After keeping for ten days at 20° C., the rusks will be overgrown by the mould; these mouldy rusks are then shaken vigorously with water, in which the cheeses are dipped immediately before salting. The French mode of procedure is more scientific; the *Institut Pasteur* (Service des vaccins, 35, Rue Dutot, Paris) supplies three different cultures for the purpose, consisting of an ordinary lactic acid starter, a culture of moulds, and a culture of the ammonia-producing bacteria. The two first-mentioned of these, or sometimes all three, are added to the milk before the rennet. The mould culture need only be used for the first ten days, after which it will have established itself in the mats used in the drying room, from which subsequent batches of cheese will become inoculated. The third culture is applied directly to the mats on which the cheeses rest during the last stage of

ripening. The cultures may also be obtained in powder form for sprinkling on the cheeses[1].

Only the **acid curd cheeses** remain to be discussed. These include both hard and soft varieties, and the latter include both smeared and mouldy cheeses—even cheeses which resemble Roquefort in being permeated with moulds, *e.g.*, the Norwegian Gammelost. The making of the *hard acid curd cheeses*[2], the Danish cheeses, **Appetitost, Knapost,** the Norwegian **Pultost,** and the Swiss **Green Alpine Cheese (Schabzeiger)** presents several points of interest; they are scalded at a high temperature or for a long time and ripened before they are shaped (the Norwegian Pultost is not shaped at all). The scalding produces the same results as in the pasteurisation of milk: the development of the sporing bacteria and the suppression of the lactic acid bacteria; as the author has shown in the case of Schabzeiger[3], and *v. Klecki* in the case of another acid curd cheese[4], the curd develops a vigorous butyric acid fermentation; in both cases the butyric acid bacteria were motile; the butyric acid gives these cheeses a sharp taste. The ripening proper is due to lactic acid rod bacteria, which possibly act in conjunction with other microorganisms. As regards the decomposition undergone by the casein, the hard acid curd cheeses do not differ greatly from the hard rennet curd cheeses (see table, p. 133). According to *Olav Johan-Olsen*[5], the ripening of Pultost, in which the author has found a fair amount of valerianic acid, is accomplished by yeast, *Oidium lactis*, and especially by a species of Mucor. *Johan-Olsen* has also carried out a detailed investigation of the ripening of the **Norwegian Gammelost.** Here the active moulds are especially species of Penicillium and Mucor, which turn the cheese green and brown respectively. As the curd or the cheese itself is strongly heated, it is improbable that the moulds are derived from the sour milk; they must find their way into the cheese at a later stage, and gradually penetrate from the surface throughout the whole mass. This already takes place during the first six weeks, as the curd is fairly acid and porous.

If it is desired to utilise separated milk for cheese, this is best done by turning it into one of the above-mentioned acid

[1] According to our experience, it is sufficient to infect the mats with *P. Candidum*. The bacteria come of their own accord while the Oidia only do harm.

[2] Several of these cheeses contain, in addition to casein, smaller or larger amounts of albumin (*Zeiger*), which also has an influence on the ripening process.

[3] "Centralblatt f. Bakt.," 2 Abt., 1904, Bd. XIII., p. 755, and 1907, Bd. XVII., p. 225.

[4] "Centralblatt f. Bakt.," 2 Abt., 1896, Bd. II., p. 169.

[5] "Undersögelser over Ost og Ostegaering," Kristiania, 1905.

curd cheeses, for, in spite of the lack of fat, a piquant product will result, owing to the formation of the sharp-tasting substances.

In the ripening of the **soft acid curd cheeses,** *e.g.*, **Harz Cheese,** the following organisms appear to play the principal part :— *Oidium lactis*, and, possibly, also certain yellow cocci[1], a mycoderma[2], yeast, and lactic acid bacteria. In the ripe state these cheeses generally contain more amino-acids and less ammonia than the soft rennet curd cheeses. *Oidium lactis* has a more pronounced action on casein in presence of large amounts of lactic acid ; it forms only small amounts of ammonia.

[1] *Eckles*, "Landwirt. Jahrbuch der Schweiz," 1905, p. 503.
[2] *Rahn*, "Centralblatt f. Bakt.," 2 Abt., 1906, Bd. XV., p. 786.

Chapter VII

Defects of Cheese

FROM the scientific point of view, *the defects of cheese must be classified according to their origin.* They may be due to *milk of abnormal composition, faulty treatment in the manufacture* (including production and treatment of the curd, pressing, salting, and treatment during ripening) or to *bacterial action.* Only the last-mentioned cause comes within the scope of this work. In practice, however, the various causes are so interdependent that it is difficult to make any hard and fast distinction. By judicious treatment of the milk and the cheese, it will generally be possible to avoid the development of harmful organisms, whereas with careless treatment, even when starting with good milk, their development may easily be encouraged.

To take an instance, if the milk has curdled badly (a fault which might have been corrected by raising its temperature or adding a little calcium chloride), owing to the presence of raw milk or milk from cows which are getting towards the end of their lactation period, the curd will not dry readily and the cheese will tend to become spongy, even though the milk used could be described as good from the bacteriological point of view. **Sponginess** may thus be due, not merely to the presence of large numbers of gas-producing organisms, but to an excess of lactose in the curd. As has been mentioned, gas-producing organisms may come from the udder (*Aerogenes mastitis*), though in the great majority of cases they owe their presence to diarrhœa among the cows and unclean milking; the acuter the digestive trouble, the richer will the manure be in gas-producing bacteria and the greater will be the difficulty in keeping the milk free from infection through the manure. The gases which are formed consist chiefly of hydrogen and carbon dioxide, the former being the more objectionable, for the water in the cheese will absorb large amounts of carbon dioxide before the slightest tendency towards sponginess becomes apparent. At 15° C. and at atmospheric pressure water will absorb its own volume of carbon dioxide, at two atmospheres pressure twice, and at three atmospheres three times its volume,

etc.; not only are the hard cheeses submitted to great pressures in the press, but any tendency towards expansion from within will meet with considerable resistance, owing to the close texture of the curd, and particularly the rind. By storing at a low temperature, a tendency towards sponginess may be kept in check in two ways, for not only is the absorption of carbon dioxide by water increased by lowering the temperature, but the development of the gas-forming bacteria is checked. It will thus be understood why sometimes bacterial growth may appear to commence afresh when the cheese is taken out of a cold cellar on a warm day, even though no new development of gas-forming bacteria actually takes place. It will also be understood why eyes are more readily formed in Emmental cheese when the curd has been saturated with carbon dioxide during the fermentation of the lactose; under these conditions the distinction between normal and abnormal eyes may not become so sharp as might be expected, even though the two processes are due to totally different bacteria. Unlike carbon dioxide, hydrogen is only very sparingly absorbed by water; it follows that those organisms which produce the most hydrogen are capable of doing the most harm. Thus the butyric acid organisms may transform the cheese into a large-holed, spongy mass in the course of two days; the non-motile butyric acid bacteria are especially dangerous, owing to their ability to ferment calcium lactate, and thus to cause damage after all the lactose has been fermented. The aerogenes bacteria, especially the colon bacteria, also produce hydrogen. On the other hand, these organisms are able not only to effect respiration by means of atmospheric oxygen, but they will transfer loosely-bound oxygen from oxidising agents, like saltpetre, to sugar, and thus consume the sugar completely, so that no hydrogen is liberated or lactic acid formed. Saltpetre is thus an excellent preventative of the harmful effects of these bacteria; as a rule it will be sufficient to add 30 to 50 grams of potassium nitrate per 100 litres of milk[1]. As the propionic acid bacteria and the lactose fermenting yeasts do not form other gases than carbon dioxide, they are less dangerous to the fresh curd. The yeasts can, however, blow the soft cheeses in which there are particularly large amounts of sugar to be fermented; their growth is promoted by small amounts of free lactic acid. The most natural means of preventing sponginess, as well as most other cheese defects, is the use of a vigorous lactic acid starter consisting, as

