AGRICULTURAL BACTERIOLOGY

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A STUDY
OF THE
RELATION OF BACTERIA TO AGRICULTURE

WITH SPECIAL REFERENCE TO THE BACTERIA IN THE SOIL, IN WATER, IN
THE DAIRY, IN MISCELLANEOUS FARM PRODUCTS, AND IN
PLANTS AND DOMESTIC ANIMALS.

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PREFACE.

To set any exact limits to Agricultural Bacteriology is difficult. Primarily the subject includes only phenomena produced by bacteria, and phenomena that especially affect agriculture. But some agricultural processes are so closely bound with other industrial phenomena that they cannot be separated. Agriculture grades by imperceptible degrees into numerous secondary industries. Quite a number of the phenomena which will be considered in these pages have a closer relation to these secondary industries than they do to agriculture proper, but nevertheless they do have, at least, an incidental relation to the farm and must, therefore, be included in a discussion of Agricultural Bacteriology.

It has, moreover, in recent years, been a growing conviction that a considerable number of phenomena, hitherto attributed to bacteria, are directly due to a class of *chemical ferments* called *enzymes.* These enzymes are sometimes produced by bacteria, but in other cases by organisms totally unrelated to bacteria. When the latter is the case the fermentations produced by them have, of course, nothing to do with bacteriology proper. But we do not know as yet how commonly these enzymes, or chemical ferments, are concerned in agricultural processes, and even where they do occur it is found that, in some cases, they are intimately associated with true bacteriological action. It is impossible to separate chemical from biological fermentations by a hard and sharp line, nor can we tell to-day how far both of them may be concerned in any particular type of fermentations. In the following pages it will, therefore, be necessary to consider, to a certain extent, both types of fermentation. While both must be described and discussed, the bacteriological fermentations will demand most of our attention. For all of these reasons the limits which we shall draw to the subject of agricultural bacteriology are somewhat arbitrary, and some of the topics here considered may perhaps not be regarded as belonging strictly to the subject. All of them, however, have at least an incidental relation to the farmer and his industry.

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TABLE OF CONTENTS.

PART I. GENERAL NATURE OF BACTERIA AND FERMENTATIONS.

PART II. BACTERIA IN SOIL AND WATER.

PART III. BACTERIA IN DAIRY PRODUCTS.

PART IV. THE RELATION OF BACTERIA TO MISCELLANEOUS FARM PRODUCTS.

PART V. PARASITIC BACTERIA.

PART I.

GENERAL NATURE OF BACTERIA AND FERMENTATIONS.

CHAPTER I.

THE NATURE OF BACTERIA.

IMPORTANCE OF BACTERIOLOGY TO AGRICULTURE.

It is about two centuries since the microscopic organisms now known as bacteria were first seen. The Dutch microscopist, Leeuwenhoek, was the first to study them, in 1695, and his descriptions, considering the fact that he had only simple lenses to work with, were remarkably correct. Even his suggestions concerning their nature sound quite modern and were certainly superior to much of the speculation that followed. For 150 years after Leeuwenhoek, although the microscope became a familiar plaything, it was hardly thought that these minute organisms offered a subject for serious study. For a century, they were simply objects of speculation, and many were the exclamations which they excited as to the wonders of nature, with here and there a suggestion as to their possible importance in producing certain natural phenomena.

Not until toward the middle of the 19th century was it conceived that the microscopic organisms, grouped together under the general head of *animalculæ*, could have more than scientific import. At that time there began to appear suggestions as to their possible relations to certain diseases, and almost simultaneously they were thought of as contributing to certain general changes in organic products, called *fermentations*. One of the first real contributions to a knowledge of their significance was the demonstration of the fact that certain microscopic organisms cause an infection in dairies characterized by the appearance of *blue milk*. Thus, it is interesting to notice that, at the very beginning of the modern study of bacteria, they were associated with peculiar *agricultural* phenomena; interesting, in view of the fact that in the next quarter of a century, or more, the chief investigation, and all the interest in them, centered around the question of their agency in producing disease. Bacteria have suffered in reputation from the fact that, at the middle of this century, they were studied by microscopists chiefly from the standpoint of their agency in the production of disease. It was quite early suggested, and soon demonstrated, that these little plants have the power of producing certain dreaded diseases, and the reputation which they thus obtained has clung to them in all of the subsequent years of study. This was natural enough, for mankind is always particularly interested in the causes of disease, and the demonstration of their causal relations to certain dreaded diseases gave to these organisms at once a wide and unenviable reputation. The very word *bacteria* has become, in the minds of some, almost synonymous with disease. Their relation to the medical profession has been recognized, and more or less extended courses in the study of bacteriology have rapidly made their appearance in medical schools. Health boards and sanitary commissions have recognized that their primary duty is to deal with bacteria, and most of the regulations looking toward preservation of the public health have been, manifestly, directed toward the destruction of these organisms.

As a result the bacteria have obtained a reputation which they have not deserved. To condemn a whole group of plants because some of them are poisonous is manifestly illogical; but this is exactly what has been done in regard to bacteria. The more they have been studied the more evident has it become that they perform other important functions in nature besides that of producing disease; that, indeed, the production of disease is really only a comparatively rare property, possessed by a few of the numerous species of this group of organisms. As bacteriologists have widened their views and looked outside of the human body they have found that these organisms are not only excessively abundant in nature, but that they play a part in the phenomena of living things which has been wholly unexpected. As far back as 1860 Pasteur had shown their agency in the souring of milk, and within the last twenty years a larger and larger amount of attention has been directed to the part played in nature by those species of bacteria which have no relation to human disease and are never parasitic. As a result there has developed a new branch of bacteriology which deals with phenomena wholly separate from disease.

In particular we have learned that bacteria are related to agriculture. Not only is it true that they are the cause of certain animal and plant diseases, with which the farmer has to contend, but it is becoming manifest that they are intimately associated with many normal processes which are going on in the soil, water and elsewhere, and which are essential to agriculture.

Agricultural bacteriology is to-day the advance ground of research. It is only about two decades since our scientists have seen the importance and the greatness of the field, and, although the advance in this short period of time has been rapid, it is inevitable that many problems should be still unsettled. While we have studied, in considerable detail, many of the phenomena where bacteria are concerned in agricultural processes, it is not to be expected that the few years during which the subject has been under investigation shall have done very much more than have cleared the field for further research. Particularly is this true in connection with the practical *application* of bacteriology to agricultural processes. Scientific discovery must always precede practical application, and although the attempt is always made to apply scientific discoveries to practical processes as soon as they are made, these applications are, in the first years, sure to be tentative and uncertain. This is exactly the condition of agricultural bacteriology to-day.

The agricultural side of bacteriology is, if possible, more important than the pathological side. If the medical student needs to know something of these organisms and their relation to disease, even more does the agriculturist need to understand their relation to his industry. Agricultural bacteriology has, therefore, become a topic of importance equal to, if not greater than, medical bacteriology. It has appeared that these microörganisms play a very fundamental part in the processes of nature; that the life phenomena of animals and plants are so inextricably bound up in the functions of bacteria that without them life processes must soon cease. Indeed, it is becoming evident that the farmer, even more than the physician, should be acquainted with bacteriology. The physician, in the curing of disease, gains a certain advantage from his knowledge of bacteria, but the farmer is obliged to make use of these agents in a large number of his farming processes; and hence it is almost a matter of necessity that the agriculturist of the future should thoroughly understand the general phases of bacteriology. Many of the most vital problems which are coming up before our agricultural communities, the settlement of which is necessary if agriculture in the future is to hold its own successfully against opposing forces, are clearly to be settled along the line of bacteriology. From beginning to end the occupations of the agriculturist are concerned in the attempt to obtain the aid of these microörganisms where they may be of advantage, and in preventing their action in places where they would be a detriment. The farm cannot be properly tilled unless the farmer has, in addition to his seed crop, soil and cattle, a stock of the proper kind of bacteria to aid him in preparing the soil and in curing the crops. Farming without the aid of bacteria is an impossibility. Although attempts at a practical application of bacteriological discoveries are numerous, many, indeed most of them, are still somewhat tentative and can hardly be looked upon as having yet reached a position where they can be unhesitatingly recommended to the farmer. Nevertheless, the discoveries already made have, in some respects, revolutionized agricultural processes. The changes in agricultural methods, due to bacteriology, have been largely adopted all over the world ; but they have been generally adopted by farmers, without any knowledge that they are benefiting from bacteriological research. That these practical applications of bacteriology to agricultural processes will increase with the next few years, is certain. We have reached a point where every advanced farmer, who wishes to put himself into a proper condition to make the best use of the means at his disposal, and to profit by discoveries as they are made, must have at least a general knowledge of the fundamental factors of bacteriology as they are related to agriculture.

WHAT ARE BACTERIA ?

The term *Bacteria* is a comparatively recent one. In all the early work which was done with these organisms this word was not used as referring to a class of microscopic organisms. Indeed, it was not until about twenty-five years ago that the organisms here included were recognized as forming a group by themselves, being before that time associated with a miscellaneous lot of others, including yeasts, molds and some animals. While to-day we recognize *Bacteria* as forming quite a distinct class of plants, we also recognize that they are closely related to the yeasts, and only a little more distantly related to the molds. Moreover, in some of the phenomena which we shall find it necessary to notice in later pages, the yeasts and molds play a part equally important with the bacteria, while in others—alcoholic fermentations—the function of the yeasts is the greater. But, with occasional reference to these closely related plants, we can confine our attention almost wholly to the *Bacteria* proper.

In a work of this nature any extended consideration of the classification of bacteria is needless. It is only the microscopist who can make use of such a classification. The agriculturist is interested in bacteria solely for their *functions* and not at all for their scientific relations. For this reason we may consider the problem of the microscopic structure and relation of these plants with great brevity, selecting for consideration only such salient features as are of most importance in understanding bacteriological terms used in literature. For a fuller consideration of bacteria and their methods of culture reference must be had to some of the numerous books now available for this purpose (see list of references).

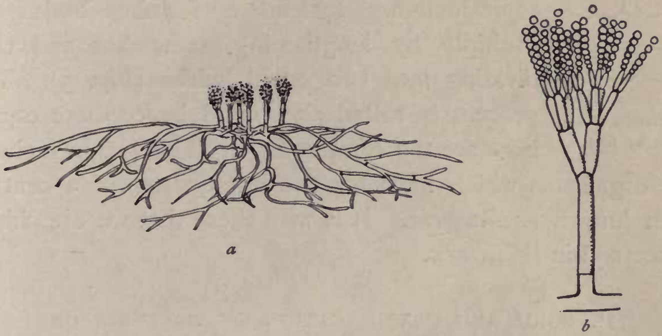
I. CLASSIFICATION OF FUNGI.

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

1. **Higher Fungi.**—Under this head we may group together a large variety of colorless plants comprising several large

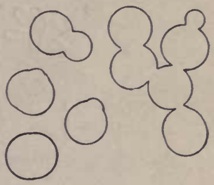
classes, including such forms as *molds, mushrooms,* etc. We are only incidentally concerned with them in this work, although many are of great importance in agriculture. They are generally characterized by the development of long slender threads (*mycelium*), which grow like delicate roots through the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

FIG. 1.



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

FIG. 2.

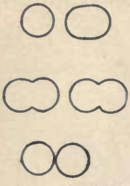


Yeast, showing method of budding.

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

is called *budding* and the yeasts are consequently called the *Budding fungi.* The importance of yeasts in the great fermentative industries, so closely related to agriculture, is well known.

FIG. 3.



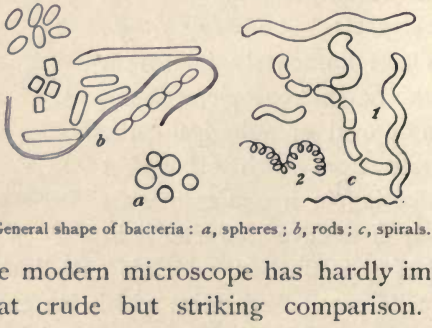
Bacteria, showing method of division by fission.

3. **Schizomycetes.** (*Fission fungi. Bacteria.*)—These plants are also microscopic. They differ from yeasts in being smaller and somewhat different in shape, but chiefly in their method of reproduction. Instead of forming buds they multiply by lengthening somewhat and then dividing into two equal halves (Fig. 3). This process is called *fission* and hence these organisms are the *Fission fungi.* This group includes the organisms which have for the last quarter of a century been known as *Bacteria.* It is with these that we are chiefly concerned in this work.

II. FORM AND CLASSIFICATION OF BACTERIA OR SCHIZOMYCETES.

Under ordinary conditions **Bacteria** are extremely simple in form. Long ago they were compared to billiard balls, lead pencils and cork screws (Fig. 4), and even the most careful work with the modern microscope has hardly improved upon this somewhat crude but striking comparison. Nearly all bacteria are either spheres, cylindrical rods of greater or less length, or spiral rods. In size they are inconceivably minute,

FIG. 4.

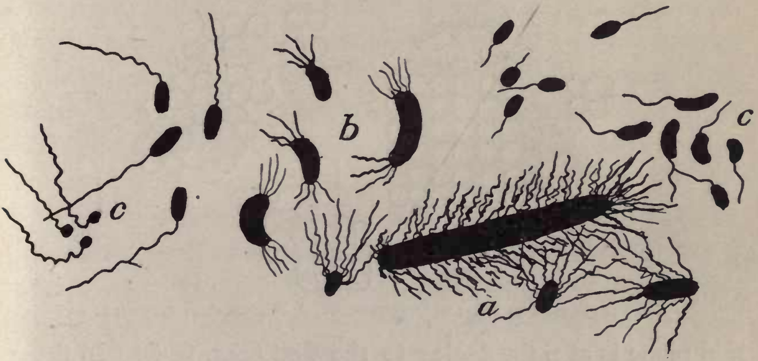


General shape of bacteria: *a*, spheres; *b*, rods; *c*, spirals.

being by far the smallest living organisms known, and demanding the highest powers of the microscope for their study. The spheres, for example, have a diameter varying from .25 μ to 1.5 μ (0.000012 to 0.00006 inch). The rods have a diameter of about the same dimensions, but their length may be considerably greater, especially when they grow into long slender threads. All are, however, far below the limits of human vision unaided by the microscope.

Many bacteria have the power of motion which is produced by slender motile hairs arising from their body and which by lashing to and fro produce a locomotion (Fig. 5). These hairs are called *flagella*. They are sometimes numerous and scattered all over the body (Fig. 5, *a*), sometimes few and grouped at one or both ends (Fig. 5, *b*), and in other cases only a single flagellum is found (Fig. 5, *c*). In many bacteria they are entirely wanting.

FIG. 5.



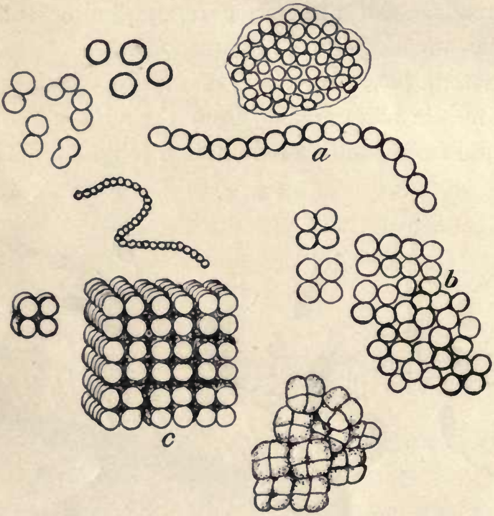
Bacilli showing flagella. (*Migula.*)

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

1. **Spherical Bacteria.** *Coccus.*—This group includes all bacteria which are spherical. They are further subdivided in accordance with their method of division. In some of them

the successive divisions follow one another in the same plane. If the spheres break away from each other after division, separate spheres, of course, arise, but it frequently happens that they remain clinging together for a time after division, so that long chains are produced (Fig. 6, *a*). In other cocci the second division plane is at right angles to the first (Fig. 6, *b*). In these cases, if the spheres remain attached they form irregular masses (Fig. 6, *b*). In a third type the divisions are in the three planes of space at right angles to each other (Fig. 6, *c*). In such cases there result solid masses of fours or some multiples of four. In accordance with these methods of division the cocci are divided as follows :

FIG. 6.



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

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

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

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

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

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

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

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

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

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

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

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

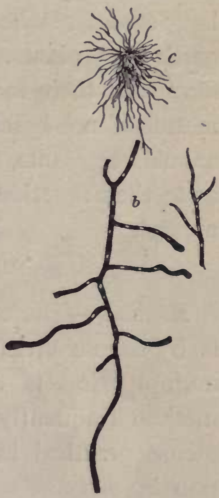
4. **Higher Bacteria.** *Cladothrix*, *Leptothrix*, *Streptothrix*, *Actinomyces.*—(Fig. 7.) Under this head are included a few forms of fungi which resemble other bacteria in some respects, but differ in others. They are composed of threads which are commonly larger than the threads of bacteria and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

This classification gives only what are usually recognized as the *genera* of bacteria. A further classification of the group into *species* is at the present time in a condition of the greatest

9

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

FIG. 7.



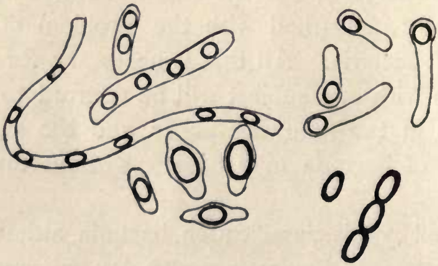
Actinomyces: *a*, a small colony; *b*, single rods. (*Boström.*)

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

MULTIPLICATION OF BACTERIA.

Method.—The common method of reproduction of bacteria is by simple division (Fig. 3). But although this method of multiplication is common to all bacteria, there is another method frequently found which, for certain reasons, is of immense practical importance. In addition to their multiplication by division some species of bacteria form *spores*. The contents of the organism collect in one or more small rounded masses (Fig. 8), after which the body of the bacterium commonly breaks up and the oval or spherical body is set free. These are *spores*. They are resting forms and their function

FIG. 8.



Showing the formation of spores.

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

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

GENERAL PROPERTIES OF BACTERIA.

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

Relation to Oxygen.—Most bacteria demand oxygen to enable them to carry on their life processes, thus agreeing with animals and plants in general. But there are some species, quite numerous and abundant as we now know, that can live without a supply of oxygen, and some indeed, that can only grow and multiply when in an atmosphere devoid of oxygen.

This property of living *without oxygen* makes them quite unique organisms, for no higher animals or plants have this power. This unusual power is one of considerable significance in explaining the conditions of bacterial life and action. The bacteria which demand oxygen, the great majority of known species, are called *aërobic.* Those that can only live in the absence of oxygen are called *anaërobic.* Some species that can live either in the presence or absence of oxygen are called *facultative anaërobic.* Between the two extremes of aërobic and anaërobic bacteria are numerous intermediate grades.

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

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

BACTERIA, AS IMPORTANT AGENTS IN PRODUCING CHEMICAL CHANGE.

When we consider the extremely minute size of bacteria, it seems strange, at first, that they can be important agents in nature. No one of them, to be sure, can accomplish very much, but when we remember their power of multiplication we can readily understand how it is that they become of great importance. Of course, the figures given above, showing a possibility of 17,000,000 offspring in twenty-four hours, are extreme and, probably, are never realized in nature. Most bacteria do not multiply as fast as this even under the most favorable conditions; but in all cases they multiply with an almost inconceivable rapidity, and this great power of multiplication makes them forces of great significance. They are like the snowflakes, each insignificant in itself, but when combined, forming the irresistible avalanche. While they are growing and multiplying with such vigor, they are sure to produce profound chemical changes in the food upon which they feed. It is to these chemical changes that their importance in agriculture is due.

Although bacteria are plants, they do not possess chlorophyll, and are not able, like green plants, to live upon mineral matter. It is true that certain species can live upon food which is much closer to pure mineral matter than that upon which any animals can feed; true also that some of them, under some circumstances, may live upon compounds simpler even than the minerals which form the food of green plants, and which are in no sense organic. This fact is a matter of the greatest significance, as we shall see later. Nevertheless, the great majority of bacteria require organic food of high complexity, in this respect resembling animals. All types of organic material may serve as food for them. Proteids, fats, starches, wood, cellulose, bone, etc., are all used by them under proper conditions. This group of organisms may be looked upon as the greatest agent in nature for destroying the organic material produced by both animal and vegetable life, either using it as food, or causing its chemical destruction and disintegration in some other way. As they feed upon this organic material, they produce within it profound chemical changes. The chemical changes thus brought about are very numerous. The chemist of to-day has hardly begun to study them and his knowledge is as yet very fragmentary. Only a very few of them are even in the slightest degree understood, and in regard to the simplest of these it must be recognized that as yet our knowledge of the phenomena is lacking in many important respects.

The great host of chemical changes which occur in organic material under the action of bacteria must be left for the future to describe and explain. At the present time we have a general knowledge of a few of them, and such of these as bear particularly upon the subject of agriculture will be explained in the following pages. We must remember, however, that those referred to are only a few of the numerous and profound chemical decompositions which occur under the agency of bacteria. These do represent, however, the chief changes produced by microörganisms which are well enough understood by our chemists to warrant a summary at the present day.

These chemical changes may be grouped under two quite distinct heads.

Synthetical Processes. *Anabolism.*—These consist in the building of complex bodies out of simpler ones. The fundamental importance of synthetical processes to the continuation of life is evident enough. The animal kingdom, in general, demands complex compounds as foods and cannot live upon such substances as are found in nature, like carbonic dioxide, nitrogen, ammonia, etc. (CO_2, N, NH_3). To make use of the elements existing in nature some process must build these elements and simple compounds into complex bodies which may serve as foods. This is largely accomplished by the green plants that furnish animals with food. But even these plants demand some of their food in a complex form, not being able, for example, to use nature's nitrogen store in the atmosphere until it has been built up into some compound like nitric acid. The constructive processes are thus of the most fundamental importance to the problem of life processes. Among the chemical changes which are brought about by bacteria some are of this synthetic character and, indeed, appear to form the connecting link between the mineral and organic world. The synthetic changes are, however, not possessed by most species of bacteria, to any considerable extent.

Analytical Processes. *Decomposition; Katabolism.*—The most common action of bacteria is that of *decomposition*. The great majority of species, like animals, live upon complex chemical foods, as already noticed, and these compounds are broken to pieces by their action and reduced to simpler molecules. Acting in this way, the bacteria are the most important agents in nature for reducing to a simpler condition the great quantity of organic matter which would otherwise accumulate upon and within the soil, or in bodies of water. The simpler molecules produced by the decomposition of the more complex substances are indefinite in number, and are understood by the chemist in a few cases only. The chemistry of the decomposition of organic substances is still in its infancy and as yet only the general nature of the changes is understood. The decomposition of these compounds in general brings the elements back to simpler conditions, and nearer to the form in which they can serve as food for ordinary plants.

Both synthetical and analytical processes are carried on, to a certain extent, by all bacteria. If they grow and multiply they must be manufacturing proteid and protoplasm out of the food products, for each new bacterium is made of protoplasm. This is, of course, a synthetic process and is characteristic of all bacteria. On the other hand, all bacteria likewise produce a certain amount of decomposition of the materials which serve them as food, giving rise to simpler products as excretions. But while all bacteria thus perform both types of chemical change, some classes give rise, as the total result of their life, to a marked decomposition of complex bodies, while others produce a marked synthesis as the total result of their chemical activity. The latter are few compared to the former.

We must notice that in still another respect the chemical changes produced by bacteria are two-fold. In some cases the new products which arise are of the nature of *excretions*. By this is meant that certain substances are taken into the bacteria and then subjected to a series of changes within their bodies. These changes are classed together under the name of *metabolism*. As a result of the metabolic changes there arise new chemical products which are eventually eliminated from the body of the bacteria as excretions. Some of the products arising in a mass of organic material undergoing decomposition by bacteria are thus of the nature of excretions (*e. g.*, *ptomaïnes*). But in other cases the new chemical bodies are apparently produced entirely outside of the body of the bacteria, and are not in any sense excreted products. It is, for example, quite certain that the alcohol which arises from the fermentation of sugar is not an excretion of the yeast cell, but that, by some agency, the sugar is broken up outside of the yeast, and that alcohol and carbon dioxide arise as products of its decomposition. Bacteria in some cases act in a similar manner (*e. g.*, *nitrification*). Just how they can accomplish such transformation outside of their own bodies has not been clear. Various suggestions have been made to explain the process and in some instances we have a partial knowledge of the method by which such changes are produced.

CHAPTER II.

FERMENTATION.

ALTHOUGH the chemical actions of bacteria are widely varied there are some points in which they all agree. They commonly result in decomposition. They begin slowly and, as the bacteria increase in numbers, they continue with increasing activity. They result in the disappearance of certain chemical compounds and the appearance of new ones, accompanying the growth of the bacteria. All belong to a type of chemical change which is called *fermentation.*

Our understanding of the relation of bacteria to fermentative processes has been greatly modified in the last few years as the result of the study of the method by which the fermentations are produced. We have noticed that some of the new substances produced by bacterial life are excretions, but that others do not seem to be the result of the metabolism, never having entered into the bodies of the bacteria. How are the latter changes produced? The answer to this question involves the whole problem of fermentations as it has been developed in the nineteenth century. Some aspects of the question are so intimately connected with our subject that a brief review of the phenomena of fermentation must be here given.

We must notice at the outset the extreme significance of fermentation in agriculture. It is not easy to define fermentative processes in a manner beyond criticism. In general they are progressive chemical changes taking place under the influence of certain organic substances which are present in very small quantity in the fermenting mass. The nature of the chemical

I need def of fermentation

changes is varied but they commonly, though not always, result in the production of simpler out of more complex compounds, frequently with the absorption of water into the chemical combination. As an example may be given the best known fermentation, that of sugar, under the influence of yeast, into alcohol and carbon dioxide. The following formula is commonly given as expressing the process:

$$\underset{\text{(sugar)}}{C_6H_{12}O_6} = \underset{\text{(alcohol)}}{2C_2H_6O} + \underset{\text{(carbon dioxide)}}{2CO_2}$$

As a second type of fermentation may be given that effected by the saliva when acting upon starch and commonly represented by the equation:

$$\underset{\text{(starch)}}{C_6H_{10}O_5} + H_2O_0 = \underset{\text{(sugar)}}{C_6H_{12}O_6}$$

It must be stated that neither of these equations is believed to-day to express the whole of the change that goes on, for the phenomena are doubtless much more complicated than here indicated.

The kinds of fermentations are numerous but they are readily grouped under comparatively few classes. The most important ones which concern agriculture are the following: The *alcoholic* fermentation; the *butyric* fermentation which produces butyric acid in butter; the *lactic* fermentation which causes the souring of milk and various other products; the *acetic* fermentation which produces acetic acid and forms vinegar; the *proteolytic* fermentation which renders soluble certain insoluble proteids, for example, in the ripening of cheese; the *oxidizing* fermentation which causes the oxidation of organic matter, as in the fermentation of tobacco; the *nitrifying* fermentation which converts ammonia into nitrites or nitrates; the *denitrifying* fermentation which converts nitrates into nitrites or simpler compounds by depriving them of oxygen. These different types of fermentation will be considered at length in later chapters. Their extreme importance to agriculture is evident from the list above given. They run all through the agricultural industries, from the preparation of the soil for plants to the production and preparation of the final food products.

Closely related to the fermentative processes are the phenomena which we call putrefaction and decay. These are also the results of life processes, and cannot be separated from fermentation by any very hard, sharp line. Putrefaction and decay are the results of chemical decompositions which are very complete and more complex and indefinite than the other fermentations. Putrefactive changes are commonly regarded as the result of the microörganisms taking certain atoms out of the organic molecules, and these molecules, thus losing some of their atoms, lose their character. The remaining atoms necessarily rearrange themselves to form new compounds which are simpler in structure. But putrefactive processes cannot be sharply distinguished from fermentations since many fermentations are apparently also the result of similar decomposition changes. We must therefore class, at least at present, the fermentations and putrefactions together. Putrefactive changes are of an importance in agriculture equal to, if not greater than, the other fermentative processes. Fermentation, putrefaction and decay together constitute nature's method of producing the slow organic changes which control the food supply of organisms.

ORGANIZED AND UNORGANIZED FERMENTS.

Although differing greatly in their nature and results, all types of fermentation agree in some few points which justify their being placed together as a single class of chemical action differing from ordinary chemical processes.

1. They are all closely associated with life processes.
2. They are all closely dependent upon temperature, ceasing

at low temperatures and also at high temperatures, and occurring with vigor within limits of temperature not far apart. Most of them occur most vigorously at temperatures between 80° and 100° F.

3. They are all produced by the stimulating action of some special body present in the fermenting material in a quantity which is very small, considering the great changes produced.

4. These bodies (*ferments*) are all rendered inert or destroyed by heat, a boiling temperature commonly destroying them so completely that they are unable to renew their action even after cooling. Their action is also checked by low temperatures, but they are able to renew their activity if warmed again.

5. Their action is commonly stopped by the accumulation of the products of their activity.

Fermentations have been known for centuries. Even in ancient Egypt the production of alcohol was familiar. Every savage tribe has its own method of obtaining alcohol by fermenting sugar solutions and the process is one of the most widely known changes in nature. Until the present century its chemical nature has been wholly misunderstood. It was formerly regarded as a purifying process, the yeasts which were found in the fermented material being looked upon as impurities which were separated from the rest. The chemical nature was determined early in the 19th century, but its relation to the yeasts was not determined until 1837, when Schwann demonstrated that fermentation would not occur except under the influence of yeasts. The conclusion that it was the result of the growth of yeasts was vigorously combated for years by Liebig, who looked upon the process as a purely chemical change, but this theory was eventually disproved by Pasteur and others, who finally demonstrated that fermentation is a physiological process accompanying the growth of yeast.

At that time there was no conception of the numerous types of fermentation which we now recognize. But even in the days of Schwann it was recognized that there was another type of chemical change which resembled the yeast fermentation in some respects. This was the sort of changes which occur in the digestion of food, and which were known, even in those early days, to be due to certain materials present in the digestive fluids. As early as 1833 *diastase* was known which could convert starch into sugar, and in 1836 *pepsin*, causing the digestion of proteids in gastric digestion, was discovered. Although these processes were seen to be different from the fermentation produced by yeast, their general similarity led to their being called *fermentations* and the active substance in each case was known as a *ferment.*

It very soon appeared that these two types of fermentation were different in some fundamental respects. It was found that whereas the fermentations akin to that produced by yeast could be stopped by various chemicals, like glycerine, the other type of fermentation could not be stopped by such materials. It was found that the second type of ferments did not contain any living bodies which could be detected by microscopic study, and they hence could not be regarded as living. Nevertheless these ferments contained some substances which were very peculiar in their nature. Like living organisms they were destroyed by high heat and they acted only at a moderate temperature. Unlike simple chemical changes these fermentations do not occur at high temperatures but become impaired and stopped when the temperature rises slightly above 100° F. Eventually it was found possible to isolate from the fermenting material (saliva, gastric juice, etc.) the fermenting body in a tolerably pure form. It was then found to be a substance which could be obtained in the form of a powder and preserved indefinitely. It contained no living cells, was not alive and clearly did not belong to the same class of bodies with the yeast plant.

These discoveries led to a sharp separation of fermenting substances into two different classes. On the one hand were those which, like yeast, were produced by organisms and were called *organized ferments*, and on the other those which contained no organisms and were called *unorganized ferments*. These latter ferments later received the name of *enzymes*, which name is now in most common use.

During the last twenty years there has been a gradual breaking down of the distinction between the two classes, which is leading toward the conclusion that the two types of fermentation may be fundamentally one. This conclusion is greatly modifying our attitude toward quite a number of fermentations of importance to agriculture, which have been previously attributed to bacteria.

THEORIES AS TO THE NATURE OF FERMENTATION.

From the beginning of the modern study of fermentation various explanations have been given as to the actual manner in which the fermentation is caused. The action of *enzymes* has from the beginning been regarded as a purely chemical phenomenon. But the *organic* fermentations have received several different explanations. Early in the century Kützig first advanced the theory that such fermentations were physiological processes resulting from the life processes of the fermenting organisms. But this view of the phenomenon was combated by Liebig who regarded the organic fermentations as purely chemical phenomena. His conception was that fermentable bodies consisted of molecules in a condition of unstable equilibrium which naturally broke to pieces under the stimulus of the presence of a little albuminous matter.

Liebig's preëminent position as a chemist lent a weight to this theory, which supported it in the face of facts, until Pasteur succeeded in establishing conclusively the relation of the phenomena to the growth of living organisms. But Pasteur's see Ferm in Ency Brit

own explanation of fermentation was hardly nearer the truth. His conception of fermentation was "life without oxygen." He thought that the microörganisms produce a fermentation only when they grow in a solution with an insufficient amount of oxygen. Under these conditions they extract the oxygen from the molecules of the fermentable compound and this causes the molecule to fall to pieces, like an arch from which the keystone has been removed. This conception of fermentation cannot stand in the light of the fact that many fermentations only take place in the presence of an abundance of oxygen (*e. g.*, *acetic* fermentation).

The next suggestion came from Nägeli who explained the phenomena upon the basis of *molecular motion*. He assumed that the ferment is endowed with a certain kind of molecular motion and that when in contact with the fermentable body it imparts its own motion to that body. The newly acquired motion then actually causes the fermentable molecule to break to pieces, shakes it to pieces as it were. This suggestion has had no adherents and cannot be regarded as of much significance, although it may perhaps be the ultimate analysis of the process.

Lastly there has risen an explanation of the organic fermentations which bases them upon the formation of *enzymes*. This suggestion has been slowly developing for many years and is the view of fermentation most widely accepted at the present time. Inasmuch as this subject has a very close bearing upon a number of the topics which we shall have to consider we must explain it at some little length.

ENZYMES.

The relation of the organized and unorganized ferments to each other has been a subject of much study and speculation. The enzymes appear to be very peculiar bodies. They cause certain chemical changes in the material fermented, but the action seems to differ from chemical actions in general in that the ferment itself is apparently *not used up in the process.* Whether this is strictly true may, from theoretical reasons, be doubted, but at all events no direct evidence exists that they are used up, and everything indicates that they can act indefinitely. A very small amount of an enzyme may produce a very large amount of chemical change, and the enzyme does not appear to enter into the new chemical bodies in any degree whatsoever. In some respects the enzymes resemble living bodies, especially in their relation to heat, and the fact that they are always produced by living organisms. But in other respects they are sharply marked off from the organized ferments. A long series of disinfectants, which wholly prevent the action of organized ferments, have no influence upon the enzymes. The latter do not increase by growth in the fermenting material, nor does their continued action depend upon their nutrition. The distinction between these two classes of fermentations has been clearly kept in mind in the development of our knowledge of fermentation in the last half century.

ORGANIZED AND UNORGANIZED FERMENTS REDUCED TO A COMMON BASIS.

The unorganized ferments or enzymes are always produced by living organisms and are therefore fundamentally based upon living phenomena just as truly as are the organized ferments. No enzyme occurs in nature except as produced by a living animal or plant, and there seems to be no more probability of chemists being able to make enzymes than of their making protoplasm. Some enzymes are produced by animals, including *ptyalin* which changes starch to sugar, *pepsin* which changes proteids to peptones, *trypsin* which has both functions, *rennet* which curdles milk, etc. A large number are produced by plants, such as *diastase* which changes starch to sugar, *invertin* which changes cane sugar to dextrose or grape sugar.

A very long list of such enzymes can now be given, each with its power of producing definite actions upon certain fermentable substances. For example, the following enzymes have been recognized as acting upon starches, sugars and cellulose compounds: *Trehalase*, *lactase*, *inulase*, *pectase*, *cytase*, *carubinase*, while *zymase*, *oxydase*, *invertase*, and *maltase* act on glycerides. The list might be easily extended. Each of these enzymes is the product of the activity of some living plant or animal cell, and each has the active power of producing chemical changes of a *katabolic* (decomposition) nature in certain definite fermentable substances.

The fact that plant cells so commonly produce these enzymes as the result of their active growth led naturally to the question whether the various so-called *organized ferments* might not act in the same way, and produce their fermentations through the action of *enzymes* which they secrete. This suggestion was a natural one, for the power of producing enzymes seems a very common one in the cell activities of higher plants. Is it not likely then, that the same power may be possessed by the lower plants as well, and if so, may not the action of the organized ferments be explained by supposing them to secrete enzymes whose direct action produces the fermentative change? If this were the case the difference between the organized and unorganized ferments would in considerable degree disappear. The so-called organized ferments would then act in just the same way as the unorganized ferments, the difference being simply in the *source* of the enzymes.

But although this would reduce the two processes to a common ground it would not in the slightest degree affect the close relation of the microörganisms to the process of fermentation. It would only place their agency one step further back. If it is true that bacteria produce enzymes and the enzymes directly cause the chemical changes, it still follows that the organized ferments, the bacteria and yeasts, are after all the cause of the fermentations. We should have simply learned that instead of producing the fermentation by a direct metabolism or the direct result of their growth, they do it by secreting an enzyme which itself produces the chemical change characterizing the fermentation.

Now this conclusion as to the agency of enzymes in the action of organized ferments is not simply a theoretical one, but one which has been demonstrated by actual observation. It has been shown in recent years that a number of the fermentations which were formerly classed as organized are produced directly by enzymes, which enzymes have been produced by living bacteria. For example, there is a class of bacteria which has the power of curdling milk without rendering it acid, an action very similar to that of rennet. It has been demonstrated that this curdling is due to an *enzyme* secreted by the bacteria and that this enzyme is quite similar to rennet. It may be wholly separated from the bacteria cells and preserved in the form of a powder, somewhat in the same way that rennet can be separated from the stomach of a young mammal. It will curdle milk as quickly as the rennet. Further, these same bacteria produce a second enzyme which has the power of digesting the curdled milk, and this second ferment is similar to that secreted by the pancreas of a mammal. Numerous other examples might be given, but these are enough to illustrate the fact that some fermentative processes, though fundamentally due to microörganisms, are directly the result of the action of enzymes. It has even been claimed in recent years that the alcoholic fermentation is thus produced. Buchner, by subjecting the yeast cell to high pressure, has separated from the cells a liquid substance which contains no living cells, but which is capable of producing the alcoholic fermentation. He has claimed that this contains an enzyme, named by him *zymase*, which has been produced by the yeast cell and is the direct cause of the fermentation of the sugar. This claim has been somewhat vigorously disputed and cannot be regarded at present as wholly demonstrated.

While a number of organized ferments have been thus found to produce enzymes, this is not yet true of them all. The *lactic* bacteria have the power of producing lactic acid from milk sugar, thus souring the milk if growing in it. This is a fermentation and there is as yet no evidence that the phenomenon is produced by an enzyme secreted by the bacteria. The lack of evidence is very likely due to the lack of proper methods of study, and it is not improbable that in future years a similar chemical ferment may be isolated in this case. At present we must still recognize that the organized ferments may probably act by two different methods. The one is an active metabolism, by which the living cell breaks to pieces the molecule of the fermented material, and the other is by excreting an active enzyme which itself induces the direct chemical change. They may, eventually, all be reduced to the latter mode of action, but even though this should be done it would not detract from the importance of the microörganism in producing fermentations.

There is one phase of this subject which puts the agency of bacteria in a different light, and is leading to a feeling that in the past the importance of bacteria has been in some cases overdrawn. As already noticed the living cells of higher plants may, and commonly do, produce enzymes which are excreted as the result of their activity. We have already noticed what a long list of such bodies are already known. Now, after the death of the plant these enzymes remain in a condition for activity for some time and may produce post-mortem changes. It very commonly happens that after the death of the plant it undergoes some kind of fermentative change. When piled into a compost heap, or stored in a silo, the plant tissues certainly show unquestionable evidence of marked fermentative changes. These phenomena are accompanied by a rise in temperature and they are apparently true fermentations. It has been assumed in the past that such fermentations are bacteriological. Bacteria are certainly sometimes present, and the rapidly widening conception of the agency of bacteria in producing fermentations has led to the conclusion that all such fermentations are due to bacteria. But the growing knowledge of the nature and abundance of enzymes is leading to-day to the conclusion that some of these fermentations are not due to bacterial action at all, but simply to the enzymes which were excreted by the plants during their life and which get a chance to act in the fermenting heap. If the plant in its life did produce such enzymes they would find their way into the compost heap and inevitably start a fermentation which would, of course, have nothing to do with bacteria. This possible explanation of some of the common fermentations has only been recently realized to its full extent. At present bacteriologists and chemists are actively engaged in investigating this problem of the relation of enzymes to fermentations, and trying to determine whether the enzymes producing various fermentations are secreted by bacteria or by the cells of higher plants. As fast as they show the latter they remove the fermentative process from the realm of bacteriology proper. Already, as we shall see later, some of the typical fermentative processes have been thus taken wholly or partly out of the domain of bacteriology.

It is necessary to point out in conclusion that even if we accept this enzyme theory of fermentations we are no nearer to a real understanding of the phenomenon than we were at the start. We have no conception of the actual nature of enzymes. We fail utterly to understand their action. How a substance can produce chemical actions by its simple presence without itself being acted upon and entering into chemical reactions is a total mystery. Whether to call these enzymes chemical bodies of a wholly *lifeless* nature, or to regard them as *semi-*

living, as some do, cannot be determined. In short we have in the enzymes substances as mysterious as the original fermentation. In explaining fermentations upon the ground of the formation of enzymes we must not, then, imagine that we are really explaining them. We are getting a little closer idea of the details of the process, but are not brought any closer to an actual knowledge of the fundamental nature of the fermentative process.

All of these types of fermentations, whether caused by the metabolism of bacteria or yeasts, or by enzymes, secreted by these organisms or by higher plants, are of vital importance in agricultural processes. Without their agency in breaking up organic compounds the soil would rapidly become unfit for supporting life. The agricultural industry is therefore not only dependent upon fermentations for many minor processes, but is fundamentally dependent upon them for its continuance. While this is true it must not be assumed that the various bacteria produce their results for the benefit of agriculture or for the benefit of the soil. There is no purpose in the matter. Each species acts its own life for its own good. If it secretes an enzyme that produces a fermentation, this is done for its own benefit and not for the farmer's. Incidentally it may result that the natural processes of life phenomena are benefited thereby, but primarily all of the enzymes secreted, and all of the fermentations produced, are for the benefit of the organisms themselves. If a bacterium secretes an enzyme into a lot of milk which causes its digestion, its purpose is to digest the milk for its own use and not for any incidental results that may accrue to agriculture.

LIST OF REFERENCES.

It is impossible to give a complete list of references of the immense literature covered by the subjects considered in this work. In the brief list at the end of each part of the book it is the endeavor to give a few of the important papers covering the topics considered, especial attention being given to recent literature.

Such lists are designed simply as an introduction for students who may desire to pursue further any of the topics discussed in the work. For this reason the lists are given topically rather than by title.

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PART II.

BACTERIA IN SOIL AND WATER.

CHAPTER III.

THE ORIGIN OF SOIL.

THE CONTINUATION OF THE FOOD SUPPLY.

THE farmer's occupation consists in converting soil and air into human food. While his duties seem to be varied they all resolve themselves into extracting from the soil and from the air certain ingredients which are induced to combine into such a form as will serve for food. Whether it be the potatoes that he digs from the ground, the fruit that he plucks from the trees, the wheat that he gathers from the fields, the milk that he draws from the cow, or the flesh that he sends to the butcher, in all cases the product has been enticed by the farmer from mother earth. Chemistry has not advanced to a stage where the elements can be made to combine directly, to produce the materials wanted for food, and the agriculturist, therefore, is obliged to depend upon secondary agents. Throughout nature, life is the agent which he uses for producing this combination of soil and air into human food. Plant life and animal life both contribute to the process, but it is through life alone that the chemical combinations which result in the production of food are possible.

The larger part of the material from which food is manufactured is derived from the inexhaustible supplies in the atmosphere, but a considerable portion, and an extremely important part of it, is derived directly from the soil. While the atmosphere is practically inexhaustible in its supply of plant food, this is not true of the soil. When the soil is chemically analyzed it is found to contain a certain amount of material that can serve as plant food, but this material is limited in quantity, and whenever a plant grows upon soil it extracts a certain amount of this limited food supply. This being the case, it would seem that the soil, in the course of time, would become exhausted of all possible supply of plant food, and if such a condition were reached the earth would become absolutely barren. But nothing is more evident than that this has not been the history of nature. Plants have been growing on the surface of the earth for countless centuries, and, so far as can be determined, they grow at the present time with no less vigor and no less luxuriance than they did in earlier times. The soil certainly has not become exhausted by the growth of vegetation and probably contains as much food material for plants now as it ever did in the history of the world.

How is it that nature thus remains ever fertile and that vegetation has continued through the ages with undiminished vigor? This is, evidently, simply a question of the food supply of plants in the soil. In brief, the answer to the question is as follows: The processes of nature are such that the same material can be used over and over again as food, passing from plant to animal and from animal to plant in an endless cycle, and as long as the energy of sunlight falls upon the surface of the earth to keep food supply in motion through this cycle, so long is it possible for the fertility of the soil to continue undiminished. It is upon the continuance of this food circulation that agriculture is dependent. We have been using the same soil year after year, and, in the longer settled countries, century after century. Plant foods are being drained from the soil. The great wheat fields of the new western lands are yielding, at present, large returns without trouble. But these crops are using up the foods in the soil, and in time the wheat-grower must face the problem of the renewal of the supply. If we can learn the method of bringing back to the soil the lost material, we shall have solved the great problem of agriculture. If we cannot learn these methods and find out how to control them, we cannot believe that agriculture will continue after the few mines of fertilizers (the nitrate beds of Chili) upon which we are now drawing shall be exhausted.

The importance of any discoveries looking toward the continuation of this food supply is evident. It is in the study of the phenomena of this food cycle with which we are chiefly concerned in considering the problem of the relation of bacteria to the soil ; for we shall learn that the most vital factor in this food cycle is the action of *soil bacteria*. The study of nature's food cycle, therefore, is largely the study of the functions of bacteria in the soil.

BACTERIA IN THE SOIL.

We must first notice that the soil is full of living organisms. Not only are the higher plants numerous, but all types of the fungi are abundant. Bacteria are extremely numerous in all soil which is supplied with moisture.

The very superficial layers of earth are extremely rich in bacteria, the number varying according to conditions, from a few thousands (10,000) to several millions (5,000,000) per gram. In sandy soil the number is very small. When the soil is polluted with decomposing organic matter the number may rise to 100,000,000 per gram. As we pass below the surface the number rapidly diminishes. At a distance of three or four feet the bacteria are few, and at six feet they have mostly disappeared. Below this they are rarely found and in the strata of soil at moderate depths of ten or fifteen feet they are practically absent ; in lower layers they are never found. The number of species of bacteria in the soil is also very great, although we have as yet no idea how great a variety of microörganisms do here exist.

The actual number of bacteria, or even the number of species, is a matter of no special interest or significance. With their wonderful powers of multiplication the question of their abundance is only one of proper conditions for growth. Our interest in soil organisms comes not from their number or species, but from their functions. The agriculturist's concern in them is wholly centered around the question of what they are doing in the soil. The fact that the soil is full of living bacteria, ready to grow and multiply if proper conditions are furnished, is the only fact we need to notice concerning their distribution. To the *activities* of these organisms in modifying the constituents of the soil we must therefore turn our whole attention.

ORIGIN OF SOIL.

Inasmuch as vegetation is dependent upon the soil, the fundamental question to consider is the origin of the soil. This question resolves itself into two topics, with one of which bacteriology has very little to do, while with the other it is very intimately associated. These two problems are connected with two somewhat different classes of ingredients present in ordinary earth. Soil contains large numbers of chemical compounds, but these may be conveniently and, for our purpose, satisfactorily, divided into two classes. The first includes the so-called *mineral ingredients*, consisting chiefly of the ground up rocks, and chemically composed of salts, bases and acids of the various elements. The second consists of the *organic ingredients* of the soil, frequently known as the *humus*, the origin of which, as we shall see, must be traced to life. For the continuation of plant life both mineral and organic ingredients are needed, since plants feeding upon the soil consume both mineral and organic substances. It is, however, the organic ingredients with which the fertility of the soil is most distinctly associated. The mineral constituents of soil are abundant enough almost everywhere, but the organic constituents are limited. Clear sand, even though it contain large amounts of the necessary mineral matter for plant food, will not sustain vegetation, and for the proper life of plants the organic materials, or their equivalents, are necessary and fundamental. Indeed, we generally look upon the fertility of any soil as strictly parallel with the amount of organic matter, or humus, contained in it. It will be the most convenient way of considering our subject to divide it into two divisions, one relating to the origin of the mineral and the other to the origin of the organic factors, although no sharp line can be drawn between them.

MINERAL MATTERS.

The mineral compounds in the soil, upon which plant life depends, are numerous but they are chiefly salts of *potassium*, *magnesium*, *calcium*, *phosphorus*, *iron* and *sulphur*. These salts exist in the soil in varying amounts, and each contributes more or less to the substance that goes to make up plant foods. It is true that plants need only small amounts of these minerals, but these small amounts are none the less necessary. The mineral matters in our soils are derived chiefly from the decomposition of the rocks, for the basis of most soil on the earth appears to be decomposed rock. The decomposition of the rocks is brought about chiefly by purely physical agencies. The effect of "*weathering*" upon the rocks, as is well known, results in slowly crumbling them into fine fragments. The weathering has been, in general, attributed to physical and chemical phenomena. The direct mechanical action of the rains, the effect of the freezing and thawing of water, the solvent action of various waters, especially if they contain carbonic acid in solution, the direct oxidation of the rocks by the oxygen of the atmosphere, all of these are the important factors in the disintegration of the rocks. We have, indeed, in past years, regarded these physical and chemical forces as the sole agencies in the breaking up of the crystalline and, later, of the stratified, rocks to form soil.

So far as this soil formation is due to physical agencies, the matter does not concern our general subject, and, if they explained the whole, the question of the origin of soil minerals would not concern bacteriology. But there is beginning to be evidence for believing that in some respects the microörganisms may be directly concerned in the problem. Doubtless, the part they play may be subordinate, but it is none the less of some considerable significance.

Rock Disintegration.—Although this weathering of the rocks, which results in their being broken into a powder and somewhat changed in chemical nature, is to be attributed chiefly to chemical and physical agencies, we are fast learning that vital agencies play an important part even in these processes, and that in the slow chemical changes which occur in the soil, microörganisms have a considerable significance. Even in regard to the primary process of rock disintegration it is becoming evident that the action of bacteria has had its influence. We shall learn on a later page that there are some species of bacteria which require no organic food for their sustenance, being able to live wholly upon mineral matter, together with ammonia salts as a source of nitrogen. Such organisms are capable of living and growing upon the surface of bare rocks. Even before this fact was known it had been suggested that microörganisms contributed to the phenomena of rock disintegration, since bacteria have been found in abundance upon the surface of disintegrating rocks. If the organisms do grow in such localities it is certain that the chemical changes resulting from their life will have an important effect upon the process of weathering. These bacteria are active oxidizing agencies and they produce a variety of by-products which cannot fail to have some influence upon the rocks. It is as yet too early to determine what this action is, or whether the action of the bacteria in this matter is of primary importance or wholly secondary. But that they do assist in rock disintegration is quite certain. The famous Alpine peak *Faulhorn* (rotten horn) was rightly named. The rock of this peak appears actually rotten. It is full of bacteria, which are easily found, and it appears certain that the rottenness of the rock in this mountain is due in considerable measure to bacteria. The significance of these peculiar bacteria, which require neither organic food nor light for their growth, will be considered on a later page.

Whatever be the agency of bacteria in rock disintegration they certainly play an important part in the subsequent changes which occur within the soil formed from the debris. Chemical transformations are going on constantly in soil and many of them are produced wholly by the action of the soil microörganisms.

Sulphur Bacteria.—In regard to some of the mineral products of the soil, bacteria have a more intimate relation. Among the mineral foods of plants *sulphates* play a very important part, and certain iron salts of potassium and silica are perhaps of equal importance. Now it is certain that the formation and destruction of these compounds in our soils is largely dependent upon bacterial action. This agency of bacteria in the transformations of sulphur is a most intimate one, since different bacteria are able both to build up and to pull to pieces sulphur compounds. Sulphur springs which deposit sulphur, and other waters and soils which develop sulphuric acid are chiefly explained by bacterial action.

In the first place many bacteria produce a decomposition of proteid material. Proteid always contains a certain amount of sulphur. The amount is not large, but this element is always present. As the proteids are decomposed the sulphur is loosened from its combinations with the other elements and set free in the form of sulphuretted hydrogen (H_2S), a gas which may almost always be detected in decomposing organic matter. Proteids are not the only source of this gas. Some species of bacteria are able to decompose sulphates or sulphites and other low sulphur compounds. The sulphites and low compounds are easily broken up by a variety of bacteria, but only one definite species is yet known with the power of decomposing sulphates, *Spirillum desulphuricans*. Some species of bacteria can even produce H_2S from pure sulphur, and it thus appears that a great variety of sulphur compounds may, under the influence of different bacteria, liberate sulphur in the form of sulphuretted hydrogen.

This gas, however, readily enters into new combinations, partly by simple oxidation and partly through the agency of bacteria. It may be directly oxidized by uniting with oxygen and then with water to form *sulphuric acid*.

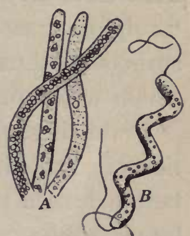
$$2H_2S + O_2 = 2H_2O + S_2$$

$$S_2 + 2H_2O + 3O_2 = \underset{\text{(sulphuric acid)}}{2H_2SO_4}$$

The sulphuric acid thus formed is an active agent in the soil, especially in the presence of decomposing organic bodies. It will readily combine with various bases to form sulphates. It can unite with ammonia to form *ammonium sulphate*, or with calcium carbonate to form *calcium sulphate*, two salts of much significance in the soil and contributing to its fertility. The H_2S instead of being oxidized may combine with iron to form *iron sulphide*.

But this direct oxidation is not, apparently, the chief method by which the H_2S is converted into sulphuric acid and sulphates. There are certain bacteria, now known for some fifteen years, which have such a close relation to this sulphur problem that they have been called *sulphur bacteria*. Some of these, which are the best known, belong to the higher bacteria (see page, 28), the two best known genera being *Beggiatou* (Fig. 9) and *Thiothrix.* In addition to these are others frequently called "red bacteria" which belong to the more common types. Both of these classes of bacteria make use of H_2S. This gas they oxidize, and the energy thus liberated serves the bacteria for their life energy. These organisms use H_2S in much the same way as other bacteria, and other plants in general, use carbohydrates, as a source of energy liberated from the compound by oxidation. The red sulphur bacteria can endure more H_2S than the others and these alone are found where the amount of this gas is excessive. In certain waters there have accumulated great quantities of iron sulphite which covers the bottom in a thick, black layer. This is especially the case in some localities on the Black Sea. This sulphite is constantly liberating H_2S and in the waters thus saturated the red sulphur bacteria grow luxuriantly. The other type of sulphur bacteria, on the other hand, are more common in the ordinary sulphur springs where H_2S is not so abundant.

FIG. 9.



Sulphur bacteria: *A*, Beggiatoa; *B*, Ophidomonas. Both show sulphur masses in the rods. (*Winogradsky.*)

This H_2S is oxidized by the bacteria, probably according to the formula:

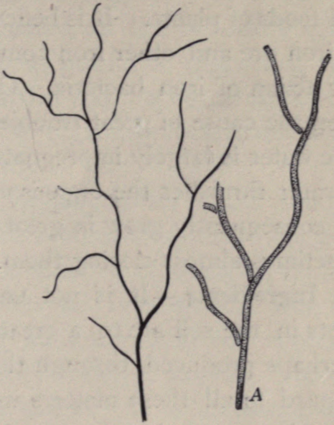
$$H_2S + O = H_2O + S$$

In accordance with this reaction sulphur is set free and it is seen to be deposited within the body of the bacteria. Small, round grains of sulphur appear in these organisms when they grow in the presence of H_2S (Fig. 9). The sulphur appears to be, not pure sulphur, but rather an amorphous form composed mostly of CS_2, but when the bacteria die this is converted into pure crystal sulphur. When the bacteria are growing, however, the sulphur does not remain long within their bodies. After a little it is oxidized by the bacteria, becoming converted into sulphuric acid, the acid being liberated from the bacteria to enter into combinations with the soil bases as already described. Sometimes the action of these sulphur bacteria results in the deposition of large amounts of pure sulphur which adheres to all sorts of solid objects in sulphur waters, forming "sulphur grasses," etc. The sulphur deposits from sulphur springs are thus, in considerable degree at least, due to bacterial action which oxidizes the H_2S, thus liberating the sulphur.

From these facts it will be seen that sulphur is in a condition of circulation in nature and that bacteria are very important factors in that circulation. Pure sulphur itself is utilized by certain bacteria converted first into H_2S, and eventually into sulphuric acid which unites with soil bases to form sulphates. The sulphates are seized by the common plants and built into the proteid molecules. At the end of the life of the plant the proteid suffers decomposition under the action of bacteria and the sulphur is liberated in the form of H_2S. The sulphur bacteria in the soil and water seize the H_2S, using it as a source of energy, oxidizing the gas and setting the sulphur free. The same bacteria, as well as others, oxidize the free sulphur into sulphuric acid which enters the soil again as sulphates. Meantime some of the soil sulphates may have been reduced to H_2S by different species of bacteria, the H_2S thus liberated furnishing more food for the sulphur bacteria. Various other low sulphur compounds (sulphites, etc.) are also utilized by the sulphur bacteria, and the total result of the whole action is that the sulphur is in the end in considerable degree reduced to soil sulphates where it can be utilized by the plants as a source of the necessary sulphur for building proteids. The cycle is complete.

Iron Bacteria.—Iron compounds are also metamorphosed by bacterial action. One class of bacteria has such a close dependence upon iron that it is called the class of *iron bacteria*. The organisms belong to the higher bacteria and there are three well-known genera, *Crenothrix*, *Leptothrix* and *Cladothrix* (Fig. 10), quite a number of species of each being known. These bacteria make use of iron compounds as sources of energy just as the last group makes use of sulphur compounds. Their chief source of iron is from iron carbonate which comes from at least two sources. Some of it is originally from the soil where it occurs in considerable quantity, especially at some depths. Some of the iron comes from the decomposition of organic compounds, the liberated iron combining with the carbon dioxide to form carbonates. This iron furnishes these bacteria with practically all the food they need, for they will grow readily upon the surface of bricks if moistened with ferrous sulphates.

FIG. 10.



Iron bacteria, *Cladothrix*. A shows a bit of thread more highly magnified. (*Zopf.*)

The iron carbonates are oxidized by the bacteria as follows:

$$2FeCO_3 + 3H_2O + O = Fe_2(OH)_6 + CO_2$$

The iron hydroxide thus formed is deposited in the cell of the bacterium causing it to assume a reddish brown color, or it may enter into combinations outside of the plant. It is an active chemical agent and readily unites with phosphorus or silica which may be in the soil, to form *phosphates* or *silicates* of iron. These salts are important soil ingredients and contribute to mineral foods of plants. It is believed that the great deposits of bog iron ore and other iron compounds are to be attributed to this action of iron bacteria. Occasionally these bacteria have been the cause of great trouble in water pipes in regions where the water is largely impregnated with iron compounds. Such water furnishes the organisms with abundance of food and they consequently grow in great abundance in the water mains, sometimes almost closing them.

Other Mineral Ingredients.—It is not unlikely that other mineral ingredients in the soil are to a greater or less extent modified, and perhaps produced, through the agency of bacterial life. In regard to all these matters we are yet in great ignorance. Up to the present time these problems have hardly been touched by bacteriologists. The mineral ingredients have been so generally looked upon as the result of pure chemical and physical forces that they have not seemed to offer fields for bacteriological research. Moreover, the more important question of nitrogen has been so prominent as to have attracted most of the attention of bacteriologists interested in soil bacteria. It is only a few of the other points which are most patent, like those of sulphur deposition and iron fermentations, that have as yet been studied by bacteriologists. The problem of the exact relation of soil bacteria to the mineral ingredients of the soil remains as a subject for future investigation. We know that the rocks undergo disintegration and we know that numerous chemical changes are constantly occurring in the mineral ingredients. To what extent these are explained by chemical and physical phenomena and to what extent vital forces have contributed and are constantly contributing, cannot to-day even be conjectured.

ORGANIC SUBSTANCES.

The fertility of any soil depends chiefly upon the organic substances present. It is these that account for most of the difference between the fertility of sand and garden soil. A sandy soil may contain all the necessary mineral ingredients for plant life, but its power of supporting vegetation is very limited, while a garden soil, having, in addition to the minerals, a goodly quantity of organic bodies, is very fertile and supports plant life abundantly.

Some of these organic bodies in the soil are easily enough understood. It is evident that fertile soil contains a considerable amount of dead and decaying animals and plants which contribute to its composition. But, in addition to the apparent decaying substances, the soil contains a considerable quantity of a material called *humus*. This is an ingredient rather difficult to define. It is abundant in fertile soil and scarce, or wanting, in barren soil. It represents the final condition into which the decayed animal and vegetable tissues have been brought by the series of changes they have undergone in the soil.

The humus is made up of many different chemical bodies, chiefly composed of carbon, hydrogen, oxygen and nitrogen, although containing other elements in some quantity. The most significant of these elements are carbon and nitrogen. In our study of plant food the problem of the oxygen and hydrogen need not detain us, since the plants can assimilate these two elements directly from the air and from the water of the atmosphere, and are therefore able to obtain an unlimited quantity. Carbon is the most characteristic element of organic bodies. This the plants obtain from the atmosphere in the form of CO_2, a gas which is present in small proportions, the air containing less than 0.1 per cent., and the problem of how this supply is maintained is one of some interest. But more important is the question of the transformations of nitrogen. So important is this factor that it is not an exaggeration to say that the whole problem of life centers around the question of the nitrogen compounds. Without a proper form of nitrogen compound in the soil, plants are ordinarily unable to live. They may obtain their carbon, oxygen and hydrogen from the air, but their nitrogen is furnished by the soil. It is the nitrogen element of the food supply whose exhaustion is threatened and whose replacement offers the greatest difficulty. We can therefore best understand the humus and the relation of bacteria to the soil by centering our discussion around the transformation of nitrogen. We would not imply by this that the other factors are not of great importance, but the problem of nitrogen has been so thoroughly studied, is so clearly the basis of plant life and the fertility of the soil, and withal so closely associated with the life of bacteria, that it may well serve as the center of our discussion.

The condition of life on the ordinary farm is such as to cause a constant loss of nitrogen. The sources of this loss are numerous but the most evident one is the gradual removal from the farm of the nitrogenous material in the crops which are sold. The farmer cannot retain all his crops upon his soil. If he could sell the *cream* from his milk, alone, he would sell the carbon compounds and retain most of the nitrogen, which is left largely in the skim milk. When, however, he sells whole milk he is sending off a considerable portion of the nitrogen which his plants extracted from the soil and furnished to his cattle. Everything sold removes some of it and the methods are numerous by which the farmer is getting rid of nitrogen. This produces an inevitable drain upon the soil and an inevitable tendency toward the condition of nitrogen starvation, a condition in which the soil is unable to support plant life unless supplied with high-priced nitrogen fertilizers.

But the exhaustion of the soil is certainly unnecessary. Althoguh there may be a constant drain of nitrogen it is clear that it may be replaced in some way. This is evident from the study of virgin soil. The chemical study of the water draining from virgin soil shows that it is always filled with nitrogen compounds, indicating that even such uncultivated soil is constantly losing nitrogen. But such soil remains fertile and has done so for ages. If we can explain how the nitrogen in the virgin soil is kept constant we can perhaps reach the secret of tilling the soil without exhausting it. To be sure it is quite a different problem to keep up the nitrogen supply in a tilled soil and in virgin soil. In the virgin soil many species of plants are commonly growing side by side, each making slightly different demands upon the soil, and when dying each becoming soon incorporated into the earth again. This is far less exhausting than on a cultivated land where a large crop of one kind of plant is rapidly produced and at once removed from the land. To keep up the nitrogen supply in the latter case is far more difficult than in the former. Nevertheless the two problems are closely related. The nitrates are drained from the virgin soil and demand to be replaced just as truly as those taken from the cultivated land, even though to a less extent. The forces which restore it slowly in one case may perhaps be made to restore it rapidly in the other. To these fundamental problems of the carbon and nitrogen supply and their transformations we next turn our attention.

CHAPTER IV.

THE TRANSFORMATIONS OF CARBON AND NITROGEN.

We can best make our subject clear if we try to follow, as closely as possible, the carbon and nitrogen in their transformations from one series of organisms to another as they enter into the realm of vital forces, and to learn whether they go through a complete cycle, being ultimately brought back to the condition in which they started. If they do so then the food problem is capable of solution and there need be no cessation of vegetation. But if there is any break in the cycle there will result a permanent loss and an eventual exhaustion of foods. The plan of our discussion will be then, to trace the elements of the food products through their circulation in nature.

PLANT FOODS.

The two most important ingredients of plant foods are *carbon* and *nitrogen*. The first of these is obtained from the atmosphere in the form of carbonic oxide. This exists in quantity in the air and has apparently always done so. It is fruitless to speculate as to its primitive origin. The method by which its supply is kept constant we shall notice presently.

The *nitrogen problem* is a far more important one. Ordinarily plants obtain their nitrogen from the soil. Here are found various compounds of nitrogen in considerable quantity. But not all of the nitrogen compounds in the soil will serve as plant food. Of the various nitrogen compounds in the soil there are two which stand out prominently as the chief form of the nitrogen foods of plants. There are *nitrates* and *ammo-*

nium salts. Nitrates are the most useful and most easily made use of, while ammonia salts can also be easily utilized, perhaps, however, after being converted into nitrates, as we shall see later. On the other hand plants are unable to use the highly complex nitrogen compounds, like proteids or other *organic substances*; nor are they able to make use of the simple compounds of nitrous acid, called *nitrites.* Nitrates and ammonium salts are then plant foods; nitrites, organic nitrogen and free nitrogen are useless for plants. Our problem is to discover the means of maintenance of the supply of nitrates and ammonium salts.

The primitive origin of soil nitrates is at present purely a matter of conjecture. The atmosphere, as is well known, contains nitrogen in great quantity, about 77 per cent. of the air being free nitrogen. It is hence almost certain that the primitive soil nitrates must have come from the atmosphere, and have been *fixed* by some agency in the soil in the form of nitrogen compounds. Several different processes are known which can *fix* free nitrogen and which may have contributed to the formation of the first nitrates in the soil.

1. It is known that certain alkaline bodies in porous soil can hold and fix a small quantity of nitrogen.

2. Electric discharges are known to induce the atmospheric nitrogen to enter into certain compounds which become fixed as combined nitrogen and serve as a source of nitrates.

These two factors have in past years been looked upon as the chief agencies for fixing nitrogen. But it seems somewhat doubtful whether they are sufficient to account for the great stores of nitrogen found in certain parts of the world. In recent years there has been a tendency to regard a third factor as of more importance.

3. Bacteria under certain conditions, to be noticed later, have the power of fixing atmospheric nitrogen.

The reasons for regarding this last factor as of the most importance are chiefly the apparent inadequacy of the other two, and the fact that positive experiments have shown that bacteria are certainly able to fix considerable amounts of nitrogen in the soil. At present it is a general belief that we must look to the action of microörganisms for an explanation of the primitive soil nitrates. This subject will be better understood in a later chapter and will, therefore, be postponed.

In addition to the nitrates the plant absorbs from the soil various minerals dissolved in water. With these we are not concerned. Under the agency of plant life, these various foods are combined to form complex compounds which we call organic. The energy needed for the construction is furnished the plant by sunlight. The plants thus produce large numbers of complex compounds out of its simple foods, but these compounds consist of a few well-known types of substances. By far the greatest proportion of them consist of *starches*, *sugars*, *fats* and *proteids*. So far as the nitrogen is concerned it is practically all in the proteids and similar bodies, while the carbon is contained in all of these products.

RESTORATION OF THE CARBON TO THE ATMOSPHERE.

The compounds thus built up have different destinies. Part of them are appropriated by the animal kingdom and to these we shall presently turn. Another large part is not appropriated by animals but begins at once to undergo destructive changes which bring its ingredients back again to their starting point. In regard to some of them the processes of chemical destruction are comparatively simple. The *starches*, the *sugars* and the *fats* are subject to chemical changes which take place, to a limited extent, under the direct influence of chemical forces, since they may be directly oxidized. All forms of active combustion in fires produce such oxidation, the result of which is that the carbon in the compounds is united with oxygen and liberated in the form of CO_2, the hydrogen at the same time being liberated in the form of water. These join the atmosphere while the minerals remain behind as ash. The carbon is thus brought back once more to its original source and in its original form. Thus all forms of combustion of carbonaceous material aid in keeping up the store of CO_2 in the atmosphere, upon which plants can depend.

But although direct oxidation may form a considerable part of this process of food reduction, another very large factor is due to the agency of microörganisms. Fires rarely occur in nature unless started by man, and there must, therefore, be some other means of oxidation. A slow oxidation of carbonaceous material occurs in nature at all times, which has ordinarily been attributed to direct chemical processes. It is quite doubtful, however, whether this slow oxidation would occur were it not for the agency of microörganisms. At all events, a considerable part of the so-called slow oxidizing processes are the direct result of their growth.

There are various organisms which contribute to the gradual destruction of the carbonaceous materials. The *sugars*, for example, undergo an alcoholic fermentation, a process very widely distributed in nature. This fermentation is produced by the action of yeasts, and the fermentation which goes on in nature is identical with that which occurs in the brewer's vat. The result of the fermentation is the formation of CO_2 and alcohol, the carbon dioxide passing into the atmosphere to contribute to the store of this important food. The alcohol, under normal conditions, also passes into the air and is eventually further oxidized into carbonic acid and water. Thus, the sugars, by the agency of yeasts, aided by the forces of chemical oxidation, are restored to the air. *Starches* have nearly the same history since they are readily converted into sugars and then fermented.

Cellulose.—For our subject a more significant feature of chemical decomposition concerns that product of plant life called *cellulose*, a material somewhat closely related to starches though different from them. Cellulose is a very important product of plant life, being present in large quantities in wood structures, and, indeed, in all plant growths, since it is the basis of the walls of ordinary plant cells. One of the purest forms of cellulose is the filter paper used by chemists. Cellulose undergoes a fermentation of a marked character due to bacteria. It has been determined by Omelianski that filter paper, when well moistened with water, undergoes a complete fermentation and is eventually destroyed. The microörganism that ferments it is a bacillus named from its discoverer, *B. omelianski.* It is a spore-forming organism which has been isolated and carefully studied. Pure cultures of this organism have the power of fermenting cellulose, the complete action requiring three to five months. As a result of this fermentation there are liberated considerable quantities of carbonic acid, hydrogen, and certain volatile acids, as well as alcohol, all of which readily pass into the atmosphere and become incorporated with this primary source of plant food. This fermentation of cellulose is clearly a matter of great significance. Considering how large an amount of carbon is, in the course of generations, locked up by plant life in the form of cellulose, it is manifestly important that there should be some agency for unlocking the combination if the supply of atmospheric carbon is to be kept constant. The bacteria which ferment cellulose are constantly at work pulling down the cellulose which has been built up by plants, reducing it to its simpler ingredients, to serve again as plant food.

The chemical changes which occur in the fermentation of cellulose cannot yet be given. It is quite probable that more than one species of bacterium may have this power. Some years ago it was claimed that *B. amylobacter* (a well-known species) could ferment cellulose, a conclusion which has never been very satisfactorily demonstrated. The organism of

Omelianski is quite different from this older known species. But this fermentation certainly does occur in practically every place where there is an accumulation of vegetable matter together with sufficient moisture and warmth. It occurs in swamps, in the compost heap and the manure pile. It is also possible that a similar fermentation occurs in the intestines of herbivorous animals. These animals certainly make use of a certain portion of the cellulose material in their foods. From the intestines of such animals have been isolated bacteria which have been found to possess some power of fermenting cellulose, and it has been suggested that it is through their agency that the animals make use of this material. This must certainly be regarded as doubtful since the only facts in our possession are that cellulose is utilized by these animals and that certain cellulose-fermenting bacteria have been found in their intestines. But whether this be true or not it is certain that in nature, wherever there is an accumulation of cellulose material, a fermentation is set up which returns to the air much of the carbon in the form of carbonic acid.

Wood.—Another product of plant life somewhat closely related to cellulose is *woody tissue.* The fermentation and destruction of wood is certainly a matter of necessity if the carbon supply is to be kept constant. That there is such a fermentation is evident to anyone who has walked through a forest and noticed the condition of the fallen trunks and branches. A fallen tree will remain for a time upon the surface of the ground apparently unaltered. But presently it becomes softened by some agency not at first manifest, and the hard, woody mass is slowly but surely converted into a soft friable substance, which is eventually entirely broken into a brownish powder and incorporated into the soil, contributing to the formation of the humus upon which the fertility of the virgin earth is dependent. This destruction of woody tissue is also brought about by microörganisms, but in this case several different types of fungi contribute to the process, and they are undoubtedly assisted in the action by the aid of insects.

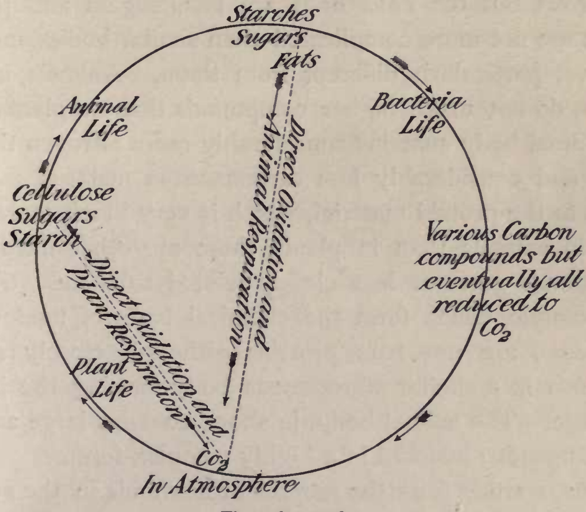
The first phenomenon that occurs in such a decaying tree trunk is the growth of common *fungi*. Various forms of mushrooms start their growth on its surface and send delicate mycelium threads into the substance of the wood. These threads grow first underneath the bark and in the superficial layers of wood; but slowly, though surely, they penetrate the hard wood, and, by the chemical excretions they produce, tend to soften this hard, tough substance. Without the growth of such fungi in the wood there seems to be no means by which the tree trunk can be sufficiently softened for decay. After the wood has become somewhat softened by the fungi, wood-eating *insects* begin their work upon it, using the wood as food. Simultaneously there begin to work upon the tree, if a sufficient amount of moisture is present, the *decomposing bacteria*, which, feeding upon the organic substances present, are able to grow in this soft wood, eventually completing its destruction into simple gaseous compounds. The result of all these processes is that the carbonaceous material in the wood is liberated, by being combined with oxygen, and passes off into the air to join the atmospheric store of carbon. The hydrogen and oxygen are converted chiefly into water, and in their turn enter the atmosphere as water vapor. In this way, by a slow process of decomposition, produced by microörganisms, the woody substance is in the course of time converted into simple chemical compounds which join nature's food supply in the air.

In these ways it is evident that large portions of the carbon, which plants have extracted from the atmosphere by their original growth, are returned again to the air in the form in which they first existed. The plant seized the carbon in the form of carbon dioxide and, combining it with water and other substances, built it up into sugars, starches, cellulose, wood and kindred compounds. The direct oxidizing power of the air may produce a destruction of part of the compounds thus formed, but the microörganisms, including yeasts, fungi and bacteria, complete the process of destruction, so that eventually most of the carbonaceous material extracted from the air is returned to it in its original form.

That portion of the plant food that is built into fats and into proteids has a future history of reduction which is practically identical with that of the same substances in animals. All these substances may, therefore, be best considered in connection with animals.

By these various transformations the cycle of most of the carbon is completed. The accompanying diagram (Fig. 11) will illustrate this cycle, showing graphically the course of transformation of the element from its origin in the air as CO_2, through the bodies of animals and plants, back again to the atmosphere.

FIG. 11.



The carbon cycle.

RESTORATION OF NITROGEN TO THE SOIL.

The problem of the nitrogen transformations is of far more significance to the fertility of the soil than that of carbon. Plants assimilate the nitrates from the soil and build them into proteids. Some of these proteids are stored in various plant products and, after the death of the plant, undergo decomposition through the agency of bacteria. But inasmuch as the further history of these bodies is precisely the same as that of animal proteids we may consider them at the same time with the study of animal proteids.

Animals appropriate a part of the materials built by plants and make further changes in them. They use the sugars, starches, fats and proteids and build them into still other compounds. The compounds built by animals are, of course, numerous, but the chief ones are fats, sugars and proteids which are not more complicated than similar bodies in plants and not particularly different from them. Animals, in other words, do not make higher compounds than do plants. But the animal body contains considerably more nitrogen than the plant and considerably less carbonaceous material. In addition to the proteid material, which is very much more abundant in animals than in plants, there are other nitrogenous compounds more or less closely related to them. *Gelatins*, for example, which form the chemical basis of tendons and ligaments, are not true proteids although closely related. *Chondrin* is a similar nitrogenous body forming the basis of cartilage. The animal body, in short, contains large amounts of nitrogenous material in a highly complex form.

Thus, starting from the nitrates or ammonia in the soil, the nitrogen has been, by the agency of vegetable life, built up into proteids, and by the animal life is more or less transformed but not changed in its essential chemical nature. It has, however, become somewhat condensed since the animal stores in its tissue the nitrogen obtained from a large amount of vegetable food. After the nitrogen has assumed this form of proteid, or allied bodies, whether in animals or plants, it has been wholly removed from the possibility of serving plants as food. We cannot feed green plants upon eggs or upon flesh. The nitrogen of these compounds is in too complex a condition for plants to use, since they must have their nitrogen in far simpler compounds than this. Our next question must be how it is that these highly complex bodies, built by plants, utilized and stored by animals, are reduced to the simple condition in which they can once more serve as plant foods. This passage downward, from the proteid to the nitrate, is controlled in large degree by the action of bacteria.

The foods taken by animals may be, so far as concerns their future history, divided into two classes :

1. Part of the food used by animals is metabolized in the animal body to furnish them with energy and material for growth. As the result of this metabolism the materials are reduced to simpler chemical forms. The sugars and starches, as well as the fats, are quite largely metabolized by the life processes of the animal. Part of these foods which the animal assimilates are, it is true, stored in its body as fat and may remain in the body for post-mortem decomposition after the death of the animal, but the larger part are oxidized in the body almost at once. The carbon present in the foods is combined with oxygen taken in by the respiratory organs, the result being that most of the metabolized carbon is eventually exhaled from the body in the form of CO_2, which joins the store of this gas in the atmosphere and thus completes its cycle.

2. The proteids consumed by the animal also have a double history, part of them being metabolized immediately, and part of them being stored in the animal and remaining there for post-mortem changes. That part of the proteid which the animal uses for its own purpose and metabolizes is broken to

pieces and reduced to much simpler compounds. It is, however, not decomposed sufficiently to bring the nitrogen back within the reach of plant life. The carbon in this proteid is in part removed from it and combined with oxygen to be exhaled as CO_2. This causes the molecule to fall to pieces and various by-products to arise, but eventually it practically all assumes the form of *urea* (CON_2H_4), a nitrogen molecule far simpler than proteid. Urea, or a closely allied compound, is the form in which nearly all of the nitrogenous material resulting from proteid metabolism in the animal body is excreted by the animal. Urea thus represents one stage in the destruction of proteid compounds, and to this stage the proteids are brought as the result of the metabolism in the life processes of animals. This urea is secreted by the kidneys and must be looked upon as the nitrogen which is no longer of any use to the animal world. But, although much simpler than proteids, this urea is not a plant food for it is still too complex for the use of plants. Common green plants are not able to utilize these nitrogenous secretions of animal bodies. It is estimated that some 38,000 tons of urea are excreted daily by the human race. To this quantity must be added the far greater amount excreted by other animals, and the total is thus enormous. What becomes of it all?

3. The second part of the animal's food is not metabolized during the animal's life, but becomes incorporated into the body and remains at the close of its life as dead animal tissue, still existing in as highly complex a form as ever. At the animal's death its body contains sugars, fats and proteids, as well as other nitrogenous substances. The sugars are readily disposed of by fermentation as already noted, but the destruction and final utilization of the fats and the nitrogenous compounds demand some further agencies than any we have yet noticed.

Thus the nitrogenous food consumed by animals has reached two quite different conditions. Part of it has become partially broken down, forming urea, while another part remains in the higher condition of proteid, gelatin, or other complex organic compounds. Neither is within the reach of plants and further decomposition agencies are needed to make them available again for life processes.

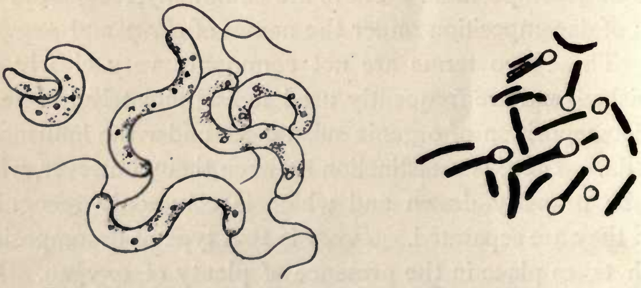
DESTRUCTION OF NITROGENOUS COMPOUNDS.

Decomposition Bacteria.—We now come to one of the most important functions of the soil bacteria. The destruction of nitrogenous compounds, urea, proteids, gelatins or other bodies, is brought about by several agencies, but the chief one is undoubtedly that of microörganisms. A small amount of the proteid appears to be decomposed in plant tissue without the aid of bacteria, and considerable is disposed of by yeasts, molds and other fungi. But it is to the bacteria that this decomposition is chiefly due and to that class of bacteria properly called the *decomposition bacteria.*

This term is a very broad one and includes a very large number of different species of bacteria and a great variety of types of decomposition. There are commonly recognized two types of decomposition under the names of *decay* and *putrefaction.* These two terms are not commonly very clearly distinguished and are frequently used indiscriminately to refer to the decomposition of organic substances under the influence of bacteria. There is a distinction between them, however, which may be properly drawn and which is commonly recognized when they are separated. *Decay* is that type of decomposition which takes place in the presence of plenty of oxygen. It is produced by *aërobic bacteria* and results in a very complete chemical disintegration of the organic substances, the carbon mostly becoming CO_2, the hydrogen mostly H_2O, etc. *Putrefaction*, on the other hand, is the decomposition which occurs in the *absence* of an abundance of oxygen. It is produced by *anaërobic* or *facultative anaërobic* bacteria and it does not result in such complete destruction. The final products of putrefaction are still quite complex. Many of them have prominent odors giving the unpleasant smell to the putrefying mass, and many of them are violently poisonous. These same bodies will, under the influence of aërobic bacteria in the presence of oxygen, become still further decomposed. Thus aërobic decomposition may not only check the odors but also destroy the poisons which develop during putrefaction under the influence of anaërobic bacteria.

The bacteria which contribute to these processes are very numerous. In the early days of bacteriology it was supposed that all such decomposition was commonly produced by the same species of bacterium, and there was described from decaying matter a bacterium which received the name of *Bacterium termo*. This species was the subject of a considerable amount of investigation, was carefully described and many of its properties known. But the improvement of methods of study has disclosed large numbers of bacteria engaged in the process, and it is no longer possible to recognize this *Bacterium termo* of older students. While many bacteria are now known to occur in decaying fluids we do not know what was meant in earlier years by *B. termo*.

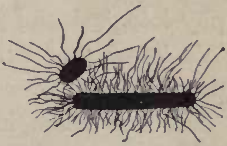
FIG. 12.



Two species of bacteria found in decaying vegetable tissue. (*Pfeiffer.*)

Of the many species of bacteria associated with putrefaction some are likely to be found under one set of conditions and others under different conditions. Some are particularly common in decaying vegetable substances (Fig. 12) and others in decaying animal tissues (Fig. 13), while some are most characteristic in fermenting urea. No systematic attempt has been made by bacteriologists to classify this miscellaneous host of putrefactive organisms, nor do we know as yet to what extent the different kinds of putrefaction may be produced by the same species of bacterium under different conditions. We know that they include cocci, bacilli and spirilli. We know that some of them produce their fermentation only when oxygen is present, while others do so in the absence of oxygen, and we know further that the by-products produced in the absence of oxygen are different from those produced in its presence, since the former are more likely to be of a poisonous nature. We know that these decomposition bacteria occur practically everywhere in nature. They are in the air, in all bodies of water and are present in extreme abundance in the soil. They are so widely distributed and so abundant that they are sure to be on hand to seize hold of any bit of nitrogenous organic matter, which, having become lifeless, can serve them as food. Every bit of excreted urea, even that secreted by the smallest insects, every dead animal body whether small or great, every bit of vegetable matter whether it be leaf, branch or fruit, provided it contain proper moisture, is sure to be appropriated as food by some of these ubiquitous putrefactive bacteria. The material is used as food by the bacteria, and, as a consequence, they multiply rapidly within the decaying substances, developing vigorously for a time, until they have used up the food; then their growth is checked

FIG. 13.



Proteus vulgaris found in decaying animal tissue.

and those remaining lie in the soil ready to grow again when more organic matter comes within their reach. By their action, then, every bit of organic matter which reaches the soil is seized and rapidly decomposed.

The chemical nature of these destructive changes is very complicated and highly varied. It will be a long time before our chemists understand them, for they involve problems in physiological and organic chemistry which are yet unsolved. We know that as a result of the decompositions many new products are formed, and that these new products must be regarded as belonging to at least two types, so far as concerns their relation to the bacteria. Some of them must be regarded as *secretions* or *excretions* from the bacteria and hence as the result of the active metabolism of the microörganisms. These are probably rather small in amount but of great significance in some connections, inasmuch as many of them are especially poisonous. Others must be looked upon as *by-products* of decomposition. By this is meant that, as the bacteria take certain atoms from the complex molecules for their own use, the rest of the molecule can no longer retain its earlier form, and consequently its atoms must enter into new relations to form new bodies. These by-products, of course, have never been actually *in* the bacteria and are not the direct results of metabolism. The new products formed in the decomposing mass are partly gaseous. This is proved by the odor that commonly arises from decaying bodies which are indications of the exhalation of volatile products. A chemical study has shown in many cases the actual nature of these gaseous products, indicating them to be chiefly CO_2, H, CH_4, NH_3, H_2S and N, in addition to others, present in much smaller amount, producing the peculiar and characteristic odors. Some of the new products are solids and may be either soluble or insoluble. If soluble they are sure to be dissolved in the course of time by the water which falls upon the decaying mass and, in solution, they pass into the soil, perhaps to be drained away in the drainage water. The insoluble bodies are also sure to be incorporated into the soil, becoming eventually mixed with the solid masses of the earth.

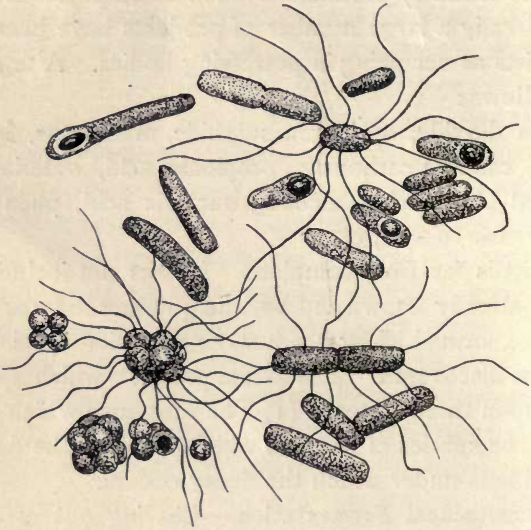
The list of the by-products of such decompositions is a long one. Already a large number of products have been isolated by chemists as occurring in putrefying bodies. A few of these are as follows:

Carbon dioxide, hydrogen sulphide, marsh gas, hydrogen, nitrogen, calcium carbonate, propionic acid, valerianic acid, acetic and lactic acids, alcohol, succinic acid, phenol, indol, leusin, tyrosin, skatol, etc.

This list is far from complete. It does not include all the products already known and beyond question there are numerous bodies, formed as by-products or excretions, which still remain to be discovered. The actual products which appear will depend upon three factors: (1) The substance which is decaying; (2) the species of bacteria which produces the decay; (3) the conditions under which the decay occurs.

The Ammoniacal Fermentation.—Leaving out of consideration these miscellaneous products, many of which are probably of no great significance in the general process, since they are present in small amount only and are certainly not yet understood, we may confine our attention to a few of the chief products of nitrogen decomposition. It is these nitrogen products which are most intimately associated with soil fertility. One of the most common and almost universal types of fermentation is that which produces ammonia. The nitrogenous body first to undergo an *ammoniacal fermentation* is urea. Urea is always contaminated with certain species of bacteria in the ducts from the bladder, and these organisms speedily induce an ammoniacal fermentation. Several species of bacteria have been found especially associated with this process. The one first described was named *M. ureæ*, but recent study has shown here, as elsewhere, the presence of a number of distinct species with this characteristic, which are almost sure to be in urine ready to accomplish its fermentation (Fig. 14). The result of the fermentation is that the urea is split up according to the following equation :

FIG. 14.

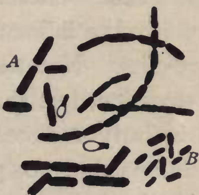


Various bacteria causing the ammoniacal fermentation of urea. (*Beijerinck.*)

$$\underset{\text{(urea)}}{COH_4N_2} + 2H_2O = CO_2 + \underset{\text{(ammonia)}}{2NH_3} + H_2O = (NH_4)_2CO_3$$

A similar ammoniacal fermentation occurs in the destruction of other nitrogenous organic bodies. Proteids almost always show the presence of ammonia while undergoing putrefaction and, indeed, the ammoniacal fermentation is one of the most widely occurring types of the chemical destruction of organic bodies. The exact details of the chemical change will rarely be so simple as that given above for the fermentation of urea. Since other organic bodies contain other chemical elements in their molecules a variety of by-products must commonly be found. Part of the sulphur present in the proteids, for example, makes its appearance as H_2S, the hydrogen as H_2O, and numerous other products appear in small amount according to the conditions. Many different species of microörganisms are known to have the power of inducing ammoniacal fermentation. It is the common character of most of the putrefactive bacteria which may be found in a manure heap, and it is also characteristic of some molds and yeasts. Prominent among these species two may be selected as perhaps contributing to the result more commonly than any others, *B. mycoides* and *Proteus vulgaris*, well-known species almost always found in soil and in decaying organic masses (Fig. 13 and Fig. 15).

FIG. 15.



Bacteria producing ammoniacal fermentation: *A*, *B. mycoides; B*, *B. stutzeri.*

The universal occurrence of such a decomposition of organic bodies is no new discovery. It has long been known and its extreme significance recognized. It is the first step necessary, in the proteid destruction, to bring the nitrogen locked up in the proteid back again within reach of plants. Its value in producing what has been called the *self-purification of the soil*, has been only recently appreciated. A little thought will show us that without the existence of some such process the soil would rapidly become unfit for the support of life simply by becoming clogged up with the remains of past animals and plants. If all the bodies of animals remained on the soil after death, and if the roots and stems of plants were not disposed of by some such process of fermentation and slow oxidation, it is evident enough, from simple mechanical reasons, that vegetation would soon cease, since there would be no room left in the soil for new plants. When we realize, in addition, that the very processes which purify the soil of these cumbersome bodies are bringing them toward a condition for further use, we can appreciate the extreme significance of these decomposition bacteria in agricultural phenomena.

Denitrification.—But this general destruction of compounds under the influence of bacteria produces evil as well as good results. Not the high organic compounds alone suffer destruction, but some of the lower compounds of nitrogen are similarly reduced, and among them, some which are of distinct value to plants. We have already seen that plants obtain their nitrogen most easily in the form of nitrates and next to this in the form of ammonia compounds. Now both of these bodies, if present in the soil under the action of putrefactive bacteria, undergo a further decomposition. We have learned in recent years that some of the decomposition bacteria are not only able to destroy the urea and proteids, but they also have the power of extracting the oxygen from any nitrates which may chance to be present, reducing them to a lower form *and rendering them useless for plant life.* Some of the soil organisms produce an even more complete destruction and so act upon the ammonia compounds as to set the nitrogen completely free from the compound.

This general process is known as *denitrification.* It always results in the removal of oxygen from the nitrogen compounds and in their consequent reduction to a lower state of organization. For example the nitrates which are acted on become reduced to nitrites. If potassium *nitrate* (KNO_3) be present and acted upon by the denitrifying bacteria it becomes potassium *nitrite* (KNO_2). This denitrification has been most carefully studied in the last five years since the importance of the phenomenon has been realized. There were first discovered two bacteria which produced this reduction in marked degree, and they were named *B. denitrificans I* and *II.* Both of them produce the reduction, but the No. II acts most energetically when in union with the very common bacterium *B.*

coli communis. The No. II acts aërobically, the latter best in the absence of oxygen. Since these two bacteria were studied quite a number of others have been found, and about a dozen have been described by bacteriologists as having especial powers in this direction. Indeed it appears that most of the bacteria which produce the putrefactive changes in decaying proteids are capable, to a greater or less extent, of reducing nitrates to nitrites if they are present in proper condition.

Not only are nitrates thus reduced but the same bacteria, or others, can act similarly upon the nitrites, taking out the rest of the oxygen, and they can even act upon ammonia salts in such a way as to free the nitrogen wholly from combination and set it loose as *free nitrogen.* The latter process is not quite so easily understood chemically, but experiment has abundantly proved that it occurs, that the ammonia compounds under the influence of denitrification are broken up and the nitrogen liberated. Apparently different species of bacteria are concerned in the different processes. The bacteria shown in Fig. 15 are common denitrifying bacteria.

The extreme significance of this denitrification for the agricultural industry is evident. Denitrification results inevitably in nitrogen loss to the soil. If the soil be stocked abundantly with nitrates and ammonia salts the denitrifying bacteria would be able, if vigorously acting, to destroy them so thoroughly as to leave the soil almost nitrogen-free. If denitrification goes on in the manure heap it may largely destroy the nitrogen compounds which the farmer intends to use for fertilizers. The importance of understanding the conditions under which this process takes place most efficiently, and the conditions under which it may be checked, is evident. In recent years bacteriologists have been earnestly investigating this phenomenon, trying to learn how the nitrogen loss which arises as its result may be prevented or diminished. This is not the place to enter into the details of those investigations. One or two general conclusions only appear, as yet, to be sufficiently established to warrant their insertion here.

1. The process of denitrification is one of extracting oxygen from nitrogen compounds and it does not occur so vigorously if the bacteria are supplied with an abundance of atmospheric oxygen. Consequently denitrification is not so prominent in soils or manure heaps which are well supplied with air as they are where air is excluded. On the other hand, these bacteria do not appear to thrive in an absolute exclusion of oxygen, some of the denitrifying bacteria acting best in the presence of oxygen, while others benefit by its absence.

2. The denitrifying bacteria need for their action a supply of some easily assimilable carbonaceous material. The presence of lactic acid greatly stimulates their denitrifying power since it furnishes them with carbon and energy. It is also a fact that the presence of hay and straw in manure increases the denitrification, a fact supposed at first to be due to the addition to the manure of many denitrifying bacteria which were adhering to the straw. It is now regarded as due to the fact that the straw supplies the carbonaceous material necessary to furnish the bacteria with carbon and energy. From this it follows that the more completely the manure heap can be kept free from straw the less the nitrogen loss by denitrification.

3. The denitrification which occurs in soil as it exists in our fields is much less than early experiments indicated. If the amount of organic matter is large, denitrification is excessive, and early experiments were conducted under conditions of large amounts of organic matter. When the organic matter is small in amount, as is the case in the ordinary soil, the denitrification is very slight. Consequently denitrification is a phenomenon of little importance in ordinary soil, while it is the cause of a large amount of nitrogen loss in the manure heap.

Nitrogenous Decomposition in the Soil.—Let us now picture to ourselves what is going on in the soil at all times. Within the soil we always find large amounts of dead organic matter, animal and vegetable. The purely carbonaceous bodies are dissipated by processes already noted. The ureas, and the more complex gelatins and proteids, are pulled to pieces and reduced to simple forms. Even such hard substances as bone undergo decomposition, for its nitrogenous ingredients furnish food for bacteria and these, in time, accomplish the complete disintegration of the mineral as well as the organic ingredients. The nitrogen from all these bodies reaches a variety of end products when the decomposition is finished. Part of it has been wholly freed from combination and this passes off at once into the air to join the great store of *free nitrogen*. Part of it has been converted into *ammonia*, and since this is also a gas, it is likewise exhaled from the decomposing soil into the air, as is clearly indicated by the odor of ammonia characteristic of decomposition. The ammonia is not all thus eliminated from the soil, especially if acids be present, for it will readily unite with various acids to form salts. If sulphuric acid be present, as we have seen it is quite sure to be, it will form *ammonium sulphate* with the ammonia, and, with the carbon eliminated from the decomposing products, *ammonium carbonate* may be formed. These salts not being volatile will remain in the soil where they may be later of direct use to plants, either directly or after undergoing further modifications to be noticed presently. But the denitrifying bacteria will not allow all these ammonia salts to remain undisturbed, for, as we have seen, denitrification is constantly setting free the nitrogen from ammonia compounds. So long as this process of denitrification continues there is a loss of nitrogen. It must be borne in mind, however, that the actual loss of nitrogen by denitrification from ordinary soil appears to be slight unless the amount of organic matter undergoing decomposition is large.

A considerable portion of the nitrogen, at the end of the decomposition, reaches a different condition. In exactly what chemical form these other nitrogen portions are left need not concern us, and, indeed, is not yet accurately known; but they constitute the nitrogenous element of the *humus*. It is upon these nitrogen compounds left in the soil that the next generation of plants must feed. Now it is certain that much of the nitrogenous material resulting from decomposition is in a condition in which it is not available for plant life. Even the ammonia salts are only very slightly used by plants unless further modifications occur in them, and the other nitrogenous bodies in the soil are not directly used by plants. In other words the decomposition processes going on in the soil do not leave the nitrogen in a form in which the next crop of plants can utilize it.

If the soil in almost any locality be analyzed it will be found to contain some nitrogen. If the soil be fertile it will commonly contain a considerable quantity of nitrates or ammonia salts. But there are other soils in which the yield of crops is small from the fact that the plants seem to be *nitrogen-starved*. These barren soils will not yield good crops unless supplied with a considerable amount of nitrogen as a fertilizer. Yet these same soils may contain plenty of nitrogen. Upon an open hillside or a meadow we may find the land very poor for supporting vegetation, and yet its soil when analyzed may yield large quantities of nitrogen. In such a soil the nitrogen is simply locked up in the humus in a form useless to plants. Thus it frequently happens that a large part of the ~~oxygen~~ nitrogen is, at the end of decomposition, held in a form not available for ordinary vegetation, and plants growing in such a soil will be nitrogen-starved although growing in the midst of plenty of nitrogen compounds. Such soils might become highly fertile if there should be some agency for unlocking these nitrogenous compounds, freeing the nitrogen from its stable relations and producing compounds of a nature to be assimilated by plants. Until we have discovered such forces the nitrogen problem is not solved. When we have such agencies at our command we can understand how soils, now containing useless nitrogen, can be made fertile; and when we have learned to manipulate them we shall have learned how to restore the fertility to many a barren field.

What we wish to look for next, then, is some force that can take these end products of decomposition in the soil—ammonia salts and the miscellaneous compounds in the humus—and build them up into *nitrates* in which form they are most easily utilized by plants. This brings us to the important problem of *nitrification*, a term which, as will be noticed, is the opposite of *de*nitrification.

BUILDING UP OF NITROGENOUS COMPOUNDS, NITRIFICATION.

The process of *nitrification* is in general an oxidation of the nitrogen compounds of the soil, the final result of which is the formation of *nitric acid*. The nitric acid thus formed unites readily with the bases present in the soil to form nitrates which are the most useful nitrogenous foods. Our starting point in the consideration of nitrification is the fact that the soil very commonly contains considerable stores of nitrogen which are not available for plants. Our problem is to notice how this nitrogen is unlocked from these useless compounds and brought into a condition in which plants can make use of it.

We must first notice again that ammonia salts are certainly available for plants, though less so than nitrates. From this it follows that the ammoniacal fermentation, which, as already noticed, is the common result of the action of putrefactive bacteria upon proteids, will furnish some food for plants. When the ammoniacal fermentation occurs in the soil in connection with the formation of acids which can hold the ammonia in the form of salts, the phenomenon will render available some of the nitrogen which was previously out of the reach of plants. The ammoniacal fermentation is sure to take place wherever high nitrogenous compounds in the soil are acted upon by common putrefactive bacteria, and this is doubtless one of the means for rendering nitrogen directly available. It must be noticed, however, that it is somewhat doubtful whether the ammonia products are *directly* used by plants. In sterilized soils plants make much less use of these salts than they do in soils which are not sterilized, a fact which indicates that possibly *all* of the ammonia compounds must be first nitrified by bacteria into nitrates before they are directly available.

But, however this may be, it is certain that ammonia salts constitute only a small part of the available nitrogen in ordinary soils. Much of the nitrogen in the soil eventually reaches other forms, and in the humus there is a variety of compounds which demand change in their chemical nature before they can be utilized by plants. It is certain that these compounds can be rendered available for vegetation by a process of oxidation. It is only necessary to oxidize the lower compounds of nitrogen to convert them into nitrates. For example: ammonia (or its salts) may be united with oxygen to form *nitrous* acid as follows:

$$2NH_3 + 2O = 2HNO_2 + 3H_2O$$

By replacing the hydrogen with potassium this becomes KNO_2, or *nitrite* of potassium. By a further oxidation the nitrous acid or nitrite becomes *nitric* acid or its salts, *nitrates ;*

$$HNO_2 + O = HNO_3$$

or later KNO_3 which is *nitrate* of potassium. In this form the nitrogen is in its most useful form for plant food, nitrate of potassium being one of the best fertilizers.

This process of the oxidation of nitrogenous matter, which was known long before it was understood, is called *nitrification.* It was at first supposed to be a simple chemical process taking place under the direct oxidizing power of atmospheric oxygen. Such an oxidation would be simple and was shown experimentally to be possible. If nitrogenous material is brought into very intimate contact with oxygen the oxidation may take place. When nitric compounds are mixed intimately with platinum black they are brought into a rather peculiar and very intimate association with oxygen. That platinum black has the power of stimulating oxidation is well known, although the reason is not clear. Under such conditions nitrous bodies are oxidized into nitric acid compounds. This possibility of a purely chemical union between atmospheric oxygen and nitrous bodies led, naturally, to the belief that a similar union of nitrous acid and oxygen occurred in the soil, especially if the soil was porous so as to bring about an intimate association of the nitrogen compound and the oxygen, similar to that occurring in platinum black. Experiments were carried out along this line to a considerable extent, designed to determine the conditions under which such oxidation most readily occurs. But these experiments led to nothing, for although they were extensively multiplied they did not indicate that the possibility of such oxidation is very great. They were mostly negative in result, simply because they were looking in the wrong direction for the discovery of the chief agents in the process. While it would not be denied to-day that such direct oxidation may occur to a slight extent, it has become evident that the chief agency must be looked for elsewhere.

The Nitrification Bacteria.—The real explanation of nitrification has appeared during the last twenty-five years and has proved to be connected with the phenomenon of life. It is very easy to demonstrate that such nitrification actually takes place in ordinary soil. All that is necessary to prove this is to place a quantity of soil in a proper vessel and subject it at intervals to a chemical analysis, when it is found that there is an increase in the amount of nitrates present, after it has remained undisturbed for a few weeks. The process is slow but practically universal. The next step in the discoveries was made by Schlössing and Muntz in 1877, when they succeeded in demonstrating that this nitrification is associated with the presence of *living matter* in the soil. This can be easily proved by placing two lots of the same soil under such conditions that, in one, living phenomena may go on, while in the other they are stopped. If, for example, one lot of soil is sterilized by heating it sufficiently to destroy the living germs present, and then this soil is compared with another lot treated in all respects the same except that it is not sterilized, the latter will be found to increase its nitrates while the former shows no such increase. The same results are obtained if the soil is mixed with antiseptics which prevent bacteria growth. In short, anything which prevents the occurrence of life phenomena in the soil prevents the nitrification.

Such experiments repeated many times and verified by numerous observers soon demonstrated that nitrification is a living process. Inasmuch as such soil contains no plants large enough to be seen, such plants having been entirely removed from the experimental soils, it followed that the living agent of nitrification must be some form of microörganism. It proved, however, to be a very difficult matter to find the organisms concerned in the process. It is true enough that the number of bacteria in the soil is large and that many different species are there found. But although many of these bacteria were isolated and carefully tested, for a long time none proved to have any power of nitrification. Most of them, indeed, produced the reverse effect of deoxidizing nitrates already mentioned (denitrification), but none of them raised the nitrites into a state of nitric acid. Moreover, none of them could oxidize ammonia so as to form nitric or even nitrous acid. If a small quantity of soil is added to a solution of nitrite the nitrite soon becomes converted into nitrate, under the influence of the fermentation started by the presence of the soil. This shows that the soil must contain the nitrifying organisms. But the bacteria which were found in such soil by ordinary methods showed no power of nitrification. Evidently the reason was that the nitrifying bacteria did not make themselves evident with the ordinary bacteriological methods.

The cause of the trouble and the secret of successful study was soon learned by a Russian naturalist, Winogradsky (1890). In bacteriological studies the common method of isolating bacteria is to get them to grow in certain definite culture media made by the bacteriologist. Such media, as commonly used, contain a certain amount of organic compounds which serve as food for the bacteria. Winogradsky proved that in such media the nitrifying bacteria will not grow. The presence of a small amount of organic matter in the culture media is directly injurious to these organisms, and the media which support ordinary bacteria luxuriantly will not allow the nitrifying bacteria to grow. Having reached this conclusion Winogradsky, by a series of very ingenious experiments, was finally successful in isolating from the soil an organism, which proved to be a definite species of bacterium, and which was able, under proper conditions, to carry on this oxidation of nitrites and ammonia in such a way as to result in the production of nitrates.

While the original discovery belongs to Winogradsky it must be mentioned that precisely similar results were obtained almost simultaneously, and quite independently, by two American bacteriologists (Jordan and Richards). As soon as these discoveries were announced others followed rapidly along the path pointed out, so that in a short time the results were thoroughly verified and demonstrated.

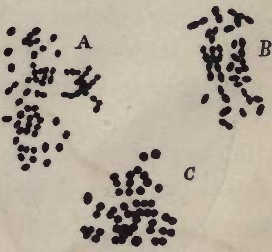
For a while the results of experiments were in some confusion since in some cases nitrates appeared to be formed while in others they did not. It became evident that nitrification was not a simple phenomenon. It was Winogradsky again, who explained the confusion by a second discovery, showing that the nitrification, as occurring in ordinary soil, is a two-fold process. The first step in the process oxidizes the ammonia into *nitrites*. In most of the experiments the nitrogen was put into the culture fluids in the form of sulphate or carbonate of ammonia and this was readily oxidized into nitrite. The second step was the oxidation of the nitrites into *nitrates*. But Winogradsky showed, not only that the two steps are independent, but that they are brought about by two different species of bacteria. The organism which he first isolated had the power of producing nitrite out of ammonia, but, when he was especially careful to obtain absolutely pure cultures, the further oxidation into nitrates did not occur. The more careful his work, the less evidence he obtained for the production of nitrates, and this led him to believe that there must be a second bacterium mixed with the first in some of the experiments. Acting upon this suggestion he was finally successful in separating the two species, and obtained a second bacterium which had the power of oxidizing nitrites into nitrates. But this second species had no power of oxidizing ammonia. When the two were together the ammonia was completely oxidized into nitrate by their combined action. His early experiments were sometimes performed with such mixtures and the confusion of the results was thus easily explained. Winogradsky's conclusions were soon verified, by others so that we now know of at least two types of soil bacteria associated with this phenomenon of nitrification. These bacteria have been called the *Nitrobacteria*. They are as follows:

Nitrous Bacteria.—Originally called *Nitromonas* and more recently *Nitrosomonas*. These organisms perform the first step in the nitrification, oxidizing the ammonia compounds into the form of nitrous acid, which forms nitrous salts with the soil alkalies. They cannot carry the process on to the formation of nitric acid. At least two species are known, one being a rod (*Nitrosobacter*) and the other a coccus (*Nitrosococcus*). (Fig. 16, A.)

Nitric bacteria, called *Nitrobacter*. This is the second species which completes the nitrification, changing the nitrites into nitrates. (Fig. 16, B and C.)

All of these species of bacteria are very widely distributed in nature and have been isolated from soils of widely separated localities. Apparently the nitrobacteria are almost universally distributed in soils all over the world. The bacteria isolated from the different soils show a certain amount of variation among themselves, but they show such decided likenesses in their general characters that they can all be properly classed together as *nitrobacteria* and divided into the few species above defined. Both types are commonly found in the soil, so that the nitrification, if it occurs, will be complete, the one raising the ammonia to nitrites and the other completing the oxidation into nitrates. Their importance in the soil can hardly be overestimated, since they complete the final transformation of nitrogen compounds into the form most available for plants, and thus unlock much of the soil nitrogen from its useless combinations.

F G. 16.

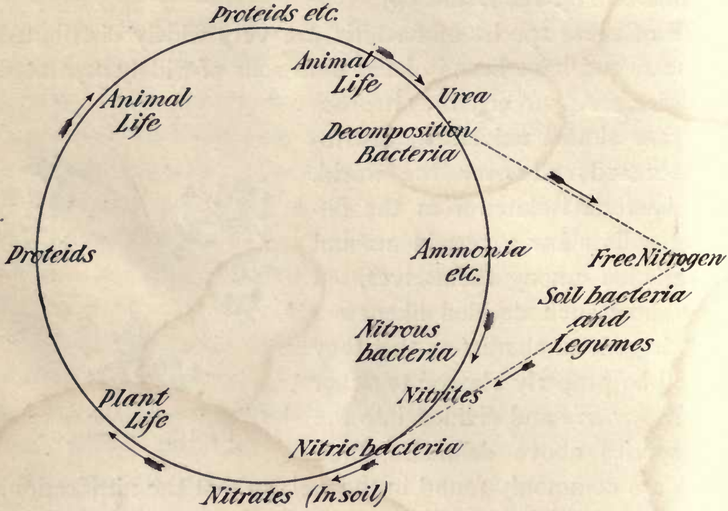


Nitrifying bacteria: A, Nitrococcus; B and C, nitric bacteria.

A graphic representation of these nitrogen transformations may serve to bring this subject more clearly before the mind. In Fig. 17 is represented the nitrogen cycle of transformation. Beginning at the bottom of the circle, in the form of nitrates, the nitrogen may be traced around the circle in its transformations through various organisms, until such parts of it as do not fly off from the circle entirely get back to their starting point.

Nitrifying Bacteria in the Soil.—What is the actual relation of these two different processes, the building-up and pulling-down nitrates, in the soil? That the nitrifying bacteria build nitrates out of lower compounds is easy enough to understand, and experiment shows that it actually occurs. But if both nitrification and denitrification were going on at all times the result would, in a short time, be a total disappearance of soil nitrogen. If the nitrification were constantly building the soil nitrogen compounds into the form of nitrates, and then the denitrifying bacteria were as constantly seizing the nitrates thus furnished and reducing them to ammonia or to free nitrogen, it is clear that the continuation of the process would in the end free the soil from all nitrogen bodies. Evidently such a condition does not exist, for no such constant dissipation of nitrogen occurs, and the soil nitrates increase rather than decrease as the total result of the action of the soil bacteria.

FIG. 17.



The nitrogen cycle.

The denitrifying bacteria are very vigorous, while the nitrifying organisms are comparatively weak. How then can it be possible that the latter produce more nitrates than the former can destroy, if both types are present in the soil and both active? It has been suggested that this may be explained by the presence of the large amount of air mixed with the soil. As we have seen, denitrification does not require oxygen, and occurs more readily in its absence since the bacteria under these conditions are forced to take oxygen out of the nitrogen compounds, producing their reduction. Exactly the reverse is true of nitrification which needs oxygen to oxidize the compounds. If then, air is thoroughly mixed with soil it would stimulate the activity of the nitrifying bacteria and check the action of the deoxidizing germs.

While this may possibly be a factor it is certainly not the whole explanation. Some of the denitrifying bacteria, as we have seen, act in the presence of oxygen and would be sure to grow in ordinary well-aired soil. The explanation of the phenomenon seems to be in another direction, and leads us to the consideration of one of the most remarkable properties of these interesting organisms.

CONDITIONS OF LIFE OF THE NITROBACTERIA.

In some respects these nitrobacteria are the most remarkable of all known microörganisms. In their conditions of life they stand in most sharp contrast, not only to other microörganisms, but in some respects to all other living organisms. It has already been stated that they do not flourish under the conditions which support ordinary life, but will flourish under conditions in which other bacteria will not live. A solution made of ammonia salts, as a source of nitrogen, and small quantities of minerals in addition, but which has no trace of organic matter, furnishes them with the proper conditions for growth. Thus they *do not need any organic food.* It has been suggested that Winogradsky's materials may not have been absolutely pure, and that even the distilled water which he used did contain enough organic matter to furnish the bacteria with a small amount. This seems rather doubtful, but whether it is true or not, it still remains that these nitrobacteria can live in a solution containing only minerals and such an infinitesimal amount of organic matter as may be contained in the purest distilled water.

Not only is this true but it appears that the presence of organic matter in the solutions, even in very small quantity, is directly injurious. The bacteria will grow readily enough in these mineral solutions, but if a small quantity of organic matter is added their growth is stopped. So sensitive are they to the presence of organic matter that these compounds act exactly like antiseptics. When present in very small quantities they are able to stop the growth of the bacteria, acting as powerfully in this respect as the antiseptic poisons act upon ordinary bacteria. For example: If peptone is present in the proportion of 1 to 4,000, the nitrate-forming bacteria are checked in their action; and 1 part to 2,000 stops their growth entirely. The nitrate-forming germ is absolutely checked by the presence of ammonia 15 parts to 100,000.

In most of these respects the nitrous bacteria are more resistent than the nitric bacteria, requiring a larger amount of organic matter to check their activities. In regard to ammonia, however, the nitrate bacteria are excessively sensitive, as above shown, while the nitrite bacteria are capable of growing in the presence of considerable ammonia.

Further, it is found that the more highly organized the compound, the more decided is its checking action upon the nitrobacteria; the more valuable the material for ordinary kinds of bacteria, the greater is its injury upon the nitrobacteria. These bacteria thus grow only under conditions in which other bacteria will not grow, and will not grow under the conditions which other species find most favorable. A more sharp contrast can hardly be conceived. Not only bacteria, but all other colorless plants, are obliged to depend upon organic food as a source of energy, in this respect resembling animals. But here is a group of organisms that not only does not need, but cannot grow in the presence of, organic matter. They do not, therefore, need any other living organisms to interpose between them and the mineral world, but may develop under conditions in which they are supplied with mineral substances alone. It is more surprising perhaps to find that they do not need light, but can utilize the mineral substances while growing in perfect darkness. This fact was at first conceived as quite contrary to our general ideas of the relation of life to physical energy. We have supposed that the only source of energy for living things is sunlight, and that this energy is stored up by green plants in the form of chemical compounds of high complexity. The animals and colorless plants use these stores as food, breaking them up and using the energy liberated for their own use. But here we have organisms which do not require organic material as a source of energy and are not able to utilize sunlight itself directly. How are these facts to be reconciled with the general views of physical energy? Evidently these nitrobacteria must obtain their energy from some other source than that which is commonly utilized by animals and plants.

Of course, these surprising and almost revolutionary facts demanded thorough verification and they soon received it from various sources. Some additions and some corrections were made to Winogradsky's first conceptions, but the general facts remained as he gave them, and it is necessary to believe that these bacteria obtain the energy for their growth from some source not available to ordinary animals or plants. That they do have a supply of energy at command is clear enough from the fact that they assimilate CO_2 and build it into carbonaceous compounds. Winogradsky thought that they obtained their carbon from carbonate of ammonia, but others have shown that they can obtain it directly from the CO_2 in the atmosphere. It is still a little problematical where they obtain their energy, but it is evidently from the oxidizing processes which they bring about. Oxidizing processes, wherever they occur in nature, furnish energy. These nitrobacteria, as we have seen, induce the oxidation either of ammonia or of nitrites, and such oxidation would yield some energy. Inasmuch as this appears the only source of energy which we can detect in the growth of these organisms, it has been believed that this oxidation itself furnishes the nitrobacteria with the energy they need for their carbon assimilation. Whatever be the exact source, it is clear enough that these bacteria have a power of utilizing some source of physical energy not possessed by any other animal or plant so far as known to-day. Here, again, we see them in the sharpest contrast to all other organisms.

It is evident that with these nitrobacteria we are brought very close to the mineral world and that such organisms may be of considerable significance in explaining the beginning of soil formation. It has long been suspected that bacteria do actually play some part in the process of the disintegration of rocks, although the suggestion has hardly been credited from the fact that there seemed to be no source from which bacteria could obtain their carbon and energy upon the surface of bare rock. It was even suggested at one time that small traces of alcohol in the air furnished the carbon and that, nourished by such minute traces of alcohol, bacteria are engaged in producing the disintegration of rocks even on mountain peaks where no vegetation is found. With our present understanding of the nitrobacteria we can gain a further support for this general agency of bacteria. If they can grow on the bare rocks, using the minerals of the rocks and the carbon of the atmospheric carbon dioxide, and obtain ammonia for oxidation from the air, it would certainly seem that they might be the means of building up a certain amount of organic material which might subsequently serve as nourishment for higher plants. They may thus be a not unimportant factor in the disintegration of rocks, and a more important factor in the beginning of the deposition of foods in the soil upon which vegetation may subsequently depend. They would thus seem to be an even more fundamental link between the organic and the mineral world than the green plants, since they are able to make use of even simpler forms of nitrogen. Ammonia and nitrites are of little use to ordinary plants, but they furnish these bacteria with their nitrogen and energy. Hence we must look upon these nitrobacteria as of great significance in the formation of soils, and in their extraordinary power of living without organic food or sunlight, they are in the sharpest contrast to all other living organisms known at the present time. (except sulphur and iron bacteria)

RELATION OF DENITRIFICATION AND NITRIFICATION IN THE SOIL.

These peculiar properties of the nitrifying bacteria enable us to understand, in part at least, their relation to the denitrification process in the soil. The building of nitrates will not take place in the soil so long as there is any considerable amount of organic material or any considerable amount of free ammonia present. If there is much organic material rapidly decomposing so as to produce ammonia, this will completely check the formation of nitrates, for these nitrifying bacteria will not grow in the presence of either organic material or ammonia. It is not until after decomposition has been completed and practically all the organic compounds used up that the nitrifying germs can begin to act. The denitrifying bacteria cease to grow after they have used up all the organic material which nourishes them, and, therefore, even if nitrates are subsequently produced they will not be reduced by the de(?) nitrifying organisms which have no power of active growth unless supplied with a good quantity of organic food.

On the other hand at the end of the decomposition changes, when the high organic compounds have all disappeared, and in their place is found a quantity of ammonia salts and other simple nitrogen compounds, we have just the proper condition for the action of nitrification. So long as there is any trace of free ammonia left the only nitrobacteria which can act are those which produce *nitrous* acid from the ammonia, since the bacteria which form nitrates are prevented from growing by the very merest trace of ammonia. First the nitrobacteria begin to work, oxidizing the ammonia, and soon, after all traces of free ammonia disappear, the nitric bacteria themselves begin their action of completing the nitrification, producing nitrates from the nitrites formed. After these nitrates are produced they are not in much danger of being reduced by the denitrifying bacteria even though they be still present in the soil. These latter demand considerable organic material for their growth and this has been all used up by the previous decomposition. Denitrification will not begin again unless more organic material is added to the soil to furnish the denitrifying bacteria with their needed food. The nitrates will be reserved for the next generation of plants growing in the soil. If, however, more organic material, like manure, should be placed upon the soil after the nitrification has been completed, this would result probably in the destruction of most of the nitrates formed. A practical lesson from these facts is that animal manure should *never* be mixed with nitrates as a fertilizer. The nitrates are useful but are pretty sure to be destroyed by the denitrifying bacteria if mixed with manure.

NITRIFICATION NOT IN ALL SOILS.

Although only a few of these nitrifying bacteria are as yet known, these few are very widely distributed. They are found in water, in sewage and in ordinary soil. They do not appear to be present in the soil of forests or, at all events, do not here produce nitrates, for these compounds are absent from forest soil. This is attributed partly to the acid nature of such soil, for the nitrifying bacteria will not grow in an acid medium. It frequently happens, too, that the nitrifying bacteria are not present in meadows, and that the formation of nitrates is here slow or wanting. These bacteria are, however, very abundant in ordinary manure, and one of the advantages of adding manure to certain soils seems to be rather from the bacteria thus inoculated in the soil than the actual plant food in the manure. For example, one frequently sees that an open pasture or meadow supports a somewhat limited crop of grass, although nitrogen compounds may be abundant enough in the soil. If cows are pastured here it is common to find plots of brilliant green, vigorously growing vegetation, surrounding the droppings of the cow excrement. Now this may be due in part to the food contained in the excrement, which is utilized by the plant, but it is not wholly thus explained. The effect lasts for a long time, and months afterwards the oasis of green may be seen in the pasture, gradually increasing in size until it reaches far beyond what must have been the limits of the direct effect of the plant food in the excrement. The explanation seems to be that this excrement contains bacteria in abundance, and these in a short time begin the work of converting the soil nitrogens into nitrates. Their influence continues to extend through the soil as they multiply and act upon a wider and wider circle, so that an increased vegetation may continue for a long time under the influence of these nitrifying bacteria which are constantly converting the soil nitrogens into nitrates. The bacteria brought into the soil in these cases have proved of much more usefulness than the actual plant food which was in the excrement.

CAN THE FARMER INCREASE NITRIFICATION?

From all this it follows that one of the primary conditions for soil fertility is the presence of proper nitrifying bacteria in the soil, and proper conditions for their growth. To the agriculturists it has thus become a practical question to learn how these bacteria, if not already present, may be added to the soil, and how they may be stimulated into activity, if they are present but inactive. To the solution of these questions bacteriologists are strenuously applying themselves, and, although they have not answered all of the questions arising, some facts of significance have already been settled.

In the first place, the cultivation of the bacteria in the laboratory and the inoculating of pure cultures into the soil appears hardly practical. The organisms are too difficult of cultivation to promise practical results along this line of work. Moreover this is not necessary. Most soils seem to have the nitrifying bacteria already present, so that a stimulation of their action is needed rather than the addition of more bacteria of the same kind. Further, if it is necessary to supply the soil with the nitrifying bacteria, this can readily be done by the use of manure, which contains them in abundance, and this process is certainly cheaper and probably more efficient than the preparation and use of pure cultures.

It is of more importance therefore, to determine how the growth of the nitrifying bacteria can be stimulated into activity. This can only be determined by studying the conditions under which they most readily grow and applying them in practice. In this connection it has been learned that a certain amount of moisture is necessary for their proper action, wet soil showing more nitrification than soil that is simply damp or saturated with water. The process of nitrification takes place only when the temperature is more than 5° above freezing, and is more vigorous with a rise in temperature. Hence it ceases in the winter months and is most vigorous in the summer. The activity of the nitrobacteria demands an alkaline medium and the presence of even a slight amount of acid checks their action. Hence it frequently happens that the addition of a little lime to soil produces a wonderful effect, far more than can be accounted for by the fertilizing value of the lime itself. The lime neutralizes the acids present and furnishes the proper conditions for the growth of the nitrobacteria. Here too we find doubtless, the explanation of the great effect sometimes seen to follow the addition of a little manure to a poor soil. The manure is sure to be alkaline in reaction, and if it be added to a soil which is acid, it may at once stimulate the activity of the nitrifying bacteria by neutralizing the acids. The nitrifying bacteria will begin then to convert the soil nitrogen compounds into nitrates and a greatly increased yield of crops may follow. This is doubtless another factor in explaining the oases of green which surround the droppings of cows in old pasture lands.