[1] According to *Rosengren*, it is dangerous to use saltpetre in Emmental cheese, even in small amounts; 10 grams per 100 litres may produce an unclean taste and turn the cheese red. Feebly acid cheeses, like Gouda, are best able to stand the addition of larger amounts of saltpetre.

far as possible, of a culture of the specific organisms of the cheese in question; in this way the ripening of the cheese will also be accelerated. Saltpetre in small amounts is without effect on the ripening process, while a low temperature and plentiful salting delay it. The last-mentioned expedient is very effective in regulating the formation of normal eyes, but it is too slow in operation to prevent the defect of sponginess unless the curd is salted direct, before moulding, though this method is not applicable to the choicer varieties of cheese. (See the " Ripening of Emmental Cheese.")

As was mentioned above, the degree of plasticity of a cheese is determined by its content of acid and salt. If the curd is too acid or if it has been made too dry, *e.g.*, by over-scalding, it will become crisp and, therefore, easily *crack or crumble to pieces* on rough treatment, and particularly if much gas is produced. A hard coat or rind produced by injudicious salting or over-drying will, of course, easily crack. The coat will also tend to crack when large amounts of whey collect beneath it; this may occur when the cheese is pressed too hard to begin with, so that the outer layer becomes too compact before a sufficient amount of whey has been expelled, or, again, as mentioned above, the trouble may be due to slimy whey. If the cheeses are too damp without, however, being particularly acid, they will *flow* or *be liquefied*, especially if the temperature is high. *Sc. liquefaciens*, if abundant, will always cause this defect. It has a strong peptonising action on the curd and produces, at the same time, a bitter taste.

While **defects in colour** no longer play any important part as far as milk is concerned, they are of great importance in the case of cheese making, for in cheese they have ample time to develop. Distinction may be made between cases in which the colour appears evenly or in spots throughout the whole cheese, and those cases in which it only appears on the surface.

Light spots in the interior are due to the reduction of the colour which has been added to the cheese[1]. The commonest colour defect is the *turning grey or blue* of the curd, due to admixture of *salts of iron or copper*. Iron may come from the water, rusty pails or, if the milk is heated by direct steam, from the steam-pipes. Copper may come from the cheese vat or, in the case of Parmesan cheese, from the untinned copper vessels in which the

[1] According to *Campbell* (" Trans. of the Highland Agric. Soc. of Scotland," 1898) this defect may be avoided by the use of a good lactic acid starter. The reducing organisms may be colon bacteria (*Harrison*, " Revue Générale du Lait," 1902, vol. 1, p. 457) and torulæ (*Harding, Rogers* and *Smith*, New York Agric. Exper. Station, Geneva, 1900, Bull. No. 183).

evening milk is often kept for eighteen hours. In the case of iron the colour is due to ferrous salts, for which reason the outer portion of the cheese will not be coloured, while the colour disappears from a slice of the cheese which is exposed to the air. In the case of copper the conditions are reversed; as the colour is due to the green cupric salts, the outer portions will be the most affected, and a slice cut out of the cheese will only develop the full colour after exposure to air for some time. Metallic sulphides, stable in air, will be produced where hydrogen sulphide is formed. The minute coloured spots which are sometimes found distributed throughout the whole mass are of greater interest from the bacteriological point of view, as they are *colonies of chromogenic organisms*, which develop in the same way as on other solid media, such as nutrient gelatine or agar [1]. The conditions of bacterial growth consequent on the cheese being a solid medium are not so strikingly illustrated in the case of the lactic acid bacteria, which grow rapidly throughout the fresh curd, and thus appear to be evenly distributed; but the slow-growing organisms which appear at a later stage will appear in this characteristic manner. Thus *Bacillus cyaneofuscus* (which, however, dies out before the cheese has fully ripened) forms *blue spots* in Edam cheese, while the chromogenic propionic acid bacteria form *red and brown spots* in Emmental cheese. In all probability the eyes in Emmental cheese are formed in those places where the colonies of propionic acid bacteria are particularly abundant. According to *Connel* [2], the so-called *rusty spots* in Cheddar are caused by *Bacillus rudensis*, an acid-producing organism, which may possibly belong to the propionic acid group, as it is generally found in or near the eyes of Emmental cheese; being particularly prevalent in spring, it is supposed to originate from the fresh grass; if once established in the dairy, it will appear in successive batches of cheese unless all the appliances are sterilised.

The form of discoloration resulting in the production of a *red colour just inside the rind*, but not in the rind itself, may be regarded as intermediate between interior and exterior discoloration. It is said to be due to the diffusion of colouring matter from the shelves into the cheese; shelves of white pine, but not of red pine or fir, are said to be objectionable in this respect. This explanation hardly holds good in all cases, for the red zone may spread after the cheeses have been removed to another place, and

[1] The staining of cheese sections to show the natural position of the bacteria was first accomplished by *Miss Gerda Troili-Petersson*, 1904 ("Centralblatt f. Bakt.," 2 Abt., Bd. XI., p. 212).

[2] "Discoloration of Cheese," Canadian Dept. of Agric. Bull., 1897, and *Harding* and *Smith*, New York Agric. Exper. Station, Geneva, 1902, Bull. No. 225.

the coloration is generally accompanied by an unpleasant taste. In the latter event the trouble is most likely due to chromogenic organisms which have penetrated the rind. Among the bacteria which produce a red colour in cheese, both cocci and rod forms are known [1]. Several of them liquefy gelatine, and many of them are chromogenic on cheese, but not on the common media, and some of the organisms which produce a red colour on the surface of the soft mouldy cheeses are only chromogenic in presence of the decomposition products formed from the casein by the moulds which are characteristic of the cheese in question. Bacteria are known which *colour the rind yellow* [2] and *brown*; in fact, the rind always becomes brown when kept damp. Atmospheric oxygen plays an important part in all these colour changes. Many moulds also produce surface colorations, *e.g.*, *Oidium aurantiacum*, *Penicillium casei*, *Cladosporium herbarum*, and *Monilia nigra*. Red and yellow Torulæ are also believed to play some part in the process. Many moulds penetrate the rind and *make the surface uneven*. In this connection mention may be made of cheese mites and maggots, though their detailed description does not come within the scope of a work on bacteriology. In order to avoid the transference of harmful organisms from cheese to cheese, the uninfected cheeses should always be washed before those which are infected, and the cloth should be boiled for a quarter of an hour daily. The only efficient means of preventing the defects under consideration is the thorough disinfection of the ripening room and the shelves (see p. 56).