Perhaps the most important result of these studies, from the practical side, has been the conclusion that *nitrification demands a good supply of oxygen* and is, therefore, stimulated by anything which increases the thoroughness of the mixture of air with the soil. This points to the great advantage to the soil of *thorough cultivation*, and teaches that the more thoroughly the soil is stirred the better the results. Such cultivation more uniformly distributes the moisture and this stimulates the bacteria growth. The simple stirring up of the soil will thus actually increase its fertility by stimulating the conversion of its nitrogen compounds into nitrates through the agency of nitrification. The more thorough the cultivation the better, and experiments indicate that a complete pulverization of the soil has a very decided value in increasing its fertility. These facts have entirely done away with the idea that the land should be allowed to lie fallow once in a few years. Fallow land is worse than wasted. The nitrification becomes very slight, and what nitrates there are present are rapidly drained away from the soil by the rain water which easily washes through such fallow land. At the end of the fallow year the land may thus have actually less nitrogen than at the start. The nitrates have probably been removed more rapidly than they would have been if the soil had been cultivated. Nitrification has been checked, no crops have been produced and the whole year is thus worse than lost. *One of the factors of a continued soil fertility is a continued soil cultivation conducted under proper conditions.*

CHAPTER V.

THE MANURE HEAP AND SEWAGE.

AFTER this somewhat technical, but necessary discussion of the transformations of nitrogen, we may turn to some practical applications of the facts discussed. Recognizing the value of nitrogenous wastes to the soil it is evident that the farmer has sources of wealth in the manure heap, the compost pile and perhaps in sewage. Their relations to the nitrogen problem we may now intelligently consider.

THE MANURE AND COMPOST HEAP.

The above considerations of the soil bacteria have contained many references to manure and compost material. The value of the manure and compost heap to the life of the farm is recognized by every agriculturist. So thoroughly is this appreciated that in some countries the wealth of a farmer is measured by the size of his manure heap, which is commonly exposed prominently in front of his house. Everywhere one may measure quite accurately the thrift of a farmer by an examination of this somewhat unsavory product of farm life. We can readily understand its importance on the farm when we remember that in this manure heap are going on, upon a larger scale, exactly the sort of transformations of food material which we have been considering.

The manure heap is always an extremely complex mixture of organic substances, of almost every conceivable kind. It contains large quantities of partly broken down *vegetable tissues* which have passed through the alimentary canal of the cattle, partly digested and partly, perhaps, fermented by bacteria. It will contain large or small amounts of *hay* or *straw*, derived from bedding and from the *incompletely digested* food, especially if horses contribute to its formation. It will likely contain sawdust or some other form of *woody tissue*. It will be likely to contain more or less *flesh* and *bone* from dead animals, and will be sure to contain *albuminoids*, *gelatins*, *fats*, *sugars*, *starches*, and indeed nearly all types of organic matter produced by animals or plants, and these will be in various states of digestion and decomposition. Lastly, and perhaps most important, it will contain large amounts of nitrogen in the form of *urea*, or liquid manure, which represent the result of the nitrogenous metabolism of animal life. This liquid manure constitutes undoubtedly one of the most valuable parts of the whole, since it contains all of the nitrogen which has been actually metabolized by animals, and which is in a form to be readily brought back again into a condition available for plant life. Nevertheless, the condition upon our farms is almost always such as to result in a large or almost complete waste of this most useful fertilizer, since it is allowed to soak into the ground without any attempt being made to retain it. In addition to these ingredients manure always contains a large amount of *water* and an unlimited quantity of *bacteria* of a wholly unknown number of species and varieties. Some of the material in the manure heap is perhaps already in a form to be used by plants, but the most of it must undergo some important chemical transformations before it can be available for vegetation.

In this manure the bacteria find most favorable conditions for growth. Plenty of food and moisture are always found and there inevitably occurs a rapid growth of bacteria. There is a great struggle for existence among them and, in the weeks of fermentation, first one and then another species may gain the mastery. If the bacterial contents of such a mass be studied at intervals the number and variety of species which are most abundant are found to be constantly changing. Different samples of manure from the same pile at the same time or at different times are almost sure to give different results to bacteriological analyses. All of this indicates faintly the wonderful complexity of bacterial life and the intensity of the struggle for existence among the numerous species originally present in the manure. Of all this, however, we have as yet only the faintest notion and must rest content with the knowledge that bacterial life is excessively active, and that now one, and now another species, gains the upper hand and produces its effect upon the decomposing organic materials.

FERMENTATIONS IN MANURE.

Destructive.—The first chemical changes which go on are those of decomposition. An *ammoniacal fermentation* is universal, as indicated by the ammonia smell which always appears. The liquid manure is most rapidly decomposed by this fermentation, this substance in a very few days, and perhaps in only a few hours, suffering the reduction into ammonia compounds as already mentioned above. This is completed before the ammoniacal fermentation of the other nitrogen bodies has fairly begun, and indicates that the proper method of handling manure will be to treat the liquid manure separately from the solid portion. Eventually all of the proteid matter is likely to undergo ammoniacal fermentation. But this is not all. The starches, sugars, cellulose and woody tissue, in time, undergo a decomposition by which CO_2 is set free and various other substances are left. The fats and fatty acids are decomposed with the formation of CO_2 and butyric acid, together with other less known bodies. The decomposition of the proteids liberates sulphur, commonly as H_2S, and this unites with water to form sulphuric acid. The sulphuric acid may combine with the ammonia to form ammonia sulphates, or the ammonia may unite with the carbon to form carbonates. A large quantity of material is lost from the manure during these changes. The loss includes carbon in large amount, a matter of no significance, however, as it has simply gone into the air from which it can readily be reclaimed by plants. But the loss includes much nitrogen, and this loss is a misfortune, since it is the nitrogen that the farmer desires to keep. The ammoniacal fermentation results in such large amounts of ammonia that it cannot be held in the manure pile by the small amount of acid formed, and is consequently exhaled into the air. The smell of ammonia tells of this nitrogen loss. The denitrifying bacteria, of which there are many varieties in the manure, are also vigorously at work reducing any nitrates which may be formed, into nitrites, and, finally, into free nitrogen. As fast as the free nitrogen is loosened from its combinations it disappears from the manure into the air, for it is not able to combine with any substances present. The loss of nitrogen from these sources is very great. From the liquid manure the nitrogen disappears very rapidly, and from the solid manure it goes more slowly, indeed, but far too rapidly for the good of the farm. The total loss of nitrogen by these means is extremely variable and depends upon conditions only partly understood. Sometimes more than half of the whole disappears in one of these ways, before the decomposition and denitrifying processes are finished by the completion of this "ripening" of the manure.

It is of great importance to the agriculturist to check this nitrogen loss as completely as possible. Various means have been suggested and used for the purpose. One of the most obvious consists in adding to the manure enough of some acid material to combine with all the ammonia as fast as it is formed so as to make non-volatile salts. For this purpose sulphuric acid has been commonly used. Other materials sometimes used appear to have rather the purpose of checking the action of the denitrifying bacteria. Of the various materials which have been recommended for the preservation of the nitrogen of the manure the most common are gypsum, kainit, superphosphates, marl and sodium bisulphate. Each of these has appeared to be of some utility in this respect and each one has been recommended. The disadvantages which also accrue and the expense involved make it doubtful whether the use of any one of them can be unhesitatingly advised.

Of less doubtful value are suggestions arising in regard to the treatment of the manure. The loss of nitrogen appears to be greater in manure when there is a perfectly free access of air than when it is compactly massed. Hence it seems wise to keep the manure in compact heaps. As already noticed the denitrifying processes require some carbonaceous material as a source of energy to the bacteria, and this material may be furnished by straw. Hence it has been concluded that the mixing of the manure with straw in the form of bedding increases nitrogen loss by denitrification. Moreover, since the manure of horses always contains a considerable amount of such undecomposed straw, its presence in the manure heap tends to increase nitrogen loss, and the wisdom of keeping the manure of the horse and cows separate is clearly indicated. The use of sawdust for bedding as a substitute for straw is an aid in decreasing nitrogen loss. The separation of the liquid from the solid manure is recommended by those who have studied into the matter. The former decomposes very rapidly, and its presence in the manure heap is believed to hasten the denitrifying processes and to cause a large nitrogen loss which would be partly prevented if the two kinds of manure were kept separate.

All of these plans for preserving manure from loss are evidently based upon bacteriological phenomena. Each one is directed, consciously or unconsciously, either to checking the growth of bacteria in the manure or to holding in solution the products of bacterial life which would otherwise escape by volatilization. We may hold the ammonia by the use of acids if this can be done cheaply and without injury to the manure in other ways. We cannot hold the free nitrogen if it is loosed from its combinations, and the only means of preventing this loss is by checking the action of denitrifying bacteria. A satisfactory method of accomplishing this has not yet been found, but bacteriologists and chemists are now studiously at work upon this problem of discovering some practical means of protecting the manure from its extensive losses.

Constructive.—As already noticed the process of denitrification is eventually replaced by that of *nitrification.* This occurs in the manure heap as well as in the soil. Exactly when and why it begins is a little uncertain ; but it appears to start only after the high organic compounds have been almost wholly broken up into ammonia, and the ammonia formed has either united with the acids to form salts or has been dissipated into the air. Even while some ammonia is still present the oxidation of the ammonia salts into nitrites is possible, for the nitrous bacteria are not prevented from growing by the presence of free ammonia. The nitric bacteria are, however, so extremely sensitive to ammonia that they cannot begin the formation of nitric acid till ammonia gas has entirely disappeared and therefore not until denitrification has wholly ceased. When the nitrifying processes do begin they complete the ripening of the manure. They oxidize the nitrogen compounds which are left, the ammonia salts becoming first changed to nitrites and then to nitrates. As this process continues the manure heap is more and more filled with nitrates and therefore becomes a better and better food for plants. At last, when the process is ended, the manure is fully ripened and its nitrogen is so fully converted into nitrates that it furnishes a most valuable supply of food for vegetation. If put upon the soil it furnishes the soil both with plant food and with great quantities of the nitric bacteria which may be of direct value to the soil.

Of course this ripening need not take place while the manure is still within the manure heap, for essentially the same changes will occur if the fresh manure be thrown upon the soil. The changes which then occur will be more or less modified by the conditions, since there will be present less water, a more complete separation of the liquid and solid manure, and different relations to the supply of oxygen. It seems, as the result of experiments, that the nitrogen loss occurring when the manure is thus used fresh, is less than when it ripens in the heap. Certainly the loss of nitrogen is greater where large amounts of organic material are allowed to ferment in a mass together, than when only small amounts are present. The former condition is offered by the manure heap, the latter by the manure scattered fresh upon the soil. Indeed, some bacteriologists are inclined to believe that the nitrogen loss from denitrification is really very small under ordinary conditions where only a small amount of manure is spread out and mixed with the soil, a conclusion which would, of course, suggest the wisdom of using the manure fresh instead of waiting for it to ripen in the heap. While this advice may be sound it is of course impossible to follow it in climates where the soil freezes in winter weather. But it is certain that we must not apply too rigidly to the soil the conclusions which have been reached from laboratory experiments under the unusual condition of excessive organic material undergoing decomposition.

It must be borne in mind that in the ripening of manure it is not desired that the nitrates should form too quickly. Whether the denitrifying forces are active in soil or not they certainly are active in the manure heap, and if nitrates form early in the ripening these reducing bacteria will extract their oxygen and cause their loss as ammonia or free nitrogen. The nitrate formation should therefore, if possible, be checked until the denitrifying bacteria have ceased their growth. This may be accomplished, in part, by keeping the manure well packed together so as to exclude air, for the nitrifying bacteria demand a good supply of oxygen and will not produce nitrates in well-compacted manure.

In the use of manurial fertilizers it is necessary to bear in mind that the advantage from the process comes from two wholly different factors. The soil gains in fertility from the actual food materials in the manure used, but it may also gain an equal, and, in some cases, a greater advantage, from the large quantities of bacteria thus inoculated into the soil ready to produce further chemical transformations. But these bacteria may contain some of the species which cause a loss as well as some that cause a gain of nitrates. To check the action of the former and to stimulate the action of the latter should be the aim of the proper use of manurial fertilizers. Anything which teaches the farmer to accomplish this end brings him so much nearer to the ideal condition of continued soil fertility.

THE COMPOST HEAP.

It is evident that in a *compost heap* there must be going on a series of similar bacterial transformations. By proper means the farmer may make use for his soil of almost any organic material which contains nitrogen or the minerals needed for his crops. Vegetable tissues of all sorts contain more or less nitrogen and may readily be brought under the influence of the bacteria which are able to reduce them to plant foods. A valuable source of such material may be obtained from seaweeds, if they are at hand, or indeed any abundant vegetable substances may thus be heaped into a mass, and, if this is moistened sufficiently by rains, the bacteria are sure to work within it, gradually transforming the nitrogens, and in the end converting them into nitrates for plant food. Into his garbage heap, then, the farmer may throw all sorts of organic débris, animal or vegetable, with the confidence that his bacterial aids will in time place the nitrogenous material in some measure at his service as a fertilizer. Thus, by the aid of his invisible allies the agriculturist will be able to make use of the wastes on his farm and in time return to his soil a considerable portion of the nitrogen therein.

It is quite certain that the future study of the conditions under which these various bacteria will best act will result in such modifications of farm processes as will produce a considerable saving of the nitrogen which has been allowed to go to waste. Under proper conditions these organisms can certainly furnish the soil with larger quantities of nitrogen than they commonly do upon even the best farm. When he has them under proper control the farmer can feel sure of making use of all the available nitrogen present in the nitrogen wastes of his land, and he may greatly reduce the loss of this element which at present is going on through the dissipation of free nitrogen and ammonia into the air.

SALTPETER PLANTATIONS.

These nitrifying forces are not confined to the soil, but may occur in other localities, always resulting in the production of nitrates. Before the discovery of the nitrate beds of South America it was the custom of agriculturists to prepare their own nitrates by a simple process, not then understood, but now known to be due to nitrifying bacteria. The places where nitrates were thus formed were called *saltpeter plantations*, and the saltpeter was produced by exactly the processes we have already considered. The method was as follows :

Masses of chalky soil were mixed with various organic bodies and the whole heaped into a pyramidal pile, rendered somewhat porous by the admixture of brushwood. The heap was still further furnished with fermentable nitrogen by frequently watering it with liquid manure. In this heap occurred the various kinds of nitrogen decomposition already mentioned, and later the nitrification process began. The result was that nitrates were formed in the interior of the heap in large quantity. Eventually the nitrates were extracted by water and converted into nitrate of potassium by the addition of some potassium salt.

This method of making saltpeter was discovered before science had any idea of the real nature of the process, and it was a practical means of utilizing a part of the nitrogen in the organic substances derived from animals and plants. Whether it was the most efficient means, or more useful than the simple compost heap and manure pile, can hardly be stated. The saltpeter plantations have gone out of existence since the introduction of Chilian saltpeter, but either this or some other means of obtaining saltpeter will inevitably be adopted again when the Chilian mines are exhausted.

Nitrates are actually formed upon the walls of closets and stables where ammonia fumes are abundant. On such walls may frequently be seen a snow-white mass consisting of calcium nitrate. It is the result of nitrification of the ammonia which arises from the manure pile and which unites with oxygen and produces nitric acid. The acid combines with the calcium present in the brickwork to form *calcium nitrate*. The action is an undesirable one from the standpoint of the persistence of the walls, since it produces a corroding action tending to weaken the structure. It may of course be readily prevented by sprinkling the walls with a strong solution of some powerful antiseptic, such as formalin or corrosive sublimate.

BACTERIA IN SEWAGE.

Though a matter of great importance for the hygiene of cities, the problem of *sewage* is not very closely related to agriculture. There are certain phases of the subject, however, which bear so closely upon agriculture that at least a brief consideration is necessary. The problem of the bacteria in sewage is very closely related to that which we have just been considering; for sewage composition and sewage disposal involve all of the subjects of decomposition and putrefaction which have such an intimate relation to soil fertility. Indeed, the most recent method of sewage disposal depends upon the very same principles of chemical decomposition by bacteria which have been considered in the last chapter.

Composition of Sewage.—By sewage we commonly understand the material which collects in the sewerage system of our larger communities and which has no exact counterpart on the farm. It always contains the products of the life of men and animals, which are thrown away as no longer useful; also large quantities of both animal and vegetable foods which have passed through the alimentary canal of men and animals unassimilated. It contains a large amount of urea which has come from the animal metabolism; it contains woody matter, cellulose, fatty matter, starchy matter, and an indefinite series of other organic bodies. Almost anything which enters the city may find its way eventually into the sewers where, mixed with large amounts of water, it contributes to the sewage. The sewage thus contains exactly the same sort of material found in the manure heap and the compost pile. Evidently the problem of the various steps of decomposition of this material will be nearly identical with that already considered at length.

But the practical application of the matter is quite different. This is due partly to the fact that the chief aim is to *get rid* of sewage rather than to *utilize* it, and partly to the great modification produced by the presence of such large amounts of water. Upon the farm the problem of sewage is of little importance. The material, which in the city enters the sewage, is separated into several portions on the farm, each of which is differently utilized. Part of it goes to the manure heap, part of it is in the privy vault and is handled like manure, and part of it drains from the sink and is commonly allowed to waste itself on the ground.

Nothing further need be said concerning the first of these portions. The contents of the privy vault have practically the same relations to bacterial decomposition and denitrification as already mentioned, and should be handled in essentially the same manner. It is always emphatically necessary, however, to remember that the contents of the privy vault are far more likely to contain *pathogenic* bacteria than is barnyard manure, and it should, consequently, be much more carefully handled. That such material has been the means of distributing typhoid fever in many cases is surely demonstrated. The bacilli of this disease are voided by the patient in the excreta, and are thus sure to find their way into the vault, to be subsequently distributed over the fields where they may percolate through the soil and pollute streams and wells. It is necessary, therefore, that the contents of the privy vault should be handled with extreme care and should never be left in position where it can possibly pollute the water of either brook or well. Great precautions should also be taken to prevent its distribution around the farm by soiled boots or tools which have been used in handling it. It must be remembered that there is much more likelihood of finding pathogenic bacteria in *human* excrement than in that of domestic cattle, and that the disease germs thus found are far more likely to be injurious to *human* health. Evidently the farmer should exercise much more care in disposing of the contents of his privy vault than in the use of his barnyard manure, and the constant addition of lime thereto is certainly to be most thoroughly recommended. In other respects this material has exactly the same relations to decomposition and reconstructive processes as barnyard manure.

The portion of sewage which comes from the wash water of the sink or the dairy on the ordinary farm is so small that it may commonly be left to care for itself. The amount of solid material in such water is very slight, and it can be allowed to run out on the soil where, generally, it is rapidly absorbed and decomposed without any undue pollution. The organic matter undergoes the same type of decomposition to which all organic bodies are subjected under the influence of bacteria, and becomes eventually converted into plant food and incorporated into the soil. The drainage which comes from the large dairy or creamery may, however, be considerable, too much to be disposed of by such a simple manner. In this case some means must be adopted for disposing of it. The problem thus presenting itself for the disposal of this drainage is precisely the same as that presented to the city for disposing of its sewage, and the same means are to be used in each case.

Where the amount of sewage becomes large, as in the case of a large city, it offers quite a different series of problems and presents some important questions for the sanitary engineer. The disposal of the sewage of a city is also becoming a matter of some importance to agriculture. The chief reason for the difficulty which this problem presents is the large amount of water which is present in the sewage. It is true that sewage contains almost every conceivable kind of organic substance washed into it from the city, and the total amount of organic matter is very large, but this is always mixed with an extremely large amount of water, especially during rains. Hence, the total result is an extremely dilute but very abundant mixture. One who is not actually familiar with sewage can hardly realize its extreme dilution, especially in American cities.

SEWAGE FARMS.

The fact that sewage contains nitrogen, which is known to be of such use to vegetation, has led to a general feeling that in this waste product there is a most valuable amount of plant food which is ordinarily thrown away. To prevent this waste there has been an attempt made in recent years to utilize the material in sewage for fertilizing the soil. *Sewage farms* have been established in large numbers in the last twenty-five years, the design of which is to make use of this waste product. On these farms the sewage is distributed over the fields by proper conduits and the contents of the sewage thus restored to the soil for the use of crops. Upon the fields thus irrigated various garden crops are raised. For a time great claims were made for these sewage farms. The returns seemed to indicate an increased yield and an actual utilization of this waste product. Large numbers of such sewage farms were organized in England and in continental Europe. The great cities of Paris and Berlin have established enormous sewage farms in their neighborhood to use their sewage. Berlin in particular has many thousands of acres under such cultivation. These great sewage farms near Paris and Berlin have undoubtedly been very successful.

But at the present time one hears little in regard to this method of utilizing sewage, and sewage farms are not increasing. Theoretically the plan is ideal since it puts back into the soil a waste product. But practically it is only under special conditions that it is successful and many of the sewage farms are run at a loss. The reasons for this are chiefly the following : The organic matter in the sewage is not in condition to be utilized by plants. It must first undergo the process of decomposition and nitrification. If too much sewage is applied to the soil the bacteria cannot take care of it and the soil becomes clogged. Hence to procure good results a large amount of land is required. It is usually stated that an acre of land will take care of the sewage of only about fifty persons. Hence it is only where there are large tracts of sandy soil near a city, as is the case around Berlin, that sewage farming can be practical. Again, the manurial value of sewage, at best, is small on account of its extreme dilution. Crops can stand only a certain amount of water and, when the dilution of the sewage is great, it is impossible to use enough of it to obtain much advantage from its contained nitrogen. This is especially true of sewage in the United States where our cities are very extravagant in the use of water. A given volume of American sewage does not contain anything like the amount of fertilizing material found in the same volume of English or German sewage. As a result, sewage farming is not applicable to the conditions in America and such farms have not been established. So dilute is American sewage that scarcely better results can be obtained with it than with an equal amount of water. Lastly it is usually quite impossible to find in the neighborhood of large cities land sufficient to utilize their great amount of sewage. Sewage must, therefore, be commonly considered as a purely waste material and some means devised for getting rid of it. Sewage as a means of crop fertilizing does not offer, at least to American farmers, any very promising source of wealth.

The very great bulk of sewage, rendering sewage farming inadequate, has necessitated the adoption of some other method of its disposal. Where a community is in the vicinity of the ocean its sewage is naturally poured into the sea, a process which results in the contamination of the harbors around the cities and is certainly objectionable. In other cases it is poured into a stream or river and carried off by the natural drainage system of the country. This method is even more objectionable and is becoming intolerable. The pollution of streams in this way has become so serious in recent years that it has been absolutely necessary to discover some method of dealing with sewage, and the governments of states have been forced to take it into consideration. Many of the smaller streams have become badly contaminated by city sewage. The significance of this is evident. These streams must serve in many cases as the water supply of a farm or even a city, and the increasing contamination is a matter of very serious import. Many a stream which has in past years served the agricultural community for one purpose or another has become useless from sewage contamination. To meet this condition of things sanitary engineers have been at work. They have been aided by the bacteriologist, for the most successful method of disposal of sewage devised up to the present time depends wholly upon the agency of bacteria. Just as we have seen them at work purifying the soil, so the engineer is setting them at work purifying sewage.

The method adopted for purifying sewage is to destroy and remove a large part of the organic matter so as to leave the water comparatively pure. Various plans have been used for the purpose. The use of chemicals to precipitate the organic matter, which could then be removed by sedimentation, has been adopted extensively. But it has proved troublesome and unsatisfactory and has generally given place to a plan of treatment depending upon the destructive powers of the decomposition bacteria. The *bacterial treatment of sewage* is to-day rapidly obtaining adherents among sanitary engineers and promises to be the method widely adopted in the near future. The subject bears so closely upon our general topic that a brief account of it will be here given.

THE BACTERIAL TREATMENT OF SEWAGE.

The bacterial treatment of sewage depends upon the destructive action which decomposition and putrefactive bacteria have upon organic matter. Putrefactive bacteria, those growing in the presence and in the absence of oxygen, have the power of decomposing all kinds of organic bodies, both the nitrogenous and those purely carbonaceous. Most of the solid matter in the sewage is composed of these organic bodies, and it is evident that if the sewage can be induced to undergo a thorough decomposition under the action of microörganisms, this will produce a great effect upon the composition of solid matter present. Almost all of them will be reduced to simpler compounds. The carbonaceous material will be reduced eventually, if the process is complete, into CO_2 and water, with the liberation of *hydrogen* or perhaps *marsh gas* (CH_4). Such gases would leave the liquid and join the atmosphere. The nitrogenous material would suffer the decomposition which we have already noticed, resulting in the production of *ammonia*, and denitrification, which would be sure to occur, would still further reduce this to *free nitrogen*. Such gases also would be sure to join the atmosphere, unless held in solution in the liquids. In short, the putrefactive processes, which in the manure heap produce a loss deprecated by the agriculturist, would produce here exactly the result which the sanitary engineer desires to reach, a destruction and dissipation of organic material.

Such changes will take place as readily in sewage as in manure or in the soil. Indeed observation and analysis show that they commonly take place much more rapidly. In the first place the organic matter to be acted on is generally in a soluble or partly dissolved condition, and very easily acted upon by bacteria. Secondly, the great abundance of water facilitates the action, for bacteria require an abundance of water for their best growth. Thirdly, the bacteria are present in extreme abundance. All sewage contains bacteria in large numbers, although naturally the number varies. A common sewage contains from 7,000,000 to 10,000,000 bacteria per c.c. Among these bacteria are always large numbers of the various decomposition bacteria, ready to seize upon the organic material and decompose it with rapidity. Such sewage, if left to itself, will undergo a rapid and quite complete decomposition, which results in reducing large quantities of matter to a gaseous state. Other parts are rendered perfectly soluble and are completely *dissolved* in the water, so that the water of the sewage is left in a comparatively pure state, frequently comparing favorably with ordinary river water. This process is sometimes called *filtering*, although the name is quite erroneous. The sewage is not filtered in any sense but simply decomposed by bacterial action.

To allow this bacterial decomposition to take place as completely as possible, two different methods of treatment are at the present time employed, which may be used separately but are frequently combined to advantage. The first method is by what is called the *contact beds*, sometimes called *filters*. In this method the sewage is allowed to flow into large open basins the bottoms of which are covered with a layer of coke or furnace clinkers. The sewage remains in this bed for six to twelve hours when it is conducted into a second of a similar nature. After remaining here about the same length of time it has become so much purified by the bacteria that it may be allowed to flow into a neighboring stream without excessive pollution.

The second method is by the *septic tank*. This is a large closed chamber, perhaps below the surface of the ground, and closed upon all sides and the top, with simply a vent pipe extending from the top to allow the escape of gases. The sewage is passed into one end of the tank in a somewhat slow but constant stream, and the cavity of the tank is so divided by partitions as to insure the sewage a slow uniform passage through the tank, and a final exit at the other end by an effluent pipe. The flow is regulated so that each particle of sewage remains in the tank from 24 to 48 hours. The effluent water is very much purer from organic matter than the inflow. If this effluent is then conducted upon a contact bed the purification is still further increased.

The change which takes place in the sewage is due to bacterial action and is dependent upon the principles already pointed out. There are two quite different phases of the decomposition which follow each other without any sharp demarkation.

The first is due to the aërobic bacteria which act upon the organic matter in the presence of oxygen, giving rise to an ammoniacal fermentation and a considerable oxidation of the carbonaceous matter, and producing large amounts of ammonium carbonate. This ceases after all the oxygen in the water has been used up, and then the bacteria which grow without oxygen begin to work. The decomposition which they produce is of a different nature and results in the production of vile-smelling gases of carbon, sulphur and phosphorus, inasmuch as the destruction of the compounds without the access of oxygen is less complete. These gases partly pass off into the air, or are partly oxidized by aërobic bacteria growing at the surface, or elsewhere that oxygen may be obtained. Both of these types of fermentation go on extensively, in the contact beds and in the septic tank, and both seem to be necessary for the thorough decomposition of the organic matter.

As a result of these two types of decomposition the various organic bodies in the sewage are very largely destroyed. The same processes of decomposition that occur in the manure heap take place here. Various gases are liberated (NH_3, N, CO_2, CH_4, H_2S, etc.), and the total amount of solid matter is thus greatly reduced. Later in the process, especially in the contact beds where oxygen is abundant, a vigorous oxidation of the nitrogen compounds begins (*nitrification*) which results in the formation of large amounts of nitrates. These nitrates are, however, thoroughly soluble and become at once dissolved in the water of the sewage, which consequently clears up. In this way nearly all of the nitrogen which was held in high compounds in the original sewage, has either become dissipated into the air as ammonia or free nitrogen, or has become converted into nitrates and has dissolved in the water to form a clear solution which is not objectionable when discharged into streams. Such effluent water would be highly useful for irrigating soil, inasmuch as it contains so much nitrate material;

but no attempt has been made to utilize it. It is well for the agriculturist to remember that such effluent water derived from sewage treated by bacteria, contains useful material if he can contrive to use it for irrigation.

It might be supposed that the bacterial treatment would greatly increase the number of bacteria in the sewage. The rapid destruction of organic matter certainly points to active bacterial growth and we should expect to find bacteria far more abundant at the end than at the beginning of the treatment. But for reasons as yet little understood, the reverse is the case. The number of bacteria in the treated sewage appears to be always less than in the raw sewage. The amount of reduction in bacteria is by no means constant. Sometimes it is comparatively small. In a long series of tests upon the sewage of London, treated in this way, a reduction of only about 32 per cent. was found (7,000,000 to 5,000,000). In other cases the reduction is very much greater, and sometimes there is found a number as high as 9,000,000 per c.c. in the raw sewage, and only from 5,000 to 10,000 in the treated product. Something evidently is at work destroying the bacteria in large numbers, but its efficiency varies widely in different instances.

It must be remembered that this method of treating sewage has been designed for the purpose of producing a chemical rather than a bacterial purification. As a chemical process the method is a success even if the bacteria should be left in large numbers. If, in addition, the bacteria can be as much reduced as indicated in some of the experiments, the whole plan may be made surprisingly efficacious as a means of disposing of sewage.

This whole topic is only a part of the general subject of the transformation of nitrogen. Whenever nitrogenous matter is mixed with water and allowed to stand for a time, decomposition changes begin which result in a more or less complete destruction of the compounds. This occurs in the soil, in the manure heap, in the privy vault, in the sink drain or in sewage, the phenomena being fundamentally the same in all cases, although differing in details with differences in the kind of compounds present, the amount of water, the temperature, the access of oxygen, the species of bacteria present, and doubtless other factors. It results in a purification of the soil or a purification of sewage from similar reasons.

Such a disposal of sewage means, of course, a complete loss of the nitrogenous material, for no method is adopted for utilizing the wasted nitrates. But this fact is no longer regarded so seriously as it was a few years ago. Inasmuch as we have learned that there are efficient forces in nature for bringing back from the atmosphere the nitrogen dissipated from the soil, it is a matter of less significance to throw away the sewage nitrogen than it appeared to be when the only known source of nitrogen was supposed to be the fixed nitrogen of the soil. Since the soil can readily replace its lost nitrogen through the agency of certain species of bacteria (see Chapter VI.) it is no serious matter if some of the nitrogen is thrown away.

CHAPTER VI.

RECLAIMING LOST NITROGEN.

THE LOSS OF NITROGEN.

In spite of all that can be done to prevent it, there is a loss of nitrogen from the soil. While some of the nitrogen extracted from the earth by the plants has gone through a series of transformations and has been brought back again into the condition of plant food to be incorporated into the soil, a considerable portion of it has had quite a different history. This loss of nitrogen to the soil is brought about by several means, the chief of which may be briefly summarized as follows:

1. A considerable portion of the nitrogen is carried off to the ocean. The bodies of animals and plants that, after death, chance to fall into streams are carried to the rivers and to the sea. Much nitrogen is taken from the soil and carried to the city as human food. Most of this may eventually find its way into the sewage system which empties into the streams and, unless previously dissipated, ultimately reaches the ocean. When the nitrogen has reached the ocean it is, of course, beyond the reach of the plants growing on the soil, even if no further change occurs.

2. Denitrification. This process, as we have seen, is the source of a constant nitrogen loss, causing the nitrogen compounds to be deprived of oxygen and to be reduced to simpler and simpler conditions, until there is finally produced ammonia, which, as a gas, passes into the atmosphere, or nitrogen, which is dissipated in a similar way. This denitrification takes place in the soil at all times, though probably it is not very vigorous

in ordinary soil. It takes place, however, very vigorously and effectively in the manure heap and compost pile, and within any mass of decomposing organic matter. It is also extremely vigorous in sewage where the denitrification and similar processes are so thorough as to cause a very large dissipation of the nitrogenous compounds into a gaseous form.

3. Drainage. The rains, as they fall upon the soils, are constantly dissolving the nitrates and other soluble nitric salts and carrying them by natural drainage sources to the brooks and rivers and hence to the ocean. This occurs in all soils and is a constant source of loss.

4. Decomposition by chemical means. There is a considerable amount of the fixed nitrogen store lost to the use of plants by purely chemical processes. Every form of nitrogen explosive depends for its basis upon some of these nitrogen salts which would be of use to plant life. Their explosion destroys the organic compounds and dissipates the nitrogen into the atmosphere. Large quantities of the earth's nitrogen store are thus dissipated.

This problem of the loss of nitrogen has seemed to be a serious one to the agricultural industry. As is well known, our farm lands slowly become incapable of supporting the crops demanded of them. This loss of fertility in the soil of worn-out farms is due, doubtless, to a number of factors, but the loss of nitrogen is certainly the most prominent one. All over the agricultural world it has been found necessary to replace this lost nitrogen in the soil. For this purpose we depend mostly upon commercial fertilizers, which commonly contain nitrogen in the form of nitrates. Of such fertilizers there are a number of large stores in the world, especially in South America, and these nitrates are brought from long distances and sold at high prices. Only a few large deposits of nitrates appear to exist in the world, and these are being rapidly exhausted. The high prices of nitrates are necessary and are bound to increase as the soil needs them more and more, and as the supply diminishes. Clearly enough the supplying of the lost nitrogen will become more and more expensive as the great nitrogen stores are used up. The seriousness of this problem of a constant draining of nitrogen from the soil has been quite prominent in the minds of chemists and agriculturists as they have, in the last few years, learned the significance of nitrogen for agriculture.

The continuation of agriculture depends upon the existence of some means of reclaiming the nitrogen out of the atmosphere for the use of plants. If there is no such means it is evident that the nitrogen store of the soil will be used up and vegetation will eventually, and, in highly cultivated lands, speedily die of nitrogen starvation. If, on the other hand, there is some means of reclaiming such lost nitrogen there is no need of nitrogen starvation, since there is an absolutely unlimited store of this element in the form of the free nitrogen of the air. It is quite evident that there is some means within the reach of organic nature for making use of this atmospheric nitrogen. Vegetation has continued on the earth for an unknown number of centuries without any apparent diminution of the nitrogen supply. This would not have been possible unless there were some means by which the soil could obtain from the air a stock of nitrogen to replace that which has been lost by the processes already indicated.

It is well known that plants obtain their carbon directly from the carbon dioxide of the air, and that their water is also obtained from the rain. These facts would naturally lead us to believe that they could also obtain nitrogen from the atmospheric store. But experiments have shown that this, under ordinary circumstances, does not occur. Most careful and thorough experiments have shown that, when plants are growing under ordinary conditions, unassisted by secondary aids (which will be noted presently), and guarded from any possible source of error, they cannot assimilate their nitrogen out of the atmospheric store, but must depend upon the soil ; and that they can only use *compounds* of nitrogen which are in the soil. Free nitrogen is useless for them. But though ordinary green plants cannot use it, it is perfectly evident that there is some means by which the atmospheric nitrogen can be fixed in the soil in a combined form. Our next problem, then, is to search for the agencies which reclaim this atmospheric nitrogen.

NITROGEN FIXATION IN FREE SOIL.

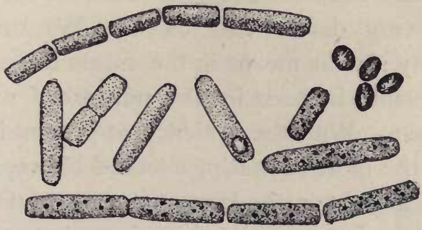
We find in the first place that soil itself if simply allowed to stand untouched for a number of weeks will gain nitrogen without any visible vegetation of plants. A lot of earth placed in a proper vessel and kept free from vegetation will, in time, be found to contain more nitrates than at the outset. Part of these nitrates, doubtless, are due to the process of nitrification already mentioned, by which the nitrogen compounds, which were in the soil, but not in the form of nitrates, are converted into nitric acid by the nitrifying bacteria. But this is not the whole explanation, because analysis of such soil shows that at the end of several weeks there is actually a larger amount of total nitrogen in the soil than there was at the start. If, then, this total nitrogen has been increased, the most probable conclusion is that it has been in some way derived from the atmosphere. One other suggestion is possible. It may be that such increase in nitrogen is due to the absorption of ammonia gas which is present in the air, at least in small quantities. This has been disproved by experiments which have shown that there is a similar absorption of nitrogen, with an increase in nitrates, in soil which is bathed in air practically free from ammonia. No one to-day questions the conclusion that the source of the nitrogen increase in the soil under these experiments is atmospheric free nitrogen.

Further, it is abundantly demonstrated that this fixation of atmospheric nitrogen is due to the *growth of microörganisms.* That it is due to the action of living organisms is proved by the effect of sterilizing such soil. Two vessels may be filled with similar soil, one of which is sterilized by heating, while the other serves as a control. The former fails to gain nitrogen, no matter how long it is kept in contact with the air; the latter slowly but surely increases its store of fixed nitrogen in the form of nitrates. This proves that some living organisms are concerned, and the fact that no *visible* plants are growing in the soil shows that the higher plants do not produce the result. The only conclusion that can be drawn, therefore, is that microörganisms are the agents for reclaiming free nitrogen from the atmosphere and fixing it in the earth in some form of nitrogen compounds, which eventually become nitrates and, thus, plant foods. To some problematical microörganisms in the soil, then, must we attribute this first process of reclaiming for the soil the lost nitrogen.

If such a microörganism exists we ought to be able to discover it and isolate it for experiment. Certainly this ought to be possible if it should be a bacterium. But the isolation from the soil of an organism having this power has not proved to be an easy task. The power of assimilating free nitrogen is not common among microörganisms. Winogradsky was the first to search systematically for such a bacterium and he tested a large number of soil bacteria. Only one of those studied by him proved to possess this power in any considerable degree. This organism he named *Clostridium pasteurianum.* From its growth there results, not only *nitrogen*, fixed in the soil by the organism in an insoluble form, but such other by-products as *hydrogen*, *carbon dioxide*, *butyric* and *acetic acids.* Like nearly all of the nitrogen-fixing organisms, this power of assimilating nitrogen is best shown when the organism is growing in a nitrogen-free medium. Since Winogradsky's first discovery of this bacterium, a few others have been found with the same power. Some bacteriologists insist that the power of fixing free nitrogen is quite a common one of microörganisms, but only very feebly developed in most cases. As yet we know very little in regard to the nitrogen-fixing bacteria, nor do we know very much in regard to their significance in ordinary soil. That they constitute one of the important agencies for keeping up the supply of nitrates in the soil is quite certain from the fact that sterilized soil does not increase its nitrogen. But beyond some general statements of this sort which can be unhesitatingly made, we have very little knowledge at present in regard to the action of these microörganisms under ordinary conditions.

Bacteriologists have been so convinced of the great importance of this function of fixing nitrogen that they have thought that the inoculation of the soil with the proper organisms would be a process of great advantage to agriculture. Acting in accordance with this idea one of the European bacteriological laboratories has, within the last few years, offered to agriculturists a commercial product called *Alinit*, which, it is claimed, has the power of fixing free nitrogen and of producing nitrogenous compounds in soil proper for plant food, and will, for these reasons, decidedly increase the yield of a given soil. This Alinit has been subjected to many experiments testing its composition, its effect upon the ordinary field crops and its function

FIG. 18.



B. ellenbachiensis, from *Alinit*.

as shown by strict laboratory experiments. The results show that Alinit consists of nearly a pure culture of a definite species of microörganism which has been named *B. ellenbachiensis* (Fig. 18) and is closely allied to a common soil bacterium, *B. megatherium*. This organism has the power of fixing free nitrogen under certain circumstances, and also of reducing nitrates to nitrites under other conditions. It may thus have two different functions in the soil.

But in spite of its theoretical utility the practical application of Alinit to the soil for the purpose of increasing the yield of crops has not been so universally satisfactory as to warrant its general recommendation. Some experimenters have announced a decided increase in yield, while others have found that the use of Alinit has not resulted in any greater harvest than that obtained from similar soil without the Alinit. On the whole the positive results have been more numerous and more emphatic than the negative, but have not been abundant enough as yet to warrant any very definite conclusions. Whether it will be possible by any similar means in the future to furnish the soil with more valuable bacteria for the purpose of utilizing nitrogen is uncertain. With the evidence at present in our hands it seems that the most promising method of preparing the soil so that plants can obtain the largest amount of nitrogen, is not by the addition of extra microörganisms, but rather by such tilling and cultivation of the soil as will stimulate the growth of the microörganisms present. The probability is that soil at all times contains all kinds of nitric organisms in sufficient quantity for all purposes. To produce nitrogen fixation the proper *conditions* are needed and these can be obtained by the cultivation and stimulation of the soil rather than by adding more bacteria.

BACTERIA AND LEGUMINOUS PLANTS.

Nitrogen Fixation by Legumes.—There is a second means at the disposal of the farmer for increasing his nitrogen store, of more practical value than the last. This also is dependent upon bacteria, and upon a peculiar relation between bacteria and the family of *leguminous* plants. As already noticed, experimental evidence indicates that ordinary plants are unable to make use of atmospheric nitrogen. Long series of experiments were conducted to test the matter and the more rigidly the experiments were performed, the more evident did it become that such an assimilation does not occur in ordinary green plants. It was, however, shown in 1881, that this conclusion did not hold in regard to the great family of *legumes.* It was then demonstrated very conclusively that peas and beans, growing in a soil free from nitrogen and fed upon food containing no nitrogen, did, in the course of a few weeks' growth, increase the amount of nitrogenous material present in the plant, and, inasmuch as the only possible source of this nitrogen was the atmosphere, the conclusion was unhesitatingly drawn that *peas can assimilate atmospheric nitrogen.* This conclusion, so contradictory to the belief accepted at the time, was at first vigorously disputed; but, upon being subjected to further experimentation by many observers, was found to be strictly correct. Many of the plants of the great family of legumes certainly do have the power, under certain circumstances, of fixing atmospheric nitrogen and absorbing it into their tissues.

The question of the conditions under which such a fixing of atmospheric nitrogen occurs, was recognized to be one of the greatest importance. In the course of the next ten years there was gradually unfolded by the experiments of botanists, chemists and bacteriologists, a series of surprising facts which have resulted in demonstrating that this fixation of atmospheric nitrogen by the legume is dependent upon the growth of certain soil bacteria. The facts by which this conclusion was finally demonstrated are briefly as follows:

Root Tubercles.—The first step was the observation that the fixation of nitrogen by legumes is associated with the development upon the roots of little nodules known as tubercles. (Fig. 19.) These tubercles are little swellings on the roots, sometimes very numerous, and varying from the size of a pinhead to the size of a pea. They have been known for a long time, since they can easily be found on nearly any legume growing luxuriantly in the soil, if the roots are carefully pulled up from the soil in such a way as to prevent these nodules from being destroyed, and if the soil is carefully washed away. Their nature was unknown and they were in general regarded as galls upon the roots, similar to those that appear upon the leaves and branches of trees, and, therefore, were looked upon as a type of disease. It was, however, evident that if they were of the nature of a disease, they did the plants no injury, for the plants developing these tubercles were as luxurious as those without them. Indeed, as soon as the nitrogen-fixing

FIG. 19.

A leguminous plant (vetch) showing root tubercles.

power of legumes was demonstrated, it became evident that the fixation of nitrogen was associated with the formation of tubercles. Only such plants as developed tubercles were able to increase the amount of nitrogen in their tissues, and the amount of nitrogen fixation was roughly proportional to the development of tubercles. Plants without tubercles showed no increase; those with a moderate number, a slight increase; and those with abundant tubercles, a larger increase in nitrogen. (Figs. 19 and 20.)

FIG. 20.

A leguminous plant (vetch) without tubercles, showing less vigorous growth.

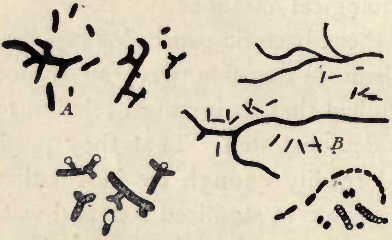
This, of course, raised the question as to the nature of these tubercles and led to a series of experiments in regard to their formation. It appeared that the tubercles would not form upon the roots of legumes if the plants were grown in sterilized soil. Under these circumstances the plants developed no tubercles, fixed no nitrogen and, unless fed with nitrogenous food, made very little growth, being stunted and small. It was next shown that if the legumes were sown in sterilized sand, without nitrogenous food, and were then watered by water which had been standing in contact with ordinary soil, results were quite different. Such water, sometimes called a "soil infusion," is made by simply soaking soil in water and then filtering off the solid particles, using the filtrate for watering the growing legumes. Plants watered with such infusions showed two interesting stages of growth. The peas, for example, sprouted readily and for a short time grew vigorously; then the vigorous growth ceased and the plant seemed to be suffering for lack of food. This has been called the *nitrogen hunger stage*, and represents a period in which the plant has used up the nitrogen in the pea, and consequently all that was within reach. Control plants, grown in similar soil and watered with pure water, never recovered from this stage, but those that were watered with the soil infusions, after a few days of such nitrogen hunger, recovered, began once more a vigorous growth and eventually produced large-sized plants with a good yield. Upon examining the roots of the plants they were found to have developed tubercles, while the control plants, watered with sterilized pure water, did not develop tubercles. These facts of course indicated that in the soil infusion some agencies were present which stimulated the development of tubercles and the consequent fixation of nitrogen, and that the power of absorbing atmospheric nitrogen enabled the plant to recover from the nitrogen hunger stage.

Tubercle Bacteria.—These facts suggested the agency of microörganisms in the production of tubercles, and naturally led to a careful microscopic study of these bodies. The microscopic structure of tubercles had been studied years before; as long ago as 1866 there had been found by Woronin small bodies inside of the tubercles, the nature of which he did not understand. A little later, in 1874, Erickson had found in tubercles certain thread-like bodies, which were not intelligible to him, but which he regarded as probably microörganisms of some sort. The conclusion reached was that these little bodies were parasitic microörganisms invading the root, producing a pathogenic effect upon the tissues, and causing the development of the tubercles. The next step was naturally the isolation of these little bodies and their careful study by more modern methods, to determine whether the conclusions reached were correct. Their isolation did not prove to be difficult. It was only necessary to remove a fresh tubercle at a certain stage of its growth, carefully wash its surface to remove accidental soil bacteria, and then to inoculate culture media with the contents of the tubercle. The result was a growth of bacteria in the culture media which could then be studied by ordinary bacteriological methods.

The study of these bacteria proceeded rapidly and in a short time the organisms in question were so thoroughly isolated and carefully studied that their causal relation to the tubercles in question was demonstrated. That they produce the tubercles was proved readily enough by inoculation experiments. Legumes were grown in sterilized soil and watered with bacterial infusion from these cultures ; the result was in all cases the growth of abundant tubercles and the fixation of nitrogen. The most striking of these experiments were made with germinating peas. Such peas, if kept moist and warm, will grow for several days, sending out their normal roots even without being planted in soil. It was found by dipping the tip of a needle into cultures of the microörganisms and then pricking the rootlets of young legumes at various points, that the development of tubercles would almost inevitably follow such slight wounds. In favorable experiments the tubercles appeared in six days after the inoculation and always at the point of inoculation. All of these facts proved that these microörganisms were concerned in the development of the tubercles.

The study of these little bodies very soon proved that they must be classed with bacteria and the name *Bacillus radicicola* was given to them by Beyerinck, who first studied them with care. They are undoubtedly organisms belonging to the general group of bacteria, but they are somewhat peculiar in their methods of growth. In the laboratory culture media they grow like ordinary bacteria (Fig. 21, *B*) but in the roots of the legume, inside of the tubercle, they produce forms unlike most of the more common bacteria. At certain stages of the development, by branching or budding, they produce what are called Y and T forms, a method of growth not characteristic of bacteria in general (Fig. 22, *A*). It is found also that after the beginning of the formation of the tubercle, long, thread-like masses, filled with bacteria, can be seen extending among the tissues of the plants. These long threads appear almost like pouches in which the bacteria are held, but they eventually disappear and the bacteria themselves diffuse somewhat through the tissues. These phenomena, the Y and T forms and the pouch-like threads, have been puzzles to the bacteriologists, for they are not characteristic of any other bacteria known. It has been doubted whether the organisms should be called bacteria. These organisms, whether true bacteria or not, still retain the name originally given them and are now well known. They have been found universally characteristic of root tubércles and many bacteriologists have made extended studies with them.

FIG. 21.



The root tubercle organism, *B. radicicola*, under different conditions. (*Mazé.*)

Up to the present time it has not been possible to isolate them directly from the soil. That they are in the soil is of course evident from the experiments already mentioned. They may be isolated with ease from the tubercle on the root, but the difficulties of separating bacteria from the soil have been so serious that no bacteriologist has as yet been able with certainty to isolate the tubercle bacillus from the earth in which it is known positively to be present. They are certainly soil bacteria, although, thus far, found only in the root nodules.

PRODUCTION OF TUBERCLES BY THE BACTERIA.

Our next question is the relation of the microōrganism to the plant in whose roots it grows. There is no doubt that these bacteria from the soil make their entrance into the roots in the young condition of the legume, passing into its root apparently through the delicate root hairs. Inside the root they find favorable conditions and multiply rapidly. Their presence serves as a stimulation to the root tissue of the plant which results in an abnormal growth of the root. The cells of the root tissues multiply more rapidly and more abundantly than usual, and the extra growth produces the tubercles characteristic of the phenomenon. The tubercle itself is thus really a growth of the leguminous plant, stimulated by the presence of the microorganism. Inside of this growing tubercle the bacteria undergo a series of transformations, which are regarded in part as abnormal growths due to unfavorable conditions, the nature of which is not well understood. They soon produce a mucilaginous material which permeates the tissues of the plant in the form of long, delicate threads containing the microorganisms. They subsequently develop peculiar forms, referred to as the Y and T forms, which are not normal characters of ordinary bacteria, nor of these bacteria under ordinary conditions. (Fig. 21.) Microscopists have puzzled over these forms and have hardly yet been able to satisfy themselves whether they are normal growths of bacteria, or degenerate forms of the organism resulting from abnormal conditions. Bacteria do not as a rule produce branches from their sides, but simply divide by cross division. The presence of these branching forms has suggested to some students that the organisms in question are really related to some of the *higher molds* rather than to bacteria proper. Others have thought that they represent a type of *yeast* plant, the side branches representing buds characteristic of this group of fungi. Other microscopists are as confident that the forms are true bacteria with peculiar methods of growth under certain conditions. Other bacteria are known to show unusual forms when placed in unfavorable surroundings. Even the *Diphtheria bacillus* sometimes shows branches. It is also certainly a fact that similar irregular growths with side branches may be induced in the organisms of the root tubercle by cultivating them in ordinary laboratory culture media in which a certain amount of acid has been added. But whatever be the conclusion on this matter there is no doubt that these peculiar Y and T forms, *bacteroids*, as they are called, are a modified growth of the *B. radicicola* that get into the root from the soil. Eventually the bacteroids also disappear and a more typical form of rounded bacteria is found in the tubercle. In all cases the bacteria which make entrance into the root of the legume appear to go through a somewhat similar series of transformations during the growth of the tubercle.

The tubercle, therefore, must be regarded as a peculiar abnormal growth of the root of the leguminous plant, stimulated by the presence of the invading organisms. They are as truly abnormal as are the galls produced on the leaves or branches of trees by the sting of insects. They are the result of the action of the presence of bacteria and clearly do not normally belong to the leguminous plant itself.

ASSIMILATION OF NITROGEN.

A still more difficult problem to settle is the relation of the whole process to the assimilation of free nitrogen. That the increase of nitrogen in the legume which develops the nodule is an assimilation of free nitrogen from the air is abundantly demonstrated, though whether the nitrogen is absorbed through the leaves or through the roots is not so easily answered. It has been recently asserted that the nitrogen does not come directly from the air but from the nitrogen dissolved in the soil water and absorbed through the root hairs. That the ultimate source is the air is certain.

There are several possibilities suggested as to the relation of the microörganism to the legume. In the first place it is perfectly clear that in the ordinary soil the association of the legume with the microörganism is necessary to the fixation of nitrogen in the form of a nitrogen compound. But whether it is the legume or the bacterium which fixes the nitrogen, or whether it is a phenomenon occurring only as the result of the two organisms combined together in a united growth, is not so clear. It has been claimed, on the one hand, that the bacterium itself fixes the nitrogen, and that the root of the legume simply furnishes a convenient and appropriate medium for its growth. This would seem to be rendered possible by the fact already mentioned, that some soil bacteria certainly do fix free nitrogen. If this is the case, the tubercle bacterium should be able to fix nitrogen, when growing vigorously under proper conditions, even in the absence of the leguminous plant. One of the more recent experimenters (Mazé) has claimed to have demonstrated this fact. Upon cultivating the *B. radicicola* in an especially favorable medium, and supplying them with an abundance of atmospheric oxygen, he has found an increase of nitrogen in the culture media, which increase he attributed to the assimilating action of the bacteria. If this result be confirmed, it is evident that we owe the power of fixing nitrogen to the microörganism. With the exception of Beyerinck and Mazé, no one has yet been able to demonstrate any considerable fixation of nitrogen by the action of bacteria growing alone in laboratory culture media.

A second suggestion is that the leguminous plant fixes the nitrogen through the agency of its leaves or roots, the bacilli in the roots merely serving as a stimulus to increase the normal functions of the plant. Those who have adopted this view have insisted that the power of fixing nitrogen is a very widely distributed function of almost all green plants. They have claimed that many other plants, wholly unassociated with microörganisms, are capable of fixing small amounts of atmospheric nitrogen. This is said to be true of many green algæ growing in water, of mustard, of oats and a host of other plants, the legumes differing only in the amount of nitrogen assimilated.

It is extremely doubtful whether this conclusion is correct. The general consensus of experimental work denies the possibility of nitrogen fixation by ordinary green plants. It is, therefore, at the present time not generally believed that the legume, or any other green plant, has of itself any power of fixing nitrogen. It is certain that if the green plants do fix nitrogen through their leaves the amount thus fixed by the ordinary plant is extremely small and not to be compared with the large quantity fixed by the legumes under the influence of the root nodules. Neither the ordinary plant under any condition, nor the leguminous plant without the agency of the bacteria in the nodules, has the power of fixing an amount of nitrogen which is appreciable to ordinary chemical analyses.

A third suggestion as to the phenomenon is that the fixation of nitrogen is the combined result of the organisms acting together. Exactly how this occurs is wholly unexplained, even by hypothesis. It is a well-known fact that there are many instances in nature where two quite different organisms are associated with each other, and, when combined, thrive excellently, each showing much more vigorous growth than when growing alone. This phenomenon of association for mutual benefit is known by biologists as *symbiosis*. It has been the usual belief that the phenomenon we are considering is one of symbiosis; that neither the legume nor the bacterium by itself is able to absorb and fix atmospheric nitrogen, but that the two together accomplish the purpose, each benefiting by the presence of the other.

Still another suggestion has been advanced in recent times. As shown on a previous page certain soil bacteria can fix atmospheric nitrogen, causing it to be deposited in an *insoluble* form. It has been suggested that the first fixation is of this character by the soil bacteria (*Clostridium pasteurianum ?*), and that the tubercle bacterium uses these insoluble nitrogen compounds, rendering them soluble for the use of the legume. Such an interpretation would involve three organisms in the phenomenon, two species of bacteria and the legume.

It is impossible at the present time to determine positively which of these suggestions is correct. This much is certain, the legume and the bacterium together are able to fix atmospheric nitrogen in quite large amounts. Neither of them can do this alone in the ordinary conditions of the soil. The pea plant without the bacterium cannot fix nitrogen, and the bacterium without the legume does not, at least in the ordinary soil or in ordinary cultures, fix atmospheric nitrogen. But when the bacterium grows in the root of the legume and stimulates the production of nodules, atmospheric nitrogen is fixed, either by the one or the other, or by the two plants acting together.

This power of fixing nitrogen is in considerable degree dependent upon the amount of nitrogenous food already in the soil. In most of the experiments, where a large amount of nitrogen fixation has been demonstrated, the plants have been grown in soil wholly deprived of nitrogenous foods, and practically free from nitrogen. In other experiments, when the legumes are grown in soils already well supplied with nitrogenous foods, the increase in fixed nitrogen is very much less, or even inappreciable. In other words, the legumes appear to prefer taking their nitrogenous material directly from the nitrogenous foods of the soil when these are present in abundance. But if the soil does not furnish the proper nitrogen, then recourse is had to atmospheric nitrogen through the agency of the tubercle organisms. From this, of course, it follows that in a soil already well supplied with nitrogenous plant foods there is little or no further fixation of atmospheric nitrogen by leguminous plants; but when the soil becomes poorly supplied with nitrogenous foods the atmospheric nitrogen is seized by the leguminous plants and the store of fixed nitrogen consequently increased. In other words, this phenomenon of fixation of nitrogen by leguminous plants in association with bacteria, occurs only in soils where such fixation is needed to renew the fertility of the worn-out soil.

DIFFERENT SPECIES OF THE TUBERCLE ORGANISM.

The question whether proper bacteria are always in the soil ready to produce tubercles is one which has caused a considerable amount of dispute and much experimentation. It is practically certain that nearly all soils contain bacteria capable of living in symbiosis with leguminous plants. Nearly all soils, unless it be the extremely sandy soils that support little or no vegetation, will support leguminous plants readily enough and develop tubercles on their roots. One cannot examine the roots of legumes anywhere without finding tubercles, a fact which shows that the bacteria in question are very widely distributed in nature.

But as the subject has been studied more carefully it has appeared that not all species of legumes are capable of developing root tubercles equally well in all soils. Some soils will luxuriantly support certain species of beans, peas or clovers, producing a large crop, developing quantities of tubercles and fixing an abundance of nitrogen, while the same soil will not support other species of legumes with equal readiness. For example: the soil of Connecticut is not adapted to the legume called the *soy bean.* When this bean is planted in the ordinary Connecticut soil it does not flourish but yields a small crop, unless heavily fertilized, and does not produce tubercles. This species does, however, grow readily in Massachusetts. Some years ago the experiment was tried of importing the Massachusetts soil, upon which this plant had produced abundant tubercles, and mixing it with the Connecticut soil, subsequently planting the soy bean. The result was an excellent growth of the soy bean and the development of tubercles in the Connecticut soil. At the present time these particular plots of land are capable of producing large luxuriant crops of the soy bean, with abundant root tubercles and a large fixation of atmospheric nitrogen. It is interesting to note that the same experiment has been repeated in Germany, where the soy bean does not readily grow. In this case soil was imported from Japan, where the plant flourishes, and was used for supplying the German soil with the proper species of organism. The result was equally successful with those of Connecticut experiments. Evidently Connecticut soil does not contain the species of bacteria adapted for producing the tubercles in the soy bean, although those which produce tubercles on the pea and the clover are abundant enough.

These experiments indicate that the root-tubercle bacteria are not all alike, and that some species of legumes are not capable of flourishing and developing tubercles under the influence of the same kinds of bacteria which produce the tubercles in other species.

Such facts as these have led to the suggestion that the different species of leguminous plants demand different species of bacteria for the proper production of their root nodules, and that there are different species of this *B. radicicola*, each capable of living with a different species of legume, and incapable of producing tubercles upon others. It has not been very easy to determine whether this conclusion is correct, although experiments designed to answer the question have been multiplied. In general it is believed to-day that the conclusion that the tubercle bacteria consist of a large number of species, each characteristic of a distinct species of legume, is not borne out by the facts.

It is, nevertheless, true that certain varieties of legume will grow in soils with an abundant production of tubercles, while other varieties of closely related legumes are unable to produce an abundant crop of tubercles in the same soil. This is evidently due to the lack of microörganisms especially appropriate to the legume in question, since inoculation with proper soil infusion produces tubercles at once. But just what this means is not so evident. It certainly means that different legumes demand different varieties of tubercle bacteria. Whether these different varieties are distinct *species* is, of course, a fruitless question, inasmuch as we do not know what we mean by a species among bacteria. But the question is of importance whether these types are quite distinct, or, whether they are simply physiological varieties of the same general species. If the former is true we should expect them to remain distinct, but if the latter is true we might expect the soil bacteria to be capable of adaptation, by cultivation, to different legumes. On the whole the evidence is decidedly in favor of the latter view, and indicates that the different tubercle bacteria are probably all one general species, but that, under different conditions, this species assumes slightly different physiological relations. It can accommodate itself to growth in one or another legume, and having become especially adapted to growth in the pea does not so readily develop in the root of the bean or clover; but, allowed to develop in the soil in which the latter plants are growing, it will adapt itself as well to the new plant as to the old. In other words, the present condition of experiments indicates that there is only one of these species of tubercle bacteria, and that this species assumes different physiological characters under the influence of the different conditions in which it grows. It may adapt itself especially for growth in one leguminous plant and consequently lose its ability to develop well in others; but, if a new legume is planted in the same soil, a slow change of physiological characters takes place, and the soil organism becomes in time adapted to the new leguminous plant. This conclusion is clearly in complete harmony with the fact that the soil may at any time contain the organisms which will support one species of legume luxuriantly, while another species will have only a scanty growth.