Defects in taste and smell, originating from the fodder, will generally disappear in time. Similarly, the bitter taste due to *Streptococcus liquefaciens* and *Torula amara*, and sometimes also that due to certain lactic acid rod bacteria [3], may disappear during a later stage of the ripening. Many of the chromogenic organisms produce unpleasant tastes. *The tallowy taste* sometimes found in rich cheeses (*e.g.*, Gouda cheese) is probably due to those organisms which turn milk and butter tallowy. Bitter and tallowy tastes are defects which chiefly occur when fresh cheeses are kept too

[1] Thus *Adametz* has described two micrococci which do not liquefy gelatine, or, at any rate, only do so very slowly; they form red colonies on gelatine and agar. *Gratz* has isolated a liquefying bacterium, *Micrococcus rubri casei*, which forms pink colonies, and *Weigmann* has isolated two liquefying organisms, *Micrococcus chromoflavus* and *Bacterium casei fusci*, which form chrome yellow and cream-coloured colonies respectively on the common media, but which turn the surface of the cheese red. The red *Bacillus firmitatis*, isolated by *Roger* from Camembert cheese, grows only in the decomposition products produced by moulds.

[2] Thus *Barthel* has found that yellow spots may be produced by *Micrococcus flavus*, a liquefying organism commonly found in air.

[3] *Harding, Rogers* and *Smith*, New York Agric. Exper. Station, 1900, Bull. No. 183.

damp and cold [1]. In the French soft cheeses *Penicillium brevicaule* produces a taste of cabbage.

The organisms which produce spongy cheese may also give rise to unpleasant, mostly *bitter-sweet tastes*. Spongy cheeses, moreover, dry too readily, so that they ripen too slowly.

As **cheese poisoning**, like meat poisoning, is chiefly due to certain colon bacteria and sometimes to non-motile butyric acid bacteria, spongy cheese must always be regarded with suspicion [2].

[1] At a low temperature the peptonising bacteria develop more rapidly than the good lactic acid bacteria, or the bacterial metabolism may be too sluggish to cause the disappearance of all the atmospheric oxygen in the cheese. Sunlight promotes the oxidation of the fat in cheese, as in butter. As already mentioned, copper salts and carbonic acid which latter is copiously produced in the ripening of cheese, may turn the fat tallowy.

[2] *H. Kühl* ("Zeitschrift f. Untersuchung der Nahrungs und Genussmittel," 1913, Bd. 25, p. 193) reports on a case of poisoning by cheese which was due to an aerogenes bacterium. A number of references to the literature of this subject are given in this paper.

Chapter VIII

The Grading of Milk

THIS chapter deals with the more important methods of judging of the *cleanness and freshness of milk and its suitability for the making of good dairy products.*

EXAMINATION FOR TASTE AND SMELL

As the senses of taste and smell are our best aids in avoiding putrid or harmful food, every careful examination of milk should include tasting and smelling. Unfortunately the test is of a decidedly subjective nature, as the senses in question are soon dulled. Further, considering that the temperature of the milk varies considerably on arrival, and that the smell and taste are most pronounced when the milk is warm from the cow, it is evident that milk can only be graded very roughly by this method, and that the detailed classification in fifteen grades according to the taste and smell alone, as was previously carried out by the Danish milk grading associations, was futile.

ESTIMATION OF DIRT

Milk which contains visible amounts of pus, blood, manure, etc., must of course be regarded with suspicion from the outset. It may justly be demanded that milk retailed in towns shall show no sediment when 1 litre is allowed to stand at rest for two hours in a vessel of colourless glass. It is advantageous to use vessels tapering to the bottom, so that the sediment may readily be collected for examination after the milk has carefully been decanted off. As only the heaviest particles will separate, it is more usual nowadays to filter a definite quantity through a disc of cotton wool, which will become more or less dirty according to the state of the milk. The discs may be dried and kept, so that a "dirt scale" may be prepared for future reference and comparison[1], and the dirtiest discs may perhaps produce some moral effect if sent to the suppliers responsible for them. As it is impossible to take an average sample of dirt from a large quantity of milk, it

[1] See, for example, *Höyberg's* Scale in "Maanedsskriftet for Sundhedspleje," 1910, p. 49.

will be necessary in dairies to filter the contents of each can or churn through a separate cotton wool disc, and also to examine the empty cans carefully, for most of the dirt usually remains in them. As has been previously pointed out, the chief impurity of milk is cow manure, which contains 80 per cent. of water, and soluble matter, both of which are completely incorporated with the milk owing to the shaking up which occurs in transit; at the same time, the bacteria which constitute an appreciable proportion of the solid matter, are distributed throughout the milk. The above estimate applies to normal dung; if the cows are suffering from diarrhœa, the dung will contain a still larger proportion of soluble matter, and consequently the filtration test will show less dirt. Further, the more liquid the dung the greater will be the proportion of dangerous bacteria introduced into the milk. The estimation of dirt therefore furnishes no measure of the bacterial contents of the milk, and it must also be remembered that very dirty milk which has been well cooled may contain fewer bacteria than less dirty milk which has been inadequately cooled. In reality, therefore, the only sure indication afforded by the dirt test is whether the milk has been properly cleaned or not, either by straining, filtering or centrifuging.

TROMMSDORFF'S LEUCOCYTE TEST

The particles of dirt and foreign bodies which are found in suspension in milk are removed much more completely by centrifuging than by sedimentation or filtration. Thus, of the white blood corpuscles or *leucocytes* which normally do not separate out when the milk is allowed to stand for a relatively short time, 3 to 50 per cent. are separated by centrifuging, or even more if the milk is warm [1]. The heating should, however, not exceed 70° C., for otherwise precipitation of the proteins may occur. As was first shown by *Barthel*, the centrifuge slime therefore consists very largely of leucocytes [2]. By direct microscopical counts, normal milk has been shown to contain ½ to 1½ million leucocytes per cubic centimetre [3]. The number of leucocytes increases as the yield of milk decreases, being particularly high at the beginning and towards the end of the lactation period. It is still higher in cases of udder disease, and on this fact *Trommsdorff* has based his test, the object of which is to ascertain whether or not the milk has been derived from healthy cows. The test is carried out as

[1] *Campbell*, U.S. Dept. Anim. Industry, Bull. 117, p. 19.
[2] "Revue générale du lait," 1901, vol. 1, p. 193.
[3] *Prescott* and *Breed*, "Journal of Infectious Diseases," 1910, vol. 7, p. 632; *Breed* and *Stiger*, *ibid.*, 1911, vol. 8, p. 361.