IMPORTANCE OF THE LEGUMINOUS CROPS TO AGRICULTURE.

Although we may not know as yet exactly how this combination of legume and bacteria works to fix free nitrogen, the fact is none the less cogent. It becomes a matter of great significance to agriculture to determine the best practical method of making use of this power. Evidently there is here a great opportunity for the fixation of nitrogen without purchasing it and, if properly used, it would seem that we have here the factor needed for making possible a cultivation of the soil without exhausting its nitrogen. Virgin soil has all its factors of nitrogen loss and gain nearly balanced; cultivated soils have a balance on debit side. If we can discover a practical method of applying these factors of nitrogen assimilation, one of the great agricultural problems will be solved. Up to the present time the matter has not been brought to a condition where we can feel that we know how to handle these nitrogen-fixing forces to the greatest advantage. At the same time so much has been learned that our agriculturists are already making use of them to a larger extent than formerly, and we may fairly expect that the next few years will see these forces more thoroughly under the control of the farmer than to-day.

In making use of this means of gaining nitrogen the following facts must be considered.

1. **Selection of a Proper Legume.**—The first thing to be done is to plant some species of leguminous plant in the soil which it is desired to enrich. In selecting the proper species of plant the individual must take into consideration all the facts within his reach which teach him what species of legume is best able to grow in his soil. Some species of legumes grow better in some climates than others, and certain soils seem to be, for some reason, better adapted for particular species, quite independent of the question of the presence of the proper soil bacteria. By the proper consideration of the facts of his experience the farmer can, without much difficulty, determine what species of legume grows best in his soil. The most vigorously growing legume is the best. The plants most commonly found useful are peas, cow peas, beans, soy beans, alfalfa, vetches, seradella, and some others. Which of these various species is to be selected must be determined by the conditions of the soil and the needs of the farmer for the particular crop which he raises. The essential feature must be that the species selected should be one that will grow well in the soil in question, otherwise the advantage of the nitrogen fixation will not be obtained.

2. **Insuring Presence of Proper Bacteria.**—In order that the soil shall increase in its nitrogen store it is evidently necessary for nodules to develop in large numbers on the roots of the legumes. For this purpose, of course, it is necessary that the

proper variety of bacteria shall be present in the soil, otherwise no tubercles will be formed, or the tubercles formed will be few and small. To ensure this result may sometimes require a little experimenting and observation. As already mentioned some species of legume find in a certain soil the tubercle organism adapted to them, while other species of legume will not find the proper organisms in the same soil. The soy bean is a most excellent crop for this purpose, since it is an extremely luxurious growing legume, producing abundant tubercles and a large fixation of nitrogen when supplied with the organisms which produce tubercles. But in order to make use of this crop it may be necessary to import the proper bacteria from other soils. On the other hand, there are some species of legumes, like most kinds of peas, which are capable of growing in most soils and producing an abundance of tubercles; and the same soil will produce clover or beans in abundance. It is sometimes found, further, that a legume, which, during the first season produces only a small number of tubercles, will succeed better the second year than the first and will fix more nitrogen. The growth of the crop in the soil during the first year apparently either increases the number of soil organisms appropriate to this particular legume, or produces such changes in the physiological character of the bacteria present that they are better adapted to the legume. In either case, the second season will show a more luxuriant growth and a more successful nitrogen fixation. In short, the question of the proper legume to grow in any soil, for the purpose of fixing its soil nitrogen, is one that must be determined largely by experiment. In all cases it should be the legume that grows most luxuriantly upon soils not particularly well fertilized, and which, at the same time, produces the most abundant crop of tubercles upon its roots. These factors will depend upon climate, the chemical nature of the soil and the variety of soil bacteria.

There has been, in the last few years, an attempt made by bacteriologists to meet the difficulty here arising, by furnishing to the agriculturist a culture of the tubercle bacteria for the purpose of inoculating his soil, and thus providing it with the proper organisms to produce the tubercles. Such a material has now been on the market and under experimental tests for several years under the name of *Nitragin.* This commercial product is found by analysis to consist of a practically pure culture of certain of these tubercle bacteria, which are capable, when inoculated into sterilized soil, of producing the tubercles upon the roots of the leguminous plants. Although the method of manufacture is not known, there is little doubt that the material comes directly from the tubercles of certain leguminous plants, and that the bacteria thus obtained have been produced in large amount in the bacteriological laboratory and eventually furnished, as a commercial product, in the form of a practically pure culture.

The use of such a product evidently may or may not be of value to the agriculturist, according to conditions. If it is true that different species of legumes require at least different physiological varieties of bacteria to enable them to produce their proper tubercles, it will follow clearly that a product like Nitragin, containing a pure culture of one variety, could not be appropriate to *all* species of legumes. It might be advantageous with some crops but not with others. Further, if it is true that ordinary soil contains the tubercle bacteria in sufficient quantity for the production of the tubercles upon legumes, no advantage could, of course, be expected from adding to that soil similar bacteria in the form of a pure culture like Nitragin.

These are not simply theoretical conclusions ; they are conclusions which have been deduced from experiments with Nitragin, carried on now in large numbers in many localities for some years. The results of the use of this material have been extremely irregular, just as would be expected in consideration of the facts given. Some experimenters have found no advantage accruing from the use of the material, no larger crops being produced, and no larger fixation of nitrogen in the soil which was inoculated with the culture, than in the soil not thus inoculated. On the other hand, quite a number of the experimenters have reported a very striking difference in two such series of experiments, and have found that the inoculation of Nitragin into the soil has increased the yield and produced a larger fixation of nitrogen. Without attempting to discuss these irregularities in detail it is sufficient for our purpose to present the general results of experience with Nitragin by the following summary :

Where it is desired to increase the nitrogen compounds in a sandy soil, which is very poor in nitrogen and does not support legumes properly, the use of Nitragin frequently results in a very greatly increased yield. In a single test with clover, inoculated soil yielded 143.7 lbs. of nitrogen, while a similar uninoculated soil yielded only 4.3 lbs. The reason for this is, evidently, that in such sandy soil the tubercle bacteria are not present in sufficient quantities to make possible a luxurious crop of legumes. Under these circumstances Nitragin furnishes the needed bacteria, and its use far more than pays for itself in the increased yield and in the considerable gain of nitrogen in the soil. On the other hand, in moist soils, in those that contain more nitrogen, and in soils where legumes have grown well in recent years, the use of Nitragin seems to result in no advantage. These soils are already stocked with proper bacteria and the addition of those in the Nitragin produces no advantage whatsoever in an increased yield. We must look upon Nitragin, therefore, as a material which is of distinct advantage in the raising of legumes and fixation of nitrogen under some conditions, especially in barren soils which do not readily support leguminous plants. But it is not a material which can be relied upon by the farmer as always producing the advantage of an increased yield. Lastly it is quite expensive and difficult to obtain in good condition.

3. **Utilization of the Nitrogen.**—The next problem is how such a store of nitrogen, fixed in the soil, may be best utilized for the benefit of the next crop. There are two methods by which this nitrogen may be made available for crops subsequently growing in the same soil. The first, which is commonly called *green manuring*, consists in allowing the crop to grow vigorously for a time, and then plowing the whole crop immediately into the soil, with the expectation that the nitrogen stored up in the plants will be available in the soil for the next crop. The method by which the nitrogen becomes available is here very simple and based upon the facts already noticed. When these crops are thus plowed into the soil they are brought at once within reach of the numerous soil bacteria, whose action upon organic products has been described. The putrefactive bacteria seize hold of the proteid products in the plants, as well as the cellulose, fats, and other organic substances, and cause their rapid decomposition. An ammoniacal fermentation begins; doubtless the denitrifying bacteria seize hold of the ammonia and other ingredients formed, extracting their oxygen to a certain extent and setting free the nitrogen. After this process is finished the nitrifying bacteria in the soil seize hold of the ammonia and the nitrates that are left after the decomposition ceases. These are oxidized by the process of nitrification, and the result is a quantity of nitrates. Thus, after a few weeks, a considerable portion of the nitrogen material which is fixed in the legume has been converted into some form of nitrogen salt, either a salt of ammonium or a nitrate, either of which is available for plant life. These salts remain in the soil and may be used by the next crop of plants sown on the same field, thus increasing its yield by means of the nitrogen which has been fixed by the legume and the

bacteria together, and converted into an available form by the soil bacteria.

A second method of utilizing the nitrogen is by reaping the crop of legumes and feeding it to animals, subsequently returning the *manure* to the soil. The phenomenon that occurs here is essentially that already noticed. Part of the nitrogenous material is metabolized by the animal body to urea, and part passes into the feces unassimilated. But whether reduced to urea or not, it is eventually decomposed by the putrefying bacteria, and goes through the same series of metamorphoses which we have already described in sufficient detail. The result is, that in the end, a portion of it is returned to the soil with the manure in a form available for plant life.

It is of course manifest that under either of these methods of treatment not all of the nitrogen fixed by the legume and the bacteria is rendered available for the next series of crops. At the very best, part of it will be lost to the soil by the process of putrefaction which liberates free ammonia, and more particularly by the denitrification which liberates a certain quantity of free nitrogen. It is impossible, by any means now at our disposal, to prevent this loss, and thus a portion of the fixed nitrogen is, even with the best treatment, dissipated again into the air. But by proper treatment this loss can be reduced to a minimum and there may always be a surplus of gain. Even taking into account all the nitrogen loss that comes from these processes the use of a leguminous crop, upon a soil poor in nitrogen, furnishes to that soil for the next crop a store of nitrogen considerably in excess of that which it possessed before.

It is clear that here is a means at the disposal of agriculture for replacing nitrogen. By the proper alternation of leguminous plants and those which cannot fix nitrogen but simply use it, it would seem to be theoretically possible to keep the fertility of the soil, so far as concerns nitrogen, in a constant high condition. The exact method by which the legumes may be alternated with other crops, the proper legume for each soil, the frequency with which the leguminous plant must be cultivated on the soil to keep up the store of nitrogen, all of these questions must be worked out in practice as the result of experience. The complete solution of the problem is no easy matter, even after a possible method of solution has been pointed out. But, theoretically at least, it would seem that there is nothing to prevent the agriculturist, after he has sufficiently experimented in the various soils, from keeping his store of nitrogen at a tolerably constant quantity. He need not, in the future, be at the mercy of commercial fertilizers for the purpose of furnishing his plants with this highly important and necessary food.

As an example of the practical utility of this method of improving the yield of soil, may be mentioned a single experiment. Half an acre of land was sown with alfalfa and the next year with wheat. Besides yielding a valuable crop of alfalfa the first year, the soil was so much improved that, when sown with wheat the second year, it yielded $16 worth more of wheat than a similar half acre which had not previously been sown with alfalfa.

NITROGEN FIXATION BY OTHER PLANTS.

A final question arises, whether there are any other plants, besides the great family of legumes, that are capable of acting, either alone or with bacteria, and fixing nitrogen. Our knowledge in regard to this subject is as yet very incomplete. Some experimenters, as stated above, have insisted that the property of fixing atmospheric nitrogen is quite general among plants. It has been claimed to exist in wheat, oats, mustards and a variety of other plants including various algæ. The claim has been advanced that the property is a general one, and that the legumes only differ from other plants in that their power in this respect is greatly increased by the stimulation that comes from the presence of tubercle bacteria in their roots. These conclusions are certainly not demonstrated and are quite generally discredited. There is practically no evidence for a belief in such a general nitrogen-fixation power.

Nor have we any reason for believing that the property of living in symbiosis with bacteria, and thus fixing nitrogen, is possessed by many plants outside of the family of legumes. There are only a few other instances satisfactorily attested. Hiltner has shown that the *alder* has a relation to soil bacteria almost identical with that of legumes. Like the legumes it develops tubercles on its roots, and these tubercles are produced by bacteria, similar to those developing in the roots of legumes. The bacteria have been isolated and successfully studied in water cultures. While similar to those found in the tubercles of legumes they are apparently incapable of producing tubercles in legumes, but they cause the development of these bodies only upon the alder, with a corresponding fixation of atmospheric nitrogen. Somewhat similar facts have been determined for three other families of plants, Myricaceæ, Elæaginaceæ and Scrophulariaceæ, in each of which families tubercles are occasionally produced; but the property is a rare one in other families, while it is practically universal among the legumes. While then the possibility of fixing nitrogen is not denied for other plants, it appears to be a property of little significance except in the great family of legumes. To this family, growing in a condition of symbiosis with the soil bacterium *B. radicicola*, we must look for the chief agency existing in the soil for fixing atmospheric nitrogen.

SUMMARY.

A brief general summary of the significance of soil bacteria in agricultural processes may now be appropriately given.

The soil bacteria have important relations to agricultural processes in at least five different directions :

1. They unquestionably aid in the *decomposition of rocks*. While the so-called weathering of rocks is chiefly a physical process, a part of it is due to the agency of microörganisms that are able to grow upon the surface of rocks without the necessity of organic foods. In the formation of sulphates and iron salts of various kinds bacteria also contribute very largely. Not a little of the slow oxidation which occurs in nature, affecting the mineral substances in the earth's crust, is attributed in a measure to the action of living bacteria, so that the formation of the so-called mineral ingredients in the soil is, in no slight degree, dependent upon the action of these organisms.

2. The bacteria certainly have the power, even when acting alone, of *fixing free atmospheric nitrogen* in the soil in the form of some chemical compounds which can later be utilized by other organisms. To what extent this is possible we do not yet know, nor do we know whether the species of organisms that produce such fixation are abundant or few ; but, that atmospheric nitrogen is fixed in this way has been abundantly demonstrated. From this fact we must conclude that the soil organisms may possibly have been important agents in primitive times in fixing the atmospheric nitrogen in the soil and thus furnishing the basis of the soil nitrates. In regard to the primitive origin of nitrates we have, of course, only speculation to guide us. But the bacteria certainly are able to fix atmospheric nitrogen, and were, possibly, one of the forces in primitive times for seizing this element and fixing the organic nitrogen in the soil for vegetation.

3. The bacteria *decompose all organic bodies* that have been built up into highly complex compounds through the agency of either plant or animal life. It is their duty to pull to pieces everything that has ceased to live. In thus pulling to pieces these organic bodies, they liberate their carbon, causing it, in

most cases, to assume the form of carbon dioxide, which at once becomes dissipated into the atmosphere, keeping up the store of this gas which forms the basis of plant life. In this destruction of organic bodies they also reduce the nitrogen to a variety of simple nitrogenous compounds. Ammonia is produced in large quantity, and not a little of the nitrogen, in the form of free nitrogen, is completely separated from its combinations to join the atmospheric supply. Other nitrogenous bodies of low state of chemical combination are doubtless also formed.

4. Bacteria of a different nature seize these end products of decomposition and *build them up into nitrates.* This process occurs, according to our present knowledge, in two stages and under the influence of two different bacteria species. By one process the ammonia is oxidized to nitrites, and by the second, the nitrites are oxidized to nitrates. This process of nitrification lies at the basis of soil fertility, inasmuch as it is the means by which the low nitrogen compounds, resulting from decomposition of organic matter, are worked over and built up into the form of nitrates when they may again act their rôle in organic life. This process of nitrification goes on only in the absence of large quantities of organic food. It occurs in the soil if the amount of organic food is not too great, but will not occur while the active decomposition, due to putrefying bacteria, is taking place.

5. Bacteria, acting *in connection with the legumes, reclaim from the atmosphere* much of the lost nitrogen. In spite of every method at our disposal there is always a loss of nitrogen from the state of combination; but this nitrogen may be fully replaced in the soil for the use of plants by the agency of bacteria. In addition to the bacteria that are capable of assimilating the free nitrogen directly and fixing it in the soil, there is a second type of bacteria which acts in connection with the legumes, forming a relation which we call symbiosis. The

result of the combined action of the legumes and the soil bacteria is the fixation of large amounts of atmospheric nitrogen.

Which of the two factors of nitrogen fixation is the more important in restoring the nitrogen to the soil can at present hardly be stated. There is beginning to be evidence that the direct fixation by bacteria, independent of legumes, is of far more importance than it was thought a short time ago. Observation has certainly shown that soil which is cultivated without the aid of legumes may hold its nitrogen content fairly well, and this points to the conclusion that the direct fixation of nitrogen, independent of legumes, is of very great significance, and possibly the greatest factor concerned in nitrogen fixation.

We thus see that the whole problem of the soil fertility is inextricably woven with bacterial fermentation. From the origin of the soil, through its use by plants and the subsequent destruction to their original condition of the products formed, we find nearly every step accompanied by bacterial action. The continued fertility of the soil is thus associated with bacterial life. In the future the problem of the proper treatment of soil for the use of agriculture will be, in a very large degree, a problem of the proper control of bacteria. Agriculturists must learn to stimulate the bacterial actions which are advantageous and check those which are disadvantageous, if they would insure the continuance of soil fertility.

CHAPTER VII.

BACTERIA IN WATER.

WHILE not very closely related to agricultural problems the subject of bacteria in water has certain aspects, particularly relating to water as a distributor of disease, which are of especial interest to the farmer who must control his own water supply. We will therefore consider the matter here, although it can be done quite briefly.

ABUNDANCE OF BACTERIA IN WATER.

All surface waters abound in bacteria. If we examine the water of the ocean, or of the pool by the roadside, we shall always find bacteria in greater or less numbers. Waters coming from far below the surface of the earth appear to be sterile. Thus water coming from artesian wells contains few bacteria, provided it can be drawn in such a way as to prevent contamination before it reaches the surface. From this it follows that spring water is also quite likely to be pure, at least purer than other surface waters, although it will rarely be sterile, since there is always a chance for contamination in the superficial layers of the soil. Rain water always contains bacteria which are doubtless washed out of the air ; and the same is true of snow and hail.

The number of bacteria in bodies of surface water is not exactly what would be expected in accordance with our ideas of pure water. We commonly look upon the water in the running brook as purer than that of the stagnant pond, and might be inclined to believe the brook freer from bacteria. But this is certainly not true, for the brook commonly contains more bacteria than the pond. The supply streams of a lake or reservoir always contain more bacteria than the water of the lake. One reason for this is evident enough. The brooks form the drainage system of the country. The rains wash the whole surface of the land and all of the dirt and dust is carried into the brooks. In this dust will always be hosts of bacteria which are thus carried by the streams into the lake in great numbers. We might imagine that they would accumulate in the lake. But this does not happen. Many of them die, for some reason, and others soon settle to the bottom, so that the water in the reservoir rapidly tends to become purified, and is always found to contain fewer bacteria per c.c. than the water brought into it by its supply streams.

It will be readily understood that the number of bacteria in a stream will depend upon the extent of the contamination which it receives from sources of active bacterial growth. The actual number found by bacteriologists is quite variable, ranging from a score or more per c.c., in very pure waters, to a few hundreds, in a moderately pure reservoir; and from this number to many thousands in streams which are badly contaminated with sewage.

DANGER OF SEWAGE CONTAMINATION.

It is common to regard water in which the bacteria are reckoned by thousands per c.c. as badly contaminated, as suspicious and unsafe for drinking purposes. While this may be a proper procedure it is very necessary to guard against a misunderstanding. The fact does not indicate that it is unsafe to swallow such large numbers of bacteria. We have most excellent proof that such is not the case. Ordinary milk, as we shall see in a later chapter, contains many more bacteria than this. Indeed, the milk of our cities frequently contains bacteria which are reckoned by millions rather than thousands, and some of the best milk has several hundred thousands of bacteria in each c.c. Yet such milk is drunken with impunity, and there is no reason for thinking that these great numbers of bacteria are in the slightest degree injurious to health. Why is it, then, that we look with indifference upon hundreds of thousands of bacteria in milk and regard as suspicious water which has them in simple thousands?

The bacteria present in milk come from sources which are not likely to furnish pathogenic bacteria, and it is known that ordinarily this vast number of milk bacteria is harmless. But in nearly all cases where water is contaminated with bacteria to the extent of several thousands per c.c., the conclusion is that the water is *sewage*-contaminated. Unless water is contaminated with either sewage or some source of decomposing organic matter we do not find the number of bacteria so high. A source of contamination sufficient to furnish a body of water with this number of bacteria must be an extensive one and in the majority of cases it is some form of sewage. Now, of all types of bacteria, those found in sewage are most likely to be injurious. When we remember that sewage contains every form of human excretions, and that it is by means of the excretions that the pathogenic bacteria find exit from the diseased patient, it will be easily understood that sewage-contaminated water is likely to contain bacteria which are pathogenic for man. Sewage-contaminated water is therefore suspicious and dangerous, a fact abundantly proved by the great prevalence of water-borne diseases in cities whose water supply is contaminated with sewage, as in the cities dependent upon river water for drinking purposes. When the bacteria in water are in the thousands they render the water unsafe, not because this number of bacteria is injurious, but because such water is commonly sewage-contaminated.

When we recognize the great chance which sewage-contaminated water has of becoming impregnated with the germs of human diseases, it is a little surprising to learn that the number of diseases actually distributed by water is very small. Only two of our common diseases are known to be frequently distributed by water. The most important of these is typhoid fever, in regard to which the evidence is abundant and most conclusive. This evidence need not be given here, but it is sufficient to demonstrate that typhoid fever is very commonly acquired by drinking water, that the danger comes wholly from water which has in some way become contaminated with human excrement, usually through sewage, and that the drinking of sewage-contaminated water is probably the most prolific source of this dreaded and serious disease.

The second water-borne disease is rarely of much significance in agricultural communities. It has been abundantly proved, in recent years, that Asiatic cholera is often distributed by means of drinking water which has become contaminated with the cholera germ.

Besides these there are one or two other obscure diseases of the alimentary canal, usually characterized by diarrhœal symptoms, believed to be water-borne.

The ordinary bacteria in water are of no significance whatever, either in their relation to health or to agricultural processes. But from what has been said it is evidently a matter of the greatest importance that drinking water should be kept free from sewage contamination. While any kind of pollution of drinking water is, of course, unfortunate, sewage contamination is almost certain to be fatal to some of the many persons using such water for drinking purposes. The inhabitant of the city has no means of guarding against such contamination. He can simply put the matter into the hands of officials and must trust to their wisdom in securing a proper supply. But the agriculturist commonly controls his own water supply, and he must depend upon himself to keep it properly guarded. If his supply is in the form of a spring, the water is perfectly trustworthy and his only care need be to keep his spring clean and the water free from secondary contamination. But if, as on many farms, he draws his supply from a well, his problem is not so simple.

THE WELL AS A SOURCE OF DRINKING WATER.

It is hardly possible to overestimate the care that should be given in locating the well, or in guarding it, if this be the source of drinking water. That many a case of typhoid fever on the farm is directly attributable to the well is sure, and such wells also prove the starting point of many an epidemic of typhoid in a community, through the distribution of milk. In locating the well it should be placed where contamination is *impossible.* The most common, as well as the most dangerous contamination of well water, comes, of course, from the privy vault. Both vault and well are, for convenience, placed near the house and frequently near each other. The well is sunken several feet below the surface of the ground, while the vault is close to the surface. The contents of the vault inevitably soak into the ground and will be surely distributed in every direction, taking naturally the course of water currents under the surface. It is almost certain that, if the well is close at hand, the water courses will lead to it and the contents of the vault, in a very diluted condition, of course, will thus find their way into the well. It requires no argument to demonstrate the danger from such conditions. Nor will anyone familiar with agricultural communities fail to recognize that exactly such conditions frequently exist. Indeed, they are sometimes even worse than this, for one may find the vault actually upon an elevated mound and the well sunk into the soil at its foot not twenty feet away.

Under such conditions one need not be surprised at the spread of typhoid. A single case of the disease on the farm will be sure to contaminate the vault, and will be almost as sure to infect the well. The rest of the occupants of the farm who drink the water are exposing themselves to this disease. But worse than this, the farmer rinses his milk pails in the water from the well and subsequently puts his warm milk in the cans. The typhoid bacilli which were in the well, thus get into the milk, where they find conditions for rapid growth, and the farmer, wholly unconscious of having done anything out of the way, distributes the bacilli to the neighboring community which he supplies with milk. A typhoid fever epidemic breaks out which remains a mystery, unless some one is sharp enough to trace it to its source in the farmer's well.

Such is not an imaginary instance but represents a type of typhoid epidemic many times repeated. It is simply illustrative of one of the sources of typhoid epidemics which has been found common, and many instances of almost exactly these conditions could be given. In the last fifteen years over fifty typhoid epidemics of considerable extent have been traced to milk, many of which are directly attributable to the well. The trouble arises partly from carelessness, but chiefly from ignorance. Certainly, for his own health and that of the community which he supplies with milk, every farmer should be impressed with the fact that the problem of his well is the most critical one on his farm. It should be scrupulously guarded, and should be located in such a place as to render drainage from the privy vault an *impossibility*. The safest thing would be to give up the well entirely and depend upon some spring or reservoir; but where this is impossible the well should be on higher ground than the privy vault, or be removed from it not less than two hundred feet.

Unfortunately, everyone who has been brought up on a farm is likely to feel that this danger is imaginary, at least for his own particular home. He has drunken water from the well all his life, and so have his fathers before him, and he cannot be convinced of any danger therein. But the fact remains that many a well of exactly this sort has been the cause of typhoid. Though used for years without suspicion it has, nevertheless, been a means of death. The trouble gives no warning when it comes, and the well which has been pure for years, may suddenly begin to distribute typhoid fever bacilli, without the least suspicion on the part of those using it. In ignorance the farmer not only drinks the water himself, but distributes the germs to the city, insisting all the while that his well has "the finest water in the country." The only safeguard is, either to abandon the well entirely, or to have such an absolute isolation between his vault and his well as to make communication between them by soil drainage an absolute impossibility. There is no subject connected with bacteria which the agriculturist should more thoroughly understand and more fully appreciate than that the water in his well is likely to become contaminated with pathogenic bacteria from human feces, if they are thrown upon the ground or are placed in a vault in the vicinity of the well which is used either for drinking or dairy purposes.

BACTERIA IN STREAMS.

The pollution of the water of streams is occasionally a matter of significance to the agriculturist since this water is likely to be used for various purposes. As mentioned on a previous page this contamination of streams has become so serious as to have required special methods and special legislature directed toward allaying the evil. But even yet, and for long years to come, the streams will be used as the natural means of disposing of sewage.

Fortunately for the purity of our rivers, these waters, though largely contaminated at one point, soon purify themselves. Although large numbers of bacteria and great quantities of organic matter may be poured into a river by a large city, it is found that after flowing for a few miles the organic matter and the surplus bacteria have largely disappeared, and the water is about as pure, so far as concerns bacteria, as it was before such contamination. For example: the city of Paris pours its sewage into the river Seine in great quantity. But below the city the water gradually becomes purified until finally it is as free from bacteria as it is above the city. Recently the city of Chicago has turned the little Illinois river into an immense drainage canal for the large amount of sewage of that city. This river is a small one and flows very slowly. It finally empties into the Mississippi river after flowing for some 300 miles. It empties a few miles above the point where St. Louis takes its water supply and has naturally excited considerable alarm in the latter city. A careful examination of the bacteria in the river shows that there is a constant decrease in number as the distance from Chicago is increased, and, when it finally empties into the Mississippi, this river has no more bacteria than have the waters of the smaller rivers flowing into it which are not sewage-contaminated. In this flow the river has apparently purified itself of sewage bacteria.

How this self-purification of streams is brought about is only in part understood. There appear to be several factors concerned in the phenomenon, each of which plays a part. But how great a share each factor has can as yet hardly be stated. Evidently the phenomenon is practically identical with the bacterial purification of sewage already referred to, modified by the different conditions.

The disappearance of the bacteria has been the subject of investigation and speculation by bacteriologists and the following factors have been advanced as explaining it:

1. The *agitation* of the water and the *aëration* it receives in its flow. This factor is manifestly confined to swiftly flowing streams and can hardly be of any significance in sluggish rivers, like the Illinois.

2. The *dilution* of the water by tributary streams. This doubtless accounts, in part, for the decrease in number of bac-

teria per c.c., but it cannot be a very important factor in cases such as shown, where the number of bacteria in the river finally becomes no greater than the number in the tributary streams. Such a reduction is not simple dilution.

3. The action of *sunlight* is known to be injurious to bacteria, and it has been thought that this may be one of the factors destroying the bacteria in the streams. But its action in muddy streams must be very slight.

4. The deleterious action of other *living organisms* in the water may have its influence. Microscopic animals certainly destroy great quantities of bacteria, actually feeding upon them. That many microscopic animals do feed upon bacteria can easily be demonstrated with the microscope and these animals may be one of the efficient means of the self-purification of streams. Possibly these animals may also use up the food which the bacteria need.

5. *Sedimentation* is certainly a factor. It is well known that bacteria generally are heavier than water and will slowly sink to the bottom. In slowly flowing streams sedimentation probably plays an important part in the purification of the water from sewage bacteria.

6. *Exhaustion of the Food Supply.*—Bacteria certainly need food and some species need large amounts of food. The food in the water is of course used up either by themselves or by other organisms, and finally becomes insufficient to support bacterial life.

Whether these factors explain the purification of streams, or whether there is some other yet unexplained factor it is hardly possible to say. But certain it is that sewage-polluted streams become freed from most of their bacteria after flowing a few miles. Whether such water is safe for drinking is at least doubtful.

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PART III.

BACTERIA IN DAIRY PRODUCTS.

CHAPTER VII.

RELATION OF BACTERIA TO THE DAIRY AND ITS PRODUCTS.

BACTERIA have an important relation to agriculture, not only in regard to the raising of crops, but also in the matter of handling them. There is no product of the farm so closely related to bacteria as milk and its products. In some respects the dairy methods in use have been almost revolutionized by the discoveries of modern bacteriologists.

BACTERIA IN MILK.

Milk, when secreted from the mammary gland of a healthy cow, is commonly, if not always, free from bacteria. It has been no easy matter to demonstrate this fact since the chances of bacteria getting into the milk from the outside are very great. But a sufficient number of careful experiments have been made to demonstrate conclusively the truth of the statement.

If the cow is not in perfect health the milk may no longer be free from bacteria. When a cow is suffering from generalized tuberculosis, or when she has this disease localized in the udder, her milk, even when secreted, is sure to contain bacteria. Indeed any udder infection due to bacteria, or even a simple inflammation of the mammary gland, is likely to result in the contamination of the milk with the bacteria which cause the trouble. Anyone who milks a cow suffering from any udder trouble must remember that, under these conditions he is no longer obtaining pure milk, but milk which is probably filled with bacteria, some of which are the cause of the diseased conditions of the udder, and may also be the source of some kind of disease to man. Udder diseases are thus a most serious menace to the purity of the milk supply.

Confining our attention for the present to normal milk, we notice next that, if the milk can be kept from being contaminated with bacteria, the ordinary and almost universal phenomenon of souring will not occur. Indeed, milk free from bacteria will remain for an indefinite period without visible change. It is a fact, recently proved, that there are some chemical changes which will slowly occur in such milk. In normal milk when secreted from the cow there is present one of the chemical ferments (*enzymes*) referred to in an earlier chapter. This enzyme is produced by the cow and appears to be a normal constituent of milk. It is called *galactase* and has the power of slowly converting the insoluble casein into soluble proteids. This "digestion" is a somewhat slow process and is a phenomenon of no significance in the handling of milk or in butter-making. In the problem of cheese-making the galactase is a factor of more importance. But in the handling of milk other phenomena, due to bacteria, are so much more prominent as to conceal entirely the action of the galactase. We may therefore take for our starting point the highly important facts that, (1) so far as concerns healthy milk, it will remain unaltered if free from bacteria, and that, (2) all of the ordinary changes occurring in milk are due to the action of bacteria which get in subsequent to the time when the milk is secreted in the mammary gland.

SOURCES OF BACTERIA IN MILK.

Bacteria are sure to find their way into the milk. Recognizing the sterile condition of the freshly secreted milk we are hardly prepared to learn that, by the time it has been drawn from the cow, received in the milk pail and removed from the cow stall to the dairy, it may be contaminated with bacteria to the extent of several thousand per c.c. But this is frequently and, indeed, commonly the case. The number of bacteria in freshly drawn milk will vary greatly with the conditions existing in the dairy. There may be only a few score in each c.c. or, under exceptional conditions a smaller number still, but it is much more likely that the milk contains many thousands of bacteria by the time it has been removed from the cow stall. The actual number is of no special significance, except as an indication of the cleanliness of the dairyman in handling his cows and in caring for his dairy.

All of the troublesome changes which occur in milk and make this product so difficult to handle are due to the action of bacteria upon the milk; hence it is a universal desire of the dairyman, the milk distributor and the consumer, to have the bacteria as few as possible. Every one wishes the milk to remain sweet, and this is possible only as the number of bacteria is kept small. It becomes a matter of much importance to learn the sources from which these milk bacteria are derived. Knowledge upon this point will enable the dairyman to adopt a few precautions in the production of and caring for milk which will considerably reduce their number. A small amount of attention given in the right direction will produce much better results than a much larger amount, misdirected and indiscriminately applied.

The Cow.—The chief sources of milk bacteria are the *cow* and the *milk vessel*. Although the cow secretes milk in a sterile condition it is by no means sterile when it leaves the milk duct. There are always some bacteria in the milk ducts ready to be washed into the milk pail with the first jet of milk. At the close of the milking enough milk is left in the duct to furnish food for bacteria which may get in through the external opening, and these bacteria, in the warm temperature of the cow's body, multiply rapidly. Bacteria are thus always abundant near the opening of the teat, although inner parts of the duct are comparatively free. In the period which elapses between the milkings they increase rapidly and are sure to contaminate the first jets of milk drawn. This fact has been abundantly proved by analyses. The first lot of milk drawn, called "fore milk," always contains more bacteria than that drawn later in the milking. Toward the close of the milking the bacteria sometimes disappear so that the last milk is actually sterile when it leaves the milk duct. This is not always the case, but the last milk is always purer than the first. For example, in two tests the results were as follows :

NUMBER OF BACTERIA PER C.C.

In fore milk, 55,000,	at close of milking 00
In fore milk, 97,000,	at close of milking 500

Wide variations are shown by different tests but these two will illustrate the general phenomenon. Hence it follows that a considerable reduction in the number of bacteria in milk may be effected by simply allowing the first few jets to run to waste. The species of bacteria in the ducts of different cows differs, but it is tolerably persistent in each cow. Indeed each teat may have its own species which remain constant for a long time.

A greater source of contamination is the dirt which adheres to the body of the cow. To one unfamiliar with the cow in the ordinary barn the amount of filth which it is common to find adhering to the animal, even in good dairies, is surprising. The cows are rarely groomed ; perhaps they are allowed to stand the whole winter without cleaning. Their flanks become covered with a coating of dried excrement, mixed with all sorts of other filth. Every motion the cow makes dislodges some of this filth. Every time she switches her tail during the milking, every motion of her legs, and every rubbing or brushing of her body by the milker, dislodges particles of this material which are sure to fall into the milk pail. All of this filth is crowded with bacteria in excessive numbers, and these find their way into the milk during the whole of the milking. Here again actual tests have shown the extent of this source of contamination, and have demonstrated that during the milking a perfect shower of dirt particles laden with bacteria are falling into the milk. Hundreds of bacteria have been counted on a single short hair. The amount of dirt can even be approximately calculated and has in some cases been found to be as much as 25 mg. per quart, equivalent to 300 to 500 lbs. per day for the supply of a city like New York. The dirt thus found includes excrement, hay, hair, skin, earth, etc., etc., and nearly every particle will be covered with bacteria. Here is evidently one of the prolific sources of milk bacteria. Anything which will improve the cleanliness of the cow will improve the milk. Even the custom of moistening the under surfaces of the cow with a moist sponge immediately before milking will be of advantage by checking this constant shower of dried particles which fall from the body.

Devices for removing the larger part of this filth from the milk have been invented and are coming more and more into use. The most common method consists in filtering the milk through deep layers of sand. Quite a number of such filters have been patented and new ones are constantly appearing. A different, and in some respects, a better method, uses centrifugal force for the purpose. By passing the milk through a centrifugal machine which does not rotate fast enough to separate the cream, a large part of the dirt may be thrown out and easily removed. The milk is thus much improved so far as concerns wholesomeness. This centrifugal purification does not materially affect the bacteria, for there seem to be about as many after treatment as before.

The Milk Vessel.—The second important source of bacteria is *the milk vessel.* Upon an ordinary farm the milk vessels are rarely washed *bacteriologically* clean. The washing in hot water with subsequent drying in the sun is wholly insufficient to remove the bacteria. These are sure to remain in the vessel, clinging in the corners and cracks, partly dried perhaps, but alive and ready to begin active life just as soon as they are supplied with the food which comes to them with the next lot of milk drawn. Indeed the ordinary farm has no really effective means of washing the milk vessels. Live steam is the only satisfactory method of doing it, and this is not commonly available to the agriculturist. Many a troublesome experience of the milk dealer in warm weather is attributable directly to imperfectly washed milk cans, and disappears at once when all the milk vessels are thoroughly sterilized by live steam. Next to the filth of the cow the milk vessel is the most prolific source of trouble.

In this connection the agriculturist should guard against one common source of troublesome bacterial contamination. It has been conclusively shown that one of the ordinary sources of troublesome bacteria is water. One of the most mischievous and widely distributed dairy infections is slimy milk (see page 199), and there is good reason for believing that this infection must in some cases be traced to water. The milk producer has also in recent years been informed of the danger of distributing typhoid fever by milk and, in some cases at all events, the source of the typhoid fever germs is the water of the dairy. It is not necessary that a milk producer should water his milk in order to lay himself open to this danger. He may wash his cans in boiling water and subsequently rinse them in cold water from his well. If he uses well water which is filled with the slimy milk bacteria or with the typhoid germ, he leaves these bacteria in the cans ready for action. Moreover, it is a common practice in many dairies and creameries to place the milk in cans and submerge them partly or wholly in water for the purpose of keeping the milk cool. Of course no bacteria can get through the cans, but experience has shown that, in spite of the care given, some of the water will usually get into the milk. There is abundance of evidence that the water in which the cans are submerged is sometimes the source from which a troublesome dairy infection is derived. Clean water, and vessels cleaned with live steam, form the second set of safeguards against troublesome dairy bacteria.

The Air.—Besides these two chief sources there are others which furnish bacteria to a less extent. Some doubtless come from *the air*. In earlier years it was thought that this was a large source of contamination, but we now know that the air is ordinarily of little importance, although sometimes it has been found to be a source of trouble. One case of *soapy milk*, for example, has been attributed to the air. If a farmer throws a lot of hay down from his hay mow just before milking and allows his cows to feed upon it during the milking, the dust thus distributed in the air will be a source of considerable contamination to his milk. The remedy is, of course, to feed earlier or later, and at all events not to stir up a dust in the milking stall by the use of dry hay. If this simple precaution is taken the air will not ordinarily be a source of sufficient numbers of bacteria to cause trouble

The Milker.—The milker himself must be regarded as a source of bacteria. The clothing of the farmer is always filled with bacteria and his hair and hands are sure to be covered with them. His motions in the barn, especially during the milking, will distribute them, so that they will be sure to fall into the milk pail. To avoid this danger some of the best dairies require the milker to wear special garments when milking, and the milking clothes are carefully washed, in some instances by steaming, each day. Of course, the milker is also required to have scrupulously clean hands.

From these sources bacteria find their way into the milk during the milking. In order to facilitate the milking there is commonly used a milking vessel with a wide mouth and this, of course, greatly facilitates the collection of bacteria. In a one-test case it has been found that bacteria fell into the milk pail at the rate of 3,250 bacteria per minute for an area of ten inches, and in another as many as 1,210 per second for an area with a diameter of three inches. For the purpose of avoiding the bacteria from these sources various special means have been suggested, such as special shaped vessels with narrow mouths, and special covers which filter the milk. For this purpose, too, artificial *milking machines* have been invented, which, by the use of a series of tubes, draw the milk directly from the cow into the milking vessel without allowing any exposure to the air. Great things were expected of these milking machines, but they have hitherto proved of no practical value for the purposes designed, and they can at present be regarded as nothing more than scientific toys. Indeed, so far as experiment has shown anything thus far, it has indicated that these machines increase rather than decrease the number of bacteria; a fact due doubtless to the difficulty of keeping such apparatus properly clean. What may be the future of such contrivances cannot of course be predicted, but at present they are of no practical value.

CONTROL OF THE NUMBER OF BACTERIA.

Cleanliness.—Thus the first practical method suggested for reducing the number of bacteria, and hence for avoiding the troubles which they produce in milk, is *scrupulous cleanliness*, directed *first toward the cow*, *second toward the milk vessels*, *third toward the milker* and, incidentally, to the air of the cow stall. In addition to this, *all abnormal milk* should be thrown away, since such milk is almost sure to have come from diseased udders which are filled with various pathogenic bacteria.

These suggestions are not simply theoretical but have proved to be distinctly practical. Careful scientific testing has shown that the number of bacteria may be reduced and the keeping property of the milk much increased merely by applications of the simplest devices for cleanliness. Sometimes, where exceptional care is given along the lines suggested, milk has been obtained that is nearly sterile. Further, it is not only in scientific experiments, but in actual dairies that these simple precautions for cleanliness have worked great improvements. Wherever a dairy has taken a pride in producing good milk and has attended to the matter of cleanliness, the effect is immediately manifest in a reduction of the bacteria. There is a very great difference in the number of bacteria and the consequent keeping quality of milk from well-kept, clean dairies and from slovenly kept dairies. Indeed, the bacteriologist is commonly able to give a very accurate judgment as to the condition of a dairy and its cow sheds, by simply making a bacteriological examination of the milk. The suggestions as to cleanliness are thus not simply the theoretical advice of the bacteriologist but the result of an abundance of practical experience. Cleanliness is the first precaution which must be taken by a farmer who desires to produce good milk.

Cooling.—The second factor of importance in the keeping quality of milk, of more importance even than cleanliness, is the matter of temperature. The actual number of bacteria which get into the milk during milking is important, but it is of less importance than their rate of multiplication in the next few hours. Bacteria grow very rapidly at high, and very slowly at low temperatures. Milk, when drawn from the cow, is at a temperature most favorable for the greatest growth. Moreover the milk contains an ideal food for most species of bacteria, nearly all saprophytes flourishing wonderfully upon milk. As a result, the bacteria that get into the milk during the milking begin to grow with great rapidity. So long as the original high temperature is retained they multiply rapidly and the few thousands are soon likely to become hundreds of thousands or millions. For example, milk tested at intervals for twenty-four hours showed the following increase :

When first received,	153,000 per cu. in.
After 1 hour,	616,000 " "
After 7 hours,	1,020,000 " "
After 24 hours,	85,000,000 " "

The rapidity of multiplication is dependent directly upon temperature, being greatest at temperatures of 80° to 100° F. At temperatures as low as 50° F., the rapidity of growth is much decreased, and below 40° F. it is very slight. When the temperature of freezing is reached the growth stops entirely. At freezing temperature the milk may be preserved indefinitely without bacterial growth.

These facts explain the statement made that the matter of temperature is of more importance in connection with the keeping quality of milk than even the original cleanliness. No matter how careful a dairyman may be in obtaining clean milk, if he allows it to remain warm the few bacteria which did get in will multiply so rapidly that they will soon be as numerous as they are in another sample of milk which at first contained larger numbers but had been cooled. Milk carelessly drawn but properly cooled will keep better than milk carefully drawn but not properly cooled. Nearly all of the phases of the gigantic milk industry of civilized communities are based chiefly upon the various devices for keeping the milk properly cool, in order to prevent the growth of bacteria until it may be conveniently delivered to the consumer. The milk dealer has discovered that the growth of bacteria ruins his milk and that his only effectual remedy against them is the use of low temperatures. The bacteriologist teaches him further that the milk should be cooled *immediately* after the milking, for the obvious reason that the milk is then at a temperature which stimulates bacterial growth to a maximum. If allowed to remain warm until it cools naturally, this will take so long, especially in warm weather, that the bacteria will become very numerous within a short time.

The milk dealer is sometimes puzzled by the fact that morning's milk will sour more quickly, after delivery to the customer, than will the milk of the night before, though the latter is actually twelve hours older. The explanation of the fact is simple enough. The night's milking is to be delivered in the morning and, meantime, is placed in a cool spring or ice chest, and its temperature consequently rapidly lowered. The bacteria growth is immediately checked. When taken for distribution the next morning it does not have many bacteria, and it warms up so slowly that it is long before it is warm enough for the bacteria to grow very rapidly. But the morning's milk is taken directly from the cow, placed in the delivery cart and, while still at a high temperature, is carried around for distribution. At this high temperature the bacteria multiply quickly, and soon their number is higher than that in the night's milk which has been cooled. The morning's milk actually sours first, simply because it failed to receive the cooling *immediately after the milking*.

The remedy for this is, of course, a cooling of the milk immediately after the milking; hence the quicker the cooling and the lower the temperature, the better. It is surely unnecessary to add that the milk should then be kept at as low a temperature as possible until it is used. This fact is fully understood by all who handle milk and the numerous devices for the use of ice, in cars or in milk wagons, are testimonials to the absolute necessity of low temperatures for the purpose of preventing an undue growth of bacteria.

From these facts it will be seen that it is impossible to make any statement as to the proper number of bacteria to be found in milk of any given age. The number is highly variable and depends upon three factors. (1) The original cleanliness of the farmer in his cow shed and dairy. (2) The age of the milk. (3) The temperature at which the milk has been kept, including the quickness of the original cooling. The *farmer* can improve the keeping quality of the milk by improving the conditions of his dairy and by better devices for cooling; the *shipper* can have no less influence upon it by controlling the matter of temperature, and the *consumer* has no less a responsibility in regulating the temperature at which he keeps the milk and the quickness with which he uses it.

The question is frequently asked, what number of bacteria milk must contain before it is to be condemned. This question cannot be answered. It may contain a very few but be extremely dangerous, or it may contain millions and be harmless. It is sometimes stated that if it contains more than 50,000 per c.c. it is not fit to drink. If this is accepted then most of the milk of our larger cities is unfit to drink, for rarely does such milk contain less than several hundred thousands. The fact is that the numbers of bacteria can tell us little as to the healthfulness of milk. On a later page it will be seen that cream which is made into butter contains bacteria by hundreds of millions or even billions per c.c., but no one regards this as indicating that the cream or the butter is dangerous. Of course we want our milk as free from bacteria as possible, but where a line can be drawn between good and suspicious milk no one can say. Provided the bacteria are of the lactic species they may be present in large numbers without doing any injury whatsoever, except to render the milk less palatable by making it sour.

The problem of supplying our great cities with milk has become a difficult one with their increasing growth. Milk must be drawn from a wider and wider territory, involving a more and more extensive use of railroads. For New York some of the milk comes from a distance of 500 miles. This problem of shipment involves chiefly the shipper and distributor, but one phase of it concerns the farmer himself. If he furnishes milk from a cleanly kept farm and if he cools his milk immediately after milking, he can furnish a product that can be transported successfully to the distant city. But unclean barns and cows, and a lack of proper cooling at the start, render the task of transporting the milk difficult, and in warm weather, impossible.

It is not the mere presence of bacteria in milk which concerns us but their effect, first upon the milk, second upon the consumer. In considering the subject of the relation of bacteria to dairy products we must study the subject from three different standpoints : *Milk*, *butter* and *cheese*. Each presents its own problems and each is differently related to the question of milk bacteria.

CHAPTER IX.

BACTERIA IN MILK.

THE varieties of bacteria which get into milk from the sources mentioned are very numerous. Some of them are of practically universal occurrence in milk in wide localities. Others are quite common, while still others are rare and must be looked upon merely as occasional visitors. Only those which are somewhat common can be regarded as distinctive dairy bacteria. It is impossible at present to give a list of even those bacteria which are especially characteristic of dairy products. Some two hundred or more different types have been described as occurring in milk and its products, a considerable number of which seem so common as to be properly regarded as dairy species. Whether this large number can properly be regarded as different species, or as different varieties, or as a few species assuming different characters under different conditions, no one would at the present day venture to assert. A knowledge of these different forms is at the present time useless to any except the technical bacteriologist.

Of the long list of bacteria some are of scientific interest only, while others are of very great practical importance. We may leave out of consideration entirely most of the types which are only rarely found in milk and whose relations to dairy matters are slight. Those which are left, and whose relations are important, may readily be divided into a few important classes according to their action upon milk. The classes which are most important and easily recognized are as follows :

LACTIC ACID BACTERIA.

By far the most universal type of bacteria in milk is the class which produces lactic acid and causes the milk to sour and curdle. This lactic acid is produced from the milk sugar by a chemical change the details of which are not known and which, indeed, may not be the same under different conditions, or when produced by different bacteria. The essential nature of the change is expressed by the formula:

$$\underset{\text{Milk sugar}}{C_6H_{12}O_6} = \underset{\text{Lactic acid}}{2C_3H_6O_3}$$

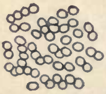
While this represents the essential nature of the change it is certain that it is a more complicated phenomenon than here indicated, and involves a number of other products, acetic acid and alcohol being frequently formed. For ordinary purposes, however, the essential effect is the production of lactic acid out of milk sugar.

The number of types of bacteria which can produce the lactic fermentation is large. Indeed, the property of producing lactic acid from milk sugar is characteristic of a large number of bacteria which are not dairy organisms. Several scores of bacteria with this power have been described by different bacteriologists, all apparently more or less distinct. But the more carefully the dairy bacteria have been studied, the more evident has it become that the long list must be considerably shortened, and to-day there is a tendency toward the belief that lactic bacteria which are concerned in the normal souring of milk are very few. The most important of these are two.

1. *Bacillus acidi lactici.*—This name was first used by Hueppe and has been subsequently applied to quite a large number of more or less different types. It is still in use and applied now to a group of related bacteria, slightly different from the original described by Hueppe, but a group which

is so common as properly to be regarded as *the lactic bacteria, par excellence.* The species, first described by Gunther, and later studied by many others, sometimes under the same name, and sometimes under different names, is the most widely distributed lactic organism in the dairies both of this country and of Europe (Fig. 22). It shows a considerable variety and while the different types described by different observers do not agree in every respect, they are all so much alike as to be regarded properly as a single species.

FIG. 22.

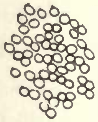


B. acidi lactici.

2. **Bacillus lactis aërogenes.**—This species is almost as widely distributed as the first, and in some places appears to be the cause of the spontaneous souring of milk. Microscopically it is almost identical with the first species (Fig. 23), but it differs considerably in its action on milk and in other characteristics.

There are perhaps a dozen other types of bacteria described by bacteriologists as causing the normal souring of milk, and among them are some which are clearly different from either of the two mentioned. But these two, with their varieties, must certainly be regarded as the lactic bacteria of highest importance and widest distribution. A description of the long list would be out of place in this work.

FIG. 23.



B. Lactici aerogenes.

One fact of the greatest importance in regard to the lactic bacteria is that, so far as known, they never form spores. This is of great significance, since it makes it possible to destroy them by the use of moderate heat of not more than 150° to 160° F., a matter of great practical importance.

The fact that milk under ordinary circumstances is sure to sour and that, if examined bacteriologically when a few hours old, it is found to contain lactic bacteria in overwhelming numbers—sometimes over 99% being *B. acidi lactici*—would lead one to expect that it is these bacteria chiefly which get into the milk during the milking. This, however, does not appear to be the case, for, when fresh milk is studied, it is found, in many cases, that the bacteria of other types far outnumber the lactic organisms. Sometimes, indeed, the lactic bacteria are so few in fresh milk or cream that they cannot be found by ordinary methods of analysis, the other bacteria outnumbering them a hundred fold. Nevertheless, when this same sample of milk is examined a few hours later, it will be found that the lactic bacteria have become more numerous than the other types and, if the milk be examined later still, when it has become distinctly acid, it will be seen that the lactic bacteria have increased so prodigiously that they constitute nearly the whole. The meaning of this fact is evident. There is doubtless a great struggle among the bacteria in the milk and, whereas many different kinds of bacteria commonly find their way into the milk at the time of milking, the lactic bacteria find the conditions very much more favorable to their growth than do the other types. They increase with a much greater rapidity than the others and finally actually crowd the other types of bacteria out of existence. In a well-soured cream all bacteria except the lactic bacteria may have disappeared, even though others were at first in abundance.

From these facts it will be seen how absolutely hopeless it is for the dairyman to try to avoid the presence of lactic bacteria. Some of them will be sure to find their way into the milk, and if so, they will find such favorable conditions for growth that they will soon become very numerous. Something may be done toward procuring milk with a high keeping quality, by trying to reduce the number of bacteria which get into the milk; but the greatest dependence must be placed upon the use of cold as a method to prevent their growth.

It is interesting and suggestive to find that the number of lactic bacteria which get into the milk from different cows is by no means the same. Sometimes, for wholly unexplained reasons, milk from certain cows, when carefully collected, will not sour. If the milking from the different cows of a large herd be collected in such a way as to avoid contamination from falling dirt and dust, it is quite common to find the milk from some of the cows without any representatives of lactic bacteria. If a large series of samples are thus collected from different cows and immediately protected from dust by cotton plugs, many of the samples fail to sour, since *they contain no lactic bacteria.* Indeed, milk direct from the milk duct does not commonly contain the ordinary lactic bacteria.

It is clear from these facts that the reason why lactic bacteria are regarded as preëminently dairy organisms, and the reason why milk is so sure to sour, are not because of the excessive number of lactic bacteria in the barn and dairy. Fresh milk frequently contains other species in just as great abundance, but the lactic bacteria find the conditions in milk more favorable for their growth, so that at the end of twenty-four hours they have gone ahead of all the rest.

The *souring and curdling* of milk is a phenomenon so nearly universal that in past years it has been regarded as a normal change due to the character of milk, just as clotting is a characteristic of blood. But this has been wholly disproved by showing that it is perfectly possible to preserve milk sweet indefinitely if bacteria can be kept out of it. But the bacteria are so common around barns that they are practically sure to get into the milk and, finding the milk an exceptionally good medium for growth, they develop until they produce a sufficiency of acid to throw the casein out of solution. Any acid added to milk will curdle it, since the casein is only dissolved in an alkaline medium, and the curdling inevitably follows the production of *lactic acid.*

THE SOURING OF MILK IN THUNDER STORMS.

If the souring of milk is always due to the growth of lactic bacteria it becomes necessary to explain the popular belief that thunder storms will sour milk. This belief rests upon a mistaken interpretation of observed facts. It is certainly true that milk is frequently found soured after a thunder storm, and the natural interpretation has been that it is the electricity of the thunder storm that produces the souring. A careful study of the phenomenon has shown that this inference is incorrect. It has been found that electricity, in the form of electric sparks, has no power to sour milk. Such electric sparks may be discharged indefinitely at the very surface of the milk without showing the slightest ability to sour it, and the force of the electricity under these conditions is certainly greater than that of lightning at a distance. Electric discharges in the thunder storm produce ozone and, since ozone is an active oxidizing agent, it has been suggested that the ozone is the cause of the souring. But this is disproved by actual experiment, which shows that ozone, even when present in large quantity, is unable to sour milk. Further, it has been found that if the milk is kept properly cool the thunder storm has no influence upon it. Lastly, it has been abundantly proved that milk which has been deprived of its bacteria may be kept indefinitely, and will remain sweet in spite of thunder storms. In short, all evidence shows that the thunder storm has no power of souring milk, unless bacteria are present to produce the lactic acid, and that the thunder and lightning have no direct effect upon the souring of milk.

What then causes the frequent souring of milk during thunder storms and the widespread belief that thunder is the cause? The answer seems to be a simple one. It is, that the same agencies which produce the thunder storm cause a rapid growth of bacteria. The thunder storm is brought on by climatic conditions, dependent chiefly upon the temperature, and these same conditions are just those which stimulate bacterial growth. It will thus happen that the same sort of warm weather which produces the thunder storm also hastens the growth of bacteria in milk if not kept artificially cooled with ice. It will frequently happen, as a result, that the milk will be ready to show signs of souring at the same time that the thunder storm appears. The two phenomena occur together, not because the one causes the other, but because the same climatic conditions which produce the storm hasten the growth of bacteria. A similar warm spell will sour the milk just as quickly even though no thunder storm appears. Whether this is the whole explanation appears a little doubtful, but it is clearly demonstrated that the thunder and lightning have nothing to do directly with the phenomenon. The souring of milk is always produced by bacteria.

PEPTONIZING AND RENNET-FORMING BACTERIA.

The second type of normal bacteria in milk is one almost universally found in milk, the number of whose species is great, but whose significance in ordinary dairying is very slight. The distinctive characteristic of these bacteria is the fact that they *secrete certain enzymes* which have a decided action upon milk. There are two such enzymes secreted by these bacteria, and some species of bacteria secrete both in considerable quantity, while others appear to produce only one.

The first of the enzymes is quite similar in its action to *trypsin* secreted by the pancreas and has been called *casease.* Its action is upon proteids which it converts into a soluble form related to peptones. In the milk it acts upon the casein and slowly renders it soluble. The milk under the action of this enzyme becomes gradually more and more transparent, as the casein is dissolved in the water, and finally it may become an almost clear, watery solution, which may have meantime developed certain colors. The essential phenomenon is, however. the solution of the casein, usually called *digestion*.

More commonly the action of these bacteria is somewhat different because of the second enzyme. This is similar in its nature to *rennet* and it produces a *curdling* of the milk. Under the action of these bacteria milk becomes in a few days a soft gelatinous curd, which is alkaline in reaction and has a sweet taste, a phenomenon sometimes called "sweet curdling." It generally happens that a few hours after the milk is thus curdled the curd begins to become softened and dissolved. The trypsin ferment (*casease*), produced by the same bacteria, digests the curdled casein, and, as fast as it is digested, it is dissolved. As the digestion goes on the whole curd may in time become dissolved and the milk become a transparent, watery fluid, of a brownish, yellowish or greenish color, or with no color at all. The final result is the same whether or not the milk first curdles. Milk that has curdled from the formation of lactic acid never becomes digested in this manner, inasmuch as the acid prevents the growth of these enzyme-forming bacteria.

The number of bacteria producing these enzymes is very large. The bacteriologist in his classification of bacteria divides them into two great divisions according to their power of *liquefying gelatin*. The dairy bacteriologist finds that the bacteria which liquefy gelatin produce enzymes as above described. All of them appear to give rise to the digestive ferment, for the power of liquefying gelatin and the power of digesting casein appear to be the same. Not all of the liquefiers, however, curdle milk for some of them digest the casein without curdling. Even these do, in some cases at least, produce rennet, but the amount is too small, or it is produced too slowly, to curdle the milk.

The study of the bacteria of cow's excrement and of water shows large numbers that have the power of liquefying gelatin, and, since these are quite sure to get into milk, it follows that ordinary milk probably contains some of this enzyme-producing class. They are so very common that they may be regarded as normal dairy bacteria (Fig. 24, *f*).

It is very doubtful whether this type of bacteria is of much, or of any, significance in ordinary dairying. Although the varieties are numerous and their number may be great at the start, greater than that of the lactic bacteria, they very rarely get an opportunity to have any considerable effect upon the milk. The lactic bacteria grow so very much more rapidly that they soon entirely outnumber the rennet-forming class, and, indeed, in most cases stop their growth. As a result, whereas the latter may be comparatively numerous in fresh milk, they become less, rather than more, abundant as the lactic bacteria grow, and finally disappear. Under such conditions their significance in the milk is probably nothing. Occasionally, however, these bacteria may become of more importance. It may happen that a sample of milk does not chance to have any lactic organisms in it, or that they are so few as to fail to get the upper hand of the others. If this occurs the other species of bacteria may find the conditions favorable to their growth. A dairyman sometimes finds, especially in the fall of the year, that his milk curdles without becoming acid, an effect produced by the growth of this class of bacteria. Some of the phenomena so troublesome in the dairy may be attributed either to the great abundance of this second type of bacteria or to a lack of a sufficient number of lactic bacteria to counteract their action. It is a fact, also, that this class of

FIG. 24.

A common milk bacillus producing rennet and pepsin-like enzymes.

bacteria plays an important part in the changes which may take place in so-called sterilized milk. Sterilized milk has been heated to a temperature of boiling water, or even higher, a temperature supposed to destroy all bacteria. Careful testing has shown, however, that, in a considerable number of samples, certain spore-bearing bacteria resist these high temperatures. Such milk will never sour, inasmuch as all lactic bacteria are killed, since they never produce spores. This class of enzyme-forming bacteria, however, are very commonly spore-bearers, and resist the temperature of boiling water. Milk which has been sterilized, therefore, not infrequently undergoes changes which affect its taste and its chemical nature, due to the class of bacteria here considered. This class of bacteria has been supposed to have important functions in cheese-ripening.

BUTYRIC ACID FERMENTATION.

A third type of bacteria, which may always be expected in milk, are bacteria which produce a *butyric acid.* Butyric acid is produced by a large number of bacteria and is in no case to be regarded as a chief product, but rather as a *by-product* of general decomposition produced by a number of species. As a rule it is produced by bacteria which grow only in the absence of oxygen, and is developed quite slowly. In ordinary dairying this type of fermentation has, therefore, no special significance in the handling of milk, since the milk is consumed before the butyric acid forms. In butter which is kept for some time butyric acid is almost sure to be produced. The chemical action is usually given as follows:

$$\underset{\text{Milk sugar}}{C_6H_{12}O_6} = \underset{\text{Butyric acid}}{C_4H_8O_2} + 2CO_2 + 2H_2$$

This certainly does not express the complete reaction.

A long list of bacteria has been described which produce butyric acid. Most of them are anaërobic though some grow in contact with oxygen. But, in spite of the long list which is known, it appears that the ordinary butyric fermentation is probably caused by a single species. This organism was first described under the name of *B. aërogenes capsulatus*, but was subsequently given the extraordinary name of *Granulobacillus saccharobutyricus immobilis liquefaciens* (Fig. 25). It appears to be very widely distributed. It is found in air, dust, dirt, milk, in the intestine of a large number of animals and almost universally in feces. It is perhaps the most widely distributed of all known bacteria. It is a strict anaërobe and produces very resisting spores. In ordinary milk, although commonly present, it never makes itself evident, since it is prevented from growing by the presence of the lactic bacteria or the presence of air. If, however, the milk is sterilized the bacillus is almost sure to be left uninjured and will subsequently produce the butyric fermentation if the milk is left without free access of air. It finds its way into butter and is doubtless one of the causes, perhaps the chief cause, of the development of rancidity. While, then, of universal occurrence, it is of little significance in the dairy except in relation to butter.

FIG. 25.

Bacillus of butyric acid. (*Shattenfroh* and *Grassberger*.)

In addition to these types of normal milk bacteria it must also be stated that other species are very commonly found in milk and are frequently so numerous that they should be regarded as normal dairy bacteria. These species, however, appear to have no noticeable effect on milk. They do not produce acid or any enzyme; neither do they change the taste, odor or appearance of milk. That they do really produce some changes in the chemical nature of the milk is very probable, but in regard to them nothing is known at present. For ordinary dairy purposes they are therefore of no significance, so far as we know, and they may therefore be omitted from our consideration.

ABNORMAL FERMENTATIONS.

The types of milk bacteria included under this head differ from those already considered merely in the fact that they are comparatively rare. Whereas milk will practically always sour through the agency of the lactic bacteria, and will always contain bacteria of the peptonizing class, as well as butyric acid bacteria, the following classes of bacteria are by no means sure to be found. Most of them are occasionally the cause of troublesome dairy infections. When they occur in milk, in numbers sufficient to cause troublesome changes, they may always be regarded as coming from some unusual source of contamination to the milk, which may be prevented. While, as we have seen, the lactic fermentation cannot be prevented by any practical means at the disposal of the dairyman, because of the universal distribution of lactic bacteria, these abnormal types of troublesome infections may always be prevented if sufficient care is taken in regard to cleanliness, and they may be checked if the dairyman simply learns from whence the contamination arises. For these reasons, in practical dairying, it is a matter of special importance to understand their sources. Of the types of dairy infections which follow, the first three are the most important.

Slimy Milk.—A sliminess in milk is not an uncommon occurrence in the dairy. It is sometimes produced by a diseased condition of the cow, slimy milk being a common characteristic of garget. In the majority of cases, however, and in all cases where a troublesome dairy infection of slimy milk appears, the trouble is due to bacteria.

In some countries slimy milk is regarded as a delicacy and is actually produced by artificial means. In Norway the people enjoy drinking milk that can be drawn out in long threads

(Taettamoelk), and they have learned to produce it by placing the leaves of a certain plant (*Pinguicula*) in the milk. This plant has not been thought to have anything to do directly with the production of the sliminess, inasmuch as bacteriologists have found upon its leaves an abundance of a certain species of bacterium which produces a sliminess in milk. In very recent times it has been claimed that there is actually a slimy secretion produced by these leaves which causes the sliminess of the milk. Whether this be true, or the more generally accepted belief, that the effect is due to bacteria, cannot at present be stated. In Holland a special ferment is widely used for the purpose of inoculating milk which is to be made into the famous Edam cheese. This ferment produces a slimy fermentation of the milk and at the same time renders it acid. Cheese made from such slimy milk ripens more rapidly and more uniformly than cheese made without such inoculation.

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

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

Slimy milk appears to be produced in dairies by one or two widely distributed bacteria. The most common one seems to be a short rod (Fig. 26), which does not produce spores and is therefore readily killed by heat. This bacterium has been found a common cause of this trouble in the United States. Further, it so closely resembles a slimy milk bacterium described in Europe by Adametz that it is probably to be regarded as the same, and is consequently known by the name given by Adametz, *B. lactis viscosus*. It appears to be a very vigorous organism and, when once present in the milk, will grow so rapidly as to make it slimy in spite of the action of the ordinary lactic bacteria which are present.

FIG. 26.

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

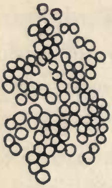
The source from which this troublesome organism is derived has been, at least in part, determined. The bacillus is an inhabitant of water, and the origin of the trouble may be originally some water supply infected with the bacillus. Its continuance in the dairy has been in one case certainly traced to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

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

Bitter Milk.—Next to slimy milk, perhaps bitter milk offers the most trouble to the dairyman. The appearance of a bitter taste is not uncommon and its cause may be manifold. There are two quite different sources of bitter milk to be distinguished. The first is something that affects the cow and causes her to produce milk with a bitter taste. Improper feeding may do this. Sometimes a cow feeds upon herbs, such as lupines, which impart a bitter taste to her milk. Bitter milk is also quite common in a late stage of lactation. In all these cases bacteria play no part. This type of bitter milk may be recognized by the fact that the milk is bitter as soon as it is drawn from the cow, and the bitterness does not increase later.

But in other cases the bitterness is a matter of slow development. The milk, when drawn, tastes as usual, and the bitterness appears after standing for a few hours, increases in intensity, and is at its maximum somewhat later. In these instances the bitterness is produced by certain bacteria which grow in the milk. As in other infections there are quite a number of species known to produce a bitter taste in milk, although probably only a small number are of practical importance. Many of the peptonizing bacteria produce a bitter taste in milk after many days, but they are manifestly not the cause of the common trouble in the dairy. A few species have been described which rapidly produce a very bitter taste, ruining the flavor of the milk in a few hours. One of these is a micrococcus (Fig. 27), and another is a bacillus described by Weigmann.

FIG. 27.



A micrococcus of bitter milk.

The source of these bitter milk bacteria in the dairy has not been made out in many cases. In one carefully investigated instance it has been traced to the bacteria in the milk ducts of the cow, a single cow in a herd being infested with the organism sufficiently to contaminate the milk of the whole herd. The entire isolation of her milk preserved the rest of the milk from the bitter taste. A disinfection of this cow's teats remedied the trouble. In another case the bitter bacterium was believed to come from some turnips which were fed to the cattle. Little as yet is really known upon the matter.

Blue Milk.—A fermentation of milk which causes it to develop a decided color is a very rare occurrence in practical dairying. Although many bacteria are known which can produce such colors, they commonly act so slowly that the milk is usually consumed before they have an opportunity to produce any noticeable effect. Occasionally, however, certain colored milks are found even in ordinary dairying.

The dairy infection longest known to be produced by bacteria is *blue milk*. This trouble is one of special interest, inasmuch as it was carefully studied and traced to its origin in a certain species of bacterium, even before the beginning of the modern study of bacteria. As long ago as 1841 blue milk was investigated by Fuchs with the best methods at his command, and it was successfully traced to the growth of a microscopic organism. This same organism has since been many times isolated, and is one of the well-known bacteria, almost the oldest definitely named species. It is known as *B. cyanogenes*. It is an ordinary bacillus, readily killed by moderate heat since it does not produce spores. Its effect upon the milk is striking, but it does not commonly appear until the milk is from one to three days old. At this time there appear blue patches, which may spread through the milk as it becomes sour, until the whole is a sky blue. The infection is by no means a common one, although occasionally it has been known to produce considerable trouble in certain localities. The remedy against it is simply the ordinary one of thoroughly sterilizing the milk vessels and cleaning the surroundings, which have likely become badly infested with the germs in dairies where the trouble has been noticed. In recent years another species of bacterium has been described having the same property of turning milk blue.

Red Milk.—Red milk is by no means uncommon in the dairy, but the red milk ordinarily seen has nothing to do with bacteria. It is generally the result of a mixture of some blood with the milk, and is due to some injury to the udder, or even to the cows having eaten sedges and rushes which have a large amount of silica in their tissues. Rarely milk may be colored red because the cows have been feeding upon madder root or other plants with red pigment. These, which are the ordinary causes of red milk, have nothing to do with bacteria.

But occasionally the red milk is of the nature of a fermentation and is produced by bacteria. Such effects, of course, do not show themselves in the milk when freshly drawn but appear after some hours. Red milk due to bacteria is, however, very unusual. Several species of microörganisms have been found with this property, the best known being *B. erythrogenes*, a species found by many bacteriologists both in this country and Europe. This species acts so slowly that it is of no practical significance in dairying. When allowed to act fully on milk it makes it blood red. Several other species have been described as a cause of red milk. *B. prodigiosus*, the cause of the so-called "bleeding bread," is said occasionally to produce a red color in milk, especially at its surface. Menme has described a *Sarcina* which produced such a trouble in Rendsburg, Germany. But, while it is common to find old milk showing patches of red due to bacterial growth, an infection of red milk is rare, and the trouble is practically unknown to the milk dealer. It is an experimental rather than a practical fermentation.

Yellow Milk, Green Milk, etc.—Other pigments are occasionally produced in milk. Indeed, milk is such a good medium for bacterial growth that almost any saprophytic bacterium may develop in the milk if opportunity occurs, and pigment-forming bacteria will be sure to produce their pigments in milk. *Orange-colored milk*, *green milk*, *yellow milk*, *amber-colored milk*, *indigo milk* have all been described by bacteriologists as well as *chocolate milk* and *black milk*. In each case the pigment has been produced by bacteria which the bacteriologists have isolated and studied. But these various pigments are produced in milk only when the specific bacterium is growing in the milk by itself and not contending with the ordinary milk bacteria. The pigments commonly appear after many days' growth, and do not develop when the milk is filled with the lactic bacteria. These various pigmented milks are therefore simply experimental but not normal infections. In ordinary dairying they do not occur, and are, at present, of interest only as showing the variety of changes which may occur in milk under the influence of different bacteria.

Alcoholic Fermentation.—The *alcoholic fermentation* of milk is one of some importance although it does not occur normally. Most sugar solutions are liable, under the influence of yeasts, to undergo a fermentation resulting in the production of alcohol. But milk sugar does not easily undergo such fermentation. It is readily converted into lactic acid, but not into alcohol, and, consequently, alcoholic fermentations of milk are produced only by special conditions and special treatment. Nevertheless alcoholic milks are frequently prepared and are articles of diet of no little value, especially for invalids. There are several methods of producing such fermentations. A beverage called *koummys*, originally prepared from mare's milk which readily undergoes the alcoholic fermentation, is now somewhat closely imitated with cow's milk by a simple device. A certain amount of cane sugar is added to the milk and yeast is placed in the mixture. The yeast quickly starts an alcoholic fermentation of the cane sugar, and the souring of the milk that occurs at the same time adds a peculiar character to the product. Another beverage called *kefir* is not so common and requires for its manufacture some special bodies called *kefir grains*. These are hard nodules of various sizes which have the power of starting an alcoholic fermentation in ordinary cow's milk. The origin of these kefir grains is unknown. To-day they are handed from person to person, taken out of the milk after the fermentation and dried to be used again. During the fermentation in the milk they increase in size and new grains may be obtained from fragments of the old ones. These kefir grains prove to be a mixture of yeasts and bacteria (Fig. 28). There are at least two species of bacteria present, and one species of yeast, and all acting together produce the fermentation. The bacteria appear to change the nature of the milk sugar by inverting it, after which it is readily acted upon by the yeasts to produce alcohol. The lactic fermentation occurs simultaneously, producing more or less of a curdling. Kefir is a beverage originating in the Caucasus mountains and is now distributed somewhat widely. It never occurs in the dairy and has no special significance in dairying. Although alcoholic milks are of some dietetic value and of considerable commercial interest, we need not consider them further.

FIG. 28.

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

SUMMARY.

From the facts outlined we may make the following summary: Although milk, when secreted from the milk gland, is sterile, it is sure to become contaminated with bacteria while being drawn into the milk pail. The chief sources of these bacteria are the milk ducts, the outside of the cow and the milk pail. The bacteria which are liable to get into the milk are capable of producing a large variety of changes in it. Ordinarily, some of the few varieties of lactic organisms are pretty sure to get into the milk, and these find the milk such a favorable medium for their growth that they increase rapidly and soon develop lactic acid and cause the milk to sour. They grow so rapidly as to leave all other species behind and, by the time the lactic acid begins to appear, the growth of other bacteria is commonly checked. The other species thus ordinarily do not produce any considerable effect on the milk. Sometimes, however, some of the other varieties of bacteria may be especially numerous, or sometimes other vigorously growing bacteria besides the lactic organisms may get into the milk. If this occurs these unusual species may develop to such an extent as to stop the growth of the lactic bacteria and prevent the milk from souring. These unusual bacteria then produce certain changes of their own in the milk, resulting in the various types of abnormal milk fermentations which are sometimes so troublesome in the dairy. Such unusual fermentations are always due to unusual causes, and by exercising a little care in the way of cleanliness, cleansing the milk vessels, using pure water and allowing no cold water of any kind to stand in the milk vessels, they may generally be avoided. If once in the dairy, they must be removed by finding, if possible, their source, and then using simple means of disinfection and thorough cleansing. Cleanliness must be the dairyman's watchword.

A few most important conclusions should be remembered by every one who handles milk, either as producer or shipper.

1. Practically all the difficulties experienced in the distribution of milk are to be attributed to the great *multiplication* of *bacteria* in the milk.

2. The remedies for these universal troubles are first, *cleanliness*, directed to the cow, the milk vessels and the milker; and second, the application of *low temperatures* as soon as possible after the milk is drawn.

3. Even with the best of precautions the lactic bacteria are sure to multiply in the milk and against them the only defense is *low temperature*.

4. *Preservatives* which are sometimes added to the milk, *boracic acid*, *salicylic acid*, *formalin*, or the various commer-

cial articles with special names, are to be unhesitatingly condemned.

5. The unusual dairy infections, slimy milk, bitter milk, etc., are due to *uncommon* sources of bacterial contamination and may be prevented by proper cleanliness, and removed by the application of disinfection, just as soon as the dairyman can find out the source from which the contaminating bacteria find entrance into the milk.

PATHOGENIC BACTERIA IN MILK.

Most of this immense number of bacteria are perfectly harmless to human health. The presence of lactic bacteria, which form the vast majority in milk that has become a few hours old, is perfectly consistent with the wholesomeness of the milk. Indeed there are some reasons for believing that lactic bacteria are of direct advantage to the human stomach and intestine, aiding in the proper control of digestive processes. At all events they do not appear to be harmful and, except for the fact that they produce a souring, the lactic bacteria are desirable rather than undesirable inhabitants of milk. Their presence commonly prevents the growth of other bacteria which would be more harmful, even pathogenic bacteria being sometimes prevented from growing by the development of the lactic organisms.

But other bacteria are sometimes present in milk, which are of a very decided injury to the consumer. The possibility of disease bacteria getting into milk and by this means being carried to the consumer, has long been recognized. That this is possible is evident, but to what extent it is an actual occurrence is not yet fully settled. A large amount of evidence has been accumulating on this matter in recent years and some very definite facts are known. The number of diseases which are distributed by milk is not large and is chiefly confined to the following:

Tuberculosis.—If milk is drawn from a cow suffering with udder tuberculosis it will be almost sure to contain the tubercle bacilli, and if this milk is subsequently drunken by a susceptible person it may give rise to a case of this dread disease. This matter is more fully discussed in a later chapter and need not be considered here.

Typhoid Fever.—Inasmuch as the cow is not subject to typhoid fever, milk, when freshly drawn, can contain no typhoid bacilli. This disease, therefore, bears quite a different relation to dairy matters from tuberculosis. Milk, if infected with tuberculosis bacilli, contains them when freshly drawn, and secondary infection is a matter of no significance. But fresh milk never contains typhoid bacilli and if they are present in the milk they come wholly from *secondary contamination.* The chief source of such secondary contamination we have already considered when referring to the bacteria in water ; for this chief source is doubtless a well, or perhaps a stream of water, that has become contaminated with the excreta from typhoid patients. But water is not the only source for the contamination of milk with typhoid bacilli. In some cases the milk contamination has been traced to the fact that the milk has been cared for by some one who has been nursing a typhoid patient, or has handled the discharges or the soiled linen from such patients. In one case it is claimed that milk was contaminated by simply standing in the room of such a patient. There is little question that any one who has any intimate contact with a typhoid patient may carry away the germs of this disease and, if employed in the dairy, will be likely to transfer some of them to the milk. It cannot be too strongly emphasized that *no person on a farm who has anything to do in caring for a typhoid patient should be allowed to have any contact with the dairy.* In no other way can the farmer be sure that he is not subjecting the community that consumes his milk to serious danger.

The special reason for danger from this bacillus is the fact that it finds in milk such a favorable medium for growth that, although the milk may have been at first contaminated with only a very few bacteria, these multiply so fast that in a few hours they are very abundant. Hence it follows that an extremely slight original contamination may become a very serious matter, and the few bacteria which originally got into the milk by multiplying may, under favorable conditions, distribute the disease along a whole milk route. This is quite different from the tuberculosis disease, where the bacilli do not multiply in the milk and where only those originally finding their way into the milk from the cow are to be feared.

The danger of distribution of typhoid fever by milk is not simply theoretical but is, unfortunately, only too real. The number of instances where there is abundant proof of such distribution is already very large and each year adds to the list. Indeed, most of the violent epidemics of this disease, which have been successfully traced to their source, are found to have originated in the milk supply. To-day two chief sources of typhoid fever are recognized, viz., the water supply and the milk supply. The water doubtless produces the larger number of cases, but it is rarely the cause of the sudden, severe epidemics which sometimes strike a community, producing many cases within a short time of each other, and soon passing away, leaving traces behind in the form of many deaths. Such sudden epidemics are commonly traceable to the milk supply. For these inflictions the farmer is commonly at fault. Through ignorance, perhaps, he has put his milk under conditions which cause it to become contaminated with the bacilli and disastrous results follow. Wholly unwittingly has he done this, for no one would willingly originate such epidemics.

For these reasons it is impossible for the farmer to be too careful in guarding his milk from a possible contamination from all fever patients. Typhoid fever is, in its early stages, such an obscure disease that it may not be recognized. The only safeguard is to make it absolutely impossible for the drainings from the privy vault to contaminate, by any possibility, the water used in the dairy, and to have milk handled only by healthy individuals. Accidents may of course happen, but if the milk-producer remembers these few facts he will be far less likely to furnish contaminated milk to the city than he has been in the past.

Scarlet Fever and Diphtheria.—There is good evidence that these two diseases may be distributed by milk and that some epidemics are attributable to the milk supply. Very little is however, actually known in regard to the matter. The cause of *scarlet fever* is yet uncertain, and it is not known whether cows can contract the disease and then produce milk already contaminated, or whether, as in typhoid fever, the contamination of the milk is wholly secondary. A few epidemics of scarlet fever have been traced to milk with more or less certainty. It is pretty certain that the milk may become infected with the cause of this disease by *secondary contamination*, and the farmer should consequently take precautions to prevent a case of scarlet fever on the farm from becoming a cause of contamination to the milk.

Diphtheria is produced by a bacillus which is well known. But here again there seems some doubt whether cows have the disease. On the whole the evidence seems to show that the diphtheria bacillus may produce certain local infections in cows and possibly thus infect their milk. It is certain, however, that the milk may become *secondarily infected* through convalescent diphtheria patients working in the dairy and handling the milk. Some instances of diphtheria have been traced to such a cause.

Cholera.—There is good evidence that cholera may be distributed by milk ; but this disease is so rare and so unlikely to occur on the farm, that it is hardly a matter of significance to agriculture.

Diarrhœal Diseases.—Besides the diseases mentioned the only others of importance commonly distributed by milk form that somewhat obscure class of diseases characterized by diarrhœal troubles, especially prevalent in warm weather. Of these by far the most serious is *cholera infantum* which is responsible for the death of so many children. These troubles are less understood than most bacterial diseases. They do not appear to be caused by any single specific bacterium, but are probably due to the excessive multiplication of a number of different bacteria in the milk. That they are due to the milk bacteria is proved by the fact that they occur in greatest abundance at the seasons of the year when milk bacteria are most numerous, that they are chiefly confined to infants fed upon cow's milk, and lastly, by the fact that they become greatly reduced in numbers when the custom of sterilizing or pasteurizing milk is adopted. What kind of bacteria are at fault in the production of these diseases we do not know. Quite a number of bacteria are found in milk which produce poisonous secretions and which may be agents in the production of these obscure diseases. For the purpose of our discussion it is sufficient to state that they are probably to be regarded as the *bacteria of filth*, and that anything which increases the amount of filth in the milk will have a tendency to increase the amount of such troubles, while any advance in cleanliness will have an influence in the opposite direction.

From all these reasons it is evident that it is of the greatest importance, both to the farmer and the public at large that: (1) The number of bacteria in milk should be kept as low as possible; (2) the greatest care should be exercised to prevent secondary contamination of milk with typhoid bacilli, either through water or by contact with infected persons; (3) persons suffering or recovering from scarlet fever or diphtheria should not be allowed to handle the milk ; (4) so far as possible the filth of the cow shed should be kept from the milk, since this is probably the cause of the diarrhœal diseases. Two general rules may be given and easily followed. *No milk from cows that have any form of udder disease should be distributed for drinking purposes. No person suffering from or recovering from a contagious disease should be allowed to have anything to do with the dairy that furnishes the public with milk.*

STERILIZATION AND PASTEURIZATION OF MILK.

The bacteria in milk are clearly the source of a large amount of trouble to the dairyman. It was inevitable that the endeavor should be made to get rid of them. It is easy to add to the milk various chemicals which will prevent the growth of bacteria and consequently preserve the milk. Many such substances have been used. There are quite a number of "preservatives" on the market, which are sold to the farmer to assist him in preserving his milk. The basis of most of these is either boracic acid, salicylic acid or formalin. All of these substances are injurious to man and their use should not be allowed in preserving an article so freely used as milk. Such "preservatives" are to be unhesitatingly condemned.

A more legitimate method of obtaining the same result is by the use of heat. All bacteria are destroyed by heat and it is therefore possible, by this simple means, to kill the living organisms in milk and thus preserve the milk from their subsequent action. This has given rise to two chief methods of treating milk, *sterilization* and *pasteurization.*

To understand the meaning of these two processes it is necessary to notice at the outset the ends aimed at in the treatment. They are three in number.

1. It is desired to reduce the number of bacteria present in the milk so that it can be preserved longer without souring or

undergoing any other deleterious change. This appeals to the producer, the distributer and the consumer.

2. It is desired to destroy the pathogenic bacteria which may be present so as to remove all danger to health in drinking the milk.

3. It is sometimes desired to remove the bacteria already present so that others may be added to produce certain desired effects, as in the ripening of cream (see page 231).

There is no difficulty in accomplishing *all* of these purposes by the use of heat, for a sufficiently high temperature will destroy all bacteria and attain all the ends at once. The practical application of heat to milk, however, presents certain important problems not easily solved.

Sterilization.—The most obvious suggestion would be to heat the milk to a temperature which would destroy *all* bacteria at once. This is perfectly possible, but, owing to the fact that milk always contains spore-bearing bacteria, it requires a high temperature for the purpose. A temperature of boiling water will not destroy the spores, and for this purpose it is necessary to heat the milk to several degrees above boiling. This involves the use of special apparatus, in which bottles of milk can be inclosed in special vessels, subjected to steam under pressure, and subsequently hermetically sealed while still within the closed vessels. Such apparatus can be used only where there is a steam supply on hand and it inevitably makes the milk rather expensive. The milk so prepared is, however, supposed to be germ-free and consequently, to keep indefinitely. Unfortunately, it has been shown that even these temperatures of superheated steam do not always destroy *all* the spores, and some of the samples of milk thus treated will subsequently undergo fermentative changes due to the spores that are left. Further it has appeared that these later changes, due to the resisting spores, are frequently such as do not change the appearance of the milk to the eye, so that such milk, though containing bacteria in quantity, will be drunken as pure milk. The fermentation has, moreover, filled the milk with bacterial products of more or less injurious nature, and consequently the drinking of such milk is far worse than drinking fresh milk which is most likely supplied chiefly with lactic bacteria. The sterilized milk, if it does retain a single spore, is, therefore, more dangerous than ordinary fresh milk. For this reason, among others, this practice of treating milk to superheated steam for the purpose of *absolute sterilization* is not popular and is clearly becoming less so. It is used to-day less than it was a few years ago and is bound to disappear.

A modified form of sterilization, which consists in simply boiling the milk, has been far more extensively adopted. This was recommended by physicians long before its real significance was understood and has been very widely used in all civilized countries. Its simplicity of application explains the reason for its popularity. It is only necessary to place the milk upon a stove and allow it to come to a boil, and the end is reached. Such treatment of milk has been very widely recommended, and has been put into use very extensively in the countries of continental Europe. In some of these countries very little milk is used without such previous boiling, and even the children are taught in school that it is dangerous to drink milk without such treatment. The purpose aimed at in this wide use of boiling, which is commonly, though not properly, called *sterilization*, is simply to destroy the danger of distribution of disease, by the destruction of the pathogenic bacteria. This purpose is certainly achieved, for the boiling temperature does destroy all the pathogenic bacteria which are likely to be in milk, since none of these are spore-producers. Boiled milk thus offers a food which contains no pathogenic bacteria.

But there are several practical objections which have arisen against this method of treatment.

1. The bacteria *spores are not destroyed*, and such milk will subsequently undergo a fermentation even more surely than if treated by superheated steam. This is, however, of little importance, since the milk which is simply boiled will not be kept for weeks, as will the "sterilized" milk put up in bottles, and if it is used within a day or two these spores will not have had opportunity to do any injury before the milk is consumed.

2. The milk acquires the well-known *taste of boiled milk* which is, to most people, unpleasant. People are willing to take boiled milk upon an emergency as an invalid diet, but few will continue its use. The taste is not enjoyed, and rather than drink boiled milk the majority of people will give up drinking milk altogether. This is certainly not desirable since milk forms one of the best and cheapest foods. Any treatment which greatly reduces the amount used is in itself undesirable, and the practice of boiling milk certainly does greatly reduce the amount used.

3. Milk treated to a temperature as high as boiling becomes somewhat *less easy of digestion and assimilation*. The heat produces several important changes which result in its being less easily handled by the digestive organs. The difference is not very great and a strong individual is able to handle such milk well enough; but delicate children and invalids are not so well nourished upon boiled milk as upon raw milk.

4. Boiling milk is a treatment which has *not proved practical to adopt on a large scale* at a central source of supply. It offers, therefore, no assistance either to the producer or to the distributer in enabling him to furnish milk which, since it keeps longer, gives greater satisfaction.

For these various reasons the practice of treating milk with a boiling temperature has not increased in recent years as it was at first believed it would. It is certainly very extensively used in private families, and is still widely recommended as a means of guarding against disease distribution through milk.

But at present, and for some time past, there has been a tendency to recommend a different method of treatment, which is theoretically far less satisfactory, but which practically proves more useful. This method is called *pasteurization.*

PASTEURIZATION.

This treatment, originally devised by Pasteur for the treatment of wine, consists in heating the milk to a much lower temperature than boiling and then cooling rapidly. The temperature used has varied considerably, from 140° F. to 180° F. In choosing a temperature two factors have been taken into consideration.

1. It is desired in this treatment to use a temperature which produces the minimum change in the chemical character of the milk and consequently does not give the taste of boiled milk. This has led to the endeavor to find the highest temperature to which milk can be subjected without acquiring these tastes. This temperature has been found to be not higher than 156° F.

2. It is desired to use a temperature which will destroy most of the bacteria in the milk. That such temperatures will not destroy all the bacteria is evident enough. But we must remember that the two chief objects to be accomplished are the increasing of the keeping quality of the milk and the removing of danger from disease bacteria. Will these low temperatures produce these two desired results?

Such moderate temperatures certainly do increase the keeping quality of the milk. While a temperature of 156° F. does not destroy spores, it does very largely destroy the active, non-spore-bearing bacteria. Now, as we have seen, the lactic bacteria, which are the chief causes of trouble in milk, contain no spores and they are consequently very largely killed by such moderate heat. Hence the total number of bacteria in the milk is immensely reduced and the milk has its keeping quality much increased. Experiment has shown that milk thus treated will frequently remain good two days longer than similar milk not pasteurized. Thus one purpose of the heating is certainly accomplished, for, although the milk will not keep indefinitely, it will remain sweet so much longer as to render it easy to handle.

Whether such temperatures destroy disease bacteria so as to remove all danger of distribution of disease has been the subject of much experiment and dispute. Of the diseases which we have mentioned above as liable to distribution by milk there is only one in regard to which there has been any disagreement. It is admitted on all sides that typhoid and diphtheria bacteria are killed by this heat (156° F.); the same is probably true of scarlet fever and certainly true of cholera. The tuberculosis bacillus, however, it has been claimed, will stand this heat without injury, and hence, in order to be sure of destroying these organisms, it is necessary to heat the milk to a temperature of 185° F. At this temperature the cooked taste and chemical changes begin to appear. Over this question of the temperature necessary to destroy the tubercle bacillus there has been much experimentation and dispute. Into the details of this matter we cannot enter. The present conclusion, which is the result of the most recent and most careful experimenting, is happily a satisfactory one. It appears that if the milk is heated in such a manner as to avoid the formation of a scum on its surface, a comparatively low temperature is sufficient to destroy the virulence of the tuberculosis bacillus. A temperature no higher than 140° F., continued for twenty minutes, has been found sufficient to reduce the virulence of the tubercle bacillus so much that milk which originally contained the bacilli is rendered harmless. This temperature is considerably below that at which the chemical changes in the milk take place. Hence it follows that milk may be thus deprived of its danger of distributing disease germs without having its physical or chemical nature noticeably changed. Such milk, when cooled, cannot be distinguished from fresh milk. It must be stated that the efficiency of such a moderate temperature in rendering harmless the tuberculosis bacillus is not fully admitted by all observers.

This conclusion is of immense value and this method is of great practical utility. The value of pasteurization is becoming rapidly recognized and this method of treatment is becoming widely adopted. The advantages of pasteurization lie in the following facts:

1. It produces milk which cannot be distinguished from fresh milk and will be used as freely.

2. It increases the keeping property of the milk, but not to the extent of leading the consumer to believe he can keep it indefinitely. The consumer is thus forced to use it up before the spore-bearing bacteria get an opportunity of multiplying sufficiently to produce the injurious secretions which occasionally render sterilized milk dangerous. The very fact that the method does not destroy all bacteria is a safeguard.

3. It removes the danger of distributing pathogenic bacteria. This is certainly true of the typical diseases mentioned. Whether it similarly removes the danger of diarrhœal diseases, not dependent upon any known specific bacteria, is not yet positively known by experiment, inasmuch as we do not know the actual cause of the diseases. But the practical experience of physicians tells us that pasteurized milk acts as efficiently as sterilized milk in reducing these diseases.

4. This method of treatment is perfectly applicable upon a large scale. Already pasteurization has been adopted in some places by large dairy companies whose milk is distributed around cities, and the plan is becoming more popular each year. At the present time there promises to be, in the near future, an extensive adoption of this treatment into at least a part of the milk supply of large communities. Pasteurizing machines which can treat a thousand quarts at once are easily

made and readily handled, and it is perfectly feasible thus to treat a very large quantity of milk for general distribution. Particularly has this treatment been useful in preparing *cream* for shipment. Cream thus treated will keep several days and may readily be shipped for a thousand miles to find a purchaser. At the present time large amounts of cream are thus marketed in this country. Pasteurization is thus a practical success and, according to the present outlook, it promises to displace the process of sterilization. While sterilization is theoretically better, since it destroys all bacteria, pasteurization is the more practical method of treatment. It seems as if an extension of pasteurization might make it possible, in the future, to handle this extremely perishable product in such a way as to avoid most of the troublesome phenomena due to bacteria growth, and to remove *all* dangers of disease distribution. Pasteurization seems at present to be the most promising method of treating milk for nearly all purposes.

The individual farmer can hardly apply this treatment to his milk, inasmuch as it demands special apparatus and can be very much better performed in a large central station. The milk industry is one in which far better results can be attained by a concentration of interests than by keeping them separated into numerous isolated farms. A central organization can easily and cheaply collect milk, and properly treat it so as to produce the best product. Such a central organization, which will first use the centrifugal force to remove the dirt, and then pasteurize its milk, will furnish a product as nearly perfect as seems to be possible with our present knowledge.

Within recent years a new method of handling and shipping milk has been adopted in some of the northern countries. The milk is frozen into ice, and in this form it may be transported to any distance without trouble. Upon being subsequently thawed out it is practically as good as fresh milk. This industry is quite new and its practical value can not yet be determined.

CHAPTER X.

BACTERIA IN BUTTER-MAKING.

TURNING to the consideration of bacteria in butter-making, we find a very different story ; for the bacteria, instead of being the dairyman's foes, are here his allies. It is true that here, as well as in milk, the presence of too large a number of unusual kinds of bacteria may produce undesirable results ; but it is also true that the butter-maker always, even though unconsciously, makes direct use of bacteria when he subjects his cream to a process, almost universally adopted in butter-making, called the "ripening" or, in Europe, more commonly called the "souring" of cream.

It is well known that butter is made from cream which has been separated from the milk, either by gravity or centrifugal force. It is subsequently agitated vigorously (churning) until the fat drops are shaken together and adhere in masses large enough to be removed bodily from the liquid in which they float. But in most processes of butter-making the cream is not churned immediately after it is separated from the milk. It is allowed to lie in a moderately warm vat for a period of twelve to twenty-four hours, or even longer, to undergo a "ripening," and it is not churned until after this ripening. It is true that in some places there is a demand for what is known as *sweet cream butter*, which is simply butter made from fresh cream without ripening ; but such a demand is very limited and the vast majority of butter is made from ripened cream.

The custom of ripening cream is an old one, doubtless as old as the process of butter-making. Upon a farm where the amount of cream is small, it is always necessary to allow it to accumulate for some days, till there is sufficient for a proper churning; during this period it is sure to undergo ripening without any intention on the part of the farmer. This necessity started the process of ripening. In ordinary farms the cream is left to care for itself, and it is sure to be ripened by the time there is enough to churn. But the centralization of butter-making into creameries, where large quantities of cream are daily handled, has put a new aspect upon the problem of cream-ripening. The ripening will no longer care for itself, but must be carefully attended to by the butter-maker, and, since it is conducted on a large scale, it has demanded new apparatus and new devices. The necessity for some accurate means of controlling the ripening is becoming more and more apparent with each step toward concentration of the butter-making. The farmer may perhaps allow his cream to care for itself since his product is so small. But such a plan would ruin a creamery where there are thousands of pounds of butter made each day. Only as the ripening can be controlled is concentration of butter-making successful.

There appear to be three chief purposes in cream-ripening.

1. Ripening is believed to *increase the yield* of butter, for it has been found by numerous experiments that the return in the form of butter is larger from cream properly ripened than from unripened or improperly ripened cream. This is true, at all events, for gravity cream; it is less significant, and perhaps not true, for separator cream. In a large dairy business this is, of course, highly important, for a saving of even a fraction of a per cent. means much to a large creamery in the course of a year.

2. It is thought that butter made from properly ripened cream has better *keeping qualities* than that made from cream improperly ripened. This factor, however, is one of no very great importance, and, moreover, is perhaps a little uncertain.

3. By far the most important purpose in cream-ripening is the production in the butter of a desirable *flavor* and *aroma*. It has been demonstrated over and over that butter made from unripened cream lacks the peculiar flavor and aroma which are characteristic of high-grade butter, and that these characters appear as the result of the ripening. If the ripening is not satisfactory, the flavor and aroma of the butter are sure to be inferior. This is the chief reason why cream-ripening is so universal.

The importance of this factor in butter-making for our creameries is very great indeed, more so than is commonly appreciated by the butter-makers themselves. When we remember that the market price of butter depends largely upon the flavor, we can easily appreciate how much the butter-maker is dependent upon this process of cream-ripening. Butter without flavor, or with bad flavor, brings a price in the market which hardly pays for the making, while a product with a good flavor and aroma will sell for at least three or four cents more a pound, and the exceptionally fine-flavored product of special creameries brings a fancy price two or three times that of poor butter. The flavor will add at least two or three cents, and sometimes one-third or one-half to the price which could be obtained for poorly flavored butter or for butter without flavor. In ordinary dairying, then, the success or failure of a creamery business depends, in large measure, upon this factor. A creamery which fails to ripen its cream properly fails to obtain a desirable flavor in its butter. It inevitably procures a lower price for its butter and may hardly meet expenses; while a neighboring creamery, that is more successful in its cream-ripening, obtains a good-flavored product, and, consequently, a price for its butter which makes the business a financial success. This matter is of more significance to-day than in earlier years, because our butter-making is coming to be concentrated in large creameries.

THE CAUSE OF CREAM-RIPENING.

The chief agency in the ripening process is the growth of bacteria. Many of the bacteria in the milk collect in the cream, and, during the ripening period, have an opportunity to multiply rapidly. The cream is kept at a temperature which favors their growth, and at the end of the ripening they are present in surprising numbers. Two actual experiments in cream-ripening will best indicate the growth of bacteria in the ripening of cream. The two samples of cream which gave the following results were collected in cold winter weather, when the number of bacteria was small at the outset and the ripening very slow.

No. of Bacteria per c.c.

	At beginning of ripening.	Half ripened.	Fully ripened.
1st expt.,	309,000	300,000,000	1,500,000,000
2d expt.,	44,000	303,000,000	1,300,000,000

The cream giving the highest numbers was somewhat over ripened and the numbers somewhat large. Normally ripened cream usually has from 300,000,000 to 600,000,000 bacteria per c.c.

These numbers are inconceivably great, far surpassing the numbers found in sewage, or indeed, in *any other natural material where bacterial analyses have been made.*

Such rapid multiplication of bacteria clearly shows that the chief feature of cream-ripening must be the growth of bacteria.

Whether this process is wholly one of bacterial growth is not certain. Babcock and Russell have shown that certain enzymes are present in milk, and that they play an important part in the ripening of cheese. If such enzymes are present in the cream they may have a share also in cream-ripening. At present, however, we have no evidence of this, but the evidence of the agency of bacteria in the process is abundant and conclusive. At all events, no one questions that the flavor produced by the ripening of cream comes from bacterial action and not from the action of unorganized ferments.

This extraordinary growth of bacteria cannot occur without producing great changes in the nature of the cream. The changes are in general similar to those already mentioned as occurring in milk. The cream sours, thickens, and acquires a pleasantly sour taste and characteristic odor. Butter made from such cream is delicately flavored and of high quality.

There is no difficulty in understanding how the growth of bacteria in the cream accomplishes the purposes aimed at in the ripening. Churning consists simply in shaking the cream until the globules of fat are brought into contact with each other. As soon as they come in contact they fuse together and, as the agitation continues, the masses of adhering globules become larger until they are finally large enough to be removed from the liquids as grains of butter. Anything which prevents the fat globules from coming in contact with each other delays the churning and decreases the yield. Now in fresh cream the fat globules do not float around freely in the liquid but appear to be held by some albuminous substance. Apparently they are partly entangled in a very minute, fibrous mass, somewhat similar to the fibrin of the blood, and this has a restraining effect upon the adhesion of the globules. This fibrin is softened by the action of the bacteria so that, after ripening, the fat particles are more free to unite, the churning is made easier and the yield of butter larger. When cream is separated by the separator instead of by gravity this fibrin material is removed from the cream by the force of the centrifugal motion. Hence separator cream does not need ripening for the purpose of making the churning easier, and it churns readily enough even when fresh.

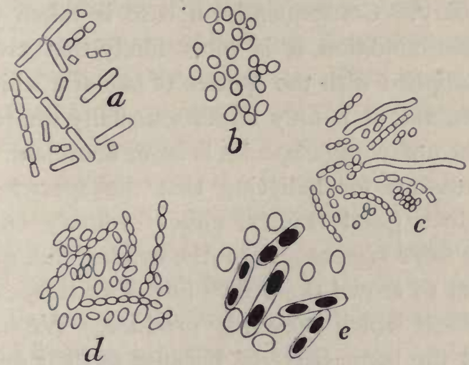
But separator cream does need ripening for the purpose of developing the flavor, which is the most important result of ripening. Sweet cream butter has very little taste and the chief purpose of ripening is to obtain the butter flavor. It is evident that the flavor is due to the products of the bacterial growth during ripening. The enormous multiplication of bacteria must produce a considerable number of chemical changes in the cream, the nature of which has been only partly determined. One change is, of course, the formation of lactic acid. A second class of changes produces products with high flavors and odors. Of the nature of these flavoring products we know at present practically nothing. They are certainly something different from the acid, since lactic acid has no odor, and its flavor, being simply sour, is not the flavor of ripened cream or butter. We do not even know whether they are the by-products of chemical decomposition or are excretions from the bacteria. But whatever be their nature, and whether they be by-products or excretions, they are present in small amount only, though strong enough to impregnate the butter with the characteristic butter flavor.

THE EFFECT OF DIFFERENT SPECIES OF BACTERIA.

We have already noticed that different species of bacteria produce very different types of chemical decomposition in milk. All of the varieties of bacteria which get into the milk are likely to get into the cream. When cream is collected for a large creamery and comes from many patrons, there are sure to be in it large numbers of different varieties of dairy bacteria. Each patron contributes his quota, and the resultant mixed cream is found to contain many different species. Each of these may be expected to have its effect upon the cream during the ripening, and the resulting butter will have a different character where the cream is ripened with different bacteria. Indeed, actual study shows that different species of bacteria, when allowed to grow in the ripening cream, produce very different types of butter. Some species produce *bitter* butter, others *tainted* butter, others *insipid* butter and others a *strong* *odor*, almost like that of putrefaction. Some produce a *tallowy* butter, others *turnip*-tasting, or *putrid* butter. In general it is the *lactic bacteria* which produce the desired results, while other types, if excessively abundant, give rise to the abnormal flavors (Fig. 29).

Careful study with the common species of dairy bacteria has shown that the majority of the species appear to have no especial influence and may grow in the cream without any marked effect upon the butter. Some of them, however, produce changes in the cream which give rise to the products imparting the desired flavor and aroma. Others produce changes which give rise to an abnormal ripening, resulting in an improper consistency in the cream, and more especially in unpleasant flavors and aromas. These, of course, the butter-maker desires to keep out of his cream, for it is these, in large degree at least, which produce the inferior qualities of butter coming from improperly ripened cream. According to our present knowledge, the majority of the species of dairy bacteria may be looked upon as *neutral*, so far as concerns their

FIG. 29.



Several varieties of bacteria from ripening cream: *a* and *b* produce unpleasant-tasting butter, *c* and *d* produce good butter, *e* has no effect on butter.

effect upon butter flavor. A few may be looked upon as *favorable* and a few as *unfavorable*.

Since the bacteria in cream are so varied in their action, it may, perhaps, be a matter of little surprise that the ordinary process of cream-ripening is so likely to give a good result, and that without any artificial means of controlling the species of bacteria, a butter-maker can so commonly obtain a good product. The reason for this is apparently three-fold. In the first place, although the number of species which produce a favorable cream-ripening is apparently not so very great, they are species which are most common around an ordinary well-kept dairy. Consequently, if care is taken to keep the dairy in good condition, it is most likely that cream will be especially supplied with the species of bacteria which produce good results, and it is only under unusually improper conditions that the unfavorable species become abundant. Secondly, there are reasons for believing that the species of bacteria which produce good results, under ordinary circumstances prove to be *more vigorous* than the others and grow so rapidly as either to crowd the others out of existence or to counteract any effect which they may produce. We have already noticed how the lactic bacteria, because of their greater vigor, grow so rapidly in the milk as to overcome the other bacteria. This occurs also in cream and to such an extent that cream, which at first has only a few lactic bacteria, may, at the end of ripening, contain over 99 per cent. of these organisms. This is a very significant fact in cream-ripening. Thirdly, the temperature used in ripening cream is such as to stimulate the growth of the favorable species, while it checks the growth of many other bacteria. Thus, the process of cream-ripening is commonly satisfactory. But although this is the case, butter-makers, the world over, are ever and anon troubled with an improper type of cream-ripening, and this makes it very desirable that, if possible, some means of controlling this process should be placed in their hands.

CONTROL OF CREAM-RIPENING.

The butter-maker has no control over the kinds of bacteria which find their way into his cream. In a creamery, he must take what the different farmers furnish him in their cream. If the cream chances to have a majority of species which can produce good results his butter will turn out well, but, if the cream chances to have more species unfavorable to the development of proper flavors, his butter falls off in quality and no care he may take in the butter-making can raise the flavor. It is evident from these facts that if there could be devised a means by which the butter-maker could be assured of his cream having the proper species of bacteria, it would result in a much greater uniformity in the product. That such a method is possible seems to be suggested by the success with which a similar device has been adopted by brewers in regulating their fermentation. In past years the fermentation of malt was liable to similar variations, due to irregularities in the microörganisms (yeasts) which produced it. The introduction of the microscope and pure cultures of yeast have revolutionized brewing. Bacteriologists have thought that the same may be possible and feasible in butter-making.

About ten years ago it was suggested that an artificial means of controlling this process might be devised. Professor Storch of Copenhagen first conceived that it might be possible to furnish butter-makers with cultures of the proper species of bacteria to add to their cream for the purpose of ripening, somewhat as yeast is used in brewing. This experimenter was one of the first of dairy bacteriologists, and not only conceived the method but put it into practical operation in Denmark. His method consisted, (1) in treating the cream by the process of pasteurization, at about 165° F., for the purpose of destroying most of the bacteria that might be present, and (2) in adding to it a properly prepared culture of bacteria, whose value in producing a good flavor had been determined by experiment. This method is, of course, logically, perfectly satisfactory; for, since pasteurization destroys most of the bacteria present in the cream, it follows that the ripening will be produced by the species of bacteria of which a *pure culture* has been added. Professor Storch was soon followed, in north Germany, by Professor Weigmann and others, and the method adopted in Copenhagen was soon extended more or less widely in north Germany and Denmark. In Denmark it is now used almost universally, and in north Germany quite widely, in general dairying. Although it has occasionally been adopted elsewhere, it can hardly be said to be used in any other countries, except, incidentally, in scattered creameries. It is rarely used elsewhere in ordinary dairying, although resorted to occasionally for the purpose of remedying butter "faults."

In the United States the use of pure cultures for cream-ripening has had a somewhat different history. It was introduced to dairymen shortly after its development in Copenhagen; but for some time little attention was paid to it, so that it was hardly brought to the notice of the ordinary butter-maker. Our butter-makers have not been in condition to pasteurize their cream. For pasteurization there is needed special apparatus, and the process involves considerable expense. For this reason the method as suggested in Copenhagen was not very feasible in this country. About five years ago a slight change was made in the process. In order to bring the subject more widely to the attention of dairymen, our butter-makers were advised to use the cultures *without previously pasteurizing the cream.* This, of course, is an illogical method, since the cream is already filled with bacteria, and the addition of a new culture could hardly be supposed to give entirely satisfactory results. With this change in the method of the use of pure cultures, our butter-makers were willing to try them, and in a short time American butter-makers learned of their meaning and experimented with them quite widely. At the outset too great expectations were raised as to the value of such cultures, and dairymen were naturally disappointed in the results. As a consequence, the use of such commercial cultures has not advanced very much in the last few years. But one thing has resulted from the attempt to introduce the use of pure cultures for cream-ripening among American dairymen. Nearly all butter-makers, at least all who have a pride in the quality of their product, have learned of the absolute necessity of greater attention to the process of cream-ripening, and nearly all have adopted such means as are easily within their power for *controlling* this ripening.

PREPARATION OF PURE CULTURES.

The first step in the preparation of *pure cultures* must be taken by the bacteriologist, who obtains, from some lot of milk or cream, a certain species of bacterium which experiment has indicated produces the desired results. Different bacteriologists have proceeded differently in searching for such a culture, and the various "cultures" placed on the market show much variation. They are commonly *pure* cultures, *i. e.*, consist of a single species of bacterium, but in some cases they consist of mixtures of several species. In practically all cases a lactic bacterium is the basis of such cultures. It must be admitted that none of the cultures which have been used have been found to be perfectly satisfactory, for, although they ripen the cream normally and produce a proper amount of acid, they all appear to be lacking in flavor.

The bacteria as obtained from the bacteriologist are in the form of a pure culture but in small quantity, insufficient to use directly in the cream, and they must consequently be "built up." For this purpose the butter-maker sterilizes a small amount of milk by heat, and, after it is allowed to cool, the commercial culture is placed in it. The mixture is then allowed to stand for about one day, when it is poured into a larger amount of milk or cream, for the purpose of still further increasing its bulk. The bacteria grow rapidly in the milk and there is thus obtained a considerable quantity of typically ripened milk or cream, thoroughly filled with the bacteria of the commercial culture. The cream thus ripened is now used as a "starter" in the general mass of cream to be churned, in proportions which vary with the different butter-makers, according to experience, but which range from 2 to 10 per cent.

I. THE USE OF PURE CULTURES WITH PASTEURIZATION.

Two quite different methods of using these *starters* have been employed. The first method involves the pasteurization of the cream before the culture is added. The cream is pasteurized at a temperature of 155° F. to 165° F. for a few moments and then cooled. This temperature destroys most of the bacteria present and prepares the cream so that the pure culture, when added, has an opportunity to grow unhindered by the numerous bacteria which were originally present.

This was the original method of Storch, and is that which has been quite generally adopted in northern Europe. Starting in Denmark it has, in that country, become very popular, and at the present time practically all the butter of that great butter-making country is made by the use of pasteurization, followed by inoculation with commercial cultures. The object of this is two-fold. In the first place the pasteurization of the milk received at the creamery is generally adopted for the purpose of decreasing the danger of distributing tuberculosis among the farms. There is a law in Denmark requiring the pasteurization of all milk which comes to a creamery. This law has been introduced as an attempt to check the spread of bovine tuberculosis which has become very prevalent in Denmark. The pasteurization makes it necessary to inoculate the cream subsequently with some form of culture. Secondly, the process seems to be very favorable in its results upon the character of the butter. Through a series of years a careful record has been kept, and it has been found by those who are cognizant with the butter industry that the use of pasteurization, followed by inoculation with commercial cultures, has produced a very general improvement in the character of the butter made. This improvement is a general one, the butter made throughout the country being more uniformly good. There is thus a greater uniformity, with fewer examples of poor butter. It is also the belief, though this is difficult to prove, that the quality as well as the uniformity of the butter has improved. That the process is generally satisfactory, and is believed by Danish butter-makers to be of practical value, is clearly enough proved by the fact that it was adopted by over 95 per cent. of the butter-makers of that country, even before the introduction of the law that has forced this method upon them. That the butter so made is exceptionally good is shown by the fact that it has been in greater demand in England than any other grade of butter. For Denmark pure cultures have been a decided advantage.

Nowhere else has this method of using pure cultures been adopted so extensively as in Denmark. It is hardly known in southern Europe, is not used in England and has been only slightly adopted in the United States and Australia. The improvement in the product of the Denmark butter-makers has been decided, but, for various reasons, other butter-making countries have not found the use of pure cultures with pasteurization of sufficient value to warrant their extended adoption. But, although the other countries have not adopted these methods as a regular procedure, this method of pure cultures is coming to be quite widely used as a means of getting rid of certain dairy troubles. All creameries are, at times, subject to the occurrence of unpleasant tastes in the butter, due to improper ripening. Under these circumstances butter-makers are now recognizing that the pasteurization of the cream, followed by the use of pure cultures for ripening, is a valuable aid in remedying the troubles.

The experiment stations have tested the process in the United States with a considerable degree of accuracy, and, while there is some difference in results, they do not find such an improvement in the character of the butter as to warrant the use of the process for the American taste. The butter made by the process in this country, according to these tests, does not grade very high. The experimental tests certainly give at present no good ground for recommending to the American butter-maker the adoption of pasteurization and pure cultures. There can be little question that the chief cause for this lies in the fact that the American taste demands a butter with a rather strong flavor. Denmark butter is sold chiefly in England, where the people are accustomed to a very mildly flavored and slightly salted butter. There is no question that butter made from pasteurized cream, even when inoculated with a culture, is less highly flavored than butter made from unpasteurized cream. For the American public, demanding a highly flavored product, the process of pasteurizing with pure culture inoculation does not seem very well adapted. This will be true until either the bacteriologists produce a pure culture of bacteria capable of giving higher flavors than any which they have yet offered butter-makers, or until the public taste is satisfied with a milder flavored butter.

PRACTICAL RESULTS.

When attention is turned to experimental tests which have been carried on in Europe as to the comparative value of butter made with and without pure cultures, it is observed that the process in question does not apparently produce butter quite so good as the very best type of butter sometimes produced in dairies that do not use this process. In other words, the best quality of butter is made without pasteurization or pure cultures. However, these exceptionally fine grades of butter do not represent the ordinary product, and the average is judged to be somewhat inferior to "culture butter." While then, the highest grade of butter is not improved, the general average of the butter made by the process in question is higher than the general average of butter made without cultures. But it has become very clear that the use of pure cultures will not make the highest quality of butter if the cream is of poor quality. It is just as desirable to have good cream now as it was before the use of pure cultures. While the quality of butter made from poor cream may be improved by the use of pasteurization followed by pure cultures, some effects of the inferior quality of cream will remain. Experiments have shown that the use of pasteurization with pure cultures will not correct the errors due to slovenliness in dairy matters. It is just as necessary that care should be taken in all details as it was before cultures were adopted.

In general dairy practice, the results have been, perhaps, more favorable than in experimental tests. This is doubtless due to the fact that the dairy has a great variety of difficulties to contend with which can be more readily eliminated in experimental tests than in general practice. If the use of pure cultures, therefore, is of any value in correcting errors that come from carelessness, it is very clear that the use of such cultures in general practice, where carelessness is to be expected, would be more valuable than would appear from the experimental tests where carelessness can be eliminated. Several years' use of such cultures have shown that an advantage is gained in at least three different points.

First: this method enables the butter-maker to handle his cream much more easily and uniformly. He can regulate the ripening in such a way that his cream will always be of a certain grade of ripeness at a certain time of day; for a little experience tells him how much of his culture, under proper conditions, should be added to the cream to produce the proper grade of ripening at the particular time when he desires to churn. The advantage of this is evident to any one who has managed a creamery, and in itself is regarded by many as of sufficient importance to warrant the use of cultures, entirely independent of any other effects.

Second: there has resulted, as already mentioned, a decided increase of uniformity in the grade of butter produced. The butter-maker can depend more certainly upon producing butter of a certain grade, month after month, than he can without commercial cultures. It must not be understood, however, that there is absolute uniformity, for variations will appear. But general dairy practice in Europe has certainly warranted the conclusion that the use of pasteurization and pure cultures gives a greater uniformity than the making of butter without their use. In addition to this increase in uniformity, it is a general belief on the part of the dairymen who have used the cultures, and of those who have tested the butter in countries where they are widely used, that there is an improvement in the average quality of the butter as well as in its uniformity.

Third: it has become pretty definitely agreed that the flavor of butter is improved by the use of such cultures. It is somewhat difficult to obtain definite proof of this, owing to the uncertainty of scores in butter tests. Probably the most striking indication of the value of these methods is the fact that the use of pasteurization and pure cultures of bacteria is being adopted by the manufacturers of oleomargarine products. (See p. 248.) It is evident enough that this method of the use of bacteria cultures would not be adopted, at such an expense, by the manufacturers of oleomargarine products if they had not satisfactorily proved to themselves that an improved flavor is thus obtained. In butter-making the business of individual makers is usually so much smaller than that of oleomargarine manufacturers, that the necessity of the new process has not been so generally felt, and certainly has not been so widely adopted.

It has been questioned whether butter made in this way has as good keeping qualities as that of ordinary butter. This matter is not as yet settled, since some experiments seem to indicate that culture butter does not keep well, while others reach the conclusion that the butter keeps perfectly well. Certainly the difference in the keeping quality is not great. But the butter-makers say it makes little difference, since their product is consumed almost immediately.

2. THE USE OF STARTERS WITHOUT PASTEURIZATION.

Pasteurization of cream involves a great deal of trouble and expense, and American butter-makers have, as a rule, been unwilling to adopt it. They have, however, felt the necessity of some means of controlling the ripening, and have, therefore, worked quite widely upon the plan of using special starters, but without pasteurization of the cream. This is undoubtedly a makeshift and is, logically, an incorrect process. The cream which they obtain is already well inoculated with bacteria, and it is clear enough that to obtain the proper results from the inoculated culture, the bacteria already present should be destroyed. But we have already seen that the bacteria present at the end of the ripening are widely different from those present at the outset. Some species are much more vigorous than others, and if present, even in small quantities, will increase so rapidly as very soon to surpass all others, in numbers. It is, therefore, theoretically possible that a pure culture of vigorous bacteria added to *unpasteurized* cream would hold its own and soon outnumber the other bacteria present. At all events, butter-makers in the United States have to a considerable extent adopted this method, with what seems to be at least a partial success. It must be remembered that, in using this method, success depends upon inoculating the cream with a large quantity of an exceptionally vigorous organism, the effect of which shall be greater than the effects of those already present. For this reason we can not expect the uniformity which would come from the use of pasteurization. We should anticipate that, whereas the process might be useful in many, perhaps the majority of cases, there would be frequent instances where the method would fail.

Results.—The results from the use of cultures without pasteurization are not uniform. If we try to compare the results with those of *spontaneous ripening*, *i. e.*, without the use of "starters," we can obtain our data only from the experiences of butter-makers. We can hardly expect to learn much from data given by individual experimental tests, since the question is not, whether the method is useful in any individual case, but whether it is of any practical value under the widely diversified conditions of general dairying. Experimental tests upon this subject must, therefore, always be regarded as of less value than the general verdict of dairymen. The use of some kind of "starters" in this way has been more and more widely adopted in the last five years, until it is probably true that a majority of the better creameries and dairies have adopted this process. Butter-makers emphasize in dairy papers, over and over again, the necessity of good starters. The advantage in the use of such starters is three-fold. In the first place, an improvement in the quality of the butter has frequently been produced. In very many instances, the butter almost immediately becomes of such a character as to demand appreciably higher market value. In general dairying, then, whatever may be the results of individual experiments, there has been so satisfactory an improvement as to lead to a constantly extending use of starters. Second, there is little question that there is a greater uniformity in the butter, although the butter is less uniform than when pasteurization is used. Third, the butter-maker certainly has a better control over the ripening process than by the method of spontaneous ripening; for by the addition of the starters he can, with very great accuracy, determine beforehand the rapidity of the ripening and the time of churning.

Because of these results this method of making butter has been very widely adopted. Ten years ago the use of special "starters" was frequently resorted to, but they were reserved practically for cold weather, when the ripening took place slowly, and no very special attention was given toward obtaining the proper starter. But to-day starters are used very extensively throughout the year, even in hot weather, and special attention is given toward obtaining the proper kind of starter. There are, however, two quite different methods of preparing these starters, one by the use of *pure cultures*, and the other by the use of *natural starters.*

Use of Non-pasteurized Cream with Commercial Pure Cultures.—This method of preparing starters is by the use of commercial cultures which are so easily purchased. These can be obtained at regular intervals from different supply centers, and then, by the use of very simple methods already described, they can be "built up" into proper cultures for adding to the cream. Such a starter is practically uniform in all cases.

Use of Non-Pasteurized Cream with Natural Starters.—A far more common method of obtaining a starter for the purpose, one that is now widely adopted in American dairies, consists in using what is known as a *natural starter*. A natural starter is within the reach of any butter-maker, independent of commercial cultures, for it is something that can be made in any dairy, entirely without the aid of a bacteriologist. It does not involve the expense of purchasing a pure culture, and for this reason has been adopted in hundreds, perhaps thousands, of dairies, where commercial cultures have been adopted in one.

The method of making this natural starter is simple. There may be various plans, but one which is satisfactory enough is as follows: A perfectly healthy cow from a cleanly, well-kept dairy is selected. After the under parts of the body are carefully brushed, and the udder moistened with a damp cloth, the first few jets of milk from the teats are rejected, and the rest are drawn into a sterilized vessel. This is then covered at once and taken to the dairy, heated to a proper temperature and passed through a separator. The skim milk thus obtained is again collected in a sterilized vessel, carefully covered and set aside to sour. After it has become properly soured it serves as a *starter* for the cream-ripening process. Of course, there are many other ways of obtaining a natural starter, for a natural starter is nothing more than a lot of skim milk or whole milk, obtained under specially cleanly conditions from an exceptionally good dairy, and allowed to *sour naturally*.

It is impossible for the dairyman to be sure that a natural starter contains the species of bacteria desired for ripening. Sometimes it may contain the proper species, and at other times an unfavorable species. Logically, then, the use of a natural starter is very unsatisfactory. But dairymen are not so much interested in the logic of the method as in the practical results, and care not whether the process they use is theoretically the best, provided it gives them a good quality of butter. There can be no question that the use of natural starters thus made has been a very decided advantage to the butter-maker in the last ten years. If it were not so we would not expect this method of ripening cream to have been so widely adopted and so generally, one might almost say uniformly, recommended by butter-makers.

We may properly ask why a natural starter, thus prepared, should commonly be a good one, and produce a desirable type of ripening, when we remember that the milk obtained in this way has opportunity for contamination with many kinds of bacteria. A partial answer is, that the bacteria which get into the milk, from a cleanly kept dairy and a cleanly kept cow, are most likely to be species of a desirable character. But the more important reason is that already referred to on a previous page (see page 190). It was there seen that the common species of lactic bacteria are much better adapted to the conditions occurring in milk, when it is kept at a temperature of about 65° to 70° F., than the great majority of milk bacteria. When milk is maintained at this temperature these lactic bacteria grow with excessive rapidity, while the other species are evidently less favored, as indicated by their slow growth. In milk maintained at such a temperature the lactic bacteria present grow so much more rapidly than the other species, that they soon outnumber them many fold, and by the time the milk has soured they outnumber the other species, ordinarily one hundred to one. This is very frequently true even when the original milk or cream had very small numbers of lactic bacteria. In some actual analyses of cream it has been found that, although in fresh cream the miscellaneous bacteria outnumbered the lactic bacteria perhaps ten fold, in the soured cream the lactic bacteria were considerably over ninety per cent. From these facts it will follow that the milk of an ordinary cleanly dairy, if left to sour at such a temperature, will show, at the time of souring, a vast majority of the normal lactic bacteria, even though it may have had at first a considerable majority of miscellaneous species. Now, it is these lactic bacteria which perform the chief part in the ripening of the cream, although perhaps not the whole, and it consequently follows that such a lot of soured milk will make a favorable starter for cream ripening, simply because the lactic bacteria which were present at the proper temperature, showed so much greater vigor that they overcame the miscellaneous bacteria which were there at the outset. A natural starter is thus not a *pure culture*, but is made up commonly of *over 95 per cent. of two or three species or varieties of common lactic bacteria.*

If we compare the results obtained by the use of commercial cultures and those obtained by natural starters in general dairying, we find little to choose between them. Both methods are useful, and in many cases there has been a general improvement in the quality of the butter obtained. As a result of experimental tests in some cases the natural starter has produced the best results, and in others the commercial starters. But, so far as present experience of dairymen has gone, there is nothing special to choose between the two methods of preparing a starter. It may be possible that, in time, bacteriologists will be able to prepare a starter which is superior to a natural starter, but up to the present they have not succeeded.