follows : 10 c.c. of the milk are introduced into a glass tube, the end of which is drawn out into a capillary graduated in thousandths of a cubic centimetre. The tube is closed by a rubber stopper and whirled at a speed of 1200 revolutions per minute. Normal milk will generally yield a sediment measuring 0·002 to 0·004 c.c., while milk drawn from diseased udders will yield a sediment measuring 0·01 c.c. or even more. The amount of the sediment is, however, not a decisive criterion by itself ; the test must be supplemented by a *microscopic examination*, and only in cases where large numbers of bacteria characteristic of udder disease,

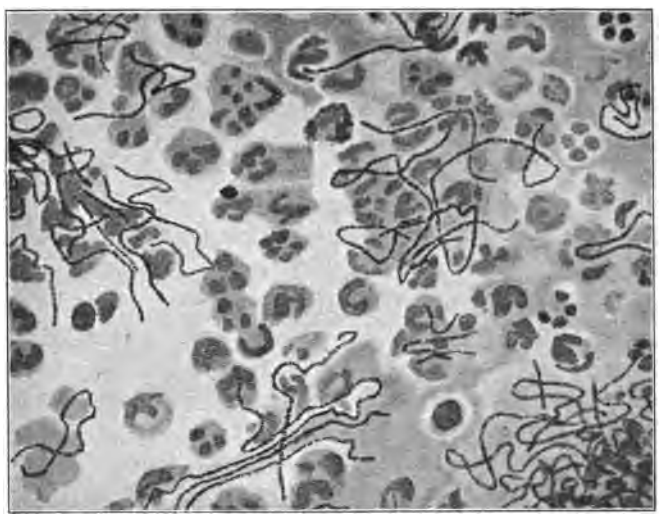


FIG. 64.—Leucocyte Sediment from the Milk of a Cow suffering from *streptococcic mastitis*. (After *Ernst*.) × 1000.

e.g., streptococci, are found can definite conclusions be drawn. As these bacteria cannot be distinguished from the harmless milk bacteria by direct inspection[1], the test is only of value when applied to milk fresh from the cow, and can therefore only be applied as an aid to veterinary control at the farm. In mixed milk the characteristic features are completely lost on account of the dilution alone.

THE CATALASE TEST

This test is supplementary to the leucocyte test. The various constituents of blood, especially the corpuscles, are rich in catalase ;

[1] Capsule formation and disc-like cells, which by some authors are regarded as characteristic of *Sc. mastitidis*, can be observed in all streptococci. On the other hand, as already mentioned under *Sc. mastitidis*, the red colour in casein starch stab cultures is very characteristic.

for this reason, milk drawn from cows with diseased udders or from cows which are approaching the end of the lactation period, or colostrum, will liberate large amounts of oxygen from hydrogen peroxide. As the fat globules carry with them large numbers of leucocytes, unpasteurised cream, obtained either by spontaneous separation or by the use of the separator, will be richer in leucocytes than the corresponding skim milk. Many ingenious and sometimes complicated forms of apparatus have been devised for the carrying out of the test. In the author's laboratory *Lind's* apparatus is used; this consists simply of a graduated tube of 20 c.c. capacity, into which 15 c.c. of milk are introduced; sufficient hydrogen peroxide (1 to 3 per cent.) is added to fill the tube, the rubber stopper carrying the bent tube is inserted, and the apparatus is inverted as shown in the illustration. The maximum amount of oxygen is obtained at 20° to 25° C., so that no water bath or thermostat is necessary. The number of cubic centimetres of oxygen evolved in six hours is taken as the catalase number. Fresh milk from healthy cows will not yield more than 2·5 c.c. In mixed milk the blood corpuscles will be too sparsely distributed to produce any recognisable effect. The milk must be perfectly fresh when tested, as many bacteria decompose hydrogen peroxide. The common sarcinæ and micrococci (see p. 38), *e.g.*, *Micrococcus candicans*, and most of the putrefactive bacteria are particularly active in this respect, while the true lactic acid bacteria and the butyric acid bacteria[1] do not produce catalase. Milk which has stood for any length of time at a low temperature, or old pasteurised milk, will accordingly show high catalase values. *Hesse*[2] has proposed to apply the catalase test to butter as follows: 100 grams of butter are warmed to 45° C. and shaken with 40 c.c. of water at this temperature; the aqueous liquid is separated and tested in the same way as milk. It is obvious that butter which has been made from pasteurised cream ripened with a pure starter of lactic acid bacteria will show a low catalase figure; if the butter has been washed with bad water it may show a high catalase figure, for *Bacterium fluorescens liquefaciens* is particularly active in decomposing hydrogen peroxide.

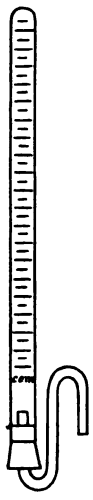


FIG. 65.—Catalase Test Apparatus.

[1] *Orla Jensen*, "Det. kgl. danske Videnskabers Selskabs Oversigter" (Danish Academy of Sciences), 1906, No. 5, p. 306.

[2] "Molkereizeitung," Hildesheim, 1912, No. 6.

THE RENNET TEST

As milk drawn from diseased udders generally coagulates badly with rennet, this test affords the same indications as the two tests just described ; it has also a practical significance, for milk which coagulates badly can hardly be made into good cheese, even if it is satisfactory from the bacteriological point of view. The test may be applied with advantage when difficulty is experienced in making the curd sufficiently dry, so that the particular consignments of milk which are at fault may be detected. In the laboratory the test is generally carried out by means of *Schaffer's* apparatus, which consists simply of a shallow water bath with a false bottom on which a number of beakers can be placed. One cubic centimetre of one of *Hansen's* rennet tablets, No. 2, in 500 c.c. of water is added to 100 c.c. of milk which is kept at 35° C. ; normal milk will coagulate in nine to nineteen minutes. *Marschall's* apparatus is better suited for use in the dairy ; this consists of a graduated enamelled iron cup having a fine opening in the bottom. The hole is closed by a finger, the cup is filled, and milk is allowed to run out until the level comes to the top graduation mark in the cup. One cubic centimetre of the rennet solution is rapidly and thoroughly mixed with the milk, and the finger is removed from the opening. The milk will cease to run out the moment coagulation sets in, so that the capacity for coagulation will be inversely proportional to the amount of milk which has run out. Conversely, this apparatus may be used for the estimation of the coagulating capacity of rennet, which is a test of some importance in the Swiss dairies which make their own rennet. The means available for correcting a deficient capacity for coagulation are discussed on p. 130.