Hence, the method which a butter-maker will adopt of obtaining a starter will be dependent upon convenience and local conditions. It may frequently be more convenient for him to purchase a commercial culture than to hunt up a proper dairy and obtain the proper milk for a good natural starter. Moreover, the starter made from a commercial culture may be relied upon as being practically constant, while this is not true of the natural starter. On the other hand, the commercial culture is a matter of some little expense, although it has been greatly reduced in recent years. For this reason the greater number of butter-makers in this country prefer making their own starters to purchasing commercial cultures.

VALUE OF PURE CULTURES.

As to the general value of the application of pure cultures to butter-making, the following conclusions may fairly represent the case: The application of pure cultures has taught butter-makers in all countries that the quality of their butter depends in very large degree upon the character of the ripening, and that this ripening is due to the growth of bacteria in the cream. It has taught them that to insure good butter with uniformity a closer attention must be paid to the ripening and that, during most of the year, perhaps all of the year, the most satisfactory ripening may be best obtained by the use of starters properly prepared. It has taught them that such a starter may easily be made by the butter-maker or it may be purchased. The use of pure cultures, with previous pasteurization of the cream, has proved, in northern Europe, of such distinct advantage that it has been adopted universally in Denmark, and widely in north Germany; but this method has not seemed to butter-makers in other countries of sufficient value to warrant the trouble and expense. The use of pure cultures has often been found most efficacious in remedying dairy troubles, which produce what are called butter "faults," and it will, in the future, be more widely used for seasons of special trouble in the creamery than it has been in the past. The use of *starters* of some kind in cream-ripening is almost sure to increase, but, according to the present outlook, the use of pure cultures does not seem likely to become adopted by the butter-makers in the United States. Natural starters appear, on the whole, to be more practical as applied to American dairying than commercial cultures.

BACTERIA IN BUTTER.

It is of interest to ask what becomes of the enormous numbers of bacteria which develop in the cream during the ripening, and whether they are subsequently of any significance in the butter. Although these bacteria continue to grow during the ripening period their growth is practically stopped by the churning and butter-making. Many of them are naturally removed with the buttermilk; others are washed away during the washing and working of the butter. Large numbers are still left in the butter. Ordinarily these bacteria do not grow in the butter, but, if it is not salted, some of them may grow and hasten its spoiling. Unsalted butter does not keep long and its destruction is largely due to bacteria. But if the butter is salted, as is the rule in most countries, the salt checks the growth of bacteria and indeed prevents the growth of most species. As a result of this and the compact condition of the butter, together with its small amount of water, the bacteria do not find the butter a favorable medium for growth and begin to diminish in numbers. A very few hours' time shows a most extraordinary reduction in numbers, and this continues until, after a few weeks, if the butter is kept so long, the butter contains comparatively few bacteria. They do not entirely disappear, for some are found even in very old butter. The ordinary species of organisms, which have been active agents in the cream-ripening, cease to grow and play no further part in the changes which may occur in the butter. The following figures of the number of bacteria in butter will illustrate the facts.

NO. OF BACTERIA PER GRAM OF BUTTER.

2 hours old.	1 day old.	4 days old.	30 days old.
54,000,000	26,000,000	2,000,000	300,000

It is important to notice that the pathogenic bacteria of typhoid fever and tuberculosis remain alive in butter for some time, and certainly long enough to render butter a source of infection should these bacteria be abundant in the original milk. Although this is a theoretical possibility, no instances are known where such an infection has actually occurred, a fact which may be due simply to the difficulty of obtaining evidence upon such a difficult problem.

It is well known that, if butter is not used immediately, certain changes occur which continue slowly for many weeks. The butter retains its fresh, delicate flavor and aroma for only a few days; but if it is kept cool and away from the light it may remain sweet and good for many months. If, however, it is not kept very cold, further changes soon begin to appear which slowly progress and eventually ruin the butter. The most noticeable feature is the appearance of *rancidity.* This change is accompanied by the development of butyric acid and frequently by a considerable change in the consistency of the butter. It finally becomes strongly rancid and tallowy, and totally ruined for use.

The cause of all these changes is still under dispute. The loss of the original fresh aroma is probably due simply to the volatilization of the products which give the aroma, a very natural phenomenon inasmuch as the aroma is necessarily due to volatile products. The later changes are more uncertain. We have already noticed that certain extremely common bacteria found in milk and butter have the power of producing *butyric acid* when growing in the absence of oxygen, and it would seem almost certain that they would produce this effect in the butter and thus explain the development of the butyric acid. This has indeed been generally assumed as the chief cause of the acid. Indeed, many varieties of butyric acid bacteria have been found by different bacteriologists and have been assigned a share in this universal change occurring in butter. There can be little doubt that they do play an important part in the process.

But the development of *rancidity* is a different matter. It is true that the rancidity commonly appears parallel with the butyric acid, but it has been shown that they are totally different and probably unrelated phenomena. The amount of acid is no measure of rancidity. Butter may, under certain conditions, develop butyric acid without the appearance of any rancidity. The rancid flavor is due to some distinct phenomenon. Whether it is to be attributed to bacteria or to chemical ferments is uncertain. If butter is made from sterilized cream it will not become rancid, but this same butter will develop rancidity if it is mixed with a small amount of normal butter. This proves that there is present in normal butter something which is the cause of the rancidity and which is destroyed by heat. It must be either *enzymes* or *bacteria*, but which cannot yet be stated. No bacteria yet experimented with will produce a rancidity in sterilized butter, a fact which seems to point toward the presence of enzymes. On the other hand, the presence of salt prevents the rancidity, and salt does not have a checking action on enzymes, a fact which seems to indicate the action of living bacteria. In short, the cause of the rancidity of butter is still *sub judice*.

From these facts it will be seen that the bacteria left in the butter are of no considerable importance in determining the subsequent changes. Most of them certainly die rapidly and the few that are left have no special significance, unless it be to contribute to the slow changes which develop in the butter after long storing.

BACTERIA IN OLEOMARGARINE PRODUCTS.

This subject is closely related to the one just considered, although possibly oleomargarine itself is to be regarded as an industrial rather than an agricultural product. The materials out of which it is made all come from the land. They consist of stearin, lard, cottonseed oil and other oils. These are mixed with the coloring matter *annatto*, warmed for thorough mixing, and then the whole mass is drawn off into cold water which chills the oils into a hard mass resembling butter. The process is certainly a useful method of utilizing quantities of oils which would otherwise be waste products. It makes a wholesome, digestible food, which could have no objection raised against it if it could only be sold upon its own merits instead of under the false guise of butter. So far as concerns the bacteria normally present in oleo products, they are commonly much less numerous than in butter, and the oleo is, on the whole, less likely to distribute infectious diseases than ordinary butter, inasmuch as the chance for contamination is much less, and the treatment is more likely to destroy the bacteria.

But, although the oleo products thus made resemble butter in appearance, they do not resemble it in taste, and the oleo factories are therefore forced to use some special method of imparting to their product a flavor as closely as possible like the butter which they are trying to imitate. To do this they depend upon the very same flavors as those found in butter and obtained from a similar source. Their method of procuring them is very simple. A certain amount of whole milk, skim milk or cream (varying according to the quality desired in the product) is placed in a large vat or in cans, and allowed to sour. Ordinarily the milk is allowed to sour normally. It is simply placed in a warm temperature where the lactic bacteria grow rapidly, and the milk sours readily enough. After the milk has properly soured, or ripened, it is placed in the mixing vat with a quantity of the melted oils, generally in the proportion of about one part of milk to four of the oils. The whole is then thoroughly mixed by violent agitation and drawn off into the cold brine. As it hardens the milk is held with the fats and thus becomes a part of the final product. Inasmuch as the milk has developed a flavor in its souring, just as cream does during its ripening, this flavor is imparted to the oleo product, and the final result is a mass of fats with the flavor of butter more or less prominently developed.

It is, of course, clear that this flavor in the oleo products is due to exactly the same factors which we have found in the production of butter flavor. The oleo-maker fully understands that his flavors are due to the action of bacteria, and he uses the best means at his disposal to favor their growth. Ordinarily he allows his milk to sour by normal lactic fermentation. In some factories, in recent years, the oleo-maker has not been satisfied to depend upon such a method, which is more or less irregular with changes in temperature, but has come to use, more and more largely, pure cultures of bacteria to introduce greater regularity in the process. In some oleo factories, indeed, so fully aware are the makers of the extreme significance of this matter of proper bacteria to the successful manufacture of oleo products, that they have actually built and furnished bacteriological laboratories, and employ bacteriologists to keep a constant guard over the bacteriological factors in the oleo-making. They know full well that the value of their product is dependent almost wholly upon their ability to obtain a flavor which resembles butter, and they have learned by experience that the flavor which they obtain is dependent upon the type of fermentation going on in the milk while it is souring. They have found it to their advantage to keep this matter of bacterial control under strict observation, and to-day they employ pure cultures in large quantities.

It is a suggestive fact that the oleo-makers have more fully come to appreciate the need of a proper regulation of bacteria in the ripening of their milk than the butter-makers have in the ripening of their cream. Every oleo-maker realizes the necessity of a strict control over the ripening of milk and he has, in recent years, come to recognize the necessity for the use of pure cultures, in order to ensure a uniformly high grade of product. Butter-makers have not come to this belief as yet, and in far too large a per cent. of instances they allow the cream-ripening to take care of itself, without any special attempt on their part to control it. It is by no means sure that the butter-maker must come to the use of pure cultures, but it is certain that he ought to use every means which can be suggested to him for properly starting and controlling the cream ripening. If butter-makers were as careful in the ripening of their cream as the oleo-makers are in the ripening of their milk, it is likely that we might hear less of the quarrel between the oleo factories and the butter-makers. Butter might perhaps be brought to a uniformly higher grade, with which oleo products could not compete.

CHAPTER XI.

BACTERIA IN CHEESE-MAKING.

METHODS OF MANUFACTURE.

In the manufacture of cheese, microörganisms are of even more value than they are in the manufacture of butter. Butter made from unripened cream has a market, though a small one, but there is absolutely no demand for *unripened* cheese. If the changes which take place in the cream during the ripening affect the nature of the butter, the changes which occur in the cheese during its ripening are vastly more profound and more significant. Indeed, it may almost be said that the whole value of cheese is dependent upon its ripening.

The fundamental phenomena of cheese-making are very simple. Milk is treated in such a manner as to precipitate the casein in the form of a curd. Commonly this is done by adding rennet to the milk, but in some forms of cheeses, the casein is thrown down by acid, the usual method being simply to allow the milk to sour, depending upon the lactic acid formed to produce the normal curdling. After the curdling, the solid curd is separated more or less from the liquid and the curd is placed in moulds for shaping and pressing. The amount of liquid left in the curd differs for different types of cheeses. After it is shaped in the moulds the cheese mass is set aside, under constant conditions of temperature, to undergo the process of *ripening*. It is the ripening which produces the changes in the cheese and which gives its typical character. With these ripening changes we are concerned in our study of bacteria.

Soft Cheeses.—There are two quite different types of cheeses, distinguished by a radical difference in the method of manufacture. The first type includes the *soft cheeses* such as Limburger, Camembert, Brie cheeses, etc. In their manufacture the curdled casein is placed in moulds for shaping, but is subjected to neither heat nor pressure. The curd is either cut to pieces by special knives, thus allowing the whey to drain off more readily, or it is simply ladled out of the curdling vat and placed at once in the shaping moulds. The whey is allowed to drain off through false bottoms or holes in the sides of the moulds, but the mass is not subjected to any pressure. At short intervals the mass is turned so as to rest upon a new surface, and by the continuation of this turning for a few days, the cheese becomes consistent enough to handle. The cheese is then placed in the ripening room where a definite and constant temperature is maintained. In this room it remains for some days or some weeks, according to conditions, and is thus ripened. Such cheeses are never made very large and they ripen rapidly. They never become hard and are sure to decay quickly after the ripening process is ended. They are therefore necessarily consumed as speedily as possible after ripening. They decay so rapidly that they cannot be transported to great distances and hence are not articles of export. They are made chiefly in continental Europe.

Hard Cheeses.—The *hard cheeses*, which are more common in the United States and England, differ from the soft cheeses in being subjected to pressure and sometimes to heat. After the casein is precipitated by the rennet, it is cut into fine bits to release the whey, and then is sometimes subjected to a moderate heat (110° F.). This heat changes the nature of the curd, making it tough and elastic. The curd is then removed from the whey as completely as possible, and placed in large moulds in which it is subjected to a heavy pressure. The pressure is increased at intervals for a few hours and there is thus produced a very compact mass of hard curd conforming strictly to the shape of the mould. The cheese thus formed is removed from the mould and set in the ripening room, where it is allowed to remain at a constant temperature until the ripening is completed. The ripening takes place slowly, several weeks, and sometimes several months, being required before it is in proper condition for the market. These cheeses are frequently made in very large sizes, three or four feet in diameter. They will keep a long time after the ripening is completed. They can be readily exported and carried to all parts of the world, and form the chief cheeses of commerce.

In the making of both types the ripening is the essential feature. The changes which occur in the cheese during the ripening are by no means thoroughly understood. They may be best considered under two heads. (1) Chemical changes, affecting the solubility of the product. (2) Changes producing the flavor of the cheese.

I. CHANGES AFFECTING THE SOLUBILITY OF CASEIN.

Although the chemical changes in the ripening cheese affect all of its ingredients in a measure, the most essential feature is a modification of the casein. Freshly precipitated casein is insoluble in water and, especially after heating, is a hard mass very difficult of digestion. During the ripening of the cheese it becomes converted, to a large extent, into soluble bodies which are much more easily digested and assimilated. As a result, the cheese becomes softer and far more readily handled by the digestive processes of the stomach. This change in the casein is the chief chemical phenomenon in the cheese-ripening.

In the production of this change in the casein there seem to be two processes at work which have different relations in the different types of cheeses. Since the modern study of bacteriology began, it has been assumed that the ripening of the cheese is a process of bacterial growth. It is known that bacteria multiply in cheese and it is also known that certain species of bacteria have the power of producing exactly those changes in the casein which render it soluble. The peptonizing bacteria (p. 194) certainly have this power, and these have been given the chief rôle in cheese-ripening. Moreover, it has frequently been shown that cheese in which the growth of bacteria is prevented by antiseptics or by previous sterilization of the milk, will not ripen. Until recently it has not been questioned that cheese-ripening is just as completely a result of bacterial growth as cream-ripening.

Action of Enzymes.—The most recent work has shown, however, that these chemical changes are not produced wholly by bacterial growth, but are in part due to the action of chemical ferments or *enzymes.* As mentioned on a previous page, Babcock and Russell have shown that fresh milk contains an enzyme, which they have named *galactase.* This galactase is a normal constituent of milk as secreted from the mammary gland, and is certainly not produced by bacteria. Galactase has an action upon casein practically identical with the change which occurs in the ripening of cheese. This has been demonstrated by subjecting milk to the action of ether vapor, which prevents bacterial growth but does not hinder the action of enzymes. When such milk is tested from time to time for soluble products, it is found that the soluble casein increases regularly, and, inasmuch as all bacterial life is checked by the ether, the only explanation is that the change in the casein has been produced by the chemical enzyme. Besides the galactase, there is always a second enzyme in the cheese, derived from the rennet used for curdling the milk. Rennet is derived from the stomach of a young mammal, and can hardly be prepared without mixing with it a considerable quantity of *pepsin.* Pepsin has also an action upon casein which changes it from an insoluble to a soluble condition.

Cheese is thus provided with two enzymes capable of affecting the solubility of the casein, entirely independent of the action of microörganisms. What part do they play in the ripening of cheese? This matter has been carefully studied by Jensen as well as by Babcock and Russell. Their general conclusions are in agreement and are, essentially, as follows:

In the ripening of both hard and soft cheeses these enzymes play a part in producing the solubility of the casein. In the soft cheeses it seems that the microörganisms, either bacteria or yeasts, are of rather more importance than the enzymes, but each contributes to the end reached. In the hard cheeses, at least in the early part of the ripening, the enzymes, both the galactase and the pepsin, are actively concerned in the increase in the solubility of the casein. While these chemical ferments are thus of much significance in the ripening, it does not appear that they are alone concerned. The ripening takes a long time and during this period both the chemical ferments and the microörganisms have an opportunity to produce their effect.

Agency of Bacteria.—It is with the action of bacteria that we are chiefly concerned, and while it is true that chemical ferments are of much importance, it is equally certain that microörganisms are concerned in a very important manner in the ripening of cheeses. In the first place there is a marked multiplication of bacteria in the cheese during the ripening. Bacteria do not find cheese so favorable a medium for growth as cream, and their growth is much slower. This is probably due to the more compact nature of cheese and the smaller proportion of water that it contains. But that they do multiply in the cheese is shown by many analyses. In the study of hard cheeses it appears that there are three periods of bacterial growth in the cheese-ripening. At first there is a decline in the number of bacteria, continuing for about a day. This is followed by an increase in bacteria which continues for two or three weeks. This increase is due chiefly to the growth of the lactic bacteria, for, while peptonizing bacteria are present, they are in small quantities and this great increase appears to be wholly confined to the lactic bacteria. After a time, the length of which is very variable, the bacteria begin to decline, until finally only a comparatively small number are left.

If the number of bacteria in ripening cheese be compared with the number in ripening cream a great difference will be found. In ripened cream, in the course of two days, the number per c.c. may be over 1,000,000,000; but in ripening cheese the number hardly rises much above 100,000,000, and this number is reached only after some weeks. The numbers thus never approach those found in normally ripened cream. This is doubtless due to the compact mass and the small amount of moisture. It follows that bacterial action in cheese will be less vigorous and less profound than bacterial action in cream.

Nevertheless this multiplication of bacteria in the cheese cannot take place without producing some effect on the product. What this effect is can only be stated in part. That the bacteria are partly concerned in the chemical changes we have already indicated. Among the bacteria are some belonging to the class referred to on page 194, which have the power of *digesting* curdled casein and converting it into soluble products. It was supposed by Duclaux that these bacteria were the chief cause of the digestion of casein which occurs in the ripening cheese. But it has appeared, as shown above, that these peptonizing bacteria do not grow to any considerable extent in the cheese, the chief increase in bacteria being in the lactic organisms. If bacteria do aid in the chemical changes it would seem to be the lactic bacteria that are concerned.

Can the lactic bacteria contribute to the advancing digestion of the casein? Freudenreich, who has done more work upon this matter than any other investigator, finds that the lactic bacteria have the power of digesting the casein, and may consequently contribute to the ripening. It is a matter of common experience that the cheese becomes acid during the progress of normal ripening. The milk sugar largely disappears and a variety of acids appear, part of which are doubtless due to the growth of the lactic bacteria. Lastly, practical experiments have shown that cheese made from milk inoculated with lactic bacteria ripens normally, while such a result does not appear when it is inoculated with other types of bacteria. All of these facts lead to the conclusion, generally accepted to-day, that in cheese-ripening, as in cream-ripening, the lactic bacteria are chiefly concerned. But whether they play an important part or a small part in producing the chemical changes of ripening cream is not yet fully determined.

2. THE PRODUCTION OF FLAVOR.

The important agency of microörganisms in the ripening of cheese is in the production of the flavor. Cheese is eaten, not because of its nutritive value, although doubtless it is one of the most nutritious of foods, but because of its flavor. The popularity of cheese in so many races of men is due to the fact that it has a strong, appetizing taste which gives relish to other foods. Although physiologists recognize no food value in flavors, they realize perfectly well that they are of the greatest significance in diet, inasmuch as they greatly aid in properly stimulating the digestive functions to handle food. A diet of tasteless food cannot be digested and assimilated. The high flavors of cheese are consequently a great boon to mankind, especially among the poorer classes who must live largely upon coarse foods. Even the coarsest bit of dry bread may be made palatable by the aid of a little cheese flavor to give it a relish. Thus the cheese flavors give this material its peculiar value as a food product. The origin of these flavors is therefore a matter of the greatest importance.

Although the chemical changes which digest the casein in ripening cheese may be due largely to the chemical ferments, galactase and pepsin, the flavors have certainly a different origin. These enzymes do not produce flavors, and casein which has been rendered soluble by them does not have the characteristic cheese taste. There is no doubt that these flavors are due in large part, if not wholly, to the agency of microörganisms. We have already noticed how common it is for bacteria to produce decomposition products with high flavors and tastes. It is these decomposition products of the microörganisms growing in the cheese that produce the characteristic cheese flavors.

Soft Cheeses.—The organisms which produce the cheese flavors are not, however, wholly bacteria. Indeed, it appears that while bacteria have an important share in the ripening, especially in the hard cheeses, other microörganisms play the more important part, at least in producing the ripening of the soft cheeses. Molds are the most important factors in some cases, bacteria in others, and probably yeasts may also contribute to the phenomenon. Indeed, the soft cheeses are best divided into three classes according to the relation of microorganisms to the process, as follows:

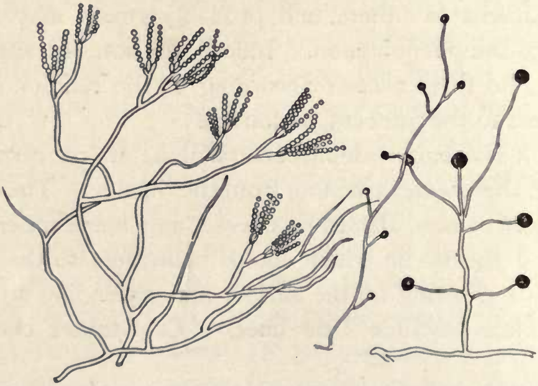
1. Soft cheeses in which bacteria alone are concerned in the ripening, the cheeses ripening from the outside. These include Limburger cheese, Backstein cheese, and some others.

2. Soft cheeses in which molds contribute to the ripening, the molds growing on the surface and extending to the interior. These include Brie cheese, Camembert cheese, and others.

3. Cheeses in which molds play an important part in the ripening and grow throughout the entire mass. These include Roquefort, Gorgonzola, and Stilton cheeses.

The general method of ripening these cheeses is always such as to stimulate and hasten the growth of the needed organisms. The moist curd is frequently wrapped in straw which has, from long use, become thoroughly impregnated with mold spores, or sometimes the form in which the cheese is placed for shaping has a straw bottom. In this way the curd is inoculated on the outside with mold spores. In the making of the Roquefort the molds are artificially planted in the cheese. The molds are first developed upon specially prepared masses of bread. After a luxuriant growth of molds appears, the bread is dried and powdered and, when the cheese is made, this powdered moldy bread is mixed with the cheese curd. The whole cheese thus becomes filled with molds. To facilitate the ripening in the interior of the mass, the cheese is pierced full of holes by a special machine in order to allow the entrance into the center of the cheese of a sufficiency of oxygen to enable the molds to grow. A somewhat similar process is used in the making of Stilton cheese. It has recently been found that the Gammelost cheese, a popular cheese in Sweden, is also due to ripening by molds and Johan-Olsen (Fig. 30) has cultivated these molds in the laboratory and used them successfully in producing this type of cheese.

Fig. 30.



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

The cheeses, thus inoculated, naturally or artificially by molds, are placed in chambers where a proper condition of temperature and moisture is maintained to stimulate the growth of the molds. These plants grow rapidly and soon cover the cheese. When they have reached a proper development the first step in the ripening is ended and the cheeses are removed to another compartment, commonly called a "cellar," where a different condition of moisture and temperature is found. In these cellars the temperature is maintained as constant as possible and is usually rather low. To a considerable extent the differences in the ripening of soft cheeses are due to differences in the temperature and moisture of these cellars. These factors and the method of handling determine the type of microörganisms which shall flourish in the cheese and complete the process of ripening begun by the molds.

In these cellars a new series of changes takes place, but, up to the present time, no cheese-maker nor bacteriologist can tell us much about the actual changes which occur. In the cellars the growth of the molds is largely stopped and, in some cases at least, bacterial growth becomes rapid. The molds have rendered the cheese alkaline in reaction and this has a tendency to check the growth of molds and stimulate the bacteria. If the temperature is too high the bacteria grow too rapidly and soon decompose the cheese into a putrid mass which is worthless. But at the low temperature of these cheese cellars such an extreme decomposition does not occur. The bacteria, however, grow rapidly and produce a new variety of chemical products, some of which contribute to the final flavor of the fully ripened cheese. These new products appear slowly, and gradually produce the characteristic cheese flavor. If the ripening is allowed to continue too long the decomposition goes too far and putrid flavors are produced which are too strong. But when the ripening has continued just long enough, and the proper stage of ripening is reached, the cheese, with its characteristic flavor, is taken from the cellar and marketed at once, highly flavored as it is by the joint action of molds and bacteria.

The different types of soft cheeses are of course handled differently. Some are made from whole milk, some from skim milk, some from sheep's milk and others from goat's milk. Some are slightly salted. Some are more completely deprived of liquid than others. Some are treated in such a way as to prevent the growth of molds, others in such a way as to stimulate such growth. Different temperatures are employed at different stages, and sometimes three ripening chambers, with different temperatures, are employed. All of these differences make the varieties which we find in the types of soft cheeses. In addition to all this it is quite sure that different species of bacteria and molds are concerned in the ripening of the various kinds of cheese.

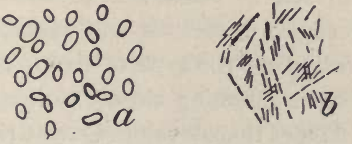
The details of these various processes are not yet understood in any single case. Of the nature of the flavoring products we are ignorant, but that they are, in large measure at least, if not wholly, due to the growth of microörganisms cannot be questioned. At present we know almost nothing concerning the species of bacteria which contribute to the ripening, nor do we know whether the different types of flavors are due chiefly to different species of microörganisms or to the same organisms acting under different conditions. We must in short at present be contented with the conclusion that the different flavors of the soft cheeses are due to different types of decomposition produced by microörganisms.

Hard Cheeses.—The ripening of hard cheeses is even less understood than that of soft cheeses. The ripening is in general similar to that of the soft cheeses but it takes place much more slowly, several weeks, or even months, being frequently required to complete it. The final result is quite different from that of a soft cheese and is plainly due to a different sort of decomposition, as is indicated by the totally different flavors developed.

The chemical changes, rendering the casein easily digestible, are generally admitted to be in considerable degree due to the action of chemical ferments already mentioned, but it is also admitted that the flavors must be due to the growth of the microörganisms. The molds, which are so important in the ripening of soft cheeses, play no part in the hard cheeses. Indeed, their growth is wholly prevented by the compact mass of the cheese and by the salting, oiling and rubbing which its surface receives. Inside of the cheese, however, the bacteria grow, although rather slowly, and to their action is attributed the chief part of the flavor production.

To determine with definiteness the species of bacteria which produce the flavors in hard cheeses, has proved a very difficult task. Some species of milk bacteria certainly do produce *cheese flavors* when they grow in the milk (Fig. 31), but their application to cheese-making has not resulted as yet in much success. It is pretty well recognized that the lactic bacteria must be looked upon as the chief organisms engaged in the ripening. At present bacteriologists are by no means satisfied with their results and have indeed reached different conclusions. Some insist that the lactic bacteria are the sole agents in the process. Others look upon the peptonizing bacteria as playing a part, either doing the whole or being the most important living factors in the ripening. Others say that the ripening is a long process in which several species of microorganisms are probably engaged. No one has been able to determine with certainty that any distinct species of bacterium

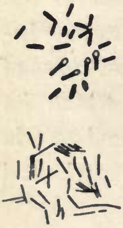
FIG. 31.



Bacteria producing cheese flavors. (*Conn.*)

is the cause of the ripening of any one of the types of hard cheeses. The difficulty lies chiefly in the fact that the process is such a long one and that so many different species of bacteria are found in the cheese at different times. This makes it impossible to say which are essential and which incidental. (Fig. 32.)

FIG. 32.



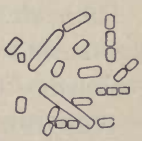
Bacteria isolated from cheese and supposed to contribute to its ripening. (*Weigmann.*)

ABNORMAL RIPENING.

Brief mention must now be made of types of cheese-ripening which are undesirable. Our knowledge of *abnormal ripening* is, in some respects, greater than our knowledge of the normal processes. It is sometimes said that half of the cheese made fails to be properly ripened. Some of the abnormal cheeses are only slightly "off" in flavor and will sell in market as passable cheeses. "Off flavors" in cheese are numerous and varied. Our taste has set a certain standard as the type of flavor which we want in cheese, and the cheese coming closest to it is regarded as the best. But the flavors that develop in the ripening are of all sorts and strengths. Some come close to the standard, others fall very slightly short, others have a slight taste which is foreign, and these pass by imperceptible grades into those whose flavor is so inferior as to render them useless. It is impossible to classify all these types of "off" cheeses. Even the cheese-maker cannot do so, although he recognizes many varieties. To reduce the proportion of these inferior cheeses is, of course, the desire of the cheese-maker, and it is for this purpose that bacteriologists are trying to discover some better means of regulating the ripening.

There are some types of abnormal cheese-ripening which are more pronounced and more easily described, and of these some of the most serious are due to microörganisms. The most common one is called "*inflated cheese*" or "*swelled cheese.*" It is characterized by the development of large amounts of gas in the cheese during the ripening. The accumulating gas causes the cheese to become swollen and filled with cavities. The amount of the swelling and the size of the holes are by no means constant. The trouble may be so slight as hardly to affect the cheese, or so marked as to ruin it completely. The holes may be a few great cavities, or they may be very numerous, minute holes. That the phenomenon is due to the growth of bacteria has been proved by a number of experiments. It does not appear that any single species produces the phenomenon, but quite a number of bacteria may be concerned in it. Several experimenters have inoculated cheeses with various species of gas-forming bacteria. (Fig. 33.) The cheese always becomes more or less swollen in the ripening and develops the cavities. There are many ordinary dairy bacteria which produce an abundance of gas in their growth in milk, and almost any one of these, if present in undue quantity in the milk, is likely to produce so much gas in the cheese as to give rise to this abnormal ripening.

FIG. 33.



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

There is no type of abnormal ripening which produces so much trouble in cheese-making as this, and various devices have been suggested for remedying it. One method is by the use of salt, which partly prevents the undue growth of these organisms. One of the most practical suggestions is a quick but effective test of samples of the milk to be used in the cheese-making. The essence of this method is to take samples of milk from different patrons, especially if the milk is in the slightest degree suspicious, and place each in a small vial of a proper form. The milk is then kept at a temperature of about 90° F., and, if gas-forming bacteria are present in abundance, they will soon make their presence known by numerous bubbles of gas. This gas can be collected, if desired, in a properly devised vessel, and measured. If it is found that the amount of gas is considerable, the milk from which the sample was taken must be rejected, since its use in cheese-making will be pretty sure to produce an undue amount of gas, with the accompanying inflation of the cheese, which it is desired to avoid. This fermentation test is in practical use in certain cheese factories and proves to be of considerable value. It is to be remembered that the normal lactic bacteria produce no gas, and do not therefore give rise to this trouble. The use of pure cultures of common lactic bacteria to inoculate the milk in cheese-making, is of value in correcting this trouble and is strongly recommended by some bacteriologists.

There are quite a number of other types of abnormal ripening known to be caused by the growth of unusual microörganisms. Cheese sometimes develops such a *bitter* taste as to be practically worthless. The fact that bacteria can produce a bitter taste in milk suggests that they may be the cause of this trouble in cheese. Only one case of such bitter cheese has been investigated by bacteriologists, and this was found to be caused by a definite species of bacterium, closely allied to one previously found to be the cause of a dairy infection of *bitter milk*. It is a coccus form, named by Freudenreich, *M. casei amari*. The organism not only produces a very bitter taste in milk in the course of two days, but, if it is inoculated into milk which is subsequently made into cheese, the cheese acquires the bitter taste during the ripening, thus demonstrating the causal connection between this organism and the bitter cheese trouble.

One of the common faults in the ripening of cheeses is a *putrefaction* of the product. This is more likely to occur in the soft cheeses than in the hard cheeses, doubtless because of their larger amount of water. Indeed the soft, moist cheese, with its high proteid content, is a most excellent medium for bacterial growth, and it seems strange that the putrefaction does not more commonly occur. Such decay does sometimes occur, beginning perhaps in the center and eventually involving the whole mass, until the cheese becomes a soft, vile-smelling mass of slime. That the trouble is due to bacteria is evident, of course, from the fact that all putrefaction is thus caused. Why it occurs in some cheeses and not in others is not known. It is most likely to be found if the cheeses are kept at too low a temperature.

Some of the common imperfections in cheese-ripening are characterized by the appearance of colored spots in the cheese. *Red spots* are quite common, chiefly on the surface ; *black spots* are sometimes found, and even *blue spots* are described among the troubles arising in the cheese-ripening. Each of these has been studied sufficiently to show that they are ordinarily caused by microörganisms. The black spots are commonly attributed to a mold-like organism, the red and blue spotting to bacteria. An imperfection in cheese-ripening, recently studied by Harding, characterized by the development of a *sweet flavor*, has been found to be due to a species of yeast.

These do not by any means exhaust the list of cheeses ripened abnormally through the action of undesirable organisms. The list is a long one and bacteriologists have as yet only touched the subject incidentally, without even attempting anything like a systematic study. A sufficient number have been mentioned to serve our purpose, showing how closely the ripening of cheese is dependent upon the species of microorganisms present in the cheese. We must conclude, not only that the proper ripening is dependent upon the presence of proper microörganisms in the milk, but that the presence of an undue number of miscellaneous bacteria is almost sure to result in some type of abnormal ripening, producing one of the numerous "off" flavors, if nothing more serious.

Cheese Poisoning.—Mention should also be made here of a phenomenon occurring in cheese which has frequently been of serious import. Cases of cheese-poisoning are frequently reported which, upon investigation, prove to be only too well founded. The poisoning in these cases is due to the development of a violent poison in the cheese, known as *tyrotoxicon*. This poison does not make itself evident in the taste, but acts very violently upon the person eating the cheese. The poison is produced by a species of bacterium which grows in the cheese during the ripening. Against such a source of trouble our present knowledge of the sources of bacteria gives us no means of guarding, beyond the general suggestion of strict cleanliness in all matters connected with the dairy or cheese factory.

PRACTICAL APPLICATIONS.

Of course attempts have been made to apply these bacteriological discoveries to the practical manufacture of cheese. Insurmountable difficulties have hitherto stood in the way of any very great success in this line. The only really practical application of cultures to cheese-making upon any extensive scale is found in Holland in the manufacture of the *Edam cheese*. The Edam cheese is a hard cheese made in a very simple manner; but the Hollander has learned that he can use an artificial culture in its manufacture to considerable advantage. The culture which he uses is known as *slimy whey* and is prepared by adding to the milk some whey which has shown a slimy fermentation. How the process started is not known. It certainly did not start with the bacteriologist, but was a simple empirical discovery. The culture is carried from farm to farm and is grown by the cheese-maker without his understanding its nature. When analyzed, the slimy whey is found to be a mixture of organisms, the most prominent of which is a slime-producing coccus which gives the characteristic sliminess. When this slimy whey is added to the milk, and the milk is subsequently made into cheese, the ripening does not take place exactly as usual. The cheese ripens faster than normal cheeses, being ready for market in about four weeks, whereas the ordinary product requires six. The ripening is also more uniform. The irregularities usual in cheese-ripening are less likely to occur, and some of the abnormal types of ripening, which occasionally appear, are largely prevented by the use of this artificial ripener. The character of the ripening is, however, somewhat different from that of normal Edam cheese, and the slimy milk cheeses can readily be distinguished, by the dealer, from the normal varieties, by means of their flavor and character. The grade of cheese is regarded as slightly inferior to that of the best cheese ripened without such a starter, and is not found to keep quite so long. It is not much used for exportation but retained chiefly for home consumption. About one third of the Holland cheeses are made by this method of artificial inoculation. It is thus an instance of the application of bacterial cultures to cheese-making, carried on upon a large scale and with evident success.

Beyond this no practical application of bacterial cultures in cheese-making has met with very wide success. It is true that certain bacteriologists have, in experimental tests, succeeded in producing normally ripened cheeses by the use of certain bacterial cultures. Lloyd, for example, has made normal Chedar cheeses (similar to the American cheeses) by the use of certain species of *lactic bacteria*, and believes that the method of manufacture may be improved and simplified by their use. Johan-Olsen has made Gammelost cheese in large quantities, and successfully marketed it, using for his ripening organisms certain species of mold. Adametz claims to have succeeded in making excellent cheeses by the use of a bacterium which he calls *B. nobilis*, a species belonging to the peptonizing rather than the lactic organisms. But none of these have as yet passed beyond the stage of test experiments.

In practical cheese-making we do not yet find pure cultures used to any extent.

POSSIBILITY OF USING PURE CULTURES.

It can readily be believed that there is a great future for the cheese industry along the lines of the practical application of bacteriological discoveries. The problem of cheese-ripening is being eagerly studied by bacteriologists. The industry involves great financial interests. The success of the cheese-making depends upon the ripening. A large per cent. of the cheeses made are greatly depreciated in value, or absolutely ruined, by their failure to ripen properly. If it is possible to discover some method by which the ripening can be controlled, so that the cheese-maker can be sure of its taking place uniformly, it would mean a saving of millions of dollars. Moreover, the development of the ripening methods would greatly extend the industry. Some of the favorite cheeses can be manufactured only in certain localities, a fact that has been interpreted as meaning that these localities are especially impregnated with the appropriate species of microörganisms which are wanting in other localities. This is certainly true in some cases, as is proved by the fact that it is possible to start new factories in new localities by simply rubbing the shelves of the cheese rooms, and the vessels of various sorts, with fresh cheese from the factory where the product is normally developed. Such a procedure would, of course, inoculate the new factory with the organisms necessary to start the normal ripening.

If it were possible to learn more thoroughly the exact nature of the microörganisms producing the desired flavors, it would certainly be possible to extend the manufacture of many types of cheeses far beyond their present homes. But the subject is complex and as yet in the experimental stage. Each type of cheese is a special problem which must be studied by itself, and the bacteriologists who are able to study such problems successfully are few. The subject is a great field for the future. It is quite certain that those interested in cheese-making will see, before many years, some decided changes taking place in the methods of cheese manufacture, based upon the investigations now going on in connection with the agency of microörganisms in producing the cheese flavors that appear during the ripening.

If it should be found feasible to make use of pure cultures of bacteria in cheese-making, it would seem that the method should prove of use both in insuring a proper development of flavor, and in reducing the chances of abnormal ripening. Whether such an application of bacteriology to cheese-making will be possible cannot yet be determined. The success of the use of slimy whey in Holland, and the experience of Lloyd and others in producing a normal and uniform ripening by the use of lactic bacteria, certainly promise considerable success in this line. One difficulty has been experienced hitherto in the application of pure cultures to cheese-making. To insure the absence of mischievous bacteria, and to give the inoculated bacteria a proper chance to act, it would seem necessary to remove from the milk most of the bacteria present. In the use of cultures in butter-making this is accomplished by pasteurizing the cream. The same method has been attempted in cheese-making but has met with difficulty. In most of the experiments conducted, the pasteurized milk has refused to ripen, even though inoculated with a great variety of bacteria cultures. Such pasteurization will of course destroy, or at least injure, the enzymes present, and this would clearly modify the normal ripening process. Indeed it has been insisted by some experimenters that it is impossible to ripen, normally, cheeses made of pasteurized or sterilized milk. If this were true it would make the application of bacteria cultures a difficult matter.

But the most recent work indicates that this objection will not hold, and that perfectly normal cheeses may be made even from pasteurized milk. If such milk is inoculated with a little normal cheese of the quality desired, the cheese made therefrom ripens normally. The normal ripening from such milk by the use of *pure bacteria cultures* has not yet been attained; but the possibility of a normal ripening after such a treatment certainly indicates that there is nothing in the way of a practical application of pure bactéria cultures to cheese-making. It must always be remembered, however, that since the ripening process is in considerable degree due to the action of enzymes, it will probably be impossible to produce a normal ripening by the use of bacteria cultures alone. The microörganisms are needed to produce the proper flavors, but the enzymes are also needed to induce the normal chemical changes which should take place during the ripening. The great financial interests connected with the cheese industry have attracted quite a number of bacteriologists to the study of the problems, and most dairy bacteriologists at the present time are working upon various phases of the problem of the ripening of cheese.

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PART IV.

THE RELATION OF BACTERIA TO MISCELLANEOUS FARM PRODUCTS.

CHAPTER XII.

FERMENTATIONS CONCERNED IN THE PREPARATION OF FARM PRODUCTS.

THERE is no phase of agriculture where bacteria play such an important part as in the dairy, although there are quite a number of other farm products in which bacteria are of much significance. For some of his produce the farmer depends wholly upon bacteria (vinegar). In the case of other products the food is prepared by a partial fermentation, while in still other cases the farmer's desire is to prevent the growth of bacteria.

In all temperate and cold climates it is necessary to keep, for the winter season, food which grows during the warmer weather. This applies equally to the farmer's own food and that of his cattle, both of which demand some sort of preservation. In many instances there is a difficulty in preserving the food because of the readiness with which putrefactive bacteria will grow in organic products, causing their disintegration and decay. As already noticed, bacteria will feed upon almost any kind of organic matter, provided there is plenty of moisture at hand. But, for bacterial growth water is necessary and some of the foods produced on the farm have such a small amount of water that bacteria are unable to grow in them. There is little difficulty in preserving these for an indefinite length of time. This is true of most grains. Nature herself, at the end of the growing season, extracts the water from these seeds, leaving the comparatively dry mass to remain over the period of rest until the growing time comes again. There is no difficulty in preserving such foods from bacterial attack.

But some of the food material which we wish to preserve contains a large amount of water and cannot be so easily disposed of on the farm. Where the amount of water is high, the farmer must find some means of protecting the food from bacterial action. This is accomplished in a variety of ways.

In the problem of the relation of bacteria to farm products we have, therefore, two different topics to consider: (1) The agency of bacteria and fermentations in preparing the crop. (2) The methods of protecting the crop from the attack of mischievous organisms. To a certain extent the two subjects overlap, since in several cases the very methods adopted for preserving the material furnished it with flavors, or other characters, which distinctly add to its value. These will be considered in the present chapter, leaving for the next chapter those subjects connected simply with the preservation of farm products.

I. ACETIC ACID FERMENTATION. VINEGAR-MAKING.

This fermentation is one of the very greatest importance. In the first place it is the basis of a large industry of vinegar-making, which is carried on extensively in all countries. It also forms the foundation of the great industry of pickle-manufacturing, which makes it possible to find a use for large quantities of vegetable products that would otherwise be a waste product, thus furnishing a market for the product of many a cucumber farm. But, besides these extensive applications of the acetic fermentation, it is found on a smaller scale upon nearly every farm. The farmer puts aside his cider, or perhaps weak wine, and allows it to remain unmolested for months. During this time the alcohol is, in part, slowly converted into vinegar by the formation of acetic acid. In all of these processes the vinegar-maker is, consciously or unconsciously, making use of bacteria.

The formation of acetic acid is essentially an oxidizing process. The acid may be produced from quite a long list of organic compounds, but in practice ordinary alcohol always serves as its basis. Various weak alcoholic solutions are used, such as cider, weak wine, beer, etc., each locality naturally using as the source of its vinegar the alcoholic solution which is cheapest and most easily obtained. That the process is essentially an oxidation of the alcohol has long been recognized. Even near the beginning of the nineteenth century it was known that vinegar-making was essentially a union of oxygen with alcohol. It is common to express the reaction as occurring in two stages as follows:

$$\underset{\text{Alcohol}}{C_2H_6O} + O = \underset{\text{Aldehyde}}{C_2H_4O} + H_2O$$

$$C_2H_4O + O = \underset{\text{Acetic Acid}}{C_2H_4O_2}$$

Such a simple oxidation can be brought about by a purely chemical process. As long ago as 1821 Davy discovered a peculiar property of *platinum black*, or finely divided platinum. When alcohol is mixed with this substance the mixture becomes very hot and an active union with oxygen takes place which results in the production of acetic acid. This is a purely chemical, and not a vital phenomenon.

For a time it was believed that the natural formation of vinegar, by the so-called *mother of vinegar*, was a similar process. This brownish mass was thought to serve, like the platinum black, to *condense* the oxygen, and even when Pasteur began his studies, he made the erroneous conclusion that the mother of vinegar acted thus chemically rather than physiologically. But the error of this conclusion was later shown by two facts. (1) The ordinary acetic acid fermentation is stopped by the accumulation of the acid, and it will not occur at all if the amount of acetic acid in the solution be more than 14 per cent. The formation of acid by platinum black is entirely uninfluenced by such an accumulation. (2) The formation of the acid by the natural process is most abundant at about 95° F., diminishing rapidly if the temperature is raised above this. The oxidation by platinum black goes on much more rapidly at higher temperatures and may in itself produce so much heat as actually to ignite the alcohol. The two processes must, evidently, be very different in nature.

ACETIC ACID PRODUCTION A TRUE FERMENTATION.

That the "mother of vinegar" is a necessary agent in the acetic fermentation has been recognized from the earliest times. This mother of vinegar is a soft, felted mass, semi-solid, and commonly forming a scum on the surface of the fermenting alcohol. Its causal connection with the phenomenon is indicated by the name given it, *mother of vinegar*. The fermentation does not occur if this material be wanting, and a very small bit placed on the surface of an alcoholic solution soon extends itself and covers the whole surface, inducing an active acetic acid formation. This mother of vinegar proves to be a mass of microörganisms. It was first named *Mycoderma* by Persoon, who studied it in 1822 without having any suspicion that the skin was the cause of the acetic acid. Its organic nature was proved a little later by Kützing, who showed that this skin was made of numerous minute, living organisms, and who positively asserted that they were the cause of the acetic fermentation. But in regard to this fermentation, just as in regard to others, the chemist Liebig checked the advance of discovery by his own theories of fermentation, which regarded the whole class of phenomena as chemical processes and which strongly opposed the physiological view of their nature. The great weight of Liebig's name, and the brilliancy of his discussions, prevented further advance along the line of fermentative discoveries for many years.

Eventually the subject was taken up by Pasteur, who soon succeeded in putting beyond dispute the conclusion that the phenomenon is due to the microörganisms present in the mother of vinegar, although his explanation of the phenomenon was incorrect. With this conclusion it becomes evident that the equation given above cannot longer be regarded as expressing more than an approximation toward the actual phenomenon which occurs. It is certain that, as the microorganisms produce this change in the alcohol, they do it for their own benefit and probably make use of part of the product. The equation above given leaves nothing for the bacteria to appropriate to their own use, and is therefore, though easily understood from the chemical standpoint, unintelligible from the standpoint of bacteria. It is quite certain that the phenomenon which occurs is far more complicated than indicated by the equation, and this must therefore be looked upon only as a useful expression of the essential nature of the process. Various by-products are at the same time formed which do not enter into the equation at all, as above represented.

The relation of the organisms in this "mother" to the process of acetic acid formation may readily be understood by a brief description of a common method of vinegar-making. In the Orleans process, oaken casks are used which are kept in a room with a temperature of about 70° F. Each new cask is first steamed and then impregnated with boiling hot vinegar to "sour" the cask. After this it is filled partly full of good clear vinegar and about half a gallon of wine is added. About a week later a little more wine is added and in another week still another lot. This is continued until the cask contains about forty gallons (two-thirds full). Then about half of the material is withdrawn as vinegar, and from this time on some two gallons of vinegar may be withdrawn at a time, its place being made good by the addition of wine. The cask when once started may continue in operation for six to eight years without interruption, but eventually it becomes so filled with tartar and mother of vinegar that it must be cleansed.

Shortly after the process of vinegar-making has started a skin of the Mycoderma grows on the surface. This soon covers the whole, and its action produces the vinegar. At the outset the proper vinegar organisms are apt to be few in number and other bacteria get a chance to develop. Consequently, in recent years, the method has been widely adopted of cultivating the mother in special cultures, on the surface of wine that is soured in open dishes or vats. A small amount of this culture is then placed in the wine to start the growth of the "mother," and from this inoculation it spreads rapidly.

After the scum is produced the vinegar formation begins. Exactly how the organisms produce the result we do not know, but in some way they cause the alcohol to unite with oxygen from the air and thus become converted into acetic acid. The oxidation is doubtless in some way a benefit to the plants, for they grow luxuriantly during the process. The oxidation does not always stop at the formation of acetic acid, but is sometimes carried further so as to split up the alcohol into simpler molecules. This results in a loss, and is one of the difficulties to be met with in the vinegar manufacture. The loss is commonly not great, for the accumulation of acetic acid will soon stop the growth of the organisms, since they are unable to grow after the acetic acid has become abundant. Different species of the organisms can endure a different amount, but when the acetic acid has reached 14 per cent. the bacteria are never able to produce any more. A serious loss to the manufacturer arises from the different powers of different species of the vinegar plant to produce acetic acid under varied conditions.

A second process of vinegar-making, known as the "quick process," does not at first sight appear to be caused by microorganisms. This process, used mostly in Germany, consists simply in an intimate mixture of alcohol with air, by means of beechwood shavings. A mass of such shavings is placed in tall vessels and thoroughly moistened with an alcoholic solution. Then the whole is inoculated with a little warm vinegar. The vinegar thus added starts the process and, in a few hours, vinegar makes its appearance in quantity. Such a process seems at first to be more like a chemical phenomenon than a fermentation induced by microörganisms. But here also a vital process is concerned. In the first place, the process does not start until a little warm vinegar has been added to the mixture, and such vinegar will be sure to contain bacteria. This of course suggests that the microörganisms, thus added, spread through the shavings, grow rapidly and soon induce the oxidation. Indeed, Pasteur and others were easily able to prove that, if the growth of the fungi is prevented in such a mixture, no vinegar is formed. Hence the quick vinegar process is also dependent upon the presence and active growth of the vinegar plants.

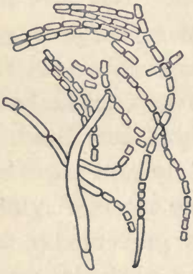
These are not the only methods adopted in vinegar-making, for this industry, which is a very large one and increasing in importance, has demanded numerous devices. Different details are followed by different manufacturers and different kinds of vinegar are made in different ways. But whatever be the process it is always fundamentally dependent upon the growth of some of the vinegar organisms which are either naturally or artificially inoculated into the alcoholic solution.

THE VINEGAR ORGANISMS.

What are these organisms which bring about this oxidation of alcohol into acetic acid? While Pasteur's investigations showed the close relation of the organisms to vinegar-making, he gave us little knowledge of the organisms which produce the fermentation. He called them by the name originally given—*Mycoderma aceti*—but he made no special attempt to determine whether this represented a single species or a complicated mixture of organisms; nor did he regard them as bacteria.

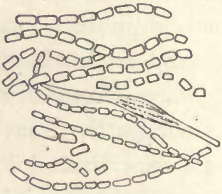
The first advance in our knowledge of the vinegar plant came from Hanson, who has done so much to increase our information in regard to fermentation processes. He found that the Mycoderma is not a single organism, and that it clearly belongs to the general group of bacteria. The name *Mycoderma* was consequently soon changed to *Bacterium*, and the specific names of *aceti* and *pasteurianum* were added, as the names of the first two distinct species isolated by Hanson. This same author soon added a third to the list, and Brown later added a fourth. As a result there came to be recognized four species of acetic bacteria as follows:

FIG. 34.



B. aceti.

FIG. 35.



B. kützingianum.

Bacterium aceti (Hanson), Fig. 34.
Bacterium pasteurianum (Hanson).
Bacterium kützingianum (Hanson), Fig. 35.
Bacterium xylinium (Brown).

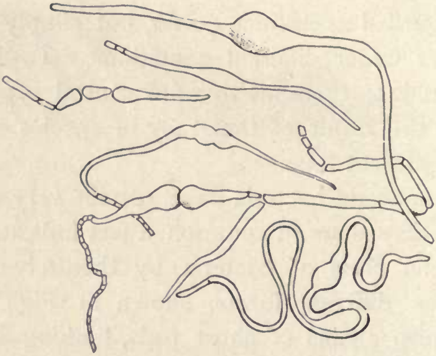
But in regard to the acetic bacteria, as in other cases, further investigation has appeared to increase the number of species very greatly. In the last few years many different organisms have been added, until to-day the list of organisms, regarded as distinct species, and having properly the designation of *acetic bacteria*, is very long.

The last few years have seen a large amount of study given to these various acetic bacteria. Not only have the species been multiplied and the characters of the different forms carefully studied, but numerous physiological experiments have been made upon their power of producing acetic acid from different materials and under different conditions. The great multiplication of species has led to a belief that the various forms are not to be regarded as distinct *species*, but simply as variations of a few types under different conditions. To-day there is a tendency to reduce them all to a few central types. Exactly what will be the result of the study of species can hardly be stated at the present time.

All of these acetic bacteria have certain very characteristic points which they share in common, a fact indicating that they form a distinct class of bacteria by themselves. They all exist in three different forms, shown in Figs. 34 to 36. They may form chains of short rods, looking like ordinary bacilli (Fig. 34). They sometimes grow out into long, slender threads, sometimes very long without any traces of divisions. These threads may subsequently break up into short elements. Finally they all have a peculiar habit of forming long threads with rounded, swollen centers (Fig. 36). When these threads break up into short forms, only the thick, swollen centers remain undivided. What significance these swollen centers have in the life of the organism is not understood. This character is such a peculiar one, and so unique, that it plainly places these acetic bacteria in a class which must remain isolated from other bacteria. It is a class of organisms with quite a large number of different varieties, distinguished not only by slight differences in structure but also by some more prominent and important differences in relation to conditions, and in their power of forming acetic acid.

The differences in the physiological characters of these varieties are considerable and are factors of much importance in the actual processes of vinegar-making. They have varying powers of fermenting different organic bodies, and differ very much in the amount of acetic acid they will produce under similar conditions. For example *B. aceti* produces 1.27 per cent. of acid at 59° F. while under the same condition *B. pasteurianum* will produce .08 per cent. But, more important still, is the fact that the temperatures which favor the different varieties are not the same. *B. aceti*, for example, produces a good fermentation at a temperature of 42° F., whereas *B. pasteurianum* at 44° will not even multiply, and produces no fermentation. Some of the species produce their maximum effect more quickly than others, and some may begin to destroy the acid produced under conditions of temperature and time in which other varieties are still active. With such a very wide difference in the optimum conditions of action, it is manifest that the best results

FIG. 36.



Acetic acid bacteria, showing long rods and rounded swollen centers.

would be obtained by the use of a single type of the bacterium, and by the maintenance of the conditions under which this type could grow the best. When there is in the "mother" a miscellaneous mixture of the various forms, of a wholly unknown nature, it is evident that no conditions can be maintained which will produce the best results.

THE USE OF PURE CULTURES.

In the manufacture of vinegar, by the processes now in use, there is always a considerable loss of alcohol and a smaller yield of acetic acid than there should be from the quantity of alcohol used. It is well recognized that this is to be accounted for largely by the fact that the fermenting organisms are not all of one species, and that the "mother" is commonly a mixture of several varieties, each demanding different conditions for its proper and most efficient action. It is inevitable that such chance methods should not result most efficiently.

For some time the bacteriologists have been insisting that the adoption of pure cultures in vinegar-making would bring about a considerable improvement in the results. Every one recognizes what a revolution has been brought about in the brewing industry by the introduction of pure cultures of yeasts into that fermentative process. It is perfectly possible that a similar application of pure cultures of acetic bacteria should be made in the vinegar industry, and it is quite probable that such an introduction would result in an improvement in the manufacture and in a considerable saving of the material now lost.

But hitherto there has been no considerable application of pure cultures to vinegar-making. To obtain absolutely pure cultures is a difficult matter, and to keep them pure from subsequent contamination is more difficult still. Vinegar-makers have not found the advantages derived from such methods sufficient to pay for the labor and trouble of applying them, and they do not yet appear to have been adopted in any practical vinegar-making plant. But vinegar-makers have, to a considerable extent, adopted rougher methods of obtaining the vinegar organism in quantity and in a comparatively pure condition. One simple method adopted is as follows: Filter two parts of white spirit vinegar through fine bolting cloth to remove the vinegar eels. Mix the liquid with $\frac{3}{20}$ part of alcohol of 90 per cent. volume, and 1 part of white wine which has been previously boiled, filtered and cooled. Place this mixture in shallow dishes and cover with glass plates. The vinegar organism appears in a few hours as a thin scum which will ordinarily be pure, or nearly so. If the scum does not show any white spots (molds) gently lower it upon the surface of the vat containing the alcoholic solution which is to be made into vinegar. The result is a comparatively pure culture of vinegar organisms and a satisfactory fermentation. When used in making vinegar from cider, this process gives a vinegar quite superior to the ordinary type, having a finer flavor and better keeping properties. Such a method of preparing comparatively pure cultures is useful and quite frequently used.

Whether strictly pure cultures will ever be used in vinegar-making cannot be predicted. If the product of any single factory were greater and compared with the amount of beer in a brewery, it is certain that pure cultures would have been long since adopted. The small amount of the product has rendered questionable the wisdom of the expense and labor of preparing and preserving pure cultures.

Of course the farmer who simply lays aside his few barrels of cider, or other alcoholic solution, to allow it to be converted into vinegar, will not be troubled or especially interested in the matter of pure cultures. A little loss is nothing to him, and the preparing and preserving of pure cultures an impossibility. He feels tolerably confident that the cider which he sets aside for the purpose will contain some of the acetic acid bacteria and that in course of time he will obtain vinegar. Whether he gets the advantage of all the alcohol, or loses half of it, does not matter much to him. Even if several barrels should not produce a proper quality of vinegar it would not be of much importance. Sometimes he finds that his vinegar is stronger than at other times, and sometimes he finds that its taste is much inferior to the ordinary grade of vinegar. Perhaps this raises in his mind a temporary question as to the reason for the differences. But he never pursues the subject further. The reason is doubtless, in all cases, the differences in the condition of the acetic acid bacteria growing in his cider ; for whether he is aware of it or not, his vinegar is prepared for him by the activity of one or more species of the acetic bacteria which he has ever at his command.

II. THE FERMENTATION AND CURING OF TOBACCO.

It is unnecessary to emphasize the great significance of the tobacco crop for the agricultural industry, or the importance of a thorough understanding of the proper methods for making the finished product. Tobacco is a crop the value of which is wholly dependent upon the success of its *curing* and final preparation for market. The green crop is valueless and many a crop is ruined in the curing. That the curing of tobacco is primarily a *fermentative* process is an undisputed fact. Until recently it has been believed that the fermentation which takes place in the curing is due to the growth of bacteria. In recent years, however, it has become doubtful whether bacteria play the important part in the process that was believed a few years ago, inasmuch as it has been shown that certain *enzymes*, produced by the tobacco leaves themselves, are the chief agents in the fermentation. It is still insisted by some bacteriologists that, although these enzymes are important agents, bacteria are after all factors of the utmost importance in the normal ripening. Whether future investigations will result in showing that this fermentation must be taken wholly out of the realm of bacteriology cannot yet be stated. But, at present, the curing of tobacco forms an integral part of bacteriology, even though the future should demonstrate that it is the result of chemical rather than bacterial fermentation.

A brief description of the process of curing will aid us in obtaining the clearest idea of the process and the dispute concerning it. The tobacco leaves are first hung up in a drying shed to undergo a partial *drying*. Sometimes they are stripped from the stem before this drying, and sometimes the whole stem is hung up with the leaves hanging downward. In this state they become partially dry, their leaves wilting and assuming a somewhat brown color. This change is perhaps akin to a sort of preliminary fermentation, but there is no reason for believing that microörganisms have anything to do with it. When it takes place normally it is purely a chemical and physical process. It may happen that certain microorganisms, molds or bacteria, attack the leaves in this condition and produce certain diseases of the leaf, doing much damage. This occurs chiefly if the leaves are too moist, and does not occur if the temperature and moisture of the air are normal.

After the drying process is completed the leaves are ready for the *fermentation* proper, the process upon which the value of the product largely depends. The methods of bringing about the fermentation vary considerably. Sometimes the leaves are left hanging a long time, and are eventually packed closely in boxes weighing several hundred pounds each. These boxes are then left to take care of themselves. They are commonly packed in cold weather and remain undisturbed several months. When the warmer weather comes in the spring, a fermentation is set up in the cases, which progresses without any attention from the owner, and, after quite a number of months, the boxes are opened to determine the success of the fermentation and the character of the product.

In a different method of fermentation, adopted chiefly in warmer climates, a plan is used which keeps the whole process under close observation and is in this respect undoubtedly superior. This method is a fermentation in open piles, and can be used in colder climates only by the use of artificial heat. The leaves are here piled upon each other, not too tightly, and a great heap is made, some three feet high, or more. This heap demands for its proper fermentation a warm, moist atmosphere, such as is found in tropical and semi-tropical climates. Within a short time the temperature of the mass in these piles begins to rise, sometimes rising as much as ten degrees a day. The temperature is allowed to rise until it reaches about 125° or 130° F.; then the piles are opened and the leaves heaped up again in another similar pile, care being now taken to put on the inside those leaves which were before on the outside. Another rise in temperature follows and again, after reaching 125° F., the heaps are thrown down and remade. This is repeated from five to eight times, several days elapsing between the successive heapings. At the end the tobacco is in the proper condition for market. The fermentations do not necessarily end here, however. The manufacturer commonly allows the tobacco to undergo a second fermentation under different conditions. The later fermentation is called "sweating" and brings the leaf into a better condition for use. A further fact is of significance. After the first fermentation the leaf slowly improves in quality if allowed to lie for a year or so before using. All of these phenomena need to be explained before our agriculturists can properly handle this product to the highest advantage.