DETERMINATION OF ACIDITY

The degree of acidity is generally understood to be the number of cubic centimetres of standard sodium hydroxide solution required to neutralise a given volume of milk, with phenol phthalein as indicator. The *Soxhlet-Henkel* degrees, which are largely used on the Continent, express the number of cubic centimetres of quarter normal alkali per 100 c.c. of milk. In British and American works, degrees of acidity are understood to represent the number of cubic centimetres of normal sodium hydroxide per litre of milk ; the titration is often carried out with decinormal alkali, using smaller quantities of milk, the results being calculated to the above standard ; as little as 10 c.c. of milk is sometimes used, but more exact results can be got by using larger quantities. No water should be added to the milk or cream before titration, as

this will lower the acidity. *Ellbrecht's titration paper* "Exact" has been designed as a colour standard in order to ensure that the same end-point is reached each time. *Richmond* gives the following method in his "Dairy Chemistry": "10 c.c. of milk are titrated with decinormal baryta, or 11 c.c. with eleventh normal strontia, using 1 c.c. of ½ per cent. phenol phthalein; as a standard, an equal volume of milk is coloured with one drop of 0·01 per cent. rosaniline acetate in 96 per cent. alcohol." The degree of acidity of normal milk generally lies between 16 and 19 (6·4 to 7·6 *Soxhlet-Henkel*). If under 15 (6 *S.-H.*), the milk is probably derived from cows which are sick or approaching the end of the lactation period, or it may have lost a portion of its natural carbonic acid by having been kept in shallow vessels, shaken or warmed. If the degree of acidity is over 21 (8·4 *S.-H.*) the milk may be derived from cows suffering from streptococcic mastitis or it may contain colostrum the acidity of which may be as high as 55. As a rule, however, high acidity will be due to incipient lactic acid fermentation. Mixed milk having an acidity of over 21 will usually coagulate on mixing with an equal volume of 68 per cent. alcohol; this is the basis of the so-called **alcohol test.** The **boiling test** is based on the fact that milk having an acidity of over 27·5 coagulates on boiling. It is, however, impossible to be certain that the milk will stand pasteurisation if the acidity exceeds 22·5 [1]. Fresh milk shows an amphoteric reaction towards litmus, *i.e.*, it turns red litmus blue and blue litmus red; if the degree of acidity is under 12·5, the reaction towards litmus will be alkaline. According to *Höyberg* [2], the *rosolic acid solution* proposed by *Hilger* for the detection of added soda may be used with advantage in testing milk from the individual quarters with a view to detecting udder disease. If 5 c.c. of 96 per cent. alcohol and 0·5 c.c. of a 1 per cent. rosolic acid solution are added to 5 c.c. of milk, an orange colour will be obtained with normal milk, and a red colour with alkaline milk. *Eugling* [3] has shown that a saturated alcoholic solution of alizarin may be used for the same purpose; if 5 to 10 drops of the solution are added to 50 c.c. of milk, a red-violet colour will be produced with normal milk, a violet-blue colour with alkaline milk, and a yellowish colour with sour milk. *Morres* [4] combines this test with the alcohol test in the **Alizarol test,** 0·05 per cent. of alizarin being dissolved in the 68 per cent. alcohol, so that an indication may be obtained as to whether the coagulation is due to acid- or rennet-producing bacteria. If narrow test tubes

[1] *Henkel,* "Milchwirtschaftliches Zentralblatt," 1907, Bd. III., p. 378.
[2] "Skandinavsk Veterinaertidsskrift," 1911, p. 23.
[3] "Handbuch f. die praktische Käseriei," Leipzig, 1901, p. 20.
[4] "Oesterreichsche Molkerei-Zeitung," 1912. A colour scale for this test is supplied by *Dr. N. Gerbers Co.*, Zurich.

are used, 2 c.c. of milk and 2 c.c. of alcohol will suffice for this test. It is recommended that the dairies should apply it to each can of milk and reject all milk which shows a precipitate or an abnormal colour.

THE FERMENTATION TEST

This test shows if the milk has become infected with an undue proportion of gas-producing organisms, which first and foremost include the pseudo lactic acid bacteria. We have already seen that these are among the most objectionable organisms that can be met with in dairy practice, as they cause trouble in various ways, including the spoiling of milk for the purpose of cheese making. As the pseudo lactic acid bacteria are usually brought into the milk with the cow dung, and are particularly plentiful when the cows are suffering from diarrhœa, the fermentation test will give evidence of undue contamination and thus give warning that the milk may possibly be dangerous for human consumption. This test is of value not only in judging of the suitability of milk for cheese making, but also in the laboratory control of retail milk, particularly that which is to be used for infant feeding. While most of the milk bacteria (excepting the thermobacteria, which, however, are rare in fresh milk) grow best at about 30° C., many of them ceasing to develop at temperatures above 38° C., the pseudo lactic acid bacteria, being typical intestinal organisms, have their optimum at blood heat, and will gain predominance most readily at a slightly higher temperature, for which reason the fermentation test is carried out at 38° to 40° C. It is important that the temperature should not be allowed to vary beyond these limits, as at a higher temperature good milk may appear to be bad, while at a lower temperature bad milk may appear to be good. If fine distinction is made between the different types and degrees of fermentation, these temperature limits are too wide, and the temperature should be kept constant at 38° C. In order that the results of the test may be strictly comparable, it is advisable always to use tubes of a certain diameter (about 2 cm.), into which 40 c.c. of milk are introduced. The shape of the tubes is shown in the accompanying illustration ; they should be strongly made and provided with a graduation mark at 40 c.c., and with a frosted square so that they can be marked in pencil. An average sample of each supplier's milk should be taken, preferably from the weighing or measuring vessel, by means of a small measure furnished with a pointed spout. The measure should be rinsed several times with the milk which is being sampled before taking the actual sample. The tubes are marked, covered with a small cap of aluminium or zinc, placed in stands and brought into the

water bath. Failing a proper thermostat, the temperature may be kept fairly constant by means of a spirit lamp or gas burner, provided that a large well-insulated water bath is used, and that the temperature of the room does not vary too much. The samples are examined after twelve and twenty to twenty-four hours. If quite fresh, the milk will remain liquid for twelve hours. The sooner visible alteration occurs, the greater the importance to be attached to the results of the test. On the other hand, milk

g_1 g_2 g_3

FIG. 66.—Gelatinous Types.

b_1 b_2 b_3

FIG. 67.—Blown Types.

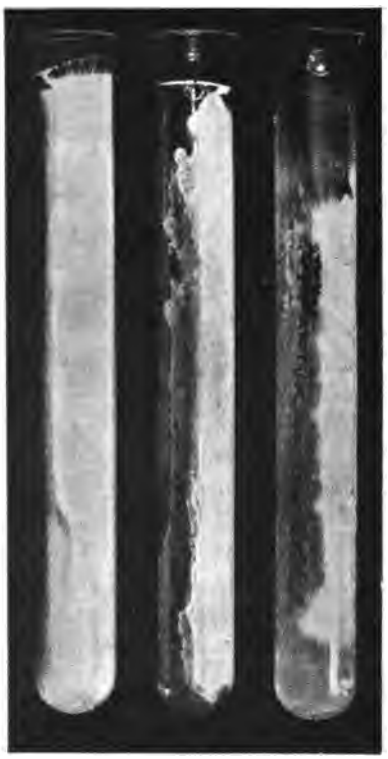
containing relatively few bacteria gives unreliable indications, duplicate tests often showing different types of fermentation.

According to *Peter's* classification, we may distinguish between the following types in the fermentation test :—*Fluid* (*f*), *gelatinous* (*g*), *blown* (*b*), *spongy* (*s*) *and cheesy* (*c*) (see his illustrations). In the gelatinous type the true lactic acid bacteria predominate ; a perfectly homogeneous coagulum is denoted by g_1, a coagulum with very few streaks and bubbles by g_2, and one with few streaks and bubbles by g_3. In the blown (gassy) milk the pseudo lactic

acid bacteria predominate; b_1, b_2 and b_3 indicate progressive degrees of intensity of gas evolution. While there is only a difference of a degree between g_3 and b_1, all the casein will have been driven to the surface in b_3. The spongy types, s_1, s_2 and s_3, differ from the blown types in being finer in curd texture. In s_1 the coagulum forms so fine a network that it may easily be mistaken for g_1. Milk which is poor in true lactic acid bacteria often becomes spongy in the fermentation test, owing to the gas production

s_1 s_2 s_3

FIG. 68.—Spongy Types.



c_1 c_2 c_3

FIG. 69.—Cheesy Types.

being in full swing before coagulation occurs. In this case the gas-producing organisms may sometimes be lactose fermenting saccharomycetes. The cheesy type is distinguished by a well-marked separation of clear whey, due to organisms which secrete rennet-like enzymes, especially peptonising lactic acid streptococci; c_1, c_2 and c_3 indicate progressive degrees in the contraction of the curd. If at the same time much gas has been formed, this type cannot well be distinguished from the spongy or the blown type. In actual practice distinction is only made between the

highly objectionable blown types b_2, b_3 and s_3 on the one hand, and all the remaining types on the other. In examining the fermentation types the following should also be watched for: sediment (and possibly pus), sliminess, and evil-smelling milk. Alkaline milk keeps fluid for a long time, and often putrefies before going sour.

The **combined rennet and fermentation test** suggested by *Fr. Jos. Herz* is a special form of the fermentation test, 2 c.c. of the rennet solution mentioned above being added to each test tube. Milk which coagulates badly will have separated comparatively little whey and formed a soft and non-coherent coagulum after twelve hours. On examination after twenty to twenty-four hours the coagulum should have assumed the form of a smooth cylinder, which only shows small holes in longitudinal section. If the coagulum contains large holes, and especially if it forms a screw-shaped sponge floating on the surface of the whey, the milk must be considered unsuitable for cheese making.

In the Emmental dairies, where a home-made rennet is used which, at the same time, is also a culture of the more important ripening bacteria, but which not infrequently contains colon and aerogenes bacteria, it is of the greatest importance to test the milk, both with and without the addition of rennet. If a bad result is obtained without rennet and a good result with rennet, the milk is certainly bad, though the lactic acid bacteria in the home-made rennet will be able to counteract the defect. The milk is only unsuitable beyond doubt if the test with rennet turns out badly. If the last-mentioned result is obtained in spite of the fact of the milk being good, then the rennet will be known to be unfit for use.

The tubes and caps used in the fermentation test must be cleaned immediately after use by rinsing carefully with hot soda solution, after which they should be placed in the stands and covered completely with water into which steam is then passed for about fifteen minutes. Finally, they are to be dried in a warm place.

THE REDUCTASE TEST

All living cells, including microorganisms, have reducing properties, which are well illustrated by their behaviour towards methylene blue. The rate at which milk decolorises methylene blue will, therefore, depend on the number of microorganisms in it. The reductase test devised by *Barthel* [1] and the author [2] is based on this fact.

[1] "Kungl. Landtbruks-Akademiens Handlingar och Tidskrift," No. 6, 1907.

[2] "Maelkeritidende," 1909, p. 359.

It must not be assumed that the time taken to decolorise methylene blue (reduction time) under standard conditions is an accurate measure of the number of microorganisms in the milk ; for, in the first place, all microorganisms do not reduce with equal rapidity, and, second, the milk itself, as obtained from the cow, contains reducing substances. Of the milk bacteria examined by the author *Streptococcus liquefaciens* appears to have particularly marked reducing powers, while the true lactic acid bacteria are among the organisms which reduce slowly. The obligate anaerobic bacteria reduce rapidly, a fact easily explained on considering that they derive their energy from reduction processes. The reduction time is shortened by the addition of a little alkali, and lengthened by the addition of a little acid. As regards the reducing substances natural to milk, the most important is *aldehyde reductase*, an enzyme which appears to be associated with the fat globules[1], but which has no significance in the present connection, as it only decolorises methylene blue in presence of formaldehyde. Greater interest attaches to the leucocytes, which, like other living cells, are able to reduce methylene blue[2]. They will, however, only exert an appreciable effect on the reduction time if present in large numbers, while it can hardly be considered a drawback to the test that milk rich in leucocytes should appear to be worse than its bacteriological condition would warrant, inasmuch as such milk should always be regarded with suspicion. Of still greater importance is the fact that milk contains substances other than enzymes which exert a reducing action in the absence of oxygen. Thus *Burri* and *Kürsteiner* have shown [3] that newly-sterilised milk which has been prevented from absorbing oxygen decolorises methylene blue rapidly, and *Barthel* [4] has shown that raw milk behaves similarly when the dissolved oxygen is expelled by a current of hydrogen or carbon dioxide, from which he concludes that the decolorisation of methylene blue in milk is due to the action of the milk itself, and that the microorganisms only act indirectly by consuming the dissolved oxygen. This

[1] *Orla Jensen*, " Uber den Ursprung der Oxydasen und Reduktasen der Kuhmilch," Det Kgl. Danske Videnskabernes Selskabs Oversigter (Danish Academy of Sciences), No. 5, 1906, and Centralblatt f. Bakt. II. Abt. 1907, XVIII., p. 211.

[2] *Olav Skar*, " Skandinavsk Veterinaertidsskrift," 1913, p. 51.

[3] " Milchwirtschaftliches Zentralblatt," 1912, p. 269.

[4] " Skandinavisk Veterinaertidsskrift," 1916, p. 155. The author has been able to confirm *Barthel's* results, and has found that milk with a low bacterial count which is kept free from atmospheric oxygen by passing a current of hydrogen through it reduces methylene blue in forty-five minutes at 40° C., no matter whether raw or sterilised. (On the addition of a little formaldehyde decolorisation was complete in ten minutes.) As pure lactose solutions were not found to decolorise methylene blue under similar experimental conditions, the reducing action of milk itself cannot be due to the lactose.

explanation, however, hardly covers the case of the obligate anaerobic bacteria, but, as far as the aerobic organisms are concerned, the reducing action of the milk itself is, no doubt, a contributing factor, so that in the reductase test the degree of aeration of the milk is a condition which must not be overlooked.