The primary *fermentation* is clearly the essential process of the tobacco-curing. During the fermentation some very essential changes in the tobacco take place. The chief of these changes are the following: A decrease in nicotine—an increase in alkaline reaction—an increase in ammonia—the disappearance of sugar—an increase in the amount of nitrate—a loss of water—a change in texture of the leaf—a change in color by which the final brown color is produced, and a change in flavor. These numerous changes are of course quite varied in their nature but are mainly due to oxidation.

NATURE OF THE FERMENTATION.

Bacteria.—It is a well-known fact that the tobacco prepared in different tobacco-raising countries shows great differences in quality. Even the same variety of tobacco when sown in different localities fails to produce the same product. Now, in considerable degree, this is doubtless due to the differences of soil and climate. But, making all allowance for these differences, it has appeared that not an inconsiderable part of the difference may be attributed to differences in the fermentation process. In particular has it been suggested that the *flavor*, arising during the ripening, is a factor which could be modified by changing the fermentative process. Tobacco lovers know that the tobacco of Cuba develops in its fermentation a flavor which is not found in tobacco prepared elsewhere. The same species of tobacco raised in other countries, although it will undergo a fermentation of a normal character, acquiring the chemical and physical properties which it develops in Cuba, does not acquire the flavor that it acquires in its own home. Cuban tobacco is now raised in United States but its flavor is inferior to that raised in Cuba. Knowing the intimate relations of flavors to microörganisms in other products, already noticed on earlier pages, it has been a natural suggestion that the tobacco flavor may be the result of bacterial growth, and that the reason that Havana tobacco has its superior flavor is because the leaves raised in that country have peculiar species of bacteria on them which induce a peculiar fermentation.

Acting upon this suggestion several bacteriologists have for some years been studying the bacteria present upon the tobacco leaves and their possible relation to fermentation. Bacteria are found in abundance upon the leaves of fermented tobacco and several distinct species have been isolated and carefully studied. These species are partly common species found elsewhere, but a part of them are peculiar to tobacco. Some of them have been especially named, *B. tobacci I., II., III., IV.* and *V.*, being names given to some of the peculiar types. These are distinct species, belonging to well-known groups (*subtilis* and *proteus* groups). It has been assumed that these bacteria have probably an intimate relation to the fermentation of tobacco, and experiments have been instituted in considerable numbers with cultures of bacteria, found upon the highly flavored Havana tobaccos, for artificially inoculating tobaccos grown elsewhere, in the hope of developing the desired flavors. The original investigator in this line was Suchsland but others have followed his suggestions. No very great success has attended these experiments up to the present time. The experimenters claim that they have succeeded in improving the flavors of the tobacco by the use of their cultures. The species *B. tobacci I.* and *II.* have been said to give the highest flavor, and to promise success in this line. The success thus far attained has not, however, been sufficient to warrant the adoption of the methods in any except these somewhat doubtful laboratory experiments.

Enzymes.—While these bacteriologists were working upon the problem of the relation of bacteria to the fermentation, Loew undertook the investigation of the subject from the standpoint of *chemical enzymes*, and succeeded readily enough in showing that, at all events, the chief part of the fermentation must be attributed to enzymes rather than to bacterial action. In the first place the extremely rapid rise in temperature is too high to be accounted for by ordinary bacterial action. Fermentation due to bacteria may certainly produce a rise in temperature, but a rise as high as 130° F. is entirely beyond anything that could be expected of living microörganisms. Not all bacteria can endure such a temperature, and there is no reason for thinking that they can produce the rapid rise that occurs in the fermenting tobacco. Secondly, while it is true that bacteria may be found upon the leaves of the fermenting tobacco, Loew found them only in small quantities, far too few to account for the fermentation which was producing a rise of temperature of 10 degrees per day. Further, the amount of moisture in the tobacco leaves is low, not over 25 per cent., and in such a condition bacteria do not readily grow; indeed Loew believed that they could not grow at all. Lastly, nicotine is generally looked upon as a means of *checking* bacteria, and hence the fermenting tobacco cannot be regarded as a favorable place for bacteria to grow. For all these reasons Loew concluded that bacteria are not the active agents in tobacco fermentation.

But Loew obtained positive as well as negative results. He found by proper study that the tobacco leaves contain certain chemical substances which are capable of exerting a strong oxidizing action, and hence of giving rise to the chief phenomena of tobacco fermentation without the aid of bacteria. These bodies he called *oxydase* and *peroxydase*, both of which he found in abundance on the leaves. They were regarded by him as enzymes and he believed them capable of producing all of the phenomena of fermentation. Loew's careful studies have subsequently been confirmed by some of the students who had been working upon the bacteriological side of the problem, and may therefore be taken as abundantly demonstrated. It may be taken as demonstrated that there are developed in the tobacco leaves certain chemical substances which carry on an active, oxidizing function, and that it is these bodies which are chiefly concerned in the prominent phenomena characterizing the tobacco fermentation. Whether these bodies are really *enzymes*, as Loew claims, is doubted by some. But this matter is immaterial for our purpose. They are certainly chemical bodies of some kind, and are produced by the tobacco leaves and not by bacteria. The most striking phenomenon of tobacco fermentation is therefore not due to the action of bacteria.

But while these facts show that enzymes, or similar bodies, play the chief rôle in the fermentation, they do not by an means exclude the action of bacteria. We have already noticed that in the ripening of cheese, while the enzymes are extremely important agents in the chemical changes going on, the bacteria are of chief importance in the production of the *flavors.* The fermentation of the tobacco by the oxydases does not satisfactorily explain the flavors. When Havana tobacco is fermented in the United States it ferments normally but does not develop the typical Cuban flavor. It is quite possible that this flavor is, after all, a matter of bacterial action. When the Cuban planter ferments his tobacco he commonly sprinkles it with special preparations, a process called "petuning." These preparations are usually secrets, and each plantation is likely to have its own. They consist of mixtures of various chemicals, of which organic fluids yielding ammonium carbonate frequently form a part. The action of this petuning is problematical, but it is believed by the planters to contribute to the production of the peculiar *flavors* of Cuban tobacco.

Now these mixtures are good culture media for bacteria, and when they are sprinkled upon the tobacco the leaves are, in a way, inoculated with bacteria. In other cases the stems are treated with a mixture of water and molasses which also stimulates bacterial growth. On such *petuned* leaves bacteria are abundant, and this accounts for the somewhat large number of species which German investigators have found upon Havana tobacco, and which they have isolated in their attempts to produce the desired flavors by bacteria cultures. Loew acknowledges that such petuned leaves have plenty of bacteria upon them. There is no evidence at hand to indicate whether these bacteria have anything to do with the production of flavor, beyond the statements made by the European investigators, to the effect that by using the cultures of these bacteria they have been able to obtain the desired flavors. It is certainly not impossible, and not improbable, that the flavor production, which does not seem to appear typically outside of Cuba, may be due in part to bacterial action, possibly to the action of the very bacteria that the planter unconsciously sprinkles over his leaves in the petuning which occurs before the fermentation begins.

The question whether bacteria have any part to play in the normal tobacco-ripening must, then, for the present, be left without any decision. That the normal fermentation occurs under the influence of the chemical ferments present in the leaves, appears to be most probable, and there can be little doubt that the chief phenomena in the ripening of tobacco must be attributed to such a chemical process, rather than to bacterial fermentations. It does not yet appear to have been proved that bacteria play no part in the process as it normally occurs. That bacteria may develop in the leaves under certain conditions is quite certain, and that these bacteria may effect the flavors is equally sure. It is certainly known that sometimes the bacteria do affect the flavor injuriously, developing an aroma which detracts from the quality. The development of bacteria thus certainly affects the flavors, and these organisms are concerned in the proper fermentation, whether or not they are necessary for the normal process of tobacco-curing. In this unsettled condition of results we must wait for more accurate experiments in the future, to determine whether bacteria are to be wholly excluded from the normal process of tobacco-curing, or whether they must still be regarded as having a prominent influence in the production of the high grades of flavor which appear in some brands of tobacco.

Whether or not bacteria play a part in the normal ripening, it is certain that they sometimes do injury to the crop and produce abnormal fermentation. The presence of too much moisture on the leaves is likely to be followed by the growth of various microörganisms. Molds are the most common injurious organisms to appear under these conditions, but bacteria may also develop and produce disastrous effects. It does not lie within our purpose to consider the various diseases of tobacco in detail. The fact is that from the time the tobacco is growing in the field, until it has reached its final state as a completed product, it is subject to a considerable number of diseases. It is a very delicate product and slight changes in moisture or temperature are almost sure to be followed by troubles of some kind, injuring or ruining the crop. Of these troubles some are produced by molds or special fungi, and some by bacteria. The consideration of these various troubles, whether bacteria or of a different nature, concerns only the person interested in raising tobacco and they are of no special interest to the agriculturist in general. We shall not, therefore, further consider them in this work.

FERMENTATION IN FOOD PRODUCTS.

We notice next a few farm products whose manufacture involves some type of fermentation for the purpose either of preventing decay or of giving a special character to the product. There are quite a number of these which are of more or less significance in the handling of food products.

We may first refer to one or two incidental products, which have a somewhat limited use in different localities, so limited that they cannot properly be regarded as agricultural products in any general sense. It is the custom of agriculturists, and indeed of other persons, to preserve some vegetable foods from putrefaction, and at the same time to impregnate them with specially desired flavors, by causing them to undergo a fermentation of which the production of lactic acid is a prominent feature. We have already noticed how the growth of lactic bacteria in milk prevents the growth of other bacteria, and delays, or wholly prevents, the putrefaction which would occur if the lactic bacteria did not grow and produce an acid. If various forms of vegetables which decay readily are caused to undergo a proper acid fermentation, they may be preserved a long time.

III. SAUERKRAUT.

Perhaps the most common preparation of this sort is *sauerkraut*, a fermented food quite popular with some people. This is made of cabbages which are packed away in proper vessels and mixed with a certain amount of salt. The nature of the fermentation which occurs in the cabbage is very little understood. Acids are developed, and the chief agency in the formation of the food has been commonly attributed to lactic bacteria. There has been at present, however, very little careful study of this type of fermentation. One species of bacterium and two species of yeast have been found in the fermenting sauerkraut and are believed to be its cause. The fermentation appears to be practically identical, whether the material is supplied with oxygen or not, either aërobic or anaërobic fermentation being consistent with the production of the typical fermentation. The bacteria rapidly develop acid up to a certain grade of acidity, but after this grade is reached the bacteria cease to grow and soon die. The final material does not contain bacteria in very great abundance. The yeasts produce some alcohol, and CO_2 and CH_4 are liberated from the fermenting material in quantity. Beyond the few facts here mentioned, little is known in regard to the formation of this material.

Certain other food materials are similarly used and similarly fermented. A form of soured food is made from beans in certain countries, which is in a similar way allowed to undergo an acid fermentation. So little is known in regard to the real nature of these products that we can at present do no more than simply mention them.

IV. SILAGE.

In the silo the agriculturist has devised a method of utilizing certain food products of which the soil yields him large crops, but which contain large amounts of water and which lose their value, in great measure, when dried. Moreover the silo not only enables him to preserve such food, but it impregnates the food with new flavors and a new character which, in some respects, enhance its value, since they make a product especially relished by cattle. The preparation of silage involves a fermentation of a very marked character. It is a fermentation which, until recently, has been regarded as due to bacterial action, although the subject was little understood and not carefully investigated. The recent, more careful studies have shown that here, as in the tobacco fermentation, bacteria play a part of less importance than formerly believed and that, under certain circumstances, the silage fermentation may take place without the aid of bacteria, suggesting perhaps that the whole process is a means of preventing their growth rather than stimulating it.

Preparation.—In the preparation of silage the material to be used, most commonly corn, although other succulent vegetable material may be utilized, is cut into moderately small pieces and packed away firmly in a solid mass in a tall air-tight compartment. Sometimes the silo is filled rapidly and sometimes more slowly, with somewhat different results in the two cases. After the silo is filled it is closed at the top, and frequently subjected to considerable pressure. The contents are thus largely deprived of air. Air, of course, gets in around the top, but little or none around the sides or bottom, so that only the superficial layers are affected by it. This is an important factor in the preparation of silage, and upon the successful exclusion of air the character of the product is, in part, dependent.

In the silo important and profound changes take place. The first phenomenon to be noticed is the development of a large amount of heat, resulting in a rapid rise in temperature. The extent of this rise in temperature is dependent upon the amount of oxygen present, and the readiness with which the heat is radiated. Under some conditions a temperature as high as 150° F. is produced, while in other cases the temperature is considerably lower. The proper production of silage does not appear to be dependent upon this rise in temperature, inasmuch as perfectly normal silage may be made in small vessels where hardly any rise in temperature is noticeable (not over 70° F.).

This high temperature lasts a few days and then the mass slowly cools. The production of heat appears to be very rapid for a few days and then somewhat quickly declines, but a less rapid evolution of heat continues for a long time, perhaps several weeks. After the reduction in temperature other changes begin which are much slower, and after several weeks the character of the material is found to be greatly changed. It develops a certain amount of acid, its chemical nature is somewhat altered, and it develops a new flavor and aroma which should be distinctly aromatic but with no signs of putrefaction or mustiness. There is found to be a considerable loss of material, a loss ranging from 4 per cent. to 40 per cent. This is a very wide range, and shows that the method of ensilage has an extraordinary effect upon the product obtained. The loss is largely parallel to the amount of oxygen which finds its way into the silo, being greatest when the amount of oxygen is great, and very slight if the oxygen of the air be thoroughly excluded. The loss is chiefly a loss of carbohydrates, although there is also an appreciable loss of albuminoids. This is an actual loss of food material and one, of course, which the agriculturist desires to avoid. Perfect exclusion of air is evidently thus the best means of preventing the loss.

In a properly prepared silo the fermentative changes do not extend beyond this, and the material will now remain sweet for months. The superficial layers may sometimes become decayed and ruined, but this does not extend into the mass. After the feeding from the silage is commenced it must be used up somewhat rapidly, for various undesirable fermentative changes may set up in the superficial layers as they are successively exposed to the air. The material is eagerly devoured by cattle, to whom the aromatic flavor appears very agreeable, and thus the silo offers to the farmer a useful aid in preserving succulent fodder for winter use.

Silage Bacteria.—The silo was an empirical discovery, brought to its present state of perfection without any knowledge of the actual phenomena occurring in the silage. Although we know more about it to-day, we are still in ignorance in regard to some of the essential phenomena. That the process is similar to an ordinary fermentation is sure enough, and when first called to the attention of bacteriologists it was supposed to be a bacterial fermentation. The initial heating of the mass was supposed to indicate a fermentative action of bacteria-like organisms. It was suggested that the bacteria first used all the oxygen which might be mixed with the mass, and, as they grew, developed the heat which caused the rise in temperature. This growth of aërobic bacteria continued until the oxygen was used up, when the *aërobic* bacteria ceased to grow and the *anaërobic* organisms began their activity. To the growth of the latter was supposed to be due the chief changes which occurred slowly in the silo, the chemical changes and the development of flavors being due to their growth here as in cheeses. The fact that the oxygen was used up prevented the growth of the ordinary bacteria of decay, and consequently the material might remain sweet for a long time.

Such an explanation has been chiefly the result of hypothesis, and has never been demonstrated by any actual study of silage. It has been the basis of various attempts to explain the origin of silage and the different changes occurring in the chemical nature of the carbohydrates and albuminoids. It is true that bacteria may be found in abundance in silage under ordinary conditions, and they are even more noticeable under unusual conditions. It has been therefore tacitly assumed that these are the real causes of the fermentation.

Non-bacterial Processes.—But here, as in the tobacco-curing, it is becoming manifest that the importance of bacterial action has been exaggerated, and that perhaps bacteria have nothing to do with the formation of silage. In the first place it is quite evident that the original rise in temperature cannot be due to bacterial action. The rise in temperature is too great. While it has been recently shown that some bacteria may live at temperatures as high as 140° F., it is very improbable that a temperature of 150° F. could be *produced* by their growth. This temperature indeed destroys most bacteria, and no one would to-day believe that this original heating can be accounted for by the growth of bacteria. Moreover, the heating takes place much too rapidly for such an explanation. If bacteria were its cause the heat would develop slowly and only as the bacteria have a chance to grow; but in reality the rise in temperature in the silo is very rapid, much too rapid to be accounted for by bacterial growth. Lastly, it has been shown by Babcock and Russell, that typical silage may be formed under conditions which prevent the growth of microörganisms. If ensilage material be placed in proper vessels and in an atmosphere saturated with ether vapor, which is supposed to prevent the growth of bacteria entirely, the material goes through a typical fermentation and becomes normal silage.

What then is the cause of the fermentation? A partial answer has been given as follows: The living plant cell is always carrying on the physiological process of *respiration*, a process quite similar to respiration in animals, and resulting in the use of oxygen and the evolution of carbon dioxide. In this respiration carbohydrate bodies are used, with some albuminoids as well, and a certain amount of heat is evolved. Now the plant cells do not die when the plant is cut down, but continue for some considerable time to carry on this process of respiration. Cutting the plant to pieces appears, indeed, to increase temporarily, rather than to decrease, the respiratory changes. These may go on for several days, until, indeed, the plant cells are fully dead. These are well-known facts, recognized by botanists for a long time.

To these respiratory changes is due part of the fermentation of silage. After the material is packed in the silo the plant cells remain alive for several days and carry on these respiratory changes as long as they are alive and have oxygen at their command. This results in the gradual oxidation of the carbohydrate material and the evolution of carbon dioxide. A study of the gas evolved from the silo while it is undergoing the preliminary heating, shows it to be almost pure carbon dioxide. This fact clearly indicates that the fermentation is not putrefactive and probably is not due to microörganisms, since they would almost always give rise to other gases along with the carbon dioxide, ammonia or some other nitrogen-holding gas being commonly the result of bacterial fermentation. These respiratory changes are thought to be fully sufficient to explain the initial changes in the silage, with the initial heating and evolution of gas.

In regard to the later changes in silage we can say less. The formation of acid, hitherto attributed to acid bacteria, is regarded by Babcock and Russell as wholly independent of these organisms. They regard it as due to some changes which take place in the protoplasm of the plant cells before their death. They find that the acid will develop normally in silage in the presence of ether, and hence it is not due to bacteria. They find, moreover, that the amount of acid developed is roughly proportional to the length of time which elapses between the packing of the silo and the final death of the plant cells. When silage is made from immature corn, the cells are more active and remain alive considerably longer than they do when made from fully ripened corn, and under these conditions the amount of acid developed in the silage is greater. Thus all of the general changes occurring in silage are attributed to the activities of the protoplasm of the plant cells, as they are slowly dying under the conditions which exist in the silo. The chief fermentations in silage are therefore not due to the action of bacteria.

The development of the flavor in the silage is as yet not understood. Neither the respiratory changes, nor the changes in the protoplasm mentioned, are such as are likely to give rise to flavor. To what the flavors are due the authors we have referred to are unable to say. They are inclined to believe them due to the action of certain enzymes which they think are liberated from the plant cells when dying. That such enzymes may be thus liberated from plant cells we have already noticed in tobacco, but that they produce the flavors found has not yet been certainly proved, or even rendered probable. In regard to this phase of the silage fermentation we are therefore in almost absolute ignorance, and cannot as yet say whether it is wholly produced by chemical enzymes or whether microorganisms may not have something to do with it.

From all these facts it becomes clear that while this method of preparing food is due to a fermentation it cannot be attributed to the growth of microörganisms. It certainly involves some other factors, and it is uncertain whether bacteria, or other microörganisms have anything to do with the process as normally carried on. Sometimes bacteria do have an effect upon the silage. Putrefactive bacteria may get an opportunity to grow and produce an abnormal fermentation, resulting in ruin to the product. The proper formation of silage would seem, according to present knowledge, to be based rather upon preventing the presence and growth of bacteria than upon stimulating it. How this bacterial growth is prevented we do not know. The material packed in the silo has plenty of bacteria within it and plenty of moisture. How the fermentation which occurs is able to prevent the growth of these bacteria and of the consequent putrefaction of the silage, cannot at present be stated.

It must finally be noted that the process, as it is thus described, is perhaps not properly to be regarded as a *fermentation.* It is not due to enzymes nor to microörganisms, but is simply the continuation of the ordinary protoplasmic activities of the plant cells, under the peculiar conditions of the silo. Although the process seems similar, and, in some respects the results are the same, the causes of the changes are wholly different from those which we have regarded as the causes of fermentation. If enzymes are produced the phenomenon may be regarded as in part a true fermentation ; but if the causes above mentioned are the chief ones silage formation is not a true fermentation.

SOUR FODDER.

A second method of preparing succulent vegetable material is sometimes employed, in order to make use of certain waste materials from different industries. The materials most commonly utilized in this way are exhausted slices of beet root from sugar factories, potatoes previously steamed, frozen sugar beets, corn stalks, etc. These materials would commonly be wasted or used only as fuel, and any plan which can utilize them as food, by preserving them in a good condition for months, is a decided saving. A method adopted for this purpose is in use in some countries though not much known in America. It consists of a process in some respects similar to ensiling, but differing in certain important points and resulting in a somewhat different product. As commonly employed the method is as follows :

Pits are prepared from 40 to 80 inches deep, and from 80 to 120 inches wide. The material to be treated is tightly packed in these pits and covered with a layer of chaff, over which is placed a thick layer of soil. Upon the whole is placed a layer of boards, which are weighted with stones sufficient to produce a pressure of nearly 2,000 pounds per square yard. In such a compactly pressed mass it is hardly possible for oxygen to enter, and the respiratory changes, so important in the silage, hardly occur. There is a slight rise in temperature but rarely over 95° F., which temperature is reached in a little over two weeks. After this the temperature falls. The changes that take place in the material have not been much studied. There is a very marked loss of material, amounting in some cases to a quarter of the weight of the dry substance. The loss is chiefly in the carbonaceous materials, the woody fibers, and also in the albuminoids. In some instances as much as 60 per cent. of the albuminoids is lost in the process of fermentation. It is also a fact that the development of acids is large, especially the development of volatile acids, which give the material a strong acid smell and taste.

It has been assumed, without proof, that in this preparation of *sour fodder*, we have a process in which bacteria are chiefly concerned and that the acid-forming bacteria are the important agents. Such is a natural supposition from the slow development of heat, and the subsequent large development of acid. But the demonstration of the large agency of other processes, in the formation of ordinary silage, throws the whole question open to debate, and suggests that here too other phenomena besides the growth of microörganisms may be concerned.

Indeed, there seems to be no very marked difference between the preparation of sour fodder and sweet ensilage. There is a greater pressure used in the former preparation, and doubtless a less free access of oxygen. The slow development of the heat, and the older nature of the material used, renders it less likely that much can be expected in the way of the respiration of the plant cells. The large amount of volatile acids also leads to a strong suspicion of the action of bacteria, rather than of chemical changes in plant cells. But all of this must be regarded as pure hypothesis until some one shall have actually made a study of the phenomenon itself from a bacteriological standpoint.

Both sour fodder and sweet ensilage are products of very great use in agriculture. Any method which will enable the farmer to make use of products which would otherwise be wasted, must be regarded as decidedly valuable. Ensilage enables a great utilization of a crop, for cattle, which could not otherwise be carried through the winter, and the method of preparing sour fodder makes it possible to utilize a considerable portion of many a waste product. While it is true that there is a large waste in material as it is prepared for sour fodder, it is a great saving on the whole, inasmuch as, without this method, the whole product would be a waste. As it is, it is converted into a food product, readily eaten by stock, and of great assistance in providing them with the proper food for the long winter. Agriculturists will be wise to develop these processes in the future further than they have in the past, and a more complete study of the actual changes going on in the formation of a usable food out of so many waste products might well deserve the closer attention of bacteriologists and chemists.

CHAPTER XIII.

PRESERVATION OF FOOD PRODUCTS FROM BACTERIA.

It is not only the fallen monarch of the forest which, after serving its usefulness, is attacked by molds and bacteria and slowly decomposed. It is as truly every bit of wood which the farmer may have built into his dwelling, unless he carefully protects it. His fence posts, the sills of his houses and barns, in short every bit of timber which he has laboriously fashioned, is sure to undergo decay if it is exposed to sufficient moisture. This slow decay causes his barn and every wooden structure to settle, and the roof to cave in unless he gives it proper attention; even with the best of care his wooden structures eventually yield to the attack of microörganisms. Moreover, his living trees are not exempt from a similar process of decay due to molds and bacteria. The fungi make their way into the living tree and cause the decay of its interior just as they do that of a fallen trunk. They may even attack the living parts of a tree, slowly sapping its strength and eventually killing it. Many a valuable tree is thus destroyed. All around his farm the farmer finds a general decay of organic matter, and it is one of his tasks to prevent it or make it as slow as possible. He tries to guard his wooden structures from moisture; he covers them with a protecting paint, and in every possible way endeavors to check the action of the decomposing organisms.

But in the preservation of food products is found the greatest difficulty, since these are so readily attacked by bacteria. Bacteria will cause the rotting of the farmer's crops and his compost heap with equal readiness, and a large part of a farmer's labor is directed toward the application of means to prevent the growth of bacteria where they are not wanted. There is no sharp line separating the process devised for preventing bacterial growth and those mentioned in the last chapter producing special fermentations. The ensiling of corn is a means of preventing its decay, and at the same time a means of furnishing it with special flavors which increase the value of the product. In the preparation of hay the chief aim is to prevent subsequent decay and decomposition by bacteria, but, as we shall see, even in this process fermentations play a part. But although the two purposes run together in some food products, they are, in general, quite distinct.

PREVENTING THE ACCESS OF BACTERIA.

If it were possible to protect various food products from the presence of bacteria they might be preserved almost indefinitely. But this is not possible except by hermetical sealing. Bacteria are so abundant in all water and air that they are sure to get into any mass of food which is simply exposed to the air.

Certain natural foods, however, do have a certain amount of protection against bacteria. Fruits in general are excellent food for bacteria and undergo ready decay. The decay of fruit has been carefully studied by bacteriologists, and has proved to be due to a considerable number of species of bacteria. No single species appears to be particularly concerned but a considerable variety may contribute to the phenomenon. (Fig. 12, page 80.) These species of decomposing organisms are so abundant in air and water that it is simply impossible to keep them away from fruit and, consequently, an indefinite preservation of fruit is impossible. But for a while the attack of bacteria is delayed by the presence of the fruit skin. A tough, smooth skin, like that of the apple, is not readily passed by bacteria, and thick-skinned fruits are in a measure protected from decay. Hence it follows, also, that the person who is desirous of preserving fruit must take especial pains to keep the skin from injuries and bruises, for through such wounds in the skin the bacteria find most ready entrance, and once within the softer parts they produce rapid decay. Hence, too, we see why one decaying piece of fruit will contaminate its neighbor, inasmuch as it will furnish a center of active bacteria, which multiply and become so numerous as to be sure to pass from one fruit to another. In these facts too do we find the explanation of the increased period of preservation which the fruit dealer can give to his fruit by careful wiping and cleaning it at intervals, since this removes many of the bacteria which would otherwise be likely to find a means of getting through the skin. In short, the whole secret of keeping fruit finds ready explanation when we bear in mind that the putrefactive bacteria, although abundant, do not with readiness pass through the fruit skin, and, consequently, that any means which keeps the skin intact and clean will delay the entrance of bacteria and hence the decay of the fruit. But in spite of the best of care their entrance cannot be wholly prevented, for some point of rupture in the skin will surely be found and all fruit is certain to undergo decay in time. To preserve fruit for any length of time it is necessary to adopt more efficient means.

DRYING.

The most easily applied means of preventing the growth of bacteria in food products is by more or less completely drying them. Bacteria demand considerable water and will not grow unless well supplied. They must all grow in a medium that is practically liquid, inasmuch as they are able to absorb food only in a liquid condition. Most bacteria will cease to grow when the amount of water falls to 30 per cent. and all stop growing when the water falls below 25 per cent. From these facts it follows that anything that can be dried without destroying its value as a food, can, in this way, be effectually protected against bacterial action. No method of preserving food products is so universally used as this, and none so effectually.

In the preservation of the valuable cereal products little is necessary beyond this simple drying. Indeed, here nature herself adopts the same plan, and, when the grain is ripening, the large amount of water which was present in the green seed disappears, leaving the ripened grain, somewhat shriveled perhaps, but with a very small water content. Such a small amount of water does the ripened grain possess that, not only will it refuse to germinate unless moistened with water, but bacteria are utterly unable to grow within it. Nature wishes to preserve the grain through the season of rest (winter) and in order to protect it from bacteria she takes most of the water out, thus preventing the putrefaction which would otherwise surely take place. In harvesting the grain, therefore, all that is necessary for the farmer to do is to collect the product after it is fully ripened, confident that it will not contain enough water to make bacterial growth possible.

Flesh.—With other foods the task is more difficult. The most nutritious food is, in general, the flesh of animals, but this contains so much water that it undergoes decay at very short notice. Such an excellent food for bacteria does this flesh appear to be, and so abundant are the bacteria on every side, that the drying of flesh by simple means is practically impossible. We sometimes read of hunters in the wilds of nature, or of savages, in cooler climates where the air is clear and dry, preserving flesh by the simple process of cutting it into thin strips and hanging it up in the sun to dry. Such a process would hardly suffice upon an ordinary farm, for the flesh would be sure to decay before it became dry enough to resist the action of bacteria. Whether this is due to the greater amount of moisture in the air, or to the fact that there is a larger number of bacteria in the air around civilized communities, cannot be stated. But it is certain that such a simple method of drying flesh cannot be adopted upon farms in general. This method of preserving is, however, still used in hot climates, commonly with the addition of *salting*, and produces a form of food known as *pemmican*, *charque* and *tassajo*. The flesh thus prepared loses considerable of its flavor, but methods of using artificial heat have been devised which, in a measure, remedy this defect. After it is once dried, flesh may be preserved in this form almost indefinitely. The drying of flesh is a process which hardly concerns agriculture in civilized, temperate countries.

The same end is very commonly reached, even on the farm, by artificial drying accompanied by *smoking*. In the preparation of smoked hams, or other flesh, the subsequent bacterial growth is prevented, partly by the drying it receives and partly by the actual germicidal action of the smoke. When the smoke is produced from certain woods—beech wood is especially favorable—various volatile products arise, such as *phenol* and *creasote*, and these act as germicides. The bacteria on the surface of the meat are destroyed, and the surface dried and affected by the volatile products in such a way that bacteria will not readily start to grow upon the flesh. Smoked meats are thus preserved in part by the drying and in part by the action of the smoke.

Fruit.—The method of preserving various fruits by drying needs hardly be mentioned. The drying of apples, squashes, pumpkins is a common process of farm life. In warmer regions of the earth the sun's rays are sufficient to dry many fruits for preservation. Raisins and figs are thus prepared. In colder regions artificial heat must be employed. By the use of artificial heat it has been found possible to preserve by drying a large number of fruits. Pears, prunes, plums, raspberries, blackberries, blueberries, and strawberries represent some of the farm products which yield readily to this method of treatment. In fruit prepared in this way the water is not all removed by any means, sometimes as much as 30 per cent. being left. In most cases there is considerable sugar in the dried product which aids in the preservation. In pears there is some 30 per cent. of sugar, while in raisins there is about 60 per cent. It must always be remembered that drying does not destroy the bacteria, but only checks their growth, and, if the fruit has been exposed to a possible contamination of pathogenic bacteria, the drying does not remove the danger. This method of preserving fruits naturally affects their flavor and is frequently quite unsatisfactory for this reason, although it does not materially affect their nutritive value. In recent years hydraulic pressure has been used to extract the water, with results, on the whole, superior to the extraction by simple drying.

Hay.—One of the most important applications of the drying process is the preparation of hay. The fresh grass contains so much moisture that it could not be preserved in masses without undergoing extensive bacterial decomposition, and to obviate this the farmer resorts to the simple method of drying out some of the water. But this phenomenon of drying hay is not always the simple thing which it seems to be, and, in some methods of preparing hay, a fermentation is certainly involved. Where the climate is moderately dry and the sun hot, the simple method of exposing the grass to the sun for a few hours is most widely adopted. But such a method is not possible in regions where there is likely to be a large amount of rain, and it is doubtful whether such a method ever produces the best results. Two other methods of preparing hay are in use in different agricultural communities, each of which is dependent upon a fermentative process.

Burnt Hay.—In this method of hay-curing the heat of fermentation is depended upon to produce the drying. The freshly mown grass is piled in heaps from 10 to 13 ft. high, the mass being trodden down as tightly as possible to prevent the admission of air. In these heaps a spontaneous fermentation soon sets in which produces a rapid heating of the mass, a rise in temperature being sometimes noticeable in twelve hours. The temperature rises rapidly and is watched with a thermometer. When it rises to about 158° F., which occurs commonly in from forty-eight to sixty hours, the heaps are opened and spread out in thin layers to the air. The heat in the hay now rapidly dries the product and with a single turning it is ready for storing. The hay thus prepared develops an aromatic odor which ordinary sun-dried hay does not possess, and is for this reason somewhat superior.

The nature of this fermentation is simply a matter of conjecture. It has, like other fermentations, been attributed to the action of bacteria, the very great rise in temperature being attributed to certain species of bacteria which are known to live at high temperatures. But this hardly seems probable. It is found that if these heaps are not opened to the air when the temperature reaches about 158°, there is a still greater heating of the mass, and finally the whole may ignite by *spontaneous combustion*. Such a phenomenon certainly cannot be produced by bacterial action, since not even the heat-loving bacteria (*thermophilous bacteria*) are able to produce such high heat as to cause their own death. It is, therefore, much more probable that the phenomenon here involved is one of the chemical fermentations due, either to respiratory changes, or to enzyme-like bodies, which bacteriologists are now learning play such an important part in fermentations hitherto attributed to bacteria.

Brown Hay.—Another method of preparing hay is extremely common in countries where the rains are too frequent to make possible the drying by the two methods above mentioned. This is frequently employed in rainy districts like those of the North Sea and the Austrian Alps, and it is occasionally used the world over. In its preparation the grass is built up into a stack or rick, 13 to 16 feet high and 16 to 24 feet in diameter. It is well trodden down, but not packed so firmly as in the last method, and the whole stack is thatched so as to shed the rain. In such ricks a spontaneous fermentation sets up and the mass becomes heated. Here too the temperature frequently rises as high as 160° F., but it does not rise much higher, and there is no danger of spontaneous combustion. The rick is not opened, but the hay remains in the mass until the farmer wishes to use it. It is immaterial whether the hay is rained upon or not, and this makes the process especially adapted to rainy districts.

The fermentation which takes place in these ricks produces a great change in the nature of the product. It becomes a firm, dry mass, of a pale or dark brown color, or may, if the heating is too great, be almost black. It has developed at the same time an *aromatic odor* which resembles freshly baked bread. There develops also a large amount of lactic and butyric acids, the amount of lactic acid being as high as 7 per cent. and the butyric acid over 2 per cent. These acids are derived chiefly from the carbohydrates, as is shown by the great reduction in the amount of these bodies in the drying hay. A considerable part of the nitrogen material is also lost, the total loss in the hay being about 14 per cent. Whether this fermentation is wholly due to enzyme-like bodies which produce the rapid rise of temperature, or whether bacteria play a part is not known. The production of the acids has hitherto been attributed to bacteria, but without any actual demonstration of their presence.

It should also be noticed that hay prepared by the simple process of sun-drying undergoes a subsequent fermentation of a limited extent, and of a nature which is doubtless closely akin to that just mentioned. After the sun-dried hay is stored away in the barn it "heats" in the mow almost universally, sometimes indeed to such a high temperature that there is danger of ignition. This is evidently due to fermentative changes. The hay changes its character while lying in the mow, and it is clear that the change is closely akin to that which occurs in the so-called brown hay.

The whole subject of the curing of hay needs further study before we can understand its real nature or the importance of these fermentation processes. Until these facts are better known we cannot determine the best method at the disposal of the farmer for curing and preserving this very important food product.

Certain phenomena sometimes seen in cotton are clearly closely akin to the fermentation just described. Sometimes cotton undergoes a spontaneous heating sufficient to render it in danger of combustion, and this must be due to processes similar to those just described. The same thing is true of hops which occasionally develop a similar spontaneous heating during the curing.

USE OF COLD.

The use of low temperature is the oldest method of preventing bacterial action. All the common species of bacteria grow more slowly as the temperature is lowered, and all cease growing entirely when it reaches freezing. The nearer to freezing a fermentable substance is kept, the greater the delay of the bacterial growth. In the large cold-storage houses the food which is to be preserved is cooled to a temperature below freezing and is, consequently, actually frozen. At this temperature the bacteria never act and the material may be kept indefinitely. It must be remembered, however, that the low temperatures do not kill the bacteria but only delay their action, and as soon as such food products are warmed the bacteria begin their action immediately.

Where such low temperatures are not feasible, a moderate degree of cold will greatly check bacterial growth and prevent decay. The ice chest keeps the food a few degrees above freezing. At this temperature the ordinary bacteria do not grow, and the food will not undergo a typical decay, but there are some species of bacteria which can grow at these temperatures and gradually ruin the food substances. The use of low temperatures is, however, of great assistance to the agriculturist in preserving many of his fruits. These are frequently ruined by freezing, but may be kept in perfectly good condition for many months if they are kept as cool as possible, avoiding actual freezing. The cold cellar thus serves the farmer's purpose by checking the growth of the common bacteria in his fruits without lowering the temperature to freezing.

PRESERVATION BY THE USE OF CHEMICALS.

Many chemical substances are destructive to bacteria and foods may frequently be prevented from bacterial action by the addition of small quantities of some material, harmless in itself, yet having a checking action upon bacteria. Such agents are called *preservatives*. If they are to be used in the preservation of food products it is, of course, necessary that they should not be deleterious to health, and also that they should not impart disagreeable flavors to the food. It is, therefore, impossible, or at least, illegimate, to use for such purposes many of the best antiseptics which are used elsewhere; and the number that can be used without hesitation is not very great.

The most common substance thus used is *salt*. Salt is not an antiseptic in any proper sense and it does not destroy bacteria. But it may be a preservative and, when it is present in quantity in a solution, it will have a decidedly repressing action upon bacterial growth and may stop the ordinary putrefactive changes. It is the common method in use for the preservation of butter, where it also adds a relish to the product. It is also in general use for the preservation of flesh of various kinds. Flesh which is to be smoked is commonly first salted, the salt adding to the efficacy of this method of preservation. Salt pork and salt corned-beef are also preserved by the large amount of salt present in the brine in which they are kept. Salt is also widely used for preserving fish. In the use of salt it must always be remembered that the bacteria are not killed, and that flesh which contains pathogenic bacteria will not be rendered safe for eating by the salting process.

Sugar is another substance which is of great usefulness in checking bacterial action. In condensed milk it is the large amount of sugar present which prevents the bacteria in the milk from growing and producing their fermentative changes. In the dried fruits, like raisins, it is the sugar, as well as the drying, which preserves the food, for there is frequently sufficient water left in the mass to make bacterial growth possible if it were not for the repressing action of the sugar. Now sugar is in itself a most excellent food and it may without danger be freely used in the preserving of food products. Practically there are some difficulties in the way which prevent the material from being used in a very large variety of food products. Pure sugar is not liable to any bacterial fermentation if it be kept in a strong solution, or if it be in the form of crystals. When in a weak, watery solution it is sure to undergo some form of fermentation, due either to bacteria or yeasts, unless special care is taken to prevent it. Weak sugar solutions may be preserved by evaporating the water.

Acetic acid is a third substance which is quite freely used for this purpose. This subject has already been referred to on a previous page and may be passed here with the simple statement that it serves two purposes, since the flavor which is added to the food is a matter of equal importance with the preserving power of the vinegar.

It is only necessary to refer to the fact that many other substances are used for the preservation of food which are more powerful in their action and distinctly deleterious. The use of *salicylic acid*, of *borax*, of *boracic acid*, of *formalin* (under various commercial names) is only too common. These preservatives must one and all be condemned as deleterious to health. It is quite illegitimate to use them in the preservation of food products.

PRESERVATION BY HERMETICAL SEALING.

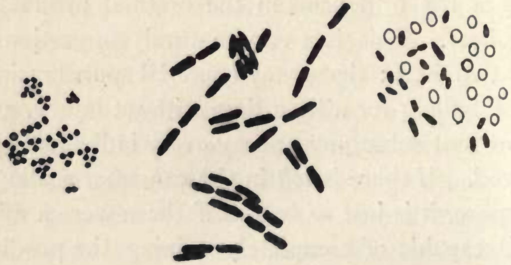
One of the most important methods of preserving perishable food products was invented a century ago, long before the significance of bacteria was known, and long before the meaning of the process was understood. It was invented by Appert, and consisted in first destroying by heat all of the bacteria present in the material to be preserved, and subsequently sealing it hermetically so as to prevent the access of more bacteria. It was at first supposed that the significance of the sealing was to prevent the access of air, but it is now known that its purpose is simply to prevent the entrance of bacteria; for if these can be kept out, the presence of air does not interfere with the preservation. It is interesting to note that this method of preservation of food products was invented and put to a wide practical use, while scientists were disputing and laboriously experimenting over the problem of *spontaneous generation*. The experiments by which scientists tried to settle this question consisted in exactly the same devices as just mentioned, viz., the heating of various organic materials to a high temperature to kill all living organisms and then, after sealing hermetically, watching to see if more life developed in the sterilized mass. While the scientists were disputing as to the results, the method of canning was put into practical use, and every can of preserved fruit was evidence against spontaneous generation.

The plan commonly adopted is well known. The material to be preserved is placed in cans of some sort and then heated very hot. Sometimes a temperature of boiling alone is sufficient, and sometimes a temperature above boiling is obtained by the use of steam under pressure. After an amount of heating, supposed to be sufficient to destroy all bacteria, the can is sealed hermetically, before it has cooled down enough to allow a secondary contamination from outside bacteria. When the process is successful in destroying all bacteria, even to the very last spore, and the sealing takes place properly, the food may be preserved indefinitely without the slightest tendency to undergo putrefactive changes. There is apparently no limit to the length of time that properly canned food products may be preserved.

The development of the canning industry does not belong to our immediate subject, but there are certain facts connected with the matter which have produced great changes in the possibilities of agriculture. It has made possible the utilization of a great quantity of food products which otherwise could not be used. Certain of our fruits are extremely palatable but very perishable, and if it were necessary to use them fresh, or in a dry condition, only comparatively small quantities could be raised. For example: before the beginning of tomato canning only a very small crop could be utilized; but the opening of this canning industry has entirely changed the conditions, and now great tracts of land can be devoted to raising this delicacy, thus opening to the farmer an entirely new outlet for his crop. The same is true of many another farm product. It is no longer necessary for the farmer to depend upon his own market, but, by the process of canning, his market may be the world, open to him the whole twelve months of the year. The canning industry makes it possible for the farmer to become a specialist where it was impossible a few years ago. He may raise green corn, or tomatoes, or strawberries as abundantly as he pleases, and that part of his product for which he does not find an immediate market may be preserved for a later season by the process of canning. It is well for the agriculturist to learn that in farming, as in all other industries, it is the *specialist* who succeeds, and that the proper utilization of this process of canning is one of the means of making a special product upon a farm yield proper returns.

A few practical points connected with the process of canning are of interest and may sometimes be of use to the agriculturist. It is one of the unfortunate facts connected with this industry that not all of the cans of food thus prepared result successfully. Many of the cans of tomatoes, and a still larger number of cans of corn, even after careful treatment and thorough sealing, undergo some of the putrefactive changes which it is the purpose of the canning to prevent. Cans of corn thus fermented become swollen from the pressure of the gases which accumulate within, and the contents are ruined. Such spoiling of the material must be due, either to the fact that the original heat was insufficient to destroy all the bacteria, or due to a secondary contamination. In most cases the chance of secondary contamination is practically ruled out by the conditions; and the cause of the trouble is, in nearly all cases, that some of the organisms in the original product have resisted the heat. This is a very natural suggestion when we remember two facts: that many bacterial spores resist the temperature of boiling for a long time without injury, and that in order to prevent subsequent decay every individual spore must be destroyed. If there is left in the can, after sealing, a single resisting spore, it is just as bad as if there were a million; for this one is capable of completely ruining the product in the course of time. If, therefore, the fruit chances to have some of these highly resistant spores clinging to it, it may very well happen that, occasionally, some of them will not be killed by the heat of the boiling. It follows, of course, from this that the readiness with which a food product yields to the canning process is largely dependent upon the question whether it is likely to contain resisting spores. Practical experience has shown that some foods are much more easily canned than others. Tomatoes have always proved difficult to preserve in this way, and the housewife has probably more trouble with this product than with any other. For a long time it was thought to be impossible to preserve green corn by canning, for, in spite of careful methods, most of the cans were found to undergo some kind of fermentation. The canning factories have mastered both of these products, but even in the best factories there is a considerable loss of the product by subsequent fermentations. In these cases it has been demonstrated, by careful bacteriological study, that the trouble is exactly what theoretically we would anticipate. In the spoiled cans are bacteria with very resistant spores which have withstood the heating of the first step in the process. It is interesting to find that the same bacteria which occur in the spoiled cans of corn are found upon the corn and its husk while still growing in the field (Fig. 37). The reason for the difficulty of canning corn is thus apparent. The corn, while growing in the field, is infested with a certain species of bacteria which produces resisting

FIG. 37.



Three species of bacteria causing the spoiling of canned corn. (*Prescott* and *Underwood.*)

spores, and the destruction of these spores by heat is difficult. The remedy is, of course, quite simple. Recognizing that the spoiled cans are attributable to bacterial spores which resist heat, the method of preventing the trouble is the use of higher temperatures or a longer exposure to the heat. Although at present little is known in regard to other instances of spoiled canned goods there can be no question that similar explanations are to be made.

So completely have these facts been known, and so thoroughly have the methods been mastered, that at the present time all foods which can stand heat can be preserved by the process of canning. Of course the flavors of the foods are frequently changed by the heating to which they are subjected, but as a rule much less than by any other method of preservation. The whole problem of human food supply has been modified by the development of these processes. Equally has the canning industry modified agricultural problems, by enabling the farmer to dispose of large quantities of perishable products which could not otherwise be handled. Canning has made possible a type of intensive farming which was impossible a few years ago, before the facts in regard to the relation of bacteria to decay were understood, and before we knew of the successful means at our command for destroying bacterial life.

BACTERIA IN EGGS.

The presence of bacteria in eggs results in trouble experienced by every farmer, and one which it seems impossible to avoid. It has been naturally supposed that eggs, when freshly laid, would be free from bacteria and hence not liable to decay. But this is certainly not the case. Bacteria are known to enter the oviduct and contaminate the mass of the egg even before its shell is deposited. Hence when the egg is laid it will frequently contain bacteria in greater or less numbers. These bacteria can obtain plenty of oxygen from the air that enters through the porous shell, and are thus able to grow readily within the egg, where they soon cause its decay. A bacteriological study of eggs has shown quite a number of different bacteria in perfectly whole eggs, freshly laid, and there seems to be no possible means of completely avoiding them. Even after the shell is deposited and the egg laid, bacteria are capable of entering it. The shell is somewhat porous and it has been proved by experiment that bacteria can pass through the pores. In short, the egg must be looked upon as a highly nutritious food product, in most cases already inoculated with several kinds of bacteria, and a body which is practically sure to undergo decay in the course of time.

It is, however, possible, by certain devices, to delay or prevent the growth of bacteria in the egg. If it chances that the egg is not contaminated at the time of laying, which may be true of some eggs, it may be preserved for a long time by any device that prevents external bacteria from entering through the shell. There is no means of keeping the fresh taste to the eggs, for this is sure to disappear after a few days, but they may be prevented from actual decay if the egg is covered with an impervious coat which prevents the entrance of bacteria and oxygen. Various devices have been adopted for this purpose, such as the packing of eggs in oats or brine, or covering them with a coat of vaseline, etc. The best method yet devised for this purpose is to immerse the eggs in what is known as *water-glass*, a material composed of sodium and potassium silicate. This is a very cheap commercial article and is bought in the form of a thick syrup. When one part of this syrup is dissolved in ten parts of water and is then poured over the eggs in a suitable vessel, it effectually prevents the entrance of bacteria, and proves to be the most efficient means of preventing the decay of eggs. A single gallon of material thus prepared will preserve 50 dozen eggs. With this method many of the eggs may be kept for weeks without decay, but some of them, even under these circumstances, will in time become rotten and worthless. There is in short no absolutely satisfactory method yet devised for preserving eggs.

BACTERIA IN THE SUGAR INDUSTRY.

A brief mention should be made of the relation of bacteria to the sugar industry, which is an important phase of agriculture. The relation of microörganisms to this industry is, according to our present knowledge, only one of injury. After the product has been harvested, bacteria may produce subsequent injury within it, giving rise to well-known troubles. The sugar beet, for example, is attacked by a disease which is called *gumosis.* It is characterized by the appearance of a yellowish color upon the cut slices of the beet, which may become dark or even black, and, later, by the appearance of a slimy material exuding upon the surface. Eventually the beet may be ruined for sugar-making purposes. This trouble has been found to be caused by bacteria, the particular species that produces this difficulty, *B. betæ*, having been carefully studied in recent years. A second source of trouble experienced in sugar-making consists in the appearance in the product, at various stages of manufacture, of jelly-like masses which may become very abundant and troublesome. These, too, have been long known, and have been studied by bacteriologists for many years, with the result of proving that they are caused by the appearance and development of certain species of bacteria. Several species are known and have been carefully studied, all of which have the power of producing a slimy secretion which gives rise to the jelly-like masses in the sugar product. The slimy secretion appears to be developed from the sugar, a conclusion proved by the fact that the same microorganism when growing out of contact with sugar develops no slime.

These subjects are of more significance in the process of sugar manufacturing than they are in agriculture, and they may therefore be passed with this reference.

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PART V.

PARASITIC BACTERIA.

CHAPTER XIV.

RESISTANCE AGAINST BACTERIA. ANTHRAX.

PARASITIC BACTERIA IN GENERAL.

In the previous parts of this work we have seen that some bacteria are capable of living upon purely *inorganic* food and are therefore wholly independent of other living organisms. These bacteria doubtless play an important part in the soil transformations and probably in rock disintegration. A second class of bacteria feed upon the *lifeless* bodies of animals and plants. These, which we call *saprophytes*, are the great agents for disintegration of organic substances, and are a vital part of agricultural processes. We have now to notice a third class of bacteria, also of great interest to agriculture, which are capable of living in and feeding upon *living* animals and plants. This enables them to exist within the bodies of animals and plants and makes them *parasitic*. Their existence involves an entirely new set of phenomena for consideration. We have learned in the previous chapters that bacteria are, in general, to be regarded as the allies rather than the enemies of the agriculturist. This is true for the first two classes of bacteria, but for the group of parasitic bacteria the reverse is the case, for these are almost universally the foes of agriculture.

The bacteria which live as parasites in domestic animals constitute one of the most serious evils which the farmer has to meet. Fortunately the number of species of bacteria capable of living a parasitic life is small. Nearly all types of bacteria which we have hitherto considered are utterly unable to live the life of parasites, and are, therefore, so far as their power of doing direct injury to animals is concerned, entirely harmless. Of the many hundreds, and probably thousands, of species of bacteria known to-day, only a very small number, a score or two, are positively known to be able to produce disease, and these organisms are commonly found only in special places and not living a free life in nature. With very few exceptions the bacteria which do live a free life in nature are utterly harmless because of their inability to live a parasitic life. The great importance of this conclusion is evident.

RESISTANCE AGAINST PATHOGENIC BACTERIA.

An extremely important topic for consideration centers around the question why the great hosts of harmless bacteria are unable to live a parasitic life. The answer, in brief, is that the active tissues of animals and plants in some way exert an influence upon the bacteria which prevents their growing. For example: the putrefactive bacteria are able to grow with the greatest readiness in a piece of dead flesh from a cow, causing its rapid disintegration. But if these same bacteria are inoculated into the blood of a *living* cow, or into its flesh, they are utterly unable to live for any length of time, and will, under ordinary conditions, die very speedily. Why should living flesh exert such a deleterious influence upon bacteria, while the same flesh, after death, offers such a favorable food for them? The complete answer to this question is one for which bacteriologists have been searching for some years and which they have only partly reached. This matter does not particularly concern us and we need only notice that the answer appears to be, briefly, that the living tissue produces certain active chemical substances which serve as germicides, actually destroying the living bacteria which get into the tissue, or at least preventing their growth ; further, that the parasitic bacteria *alone* are able to overcome the deleterious action of these substances.

One phase of the matter is of the greatest importance to our subject. This resisting power of the living tissue against the invasion of bacteria has its influence upon the true parasites as well as upon other bacteria, only its repressing influence is much less, and indeed so slight that it may frequently be overcome by the invaders. This resistance is exerted against all species of bacteria. Against the common saprophytes it is perfectly efficient ; against some parasitic bacteria it is moderately efficient and will, in many cases, prevent the development of the disease, even after the parasitic bacteria have entered (tuberculosis). Against other bacteria the resisting power is extremely slight (anthrax). The resisting power varies with different species of animals, some species having the power of absolutely resisting certain bacteria, when we call them *immune*. Man is immune against hog cholera while the hog is not. Lastly, the resisting power varies with the individual. Some members of a species will have the resisting power highly developed, while others yield easily to invasion. This we speak of as *individual resistance*.

Now this resisting power is clearly located in the living cells of the body and is dependent upon their normal functions. It is only the *living* cell which can resist the invasion of micro-organisms, either wholly or partially. From this it follows that the resistance will be greatest when the body cells are in the highest state of physical activity, and will diminish when they become somewhat impaired in vitality. Anything, then, which tends to reduce the physical health of the individual tends to reduce his power of resistance. For example, sometimes an individual shows a great tendency to develop boils or abscesses, and but little power of healing them. We say his "blood is in bad condition." By this is really meant that his body activities are so repressed that he is unable to resist the invasion of some of the common bacteria which are present on every hand, and which an individual in vigorous condition easily repels. If his physical vigor can be restored the troubles will disappear, although the bacteria which produce the boils and abscesses are just as abundant around him as before. The same principle may be applied to all attacks of parasitic bacteria. Physical vigor is the best protection against the invasion of parasitic bacteria, and a weakened physical condition invites attack.

This matter is emphasized here because it is too generally lost sight of in the combat against infectious diseases. During the past two decades there has been a very general tendency, in the attempt to avoid diseases, to place the whole emphasis upon the methods of *avoiding bacteria.* This has been very natural since the demonstration of the causal connection of bacteria and disease. If a disease is produced by a bacterium, what more natural method could be suggested for avoiding it than to avoid the bacterium? In accordance with this idea there has developed, on all sides, a long series of rules and regulations suggested by bacteriologists and adopted by health boards, all designed for the prevention of the *distribution* of disease germs. This is indeed the foundation of modern sanitation.

At the present time there is a manifest reaction against this one-sided attitude. No one fails to see the importance of this attempt to prevent the distribution of bacteria by any practical means, but there is to-day a growing recognition that there is another side of the question. The strengthening of personal vigor is of no less importance, many believe it is of more importance, than the prevention of the distribution of bacteria.

The weakening of personal vigor will do more toward increasing germ diseases than a relaxing of the rules which try to prevent the distribution of bacteria. We are beginning to believe that in regard to most infectious diseases the personal resistance of the individual will enable him to repel an attack even after he is exposed, provided his personal resistance be at its maximum; while a weakened resisting power will result in his yielding to the first attack of an invading bacterium. For some of the less violent diseases (tuberculosis) this is much more emphatically true than for other diseases (anthrax). Now it is not possible to hope that we shall ever be able to exterminate *all* pathogenic bacteria, and even if we did, other forms would doubtless take their places. Since we cannot exterminate them, it follows that all individuals will, at some time, be exposed to the attack of some of the germ diseases. Manifestly, then, the best means of elevating the healthfulness of the race is by raising the resisting power rather than by the vain attempt to destroy pathogenic bacteria. All this may be admitted without at all detracting from the value of the sanitary rules advocated by health boards.

INDIVIDUAL RESISTANCE AMONG ANIMALS.

These facts are of even more importance in the case of parasitic diseases of animals than in the case of human diseases. It is of more importance that the agriculturist should understand them, as he endeavors to make a fight against the diseases of domestic animals, than it is for the physician or the veterinarian who tries to *cure* the disease. With animals, as with man, the individual resisting power is variable, at least for some of the most common diseases. When a lot of pigs are attacked by that very fatal disease, hog cholera, some of the lot escape with no signs of the disease, showing a superior resisting power. Undoubtedly the resisting power of animals is due to a proper physical vigor, little understood, it is true, but plainly dependent upon proper conditions of life. Let the conditions be normal and the animal may resist the attack of parasitic bacteria ; but let them become abnormal, so as to reduce its vitality, and the animal is much more likely to succumb.

For example : it is a well-known fact that tuberculosis is much more prevalent among cattle that are kept stabled most of the time, than among those who live a considerable portion of the time in the open air. Now this may be due, in part, to the fact that stabled cattle have a greater chance of acquiring the contagion, since the animals are kept so close together. But this is certainly not the whole reason. Young cattle that are kept in the open for a year or two are less liable to take the disease than those kept in the stable, even though subsequently put under similar conditions. In localities where the animals run out of doors all the time the disease is rare. The more closely the animals are housed the greater the tendency to this disease, and it is practically certain that this greater tendency of stall-fed animals to yield to the disease is not because they are so much more likely to be infected, but because of the depressing influence which such a sedentary life has upon the vitality of the animal, reducing its resisting powers. It is also a general belief that highly bred cattle have a greater tendency to this disease than less highly bred stock. Stated in this way the conception may not be correct ; but it is practically certain that animals that have been bred for the purpose of producing great quantities of milk are rather more liable to yield to disease than those not so highly specialized. Such a specialization of the vitality in the direction of an abnormally high action of the milk glands cannot fail to be at the expense of other vital functions. These breeds have been developed in one direction until they have become abnormal. It is not to be wondered at if such an abnormal development should have resulted in the reduction of their general vitality, and of their resisting power against disease. It is the active, vigorous cow, which produces perhaps but little milk and is not carefully housed by the farmer, that has the power of resisting disease. At all events this is the most probable explanation of the fact that high-bred cattle seem to yield so much more readily to some diseases than do less valuable animals. In short, the prevalence and the increase of some of the diseases of domestic animals must be attributed, in no inconsiderable measure, to the introduction into our herds of conditions of life which lessen their resisting power, and not wholly to the increasing chances of contagion due to closer contact of animal with animal. That the latter phenomenon is also a factor is, of course, evident.

These facts are of the utmost importance for the agriculturist to understand. The conditions of life for his domestic animals are, to a very large degree, under his immediate and perfect control. He can regulate the amount of outdoor life they have, their active or their sedentary life, their food, their drink, and many other factors upon which depends their physical vigor. He may keep his cow housed so that it has little air; he may give it highly stimulating food and practically no chance to use its muscles; or he can make quite a different animal of it by changing its life and food. He can control the conditions of life among his animals far better than he can, or will, those of his own life. In the conditions of civilized life each individual demands his own personal freedom in regard to all matters regulating the affairs of life, and he absolutely refuses to be guided by rules and regulations, even though he may know them to be for his best physical good. No matter how good rules for living our physiologists may make, they cannot hope to get people to adopt them. But the farmer has absolute control over the life conditions of his cattle. He can regulate their life as suits him, and he can, if he will, work out among animals the problem of health and disease as it cannot be worked out among men. He may, by breeding, produce animals with some valuable feature most extremely developed; but in so doing he must remember that he is producing abnormal animals that are likely to have little resisting power against disease. He may feed them with stimulating food and force them in lines which suit him; but he must bear in mind that there is a limit to the possibilities, since all of these methods of treatment lead to abnormal conditions and greater liability to disease.

Nothing is more important than that the agricultural community should fully appreciate that resistance to infectious diseases on the part of domestic animals is in large measure an individual matter, depending upon the most vigorous health and the most normal conditions of life. The adoption of precautions for preventing the distribution of the disease germs is doubtless a matter of very great significance, but of more significance still is the endeavor so to modify the conditions of life as to increase their resisting power against these bacteria. In every case, doubtless, the plan adopted will be by the way of compromise, and will be such as to give the greatest amount of physical vigor consistent with the ends which the farmer has in view in his use of the animals. To turn them out into the fields with no attempt to produce special types, and with no high feeding would doubtless produce a vigorous breed, but it would not produce milk.

It does not fall within the scope of this work to consider at length the various animal diseases which are produced by the growth of bacteria in the living tissues. This subject belongs to the veterinarian rather than to the agriculturist. There are a few of these diseases, however, around which a monumental mass of bacteriological information has been collected, and two of these, at least, are of special significance to agriculture; one because of its scientific and historical interest, and the other because of its extremely practical bearing upon problems relating to the health of cattle, of man, and to the dairy interest generally. We shall, therefore, consider at some little length the two diseases of *splenic fever* (*anthrax*) and *tuberculosis*, and then refer very briefly to other important bacterial diseases of animals and of plants.

ANTHRAX. (SPLENIC FEVER.)

Anthrax is the disease of domestic cattle first deserving our attention. This was the first definite disease of animals proved to be produced by bacteria. It was the study of this disease which furnished practically all the data upon which the germ theory of disease was originally built. It proved to be an easy disease to study and, occurring among animals, it was possible to demonstrate the causal nature of the bacteria by inoculations, a test which is manifestly difficult, or impossible, in regard to strictly human diseases. For this reason anthrax was very well understood before human diseases were investigated. It has been a vigorously contested battle-ground, and one where the germ theory won its first, its most famous, and most decisive victory. The monumental mass of evidence which has accumulated around this disease has furnished the data and the methods by which the whole subject of germ diseases has been tested. It has presented great problems and has offered their solution. For this reason anthrax demands our first attention, even though it is a disease not very common in our own agricultural communities, and, in its ravages upon our herds, does not begin to compare with tuberculosis.