Milk will already begin to absorb oxygen freely as it comes in a fine stream from the udder, and its oxygen content will naturally be increased on pouring from vessel to vessel, and especially during any process of aeration to which it may be submitted. The dissolved oxygen will gradually be consumed as the micro-organisms increase in number; the less the milk is shaken, and the deeper the vessels in which it is kept, the quicker will the oxygen content fall off. The temperature at which the milk is kept is also an important factor in this connection, for not only

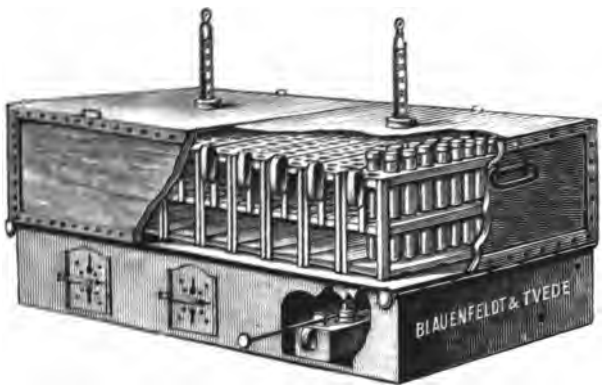


FIG. 70.—Apparatus for Reductase and Fermenting Test to take 200 Samples.

will the bacteria develop more rapidly at higher temperatures, but the individual cells will consume more oxygen at higher than at lower temperatures[1]. In order to equalise these conditions, it will be advisable always to shake the milk well before subjecting it to the reductase test.

It will be seen that the theory of the reductase test is not so simple as was formerly supposed; nevertheless, numerous experiments with mixed milk of commerce have shown that the reduction time, as determined by the reductase test, furnishes just as satisfactory a measure of the bacterial contents as the troublesome method of plate counts, which, as a matter of fact, is by no means less subject to error than the reductase test. Moreover, the differences between the reducing powers of the different species of bacteria are not greater than the differences displayed in this respect by the members of the same species

[1] This is clearly shown by *C. Lind's* work, "Reduktaseprõven sammenignet med Bakterietaellingsmetoden" ("Maelkeritidende," 1915, p. 921).

when subjected to different conditions. The more favourable the conditions for bacterial development, the shorter will be the reduction time. It follows that not only does the reductase test give an estimate of the number of organisms present, but the result will be influenced to some extent according to the vitality of the organisms, which is a factor as important as any other in determining the keeping power of the milk. As the reductase test will reveal any appreciable bacterial increase before this becomes apparent through the presence of lactic acid, it is not only the most convenient, but also the most sensitive, method for the grading of milk, whether for retail direct or for the making of dairy products. On the other hand, it must be admitted that milk from individual cows or milk that has been subjected to unusual treatment may give divergent results. The translator [1] has thus found that milk which has been kept for a long time at low temperatures appears to be better than it really is, in the reductase test carried out at 38° C., because the majority of the bacteria in it are greatly weakened at the temperature in question. Such milk is decolorised quicker at 28° C. A comparison between the reduction times of the same milk at 28° and 38° C. can thus furnish information as to how this milk has been treated. According to the author's proposal, however, the test should be carried out at 38° to 39° C., as this will, in the majority of cases, give the shortest reduction time. On the other hand, it is a wrong principle to employ a still higher temperature, as was originally done, for then the development of all the most common milk bacteria will be hindered.

The methods of sampling and the apparatus required for the reductase test are the same as have already been described under the fermentation test. Particular care must be taken to measure out exactly 40 c.c. of milk, either by means of a graduation mark on the tube or by taking the sample in a measure holding exactly 40 c.c. when filled to the rim. As the preparations of methylene blue on the market are very different in their properties, and the solutions made from them are not permanent, it is necessary to use a fresh solution of definite strength, made from a standard preparation. The tabloids prepared for the purpose [2] are readily soluble in warm water; each tabloid makes 200 c.c. of solution, 1 c.c. of which is required for each test with 40 c.c. of milk. The colour is mixed with the milk by rolling the tube in the hands, then pressing the mouth against a clean portion of the palm of the

[1] "Analyst," 1918, 43, p. 1.

[2] By Messrs. *Blauenfeldt and Tvede*, of Copenhagen, who also supply all requisites for the reductase test, including complete outfits suitable for dairies dealing with milk from 100 to 400 suppliers.

hand and shaking vigorously. For each tube a different portion of the palm should be used, and, when completely wetted, the hand should be carefully washed before proceeding any further. In actual practice, the colour is added when all the samples have been taken and the tubes are in position in the stand. After placing in the water bath, the samples should be examined at frequent intervals during the first twenty minutes, after which they need only be examined every quarter or half hour. As mentioned above, the author has found it best to employ the same temperature as in the fermentation test; in this way there is the additional advantage that the two tests may be combined. **The combined reductase and fermenting test** [1] gives information regarding both the number and the nature of the organisms in the milk.

The joint investigations of *Barthel* and the author [2] have shown that it is possible by means of the reductase test to grade milk and cream into four classes, as follows :—

Class 1.—Good milk, not decolorised in five and a half hours, containing, as a rule, less than ½ million bacteria per cubic centimetre [3].

Class 2.—Milk of fair average quality, decolorised in less than five and a half hours but not less than two hours, containing, as a rule, ½ to 4 million bacteria per cubic centimetre.

Class 3.—Bad milk, decolorised in less than two hours, but not less than twenty minutes, containing, as a rule, 4 to 20 million bacteria per cubic centimetre.

Class 4.—Very bad milk, decolorised in twenty minutes or less, containing, as a rule, over 20 million bacteria per cubic centimetre.

If samples of retail milk from different dairies are to be compared, they must, of course, be examined simultaneously; thus it would be unfair to sample one dairy on a cold morning and another on a warm afternoon. Unpasteurised milk, as sold in large towns, should retain its colour in the test for at least two hours, and the pasteurised milk for at least five and a half hours. Most of the milk retailed by the large dairies in Copenhagen fulfil these requirements [4]. The pasteurised milk supplied

[1] "Maelkeritidende," 1909, p. 359.

[2] "Milchwirtschaftliches Zentralblatt," 1912, No. 14.

[3] As the counts were found by the plating method, they were really under-estimated; milk bacteria usually occur in pairs and not infrequently in long chains, or large clumps, which are not broken up on shaking, and in such cases only one colony is obtained. The counts should at least be doubled, and in some cases they should be trebled or quadrupled.

[4] *Orla Jensen*, "Maanedsskrift for Sundhedspleje," 1909, p. 239. The translator has found the reductase and fermenting test to be of great use in the control of milk as it arrives from the farms, and that the conditions of treatment of the milk on various farms were found to correspond with conclusions which had previously been drawn from the behaviour of the samples in this test.

by these dairies is, generally speaking, not so good as might be expected; this matter deserves attention, for pasteurised milk which is rich in bacteria can only be regarded as a highly objectionable product. It should, therefore, be forbidden to sell pasteurised milk as raw milk.

In the dairies it is impossible to lay down a definite line of demarcation between first and second grade milk without unduly lowering the standard, for obviously milk cannot be expected to conform to the same standard on a close summer's day as on a frosty day. The author has therefore proposed the adoption of *the average reduction time* of all the samples tested at one time as the standard for that particular batch of samples. Only milk which is better than the average will be placed in class 1. Milk showing a reduction time equal to or less than the average, but not less than two hours, will be placed in class 2, and other cases can be dealt with as detailed above. As it may be inconvenient to have the samples examined for longer than twelve hours, the reduction time of any samples not finished in this time may be set down as twelve hours in calculating the average; such cases will only occur on cold winter days. All reduction times may be estimated to the nearest quarter of an hour, the reduction time of milk in class 4 being set down as a quarter of an hour.

If it is desired to combine the grading according to taste and smell and according to the results of the fermenting test, with the grading according to the reductase test, the following system may be adopted :—Samples placed in classes 1, 2 or 3 according to the reductase test are degraded by one class if the taste and smell are decidedly bad, and samples thus placed in classes 2 or 3 are further degraded by one class if the results of the fermentation test show b_2, b_3 or s_3. If the average reduction time should be under five and a half hours, class 1 milk which is decolorised before this time, and which is bad according to the fermenting test, should also be placed in class 2. It will be seen that, excepting in the case just mentioned, the results of the fermenting test are not taken into account in the cases of samples placed in classes 1 and 4, the reasons for this being as follows :—Milk which contains relatively few bacteria will generally show a bad result in the fermenting test as it will be particularly poor in true lactic acid bacteria; moreover, the results observed in the combined reductase and fermenting test in such cases may often be worse than would be shown in the fermenting test alone, as methylene blue exerts a certain toxic effect on bacteria, especially the true lactic acid bacteria, and the fewer the bacteria the more of the poison will each cell have to reduce. On the other hand, the fermenting results are not affected by the small amount of methylene blue

used in the reductase test, in the case of milk containing a greater number of bacteria. Conversely, milk which is very rich in bacteria generally shows good results in the fermenting test, for even if it may contain millions of gas-producing bacteria, it will generally contain still greater numbers of true lactic acid bacteria, and therefore become sour so quickly that the former type will not be able to gain predominance. No error will be committed in ignoring the results of the fermenting test in the cases mentioned, for the small numbers of bacteria in the best milk, whatever their nature, will not be able to exert any influence on the mixed milk of the dairy, while the worst milk will already have been placed in the lowest class, and cannot therefore be degraded any further. In the combined test, the fermentation need not be observed until after the lapse of twenty to twenty-four hours as the reductase test gives a far more accurate estimate of the number of bacteria present than the fermenting test after twelve hours. *It should be clearly understood that while the indications afforded by the fermenting test are purely qualitative, those afforded by the reductase test are purely quantitative, and it is only by combining the two tests that any real insight will be obtained into the nature of the bacterial contents of the milk.*

In large dairies it will only be possible to take an average sample of each supplier's milk. If the number of suppliers is not large, there is no reason why the morning and evening milk should not be tested separately provided that the cans are properly marked according to the respective meals as they always should be. When the milk only comes to the dairy in the morning, it will generally be found that the morning milk will be better than the evening milk, though if the milk comes from a long distance, the reverse may be the case during warm weather if the morning milk has not been cooled. In such cases it will also be necessary to cool the morning milk. The more frequent the test the juster will be the impression formed as to the relative goodness of the milk from different suppliers. Large dairies which of course should keep a well-equipped laboratory under the guidance of a chemist, who has been trained in bacteriology, will do best to test each supplier's milk daily. In the co-operative dairies, samples from all the suppliers should be tested once a week, and one of the members of the association should be present in turn, as an impartial witness. The co-operation of expert milk tasters is no longer absolutely necessary in view of the more objective methods which are now at our disposal ; the examination of samples in the reductase test requires no particular scientific knowledge, and may be performed by any reliable boy or girl. Samples which need not be subjected to the fermenting test should be removed from

the water bath in the evening; next morning the blown or very spongy samples may quickly be picked out. The following table has been drawn up in order to illustrate the system :—

Suppliers number.	Remarks on Taste and Smell.	Time for decolorisation.		Fermentation test.	Classification according to		
		Exact.	In round numbers.		Reductase test alone.	Also taste and smell.	Also taste and smell and fermentation test.
1		19 min.	½ hr.	g_1	IV.	IV.	IV.
2		3 hr. 31 min.	3½ hr.*	s_3	II.	II.	III.
3		24 min.	¼ hr	g_3	III.	III.	III.
4		5 hr. 30 min.	5½ hr.	b_1	I.	I.	I.
5	Acid	5 min.	¼ hr.	g_1	IV.	IV.	IV.
6		over 12 hr.	12 hr.	s_3	I.	I.	I.
7		1 hr. 57 min.	2 hr.	b_3	II.	II.	III.
8		7 hr. 5 min.	7 hr.	s_1	I.	I.	I.
9		52 min.	¾ hr	g_3s_1*	III.	III.	III.
10	Flat or slight turnip taste	5 hr. 17 min.	5¼ hr.	s_2	I.	II.	II.
11	Bitter	1 hr. 40 min.	1¾ hr.	c_1s_1	III.	IV.	IV.

The average reduction time was 3½ hours. The samples were placed :—3 in Class I., 1 in Class II., 4 in Class III., 3 in Class IV.

* In practice round figures only need be noted. To facilitate the calculation of the average, ¼ may be written as ¾. When the fermentation result is denoted by two letters, this denotes an intermediate form.

The average of the four weekly tests places the milk in the class which has to be reckoned with during the corresponding month. The figure is rounded off to the nearest unit, fractions of ½ or over being counted as 1. The grading of milk has been dealt with at length because it furnishes just basis for **payment according to quality,** the only really effective means available for improving the quality of milk. If such a system were established there would be some hope that the farmers would exert themselves to ensure the clean treatment and proper cooling of their milk, just as the more progressive of the Danish farmers have succeeded in increasing the fat content of their milk since the system of payment according to " fat units " was adopted by most of the co-operative dairies in Denmark. The two systems may easily be combined by allowing a small increase in the price per fat unit for first-class milk, and making a corresponding deduction in the case of third-class milk. Double the amount should be deducted for fourth-class milk. There would be no surer means of guarding against butter and cheese defects, and there could be no better recommendation for the dairy products than the fact that they had been made from milk sufficiently clean and fresh to be palatable

to the most critical. This reform would also have far-reaching results contributing indirectly to the welfare of coming generations; for once the principles of hygiene have gained a footing in the cow-shed they will gain admission everywhere else. *Healthy and clean cows, good milk, healthy children.*

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