Anthrax is a disease of domestic animals which has been known for many centuries. It is mentioned in the writings of Moses, and Homer refers to it in the Iliad as a well-known disease. From those early days it has been more or less constantly found among domestic animals. It occurs practically all over the globe, in all latitudes were cattle are kept, and seems to be entirely independent of climate. Every country of

Europe suffers from it constantly. Germany has lost some 4,000 cattle from this disease, in some years, and England nearly a thousand. In the United States the disease is also frequent, though generally regarded as less common than in Europe. Although widespread it does not occur in great numbers of animals, as do some of the other bacterial diseases. It may attack the animals of a single herd and produce much destruction, but it is not very contagious, and does not spread readily from herd to herd.

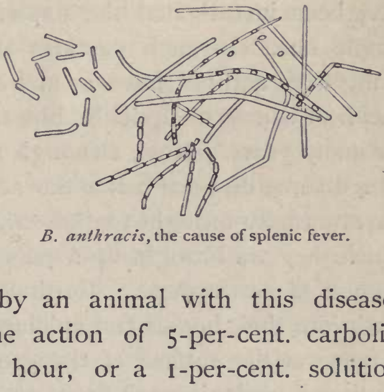
The animals chiefly attacked are cattle and sheep, and these are, indeed, the only animals in which it normally occurs as a spontaneous infection. Many other animals are, however, capable of infection. Horses, goats, deer, and mice are very subject to the disease, while the dog, cat, and white rat are not susceptible. The ordinary fowl will not take the disease under normal conditions, while the sparrow will. The disease is also found in man where it is known by various names, the most common name being *malignant pustule*. Mankind is, however, not one of the very susceptible animals and, when infected by a skin inoculation, the disease is quite apt to be local, while in sheep and cattle it is almost sure to run a fatal course. It is, however, a very serious disease in man when it finds entrance into the lungs, in the so-called *wool-sorters' disease*, and is almost sure to prove fatal. Among men the disease is mostly confined to persons who handle hides or wool, and become infected through the skins of animals who have died of the disease.

The discovery of the cause of this disease was one of the first triumphs of bacteriology. It is excited by a bacterium, *B. anthracis*, which is perhaps better known and more studied than any other species of bacterium. It was first seen in 1849 by Pollender, who mentioned little rod-like bodies in the blood of the animals suffering from the disease, but who attributed no special importance to them. A few years later, in 1863, Davaine made a further study of the organisms in the blood, and advanced the theory that they were the cause of the disease. By a series of experiments and observations he rendered it probable that his theory was correct, although he did not demonstrate it. From the publication of Davaine's conclusions the subject was vigorously disputed, the conservative students denying that the bacilli had anything to do with the disease. The difficulty of demonstrating the theory lay along two different lines. In the first place, organisms were found elsewhere in nature which looked, under the microscope, precisely like those found in the anthrax blood, but which had no power of producing the disease. Not recognizing that these microscopic bacteria might look the same and yet be totally different, the objectors to Davaine's views insisted that the presence of similar organisms in water, which would not produce this disease, disproved the theory. Secondly it was found very difficult to obtain the anthrax bacilli in a pure enough condition to demonstrate that they alone could produce the disease. A drop of blood containing them was capable of producing infection, but such a drop of blood evidently contained something besides these bacteria, and it might have been something else that caused the disease. In those early days of bacteriological study it was recognized that the only proof of a disease being caused by a definite organism, was to obtain the organism separate by itself, and then, by inoculation, to show that it could produce the disease in a healthy animal. Such a pure culture could not be obtained satisfactorily and, therefore, the proof of the theory of Davaine waited for some years.

It was Professor Koch who, in 1875, first succeeded in getting pure cultures of the bacillus in such a manner as to enable him to study it carefully and to demonstrate its power of producing anthrax in healthy animals. His work was followed almost at once by work of Pasteur who, apparently in ignorance of the experiments of Koch, undertook the investigation by quite a different method. Pasteur's work was of a more striking character than that of Koch, and a few years later, in connection with this very disease, Pasteur made an epoch-making discovery of a method of preventing it. For this reason his name has been intimately associated with the disease even though he was not the first, either to discover its cause, or to demonstrate the causal relation of the bacillus and the disease. Both Koch and Pasteur succeeded in obtaining a pure culture of the bacilli, Koch by the use of a special culture medium made of the aqueous humor from the eye, and Pasteur by the simple method of dilution produced by successive inoculations. Finding that the bacillus would grow in a solution made by steeping yeast in water, Pasteur inoculated a flask of such *yeast water* with a drop of anthrax blood. In a day or two his flask was filled with the bacilli which had arisen from the first by division. The inoculation of a second flask from the first showed like results, and, by continuing such inoculations from flask to flask, he rapidly got rid of all parts of the original drop of blood except such parts as had been multiplying in the flasks. His microscope showed him that the only thing that multiplied and remained in his later flasks was the bacillus which was present in the original drop of blood. In this way he obtained a culture of the bacillus, free from every trace of the original drop of blood. Nevertheless he found that though he continued these inoculations indefinitely, every flask was equally fatal, and a small drop of the culture would inevitably produce anthrax in a susceptible animal in a very few hours, the development of the disease being always accompanied by the growth of the bacilli in its blood in countless myriads. These results left no loop hole for criticism, proved that this bacillus was the cause of anthrax, and thus for the first time demonstrated that an infectious disease was produced by a bacterium multiplying within the body of the animal in which it grows as a parasite.

The bacterium in question, *B. anthracis*, is a typical rod-shaped bacillus (Fig. 38), of moderate size (1 μ by 3–6 μ). It is a rod with square ends and it multiplies by repeated division, the elements remaining attached to form long chains. Sometimes these long threads show no signs of the divisions and, in certain media, they form marvelously twisted and contorted masses. It is motionless and multiplies with wonderful rapidity. When in an active growing condition it is readily killed by ordinary disinfecting agents, and by a moderate heat, a temperature of about 160° F., easily destroying the rods. But this bacillus produces spores which are very resistant. The spores develop in the threads if there is an abundance of oxygen. They are of good size (Fig. 38) and may be most readily distinguished in anthrax cultures as clear, glistening bodies. It is their resistance to ordinary destructive agents that makes anthrax so persistent, and this high resistance must be borne in mind when the attempt is made to disinfect a stable which has been occupied by an animal with this disease. These spores will resist the action of 5-per-cent. carbolic acid solution for half an hour, or a 1-per-cent. solution of corrosive sublimate for about the same length of time. Few other living bodies can resist such treatment. The spores will also resist high heat. When dry they can stand a temperature of about 280° F. for two or three hours. When immersed in liquid they are much more easily killed, since the temperature of boiling, if maintained for a few minutes, is commonly sufficient to destroy them. While there are some bacterial spores which are more resistant than this, these anthrax

FIG. 38.



B. anthracis, the cause of splenic fever.

spores must be regarded as among the most resistant of all known forms of living matter. When dried the spores may remain alive for a long time, many years at least, and yet all the time retain their power of developing when placed under proper conditions. All of these facts are of the greatest importance in enabling us to understand the phenomena connected with the disease, and they evidently make the disinfection of an infested locality a matter of very great difficulty.

Although this disease is extremely fatal, animals affected rarely recovering, it is not particularly contagious, and is rarely communicated directly from animal to animal. One common method by which cattle are infected appears to be through the food which they crop in the fields. It has often been noticed that the disease breaks out in a herd shortly after it has been turned into a new pasture. In some of these cases which have been investigated the explanation of the infection is a simple one. In such pastures the dead bodies of animals dying from anthrax have been buried in earlier years. The bacilli of course die quickly, but the spores may remain alive for many years. Now, although these spores may have been buried some distance below the surface, it is certain that they are eventually brought to the surface. One of the means by which they are brought up from under ground is through the agency of earthworms. Earthworms are constantly taking earth into their bodies and voiding it at the openings of their burrows at the surface of the ground. In this way they are continually bringing soil from some depth to the surface, and it is easy to suppose that they may thus bring up the anthrax spores. This suggestion, at first tentatively advanced and much doubted, has, in later years, been demonstrated by finding the anthrax bacillus in the bodies of earthworms which were taken from pastures where the bodies of anthrax animals had been buried. The spores thus brought to the surface may certainly infect animals feeding on the grass. This too has been somewhat questioned, since it has appeared that if the ordinary bacilli be swallowed by an animal with its food they do not appear to have any power of producing disease. Probably they are so injured by the digestive juices that their pathogenic power is lost. But recent experiment has shown that this does not hold for the spores, since the anthrax spores mixed with the food are capable of inducing the disease. These spores resist the injurious action of the digestive juices and of the other bacteria present in the intestine, and make their way through the intestinal walls into the body and produce the disease. These facts readily explain the common occurrence of intestinal anthrax among cattle, and account for many of the phenomena connected with the outbreaks of epidemics.

In other cases the germs may find entrance through abrasions of the skin. When thus introduced the bacteria first produce a simple abscess in the skin which soon turns into a gelatinous pustule. This pustule does not heal, and from it as a center the bacilli spread rapidly through the body, producing a general disease which proceeds rapidly and terminates fatally. In the case of animals which, like man, are less susceptible to the disease, these abscesses may remain simple localized infections, eventually healing without spreading through the body. The name *malignant pustule* is appropriately applied to this form of the disease. In susceptible animals such recovery is very rare. There are other modes of infection, but among animals the disease is most commonly acquired either through the intestine or through skin abrasions.

In the body of the infected animals the bacilli grow with the greatest rapidity. An extremely small number of them inoculated into the body of a mouse produces the death of the animal in about twenty-four hours and, after death, the whole body is found to be filled with the bacilli in incalculable numbers. A rabbit is similarly killed in two days, and larger animals in somewhat longer periods. Even a sheep may be killed in two days by such an inoculation. The disease is marked by a high fever and much discomfort and, after death, the most characteristic symptom is a greatly *swollen spleen*, whence the name *splenic fever*. The spleen is large, hard and brittle, and contains enormous numbers of the bacilli. The blood-vessels are also found to be full of them and the capillaries may be literally crammed with bacteria. The method by which the bacillus produces its injurious effect upon the body is not simply by the mechanical presence of the numerous living parasites, but rather by the direct toxic action of certain poisons which are produced by the bacteria. These toxic bodies have been studied carefully, and may be regarded as the direct cause of the disease and death of the animal, the bacteria being the indirect cause.

This bacillus is extremely virulent in its action upon susceptible animals, so virulent, indeed, that a single bacillus, inoculated under the skin of a susceptible animal, may be sufficient to cause the disease and death. In the less susceptible animals it requires a larger dose to produce similar results. The lesser susceptibility of such animals as the dog, the horse, the bird, etc., renders them practically immune against spontaneous infection, and the disease only occurs in them as the result of artificial experiment. In man the disease is of rare occurrence, being practically confined to people dealing in or handling hides or wool, and is acquired by them either through abrasions in the skin, when it produces *malignant pustule*, or by breathing the spores into the lungs when it is called the *wool-sorters' disease*.

The bacilli, however, vary greatly in their virulence. Some cultures are extremely potent, as just mentioned, while others are far less so; and some, indeed, so weak in their action as to be unable to produce a fatal disease even in susceptible animals. It has been found possible to weaken the pathogenic powers of even the strong cultures by proper laboratory cultivation, and this fact has led to one of the epoch-making discoveries in regard to germ diseases.

PREVENTIVE INOCULATION.

One of the most important discoveries made in connection with this disease was made by Pasteur in 1881, and resulted in the use of the so-called *anthrax vaccine.* After having demonstrated conclusively the causal connection of the bacillus and the disease and its probable means of distribution, Pasteur turned his attention to the discovery of some method of prevention. In thinking over the matter there occurred to him the well-known fact that a single attack from some of the infectious diseases, if recovered from, renders the individual immune against a second attack. He asked himself the question whether this was true of anthrax and, succeeding in finding an animal that had recovered from the disease, he inoculated it with the anthrax bacillus. The animal was not affected by the inoculation, proving thus that one attack may produce a certain amount of immunity from a second. Pasteur also knew that, in general, a mild attack of a disease conveyed immunity as well, and possibly as efficiently, as a severe attack, a mild form of a disease producing immunity from a severe form. Vaccination against small-pox by the virus of cow-pox served him as an example of a mild disease protecting the individual from a more severe type. He then argued that if he could contrive to give animals a mild form of anthrax they would probably be protected from a more severe form.

To accomplish this end was not a simple task. It would be of no use to inoculate an animal with a small quantity of the bacilli, for these, by multiplying, would soon become so numerous as to produce a severe attack of the disease. It was evident that this end could be reached only by weakening the pathogenic powers of the bacilli. After continued experimenting he finally accomplished his purpose by cultivating the bacilli at a temperature somewhat above that at which they make their best growth. By the use of a temperature of 108° F. he could cultivate the bacilli, but at this temperature they lost some of their virulence and did not form spores. By continued cultivation at this temperature he obtained cultures which were so weak as to be unable to produce a fatal disease even in susceptible animals.

After reaching this result Pasteur, by an ever-memorable public experiment, demonstrated to the world the possibility of combating infectious diseases by the use of what are now known as *weakened cultures*. He inoculated half of a lot of fifty susceptible animals, including cattle and sheep, with his weakened virus. The animals were slightly indisposed, but suffered no evil consequences. In a few days he inoculated them with a second, stronger culture, with a like harmless result. Having thus prepared these test animals he summoned to a public experiment an assemblage of noted men in Paris and, in the presence of the whole, inoculated all of the fifty animals with the strong infectious material taken from an animal dead from the disease. Two days later the company assembled again to find all of the unprotected animals either dead or dying from a violent case of anthrax, while of the protected animals not a single one showed the slightest evil result of the inoculation. A more successful experiment has hardly ever been recorded.

The announcement of this discovery flew rapidly over the world, and there was at once a great demand for this anthrax vaccine from countries especially affected with the disease. Since that date these weakened anthrax cultures have been made in large quantities and sent all over the world and have been used very extensively. In countries where the disease is common, thousands of animals have been inoculated, and it is thought that the lives of a great number have been thus protected. It has been found by experience that the protection thus acquired is not very lasting, and that animals must be inoculated about once every year if they are to be thoroughly protected. This, of course, reduces the value of the inoculation, and confines its use to special localities or special seasons when the disease is rife. The vaccine also deteriorates rapidly and is only efficacious when fresh. For these reasons the method of inoculation is not used so widely as at first, and has not been used to any considerable extent in the United States.

This discovery of Pasteur had, however, an importance extending far beyond its relation to anthrax. It was the discovery of a new *principle*, and not simply the discovery of a new *fact*. If it is possible to produce immunity by the use of weakened cultures, and if pathogenic bacteria may be weakened by simple laboratory methods, why could not the same principle be applied to all other infectious diseases? It was immediately perceived that this discovery marked a new epoch in the methods of studying and handling diseases, and it was inevitable that the same principle should be extended to many other diseases. It is not in place here to follow this discovery into other fields and explain its successful and non-successful application to other diseases. It may simply be stated that this famous experiment pointed out the line along which bacteriologists have been working in the last two decades, and are still working, in their endeavors to find the proper method of mastering some of our most serious human diseases. The results have borne much fruit and we may confidently expect even greater results in the future.

CHAPTER XV.

TUBERCULOSIS.

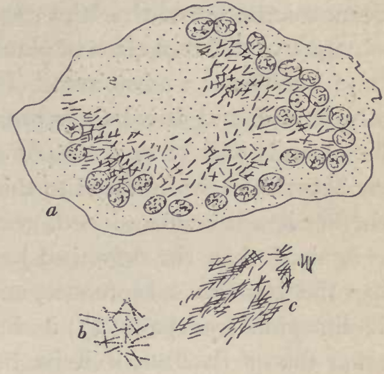
Of all the strictly pathogenic bacteria there is none so widely distributed as the *B. tuberculosis*. The great prevalence of this disease in the human race has made the subject one of the highest importance. Not only is it of great significance from the standpoint of human health, but it is the one disease of domestic animals which demands universal attention and interest among agriculturists. Tuberculosis among cattle forms one of the most vital subjects connected with modern agriculture.

THE TUBERCULOSIS BACILLUS AND ITS GENERAL CHARACTERS.

The organism which produces this well-known disease has been known to science about 18 years, having been first described by Koch in an epoch-making monograph published in 1882. Professor Koch first isolated the bacterium from the sputum of consumptive patients, and subsequently found it in abundance in animals suffering from certain diseases now known to be tuberculous. The organism itself appears commonly in the form of a short, slender rod (Fig. 39, *c*) about .2 to .4 μ in diameter and 1.5 μ to 4 μ in length. It has no motility and never forms long chains, although three or four may frequently be seen together.

In all questions concerning the distribution of an infectious disease the formation of spores is of most vital importance, as we have already seen. The problem of the formation of spores in the tuberculosis bacillus stands upon a somewhat uncertain footing. At times there have been seen within the rods certain granular masses which have been called spores (Fig. 39, *b*); but more careful study has disproved this conclusion. There are certain facts, however, which have led some bacteriologists to insist that the organism must produce spores. As we have seen, active bacteria are destroyed by a moderately low temperature and only spores resist high temperatures. Now the tuberculosis bacillus will sometimes resist high temperatures. It may even be heated to a temperature of boiling for an instant without killing it, and such resistance is only expected of spores. Moreover, the organisms may be dried and yet remain alive for many months, and probably for years, without losing their vitality, a resistance which again suggests the formation of spores. But the actual formation of spores by this bacillus has not been seen and is still uncertain. The important facts are that the organism can resist drying for a long time and is able, under certain conditions, to resist a heat close to boiling without being killed. These facts are of great practical significance, for upon them must be based all methods adopted looking toward disinfection. The question whether they form spores is one of scientific interest only.

FIG. 39.



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

The formation of spores is not the only unsettled question concerning the organism. It is even uncertain whether it really belongs to the true bacteria. Within recent years it has been found that, under certain conditions, the rods are seen to produce branches. True bacteria are not supposed to branch and, if this branching does actually exist, it suggests that the tubercle bacillus is not a bacillus at all, but belongs to a different group of fungi. It has in short been claimed by some recent microscopists that the organism is not a bacterium but belongs to a different class of plants and that, consequently, the name *Bacillus* is a misnomer. These facts have not yet been fully settled, and it is hardly possible to-day to state whether the claim is correct or not. At all events the name bacillus has become so firmly fixed to this organism that it is doubtful whether it will be abandoned, even though it should be later recognized that the organism has relations with other plants than the bacteria. Moreover, according to the most recent classification (see page 27) it should be called a *Bacterium* rather than a Bacillus, if it be retained among the Bacteria. But the term Bacillus is almost certain to stick to it, and in our discussion we shall continue to use this name, at the same time recognizing that its right to be called a bacillus is in some doubt.

Conditions of Life.—This bacillus has somewhat limited conditions under which it can grow. The temperature limits within which its development is possible are quite narrow. At first it was stated that it demanded a temperature between 96° and 105° F., but wider experimenting has shown that it will grow at much lower temperatures, even as low as 84° F. At these low temperatures it grows much more slowly than at the higher temperatures. At first it was supposed that it would not grow in any artificial media which could be prepared in the laboratory. In his original experiments Koch was obliged to use coagulated blood serum as a culture medium. But on this point, too, wider experimenting has extended the possibilities. It is now found that it can live and flourish in a variety of culture media, provided that a certain amount of glycerine is added. It was at first said to be a perfect parasite, by which term is meant that it would not live under any conditions except those of a warm-blooded animal, demanding both a temperature and a medium equivalent to the blood of such an animal. But here, too, bacteriologists have changed their views, for the tubercle bacillus will grow now in many laboratory media, and under conditions very different from those of the living body.

The facts just enumerated are of the greatest significance as indicating the possibilities of distribution of this disease. If the bacillus can live outside of the body of animals we may look to various places in nature as a source of infection, while if it demands for its existence conditions of the living body, we must look to animals alone as its source. Now although, as noticed, it can grow under conditions quite different from those of the living body, it is nevertheless a fact that, so far as our present knowledge goes, it does *not* grow outside of the body of animals under any normal conditions. It will not grow in water or in milk, two facts of the utmost importance in understanding its distribution. It is true that the bacillus may frequently be found alive outside of the bodies of animals. It occurs in sputum, in milk, in water, in dust, etc., but in these media it does not multiply, at least under any conditions to which they are normally subjected, and we must therefore conclude that its multiplication is *confined to the bodies of warm-blooded animals.* While it can flourish in the artificial culture media of the laboratory, when kept at special temperatures, it does not flourish in nature outside of the body of warm-blooded animals upon which it lives as a parasite.

Animals Subject to the Disease.—As a parasite it is able to live in a large number of animals. Besides living in man it can flourish readily in the bodies of cattle, hogs, dogs, cats, monkeys, rabbits, guinea-pigs and some other animals. In all these it produces symptoms showing great resemblances, differing, of course, slightly in the different animals. The characteristic feature of the disease is the production of *tubercles*—swollen masses of tissue—which eventually break down into a cheesy mass. These tubercles may appear at almost any part of the body. Of all the animals the guinea-pig is the most delicately susceptible to the bacillus. An extremely small infection will produce the disease in the guinea-pig, and for this reason these animals are used in experiments to test the presence of the bacillus. A little suspected milk inoculated under the skin of the guinea-pig will produce the disease inevitably, if only the smallest number of virulent germs are present. Besides these mammals a number of birds show a similar disease, with a similar bacillus present in the infected organs. The bacillus in birds is, however, in some respects, slightly different from that in men and cattle, and is commonly regarded as a different type of the organism. They are, however, closely related and there is considerable evidence that the one type may be converted into the other under proper conditions.

Most parasitic bacteria are able to grow only on certain parts of the body, diphtheria commonly in the throat, cholera in the intestine, etc. But the tubercle bacillus can live in almost any part. It is found in the intestinal organs, in the lymphatic glands, in the lungs, in the bones, in the joints, in the kidneys, in the skin and, in short, almost anywhere. When occurring in the different organs it receives different names, *consumption*, *scrofula*, *lupus*, *hip disease*, *nephritis*, being some of its common names. Knowing the wide ravages of the disease it is something of a surprise to find that it is not especially virulent and, indeed, when the individual is in good health, it will commonly produce either no trouble or only a local infection which is quickly healed. If a lymphatic gland is infected, for example, there may be simply a small tubercle which, after running a definite course, has a tendency to become hardened and dormant without producing further trouble.

Even when entering the lungs it may in a similar way be quickly checked in its growth, without the person infected suspecting its presence. In other cases the bacilli spread from the first point, and may cause many secondary foci of infection, until finally they distribute themselves through the body and produce numerous tubercles at a distance from the point of original infection. Finally they may get into the blood and be carried over the whole body, producing a generalized tuberculosis which is quickly followed by death. The disease is thus one of widely-varying virulence, from a slight local infection, which is not noticeable, to a fatal case of generalized tuberculosis. It is a certain fact that the tubercles may be healed, and that many animals, after having contracted the disease as a localized infection, perfectly recover and live out a normal life without further trouble. The importance of this fact in determining the method of treating bovine tuberculosis is evident.

The identity of the various diseases which now are classed together as of tuberculous origin was not known when the modern study first began. The diseases were long known among animals and various types recognized among men. But that the many forms of the disease, now placed together, all belong to the same type of infection has been demonstrated only in recent years, and this largely from microscopic study. It has been chiefly the discovery of the tuberculosis bacillus in these various infections that has demonstrated their tuberculous nature.

Variations in Virulence.—Not only do different animals show great differences in susceptibility, but different cultures of the bacillus show great differences in virulence. If a series of cultures obtained from different sources be tested, side by side, their power of producing disease in slightly susceptible animals is found to vary greatly. The most important aspect of this matter arises from a comparison of the virulence of the bacteria derived from cattle and from the human species. Cultures obtained from cattle behave very differently from those derived from man. If both are injected into rabbits—animals whose susceptibility to the disease is not very great—the bovine bacillus is found to be the most active. If both are inoculated into calves it is found that the human bacillus produces only a slight local infection which soon heals, while the bacillus from the cow produces commonly a serious disease which progresses, perhaps, to death. This fact has been verified sufficiently to place it beyond doubt; but the interpretation to be placed upon it is not quite so clear.

Some have thought these facts indicate that each animal has a variety of bacillus especially adapted to itself, and that bacilli from other animals are not so likely to produce the infection as those from other individuals of the same species. This suggestion led to the opinion that each species of animal is chiefly infected from other individuals of the same species, and only exceptionally from individuals of other species. This would, of course, make the passage of the disease from cattle to man an exceptional matter, and indicate that the human infection comes practically always from other men. Others have insisted that these facts show that the bovine variety of the bacillus is decidedly more virulent, and hence is more dangerous to man than the human variety. This would make the passage of the disease from cattle to man easy, while the reverse infection could hardly occur. The latest evidence on the question has shown that the bovine variety is more virulent than the human variety for several species of test animals, including rabbits, swine and cattle. Experiments to test its virulence upon man are manifestly impracticable. It is clear that the conclusion upon this matter is of the greatest importance. If the bovine bacillus is more virulent than the human variety for man as well as other animals, it will evidently point out a great danger of distribution of the disease from cattle to man. If, on the other hand, it should prove that the human bacillus is especially dangerous for man, while the bovine variety is slightly virulent for man, the danger of human infection from cattle would be slight. A positive decision between these two views cannot at present be reached. Prof. Koch has recently announced the conclusion that the bovine bacillus is different from the human bacillus, and that the disease consequently cannot pass from cattle to man.

BOVINE TUBERCULOSIS.

To the agriculturist the most important phase of this problem is its relation to cattle. It is true that in recent years, owing largely to the feeding of swine with creamery refuse, the disease is coming to be somewhat common among swine. But it is among cattle that the trouble is most widely distributed and of the most serious import. In cattle it attacks chiefly the glands of the neck, the glands of the intestinal tract, and the lungs. It may, however, be found almost anywhere. It may be located in the udder, and in these cases the milk of the animal becomes a source of danger to the animal drinking it. Fortunately, the number of cases of udder disease is comparatively small. It is impossible to give any exact statistics, but 1 per cent. is probably not far from correct. In cattle it rarely attacks the bones, joints or muscles, and is almost always confined to the glands, lungs and intestinal tract.

In cattle, as in man, it has a variable course. It may be localized in a small gland and produce no disturbance whatever. Such cases are only to be detected by tuberculin, and some of them are known to recover completely. It may, however, spread from such centers into other glands, and from these spread still more widely, until the whole body is infected and a fatal result occurs, or the animal is condemned to slaughter. The disease may take several years to run its course, the animals apparently remaining for years without special change, but eventually running down and requiring slaughter. In other cases the disease runs its course very rapidly. When it infects the intestinal tract it may affect many glands at once, and produce great numbers of small tubercles in the intestinal organs, which look like masses of grapes, and have given rise to the name *grape disease*, called *Perlsucht* by the Germans. In such cases the progress of the disease is very rapid. If it attacks the lungs it will produce coughing and difficulty in breathing. These various types, however, concern the veterinarian rather than the bacteriologist. From the bacteriological standpoint they are all the same disease, produced by the same bacillus, differing only in the point of attack, the violence of the pathogenic action and the rapidity of distribution through the body.

METHODS OF DISTRIBUTION.

It is a fact of the greatest importance, now admitted on all sides, that tuberculosis is contagious. By this is meant that the relation of the bacillus to the animal is such that there is an *easy means* of communication between one animal and another under the ordinary conditions of life. The knowledge of this fact in regard to human consumption has been of great value, since it has been followed by a steady decline in the amount of the disease. Such knowledge has not yet reduced the amount of bovine tuberculosis.

We can easily understand the methods of contagion when we remember that the bacilli are discharged from any of the open tubercles in the body and may readily find their way to the exterior. If the disease is located in an internal lymphatic gland it may not result in breaking down the gland, and there may be no discharge. Under this condition there is no contagion from this animal to another. But if it be located in the lungs the bacilli will be discharged and pass through the trachea into the mouth. They will enter the mouth and nasal cavity and infect all the discharges from the mouth and nose. It is true that the cow does not expectorate, but by putting her nose in the drinking trough she will be sure to contaminate the drinking water, and when she licks another animal, as she will be sure to do if she stands near others, she will leave some of the bacilli clinging to the second individual, ready to begin their mischief if they chance to get carried to a susceptible part, as they are very likely to do by being swallowed. Moreover, although the cow does not expectorate, she does swallow all of the secretions from her mouth. The tubercle bacilli will thus be carried to the stomach, and through the intestine, from whence, if not destroyed by the digestive juices, they will be voided with the excrement. If the disease is located in the intestine the bacilli will be sure to be discharged with the excrement. In these ways the excrement of tuberculous cattle is sure to be impregnated with the bacilli. Now the conditions of the ordinary cow stall, in even the best cow barn, are such as to make it almost inevitable that the infectious material will soon be distributed through the whole barn. The excrement is carried over the floor, perhaps, for some distance to the opening used for its exit, and the farmer's boots will always collect more or less of this excrement and carry it through the barn. The particles adhering to his boots will be sure to be knocked off when dry, and will thus be carried everywhere that the farmer goes. They will be certain to be dislodged near a healthy cow and will, most likely, become mixed with her food which is commonly thrown on the floor in front of her. If not in the food the particles will eventually become dry and be distributed through the barn as dust. In short it is inevitable that the bacilli voided with the excrement will, in time, get an opportunity to come in contact with every healthy animal kept in the same barn. These facts are quite sufficient to account for the spread of the disease from animal to animal in the same barn, under the ordinary conditions.

But even the cleanly kept barn would merely reduce, not remove, the chance of infection. When the disease is in the lungs the animal is commonly attacked by a cough. In ordinary breathing the bacilli are not exhaled with the breath, but in coughing, quantities of the organisms are dislodged from the mouth and nose, and thrown out into the air adhering to particles of moisture. These float around for some time and form a most ready means of infection on the part of other animals in the vicinity. Indeed the only method of preventing the distribution of the bacilli from an animal whose lungs or intestines are infected, is to isolate her completely from the rest of the herd, and to see that no possibility is left for her feces to be carried into the stalls of the healthy animals.

Once distributed from the infected animals, the bacilli may find entrance into the healthy animals by a variety of channels. Doubtless some of them find entrance to the lungs, either by dust particles, or by bacilli-laden moisture drops from coughing animals, which are breathed by healthy animals. If they find entrance in this way they are able to start an infection in the lungs or glands of the neck. The fact that these regions are so commonly affected in cattle shows that this is one of the common means of infection. The bacilli which get into the food or into the watering trough will be swallowed, and the same will be true of those which the animal takes into its mouth by licking its infected neighbor. Finding their way thus to the intestine they may start an intestinal disease. Such a means seems to be very common, judging from the frequency of intestinal tuberculosis. These two means of entrance are doubtless responsible for most cases of bovine tuberculosis, but there is still a difference of opinion as to which is the more common. It by no means follows that all animals receiving the bacilli in their bodies will develop the disease, for many of them may have a sufficient resisting power to drive them off. But it is very easy to understand from these facts how a single diseased animal in a barn may, in time, infect most of the herd.

ABUNDANCE OF BOVINE TUBERCULOSIS.

Tuberculosis is widely distributed among cattle, although it is by no means universally found in countries where cattle are kept. Some countries appear to be free from it. It is said not to occur in Africa, and until recently it has been absent from China and Japan, having been lately introduced with imported cattle. In the western part of the United States, where the cattle live out of doors most of the time, it is rare or absent. In general it is most abundant in localities where the cattle are housed for a considerable part of the year. It is consequently most abundant in northern countries, and appears to be most widely distributed in northern Europe, Denmark and Sweden. In warmer climates the disease is less common, due probably to the fact that the animals live in the open for a large part or the whole of the year. But even in the warmer countries of Europe it is more prevalent than could be wished. The disease is found, in short, in nearly all countries where cattle are kept, and is almost proportional to the length of time in which the cows are kept housed during the winter season.

It is practically impossible to determine the percentage of tuberculosis among the cattle of any country. There is no method of detecting its presence among living animals except by the use of the tuberculin test (see page 368), and this has not been used sufficiently in any country to make it possible to draw any conclusions as to the amount of the disease. The only reliable data for determining its presence come from examinations of slaughtered cattle, and this method is open to serious objections, the chief of which is the variation in the accuracy of the inspection in different places. The evidence from this source shows such variations that it would be rash to attempt to give any distinct figures. It may be stated that among the animals examined in the slaughter houses of Denmark it has sometimes appeared that more than half of the cows are tuberculous. From these high figures the percentage has ranged down to 10 per cent., and even lower in some cases, and, in fact, is so variable that no general averages are of any significance. In the United States the results differ so widely that figures have, as yet, little value. Sometimes every animal in a herd is found to be tuberculous, while again other whole herds are entirely exempt. In the western states the amount is small. In the eastern states it is large, and in some cases appears to approach the figures given for Denmark. One may find in different localities averages all the way from 6 per cent. to 30 per cent. or higher. When the numbers of infected animals in a herd range from 0 to 100 per cent., it is evident that no average that might be made would be of any significance.

Increase of the Disease.—The next question of vital interest is whether bovine tuberculosis is on the increase. The extreme significance of this question to the agricultural industry is so evident that it needs no comment. Upon its answer seems to depend the future of the dairy industry. For if, as has been claimed, it is rapidly increasing, the very existence of the dairy industry is threatened. But here again statistics are so uncertain as to make a conclusion difficult. Certainly we hear much more of the disease than we did a few years ago, and certainly the percentages reported to-day are much higher than they were ten years ago. The knowledge of the disease is, however, of very recent date, and it has only been in the last few years that its full import has been felt. The increasing interest in the subject has caused a more and more careful inspection of slaughtered animals, which has resulted in a constant increase in the number of reported cases. Even in the same slaughter houses and under the same management the percentage of tuberculous animals reported has been increasing year by year, in such a way as to seem to indicate an alarming increase in the past fifteen years. But a considerable part of this increase is clearly due to increased experience and carefulness in inspection. To what extent this factor explains it, and to what extent there is an actual increase in the disease, no one can pretend to say.

It is therefore impossible to state whether bovine tuberculosis is rapidly increasing, or slowly increasing, or remaining stationary. It has been almost universally recognized, however, that an increase from 11 per cent. to 33 per cent., in the returns given in the same locality for a period of 7 years (occurring in Leipzig), indicates something more than increased carefulness of inspection. The practical uniformity with which these reported percentages have increased in the last years has led to the general belief that the disease is actually increasing among our herds.

But although no definite statistics can be given, either as to the prevalence of the disease or its increase, it is evident that bovine tuberculosis is abundant enough, and that it presents a very serious problem to the farmer. Entirely independent of the question of its relation to human tuberculosis, the disease, as it exists among cattle, is a serious menace to the dairy industry. The amount of financial injury that it does to the farmer each year is very great—far in advance of that produced by the much-dreaded anthrax. The insidiousness with which it finds its way into and spreads through the whole herd, even before the farmer is aware of its presence, the large number of cattle rendered worthless through its agency, especially among high-bred and valuable animals, the suspicion which it throws upon the milk supply, the injury that it does to the animal which is to be used as food, the great cost of tuberculosis legislation by the different states, all these serve to emphasize the seriousness of the problem. Nothing can be of more importance to the farmer than the discovery of some means of controlling this disease. Legislation designed to control it has been adopted by most states in Europe and America, but such legislation has had usually in mind the protection of the public rather than the assistance of the farmer. If the latter phase of the matter had been taken into more careful consideration, such legislation would have been more successful.

THE COMBAT AGAINST BOVINE TUBERCULOSIS.

The extreme importance of this disease for agriculture makes it not out of place to give here a summary of the important facts which have been settled as bearing upon the methods of protecting cattle against this disease. Protection against tuberculosis depends upon the principles and facts already mentioned, but in their application a number of points must be borne in mind.

Resistance of Cattle.—The first thing to be remembered is that the foundation of a successful contest against the disease in a herd is that the animals should be in a proper condition to resist it. This side of the question is too commonly neglected, and nearly all of the attempts made to combat the disease have been directed solely toward devising measures for preventing the distribution of the bacillus. It is, however, admitted to-day that it is impossible absolutely to prevent the bacillus from being distributed from diseased animals, and occasional infection will occur in spite of all preventive measures. Without some efforts directed toward producing a healthy herd of resisting animals, it is quite certain that the endeavor to prevent the distribution of the disease will be unsatisfactory.

It is doubtless much more easy to give the farmer directions looking toward the prevention of the spread of the bacillus, than it is to instruct him how he may increase the resisting power of his animals. But nevertheless some suggestions may be made which, if carried out, will certainly improve the conditions and induce better health and, hence, greater resisting powers. There is little doubt that in a majority of cases the cattle need more air. Too many are crowded together in a small space in the winter season, and there is too little ventilation of the cow stalls. The treatment of the animals in the winter is doubtless responsible for most of the tuberculosis. In the attempt to keep animals warm, they have been too closely shut up in badly ventilated rooms, and the warm air is breathed over and over again by them. Such a condition, wholly independent of the tubercle bacilli which might be present, has a debilitating effect upon cattle, just as it would on men. Too frequently, even on the better farms, the cattle are shut up in the stalls early in the fall, are not allowed to go out during the long months of the winter, and never get a breath of fresh air. Sometimes the case is even worse than this, for many cows are thus shut up as soon as they begin to produce milk, and, winter and summer alike, remain in close, poorly ventilated rooms. To protect his cattle from cold the farmer makes his cow barn too warm and allows it too little air. To save trouble he keeps them housed all the time, with no out-of-door air; and to save expense he crowds them together in the smallest amount of space. These facts lie at the foundation of the large amount of tuberculosis in the colder countries. Warm rooms and a close crowding of the animals may result in a saving of food, but it invites the spread of tuberculosis, if it once gains access to a single animal. In the human race it is well known that the best protection against the disease, and the best remedy for it after it has once started, is out-of-door life. Doubtless the same is true of cattle, but this fact has been almost forgotten in the attempt to produce the most milk possible at the smallest expense. The farmer may perhaps insist that such crowded conditions are necessary and unavoidable in the modern farm, but he must also remember that, whether necessary or not, they are certainly inviting tuberculosis, and bringing his animals into a condition where they are sure to yield to the infection the first time that chance brings the bacillus in their vicinity. More outdoor life and more air are the prerequisites for a healthy herd.

The owner of cattle should also remember that anything which will induce a vigorous life will decrease the tendency to the disease. Proper food is an important factor in determining health, and, beyond doubt, the animals ought to have exercise. It may be impossible under the conditions of modern farming to allow the cattle to have exercise in the winter, but the lack of it is certainly one of the factors tending to increase the liability to tuberculosis. It must also be remembered that too great attention paid to the increase of the yield of milk lessens the resisting power of cattle. Our agriculturists, by over-feeding with certain kinds of food, and by special high breeding for the purpose of increasing the yield of milk, are trying to make a milking machine out of the cow, instead of a normal animal, and it is quite likely that this breeding out of normal characters has increased the tendency to yield to tuberculosis. The highly bred animals are, of course, too useful for the purpose for which they are bred, to be given up, but the agriculturist must remember that he cannot turn his cow into a simple milk-making machine, without suffering some evil results from the change in her nature. In short, if the cattle owner will learn that cattle are animals and not machines, and that they need something besides food and water to keep them active, he will probably soon find the tendency to tuberculosis becoming less.

PROTECTION AGAINST INFECTION.

But while the foundation of a healthy herd must be a regard for the cow as an animal, it must not end here. Methods of guarding the animal against infection are of the greatest usefulness. While we cannot hope that it will ever be possible absolutely to get rid of this disease by any method of limiting its facility of distribution, it is certainly possible to reduce it, and to protect many an animal that would otherwise contract the disease. Our brief consideration of this highly important matter must be given from two standpoints: (1) The healthy herd, (2) the infected herd.

1. Protection of a Healthy Herd.—If a farmer has a herd in which the disease has not appeared it is of especial interest to him to keep his herd in this condition; for once the disease has entered the herd it is very difficult to get rid of it. So long as he can keep his animals from becoming contaminated from without, he may be practically sure that the disease will not appear among them. All evidence shows that tuberculosis does not develop spontaneously in a herd of animals, but always comes by being introduced from the outside. A farmer who can raise his own cattle, and can properly protect them from contact with outsiders, need have no tuberculosis among them. But to protect the herd requires some knowledge and considerable vigilance. To prevent the entrance of the disease into his herd from without the farmer must exercise care in four directions.

First: In buying stock he must be sure not to purchase infected animals. This is perhaps the greatest difficulty, for it is most commonly by purchase that the disease is introduced into a herd. There is only one way by which he may be sure that he is not purchasing infected cattle and this is by a proper *tuberculin test*, under the guidance of a reliable veterinarian. Unfortunately, with the numerous conflicting rules of different states and countries, it is not always possible to make use of this test. But one thing is certain. No farmer can be confident of keeping his herd free from this disease unless he can be assured by the tuberculin test that he is purchasing animals freed from every suspicion of the disease. Carelessness in this regard will surely be regretted.

Second: He must prevent his cattle from associating with strange cattle. If put out to pasture they must be kept by themselves and guarded against chance contact with strangers. Common watering troughs, in which miscellaneous cattle are watered, must be shunned.

Third: He must not feed his calves upon milk from other herds. The way in which this is most commonly done is by the use of skim milk returned from a creamery or a separating station. From such a creamery the farmer does not get back his own milk, but always milk from another source, and, if there be a few cases of bovine tuberculosis of the udder in the neighborhood, the bacilli from these animals will inevitably soon be distributed, through the separating station, over the whole region contributing to the station. This is not mere theory but positively ascertained fact. To such milk is to be attributed the large amount of tuberculosis among swine in recent years, and it is a well-accepted fact that the mixed milk from such separating stations is one of the most prolific sources of distribution of tuberculosis among calves. The only safe procedure is for the farmer either to bring up his calves upon the milk from his own healthy herd, or to insist that all milk fed to them shall be first subjected to the process of pasteurization or boiling. So convinced have the agriculturists in Denmark become that this mixed milk is the cause of a large amount of bovine tuberculosis, that there has been passed a law forcing the pasteurization of *all* milk which is thus brought to creameries for separation of the cream. The farmer is thus protected from the tuberculosis of his neighbors' herds. The farmer's herd cannot long be maintained in a healthy condition if its calves are fed upon the uncooked mixed milk which is returned from a creamery. The feeding of such milk to hogs is nearly as injurious, for, apart from its producing the disease among swine, it will almost inevitably happen that, once on the farm and in the pig pen, the disease will spread to the cow stall.

A farmer who has a healthy herd may keep it free from this disease if he will take sufficient precautions along these lines. It has been sometimes asserted that an important source of contagion for cattle is from attendants who are themselves consumptives. It is almost certain, however, that this danger need not be feared, inasmuch as the human tubercle bacillus is only very slightly pathogenic for cattle.

THE TREATMENT OF AN INFECTED HERD.

This problem is a far more difficult one and no satisfactory solution has been reached. There seem to be no means yet devised which will eradicate the disease, except the complete destruction of the whole herd and absolute disinfection of the premises. But nevertheless much may be done in the way of reducing the trouble, and, perhaps in some cases, the herd may be perfectly freed.

The first question which will arise is whether the herd is infected, and then it must be determined which of the animals are diseased and which are still healthy. The method of doing this by clinical symptoms, which has been the only reliance in past years, is wholly insufficient for the farmer who desires to rid his herd of the disease, or reduce it to a minimum. Such a method only enables the veterinarian to pick out the worst cases, and it inevitably leaves others in the herd which are sure, in time if not immediately, to scatter the bacilli among the healthy animals. Clinical symptoms pick out only a small part of the animals infected with the disease. Whether or not most of the milder cases are likely to develop into serious ones is not in this connection of much importance, for there is positive evidence that some of them are likely to, and will soon become sources of infection to the herd. Although the farmer may feel that many of these animals are still useful, and that they may continue to be legitimately useful for a number of years, no one can fail to recognize that it is a matter of wisdom for such measures to be adopted as will prevent the dissemination of the disease from these animals to the rest of his herd. As a prerequisite for preventing such dissemination the first absolute necessity is to determine *all cases* of tuberculosis that may be among the animals. Whatever he may propose to do with such animals, it is certainly a matter of first importance that the owner recognize them.

The Tuberculin Test.—The only method of detecting all of these cases is by the *tuberculin test.* This test is certainly of use to agriculture, although, because of misunderstanding, there has been a prejudice raised against it. Tuberculin was first prepared and used by Professor Koch. The essential facts concerning its use are as follows :

Tuberculin is a product of the growth of tubercle bacilli, but it is prepared in such a way that it contains no bacilli and cannot possibly produce tuberculosis. It is made by causing the tubercle bacilli to grow in a broth containing glycerine. After growing in such broth for some weeks, the bacilli produce certain toxic products which are soluble and, of course, dissolve in the broth. The material is then treated (by filtering) in such a way as to remove the bacilli, and the clear, toxic-holding solution is *tuberculin.* Inasmuch as it does not contain the bacilli, it cannot possibly excite the disease, and its use among animals cannot incite tuberculosis as has been sometimes ignorantly claimed.

Although containing no bacilli, tuberculin does contain the toxins which the bacilli produce, and these toxins, if inoculated into an animal in sufficient quantity, would have a poisoning effect. When injected in small quantity the material has no effect upon the healthy individual ; but if the individual is already affected with the disease this inoculation produces a noticeable reaction. It results in a marked rise in temperature, which soon disappears, the animal resuming its normal condition. It was at first thought that the injection was followed by a healing process which would result in curing the disease, and tuberculin was advanced as a remedy for tuberculosis. But, while it appears to have some value in this respect, its aid in curing men from the disease has not been sufficient to warrant its use, and it has been abandoned for this purpose.

But the fact that the injection is followed by a rise in temperature makes it possible for this material to be used among cattle in *detecting* tuberculosis. Healthy animals fail to respond to this inoculation and are wholly uninjured by it. Possibly there may be a slightly decreased flow of milk for a few days, but this is temporary and unimportant. The farmer may, therefore, have his herd tested with the confidence that his healthy animals will not suffer by the test. On the other hand, the animals that have become infected with the disease will show a rise in temperature, and the test will thus make it possible to separate the affected animals from those that are yet in health.

The accuracy of the test has been the subject of some dispute and is now ascertained as the result of many thousands of cases. It has been found subject to some error. In the first place if animals are tested under abnormal conditions, as, for example, when in new barns, or taken from a cattle car and tested at once, even healthy animals may respond. But when the animals are in normal conditions the healthy animals probably never respond, or at all events so rarely as not to interfere with the accuracy of the test. Secondly, some animals very far advanced in the disease fail to respond. These cases are of little importance since they are commonly detected readily by clinical symptoms. Thirdly, all animals which are moderately attacked, and all of the very incipient cases of tuberculosis, are inevitably detected by the tuberculin. Even a single minute tuberculous gland is sufficient to cause a positive reaction to the test.

This last fact forms at once the strength and the weakness of the tuberculin test. Tuberculin does pick out with great accuracy all mild cases, and clinical symptoms will pick out the rest. But this test fails to distinguish between severe and mild forms, putting in one class the animal that may have a small tuberculous gland, which could be healed in a short time, and the animal with a severe case of intestinal tuberculosis which is scattering bacilli, to the great danger of the rest of the herd. Experience has shown that, of the animals responding to the test, some run down rapidly and require slaughtering in a few weeks, while others wholly recover, live several years of useful life, and after death show, by post-mortem examinations, that the original tubercle has been healed and the animals have come again into normal condition. There is thus a great difference between *clinical tuberculosis* and *tuberculin tuberculosis*. The former results practically always in the death of the animal, the latter may be temporary and insignificant. The former certainly *is*, and the latter *may or may not be*, a source of danger to the herd.

In the enthusiasm which followed this easy means of detection, it was claimed that it might be possible to eradicate tuberculosis completely from our herds, and some States started upon a sweeping plan of testing all cattle and slaughtering immediately *all* animals that responded to the test. This resulted in the slaughter of large numbers of very valuable cows which were so slightly affected that their owners rightly believed that they could properly have been retained for further use. A violent opposition was developed at once, which soon forced an entire change of the laws, but not before there had arisen a great prejudice against the use of tuberculin, which has not yet disappeared. This prejudice was based upon the belief that it was a useless waste to slaughter all animals that responded to this test. That belief was certainly well founded and, to-day, no one will be found who will advocate such radical measures ; hence tuberculin is no longer used as a means of condemning to slaughter all reacting animals.

But although no one to-day advocates this extreme procedure, the test, when properly used, may be of the greatest value to the farmer who is interested in protecting his herd, or in eliminating the disease after it has made its appearance. In the first place it may enable him to be sure that he is purchasing healthy animals, if he will purchase only those who fail to respond to the test. In this connection, however, he must bear in mind one fact. If an animal with the disease is tested with tuberculin and responds by a rise in temperature, she is, for a time, protected against a second test. If she is a second time inoculated with tuberculin within a few days, she will not respond. Dishonest dealers have made use of this fact to enable them to sell cattle, first inoculating them privately, and then shortly submitting them to a public test by a veterinarian. It is of course necessary to be on guard against this, as against other dishonest tricks of dealers.

Utilization of the Tuberculin Test.—The use of tuberculin is also of assistance in enabling the farmer to eradicate, or at least reduce the amount of, the disease in his herd after it is once attacked. To do this requires considerable labor and great care in the treatment of the herd. If the farmer has only a very small number of cows, or if only one or two animals react, it is by far the simplest method to remove and destroy them as soon as it can be advantageously done, following their removal by disinfection of their stalls. This will be less trouble and of less expense than the other method which is chiefly applicable to large herds, where many animals react to the test.

If the herd is a large one and the reacting animals are numerous, the following treatment is found to be of great use, and is, indeed, the only practical treatment yet devised, except that of total slaughter. The first step is to detect all tuberculous animals by tuberculin. Then all of the reacting animals which clinical examination shows to be advanced cases, and all animals that show any signs of disease in the udders, are to be removed immediately and slaughtered, since they are beyond question sources of danger to the herd. The animals that react, but show no other signs of the disease, are to be separated from the rest and placed in a barn by themselves, thus removed from every possible contact with the rest of the animals in the herd. This is not because they are necessarily sources of danger, but simply because there is no means of determining when any one of them may become a source of danger to the animals with which it associates. The healthy (non-reacting) animals are then to be placed by themselves, either in a new barn or in the old barn only, however, after thorough disinfection. By this means a practical isolation of the tuberculous animals is effected.

If, now, the farmer wishes to preserve the healthy herd from future attack he must take precautions that the isolation is thorough. The isolation may be effected by simply building a partition in his cattle shed; but if this is done there should be no door in the partition, for such a door will surely result in a carrying of bacilli from one compartment to another. The farmer must remember the facts already pointed out as to the methods of distribution of bacilli. If possible he should have separate attendants for the two herds, and at all events the boots worn in attendance on the infected herd should not be worn in the shed occupied by the healthy animals. He must remove all calves from the infected herd a few days after birth, and bring them up upon the milk of the healthy herd alone. Hereditary tuberculosis is not common, and most of the animals born of the infected herd will, at birth, be free from the disease, and may be kept so by proper care. Inasmuch as the milk of infected cattle is probably the most prolific source of the distribution of the disease among calves, the chief source of danger to the calves is removed if they are brought up on milk of healthy animals or upon milk that has been boiled. These calves must themselves be tested with tuberculin before they are allowed to mingle with the healthy herd. Lastly, the farmer must have the healthy herd tested each year, or, better, each six months, and must always remove any reacting animals to the other herd.

Such a method of treatment involves considerable labor and some expense. It is troublesome and certainly will not be adopted by the farmer without strong incentives. Where it has been tried it has resulted in a great reduction in the amount of the disease, and it has been found that a badly infected herd may thus, in a few years, be brought into a tolerably good condition. But even at its best it has not proved completely successful, for it has not resulted in a complete *eradication* of tuberculosis from the herd, and each time the healthy herd has been tested, some animals respond to the test, although in constantly decreasing numbers. But, although only partially successful, it is the only means yet suggested for protecting a farm from the disease after it has once found its way into the herd, except the radical one of complete slaughter of all reacting animals.

Whether a farmer will adopt such a method will depend upon the extent of his desire to eradicate the disease from his herd. He must remember that, as long as tuberculous animals are allowed to associate with healthy animals, he is running a risk of their being the means of distributing the disease. His only safeguard for his herd is a complete separation of the infected animals from the others. This may be most easily accomplished by slaughtering the infected individuals; but if they are especially valuable animals or very numerous, such a course would be ruinous. He may allow the infected animals to mingle with the herd, and for a time, perhaps, see no disadvantage; but he must always remember that he is running a danger of a widespread infection among such animals. Only by complete isolation can he have any certainty of protecting his cattle.

The considerable trouble and expense attending such thorough isolation of reacting animals, and the uncertainty as to its results, have led in recent years to the question whether a simpler method of treatment may not be nearly as useful. It is recognized that, of the various factors concerned in the method above outlined, the most important one is the treatment of *young* cattle, and, as a result, there has been a tendency in recent years to place most emphasis upon this factor. It is said that a practical and easily applied means of treating the subject does not necessarily involve an isolation of the reacting animals and the necessary expense. If the owner of the herd will destroy all animals that, by clinical evidence, indicate an advanced state of the disease, and all animals that have any affection of the udder, he may then greatly improve the condition of his herd by simply taking good care of his young cattle. If the calves are allowed to drink only pasteurized milk, or milk from non-reacting cattle, and if these calves are themselves tested with tuberculin before they are allowed to become members of the dairy herd, the herd can slowly be built up from healthy animals and the amount of tuberculosis reduced. How efficient this plan may be cannot yet be stated, inasmuch as there is not as yet a sufficient amount of experience to warrant any deductions. At best the farmer who adopts it must remember that it offers him no hope of getting rid of tuberculosis from his herd. It is only a palliative measure which may somewhat reduce the amount and make it possible for him to preserve a herd in which the number of diseased animals is comparatively small.

It must be recognized that the success of these measures will depend upon the individual farmer. Half way measures are useless, and no farmer will succeed in improving his herd unless he is willing to adopt thorough measures. For this reason any legislative attempt to introduce measures looking toward the improvement of the farmer's herds will be totally futile. Legislature cannot enforce such measures as promise satisfactory results. The success must depend upon the individual farmer. Isolation is a means which the farmer may adopt if he finds the disease in his herd, but it is nothing that can by any legislature be forced upon him.

THE USE OF FLESH AND MILK FROM TUBERCULOUS ANIMALS.

It is not easy to give any rules which shall be at the same time safe and practical in regard to the use of the products obtained from tuberculous animals. The essential facts which must control our decisions in the matter are as follows:

Flesh.—Tubercular matter from animals when fed to other susceptible animals may produce the disease in the animal experimented upon. From this it follows that, if the human and bovine tuberculosis are the same disease, mankind may be exposed to danger from eating the flesh of tuberculous cattle. But there is no danger unless there are tubercle bacilli in the part eaten. The tuberculous infection of cattle is commonly in the lungs, intestines or lymphatic glands, and only rarely are the muscles affected.

If an animal has simply a tuberculous lymphatic gland, its muscles are perfectly safe eating, unless they may have become infected by the knife of the butcher which has previously cut through some tuberculous mass in the animal. The danger to man from eating tuberculous flesh is therefore slight. Further, flesh is commonly cooked before it is eaten. Thorough cooking will destroy the bacteria, but even the moderate cooking which meat commonly receives, is sufficient to destroy the bacteria upon its surface, although the heat does not extend to the interior. Inasmuch as flesh is rarely the seat of the tubercular infection, and accidental contamination with the butcher's knife will be on its surface, cooking will almost always render it harmless, unless the infection is deep-seated. For these reasons the flesh of animals slightly infected with this disease need not be condemned as food. A method of dealing with this question, adopted in Germany, is an excellent one, though it would hardly be satisfactory in all countries. All meat is inspected. Carcasses which are very badly infected with tuberculosis are destroyed. Those which are only slightly infected have all the infected portions removed and the flesh is sold freely. Those which are moderately infected have their flesh sold upon what is called the "Friebank," a special part of the market where infected meat is sold, and the purchaser is given to understand that it is suspicious and not safe to eat without thorough cooking. In the United States flesh of slightly infected animals is passed by the inspectors and sold freely in the market. Whether such meat is a source of danger is very doubtful. At all events no evidence is at hand to indicate that human tuberculosis is caused by the eating of such flesh, although the lack of evidence means little, since evidence, from the nature of the case, would be very difficult to obtain, even if such infection did occur. It is universally admitted that the actual danger from this source is very small and perhaps does not exist at all.

Milk.—The problem of the use of milk from tuberculous animals is a more difficult one to settle, at least for the United States, where the people will not adopt the habit of pasteurizing milk. The milk of tuberculous cattle does not always contain the bacilli and it is an unsettled question whether it will ever contain them unless the disease be located in the udder. At all events, cows having tuberculous udders (something less than one per cent.) will produce milk infected with tuberculosis bacilli. That these bacilli are active and vigorous is proved by thousands of experiments which have shown that such milk is capable of producing tuberculosis in guinea-pigs.

It is true that the bacilli do not multiply in milk, but milk from one cow can, by being mixed with other milk, infect a large amount. It is possible that such milk may be a danger to the public health. Nor does this conclusion rest simply upon theory, for there is direct evidence to support it. Several instances, not very numerous, naturally, from the difficulty of obtaining evidence, are on record where mankind has been said to have acquired the disease from drinking milk of infected animals. It has been abundantly shown that market milk frequently contains tubercle bacilli in sufficient quantity to produce an infection in guinea-pigs, and the same is true of market butter. All of these facts certainly indicate a possible danger to the public from this source.

In regard to the extent of this danger there has been a wide difference of opinion. It has certainly been magnified by some. The danger is, beyond question, frequently overdrawn. That mankind can ever acquire tuberculosis from this source is to-day seriously questioned. It must be remembered that milk is taken directly into the stomach, and if it gives rise to tuberculosis it would be expected that the seat of the disease would be in the intestinal tract. It is, however, well known that in mankind the lungs are the most common seat of infection, and we can hardly believe that drinking milk would be the likely cause of pulmonary tuberculosis. Moreover, the number of bacilli which a person will swallow with a drink of milk will commonly be rather small, and the human individual has a considerable power of resistance against the disease. It is a further fact that, although bovine tuberculosis has been increasing, human tuberculosis has been constantly declining in recent years, and the decline has been equally great in those countries that use milk raw and in those countries that sterilize the milk before drinking it. This decrease in tuberculosis does not apply to intestinal tuberculosis among young children, indicating, possibly, that milk is a more common source of infection for children than for adults. Lastly it has recently been doubted whether the bovine bacillus, even when introduced into man, can produce the disease. For these various reasons it is a fair inference that the danger of tuberculosis from milk is not very great for adults, though it may be considerable for young children. It is quite certain that for young children it is unsafe to resort to the use of milk from miscellaneous cows, without the precaution of pasteurization.

Certainly the logical method of dealing with milk would be, to exclude from the milk supply all milk from tuberculous animals, or to allow it to be used only after pasteurization. Only thus could absolute safety be assured. But this is quite impractical, if, indeed, possible. A farmer who takes pride in his dairy and in furnishing a special quality of milk, will protect his customers by periodic testing of his cattle and by the exclusion of all reacting animals. But to enforce any regulations looking in this direction in regard to the public milk supply, is simply impossible at the present time, and will remain so for some time to come. The end could be reached through the milk supply companies, by the adoption of the simple and inexpensive process of pasteurizing all milk before distribution, and quite possibly such may be the ultimate solution of the problem. Meantime the only feasible method of treating the matter is to insist that the farmer shall rigidly exclude from the animals furnishing the milk supply *all cows with diseased udders*, and to suggest to all who have a fear of using the milk because of the slight danger existing in this food supply, that the danger may be wholly avoided by pasteurization. Whether the recently advanced views of Professor Koch, that bovine and human tuberculosis are unconnected, will soon modify our views in this connection cannot, at the present time, be determined.

CHAPTER XVI.

OTHER BACTERIAL DISEASES.

BESIDE the two already referred to, there are quite a number of diseases among domestic animals which have been beyond any question traced to the action of bacteria. None of these have as much general interest as the two mentioned, although some of them are of very great significance in certain agricultural communities. As the diseases of domestic animals have been more and more studied, it has become evident that quite a large number of them are to be attributed to the growth of parasitic bacteria. The number now known to be caused by such agency is being constantly increased, and no list given at any time could be regarded as complete. The following list includes only the most important of the diseases of domestic animals which, up to the present time, have been traced beyond doubt to a definite species of bacterium. The list is not complete. Other less important diseases could be added to the list to-day, and there are others which will probably be included in this list in later years.

BACTERIAL DISEASES OF ANIMALS.

B. septicæmiæ hæmorrhagicæ.—The bacillus that is known under this name is one that has long been studied and may well be mentioned first. It was studied many years ago by Pasteur as the cause of a disease among domestic fowls, known as *fowl cholera*. Later Professor Koch studied a disease among rabbits which he called *rabbit septicemia*. Further studies in more recent years have indicated that the cause of these two diseases is either the same or closely allied. In more recent years still, it has been found that the same, or nearly the same organism, has a much wider distribution and may be found in many animals. It occurs, for instance, in the hog, producing a widely distributed disease known as the *swine plague.* It occurs among cattle, not very frequently it is true, but common enough to be recognized as the cause of a disease usually known as *Rinderseuche.* It also occurs in the deer when it causes a disease, *Wildseuche.* In all of these cases the organisms which have been found to be the cause of the disease are so closely alike that they are quite generally regarded as identical.

The bacterium referred to is an extremely short rod and has sometimes been called Micrococcus, although now usually given the name of a Bacillus. It produces in animals a type of disease quite similar to the forms of blood poisoning which have been, in medical practice, called septicemia, and hence the specific name given to it. It is an extremely fatal disease for some animals, fowls and rabbits succumbing to the action of this microörganism with extreme rapidity and almost absolute certainty. Among the larger animals its course is not necessarily so fatal, but in all of those referred to above, the disease is a serious one and commonly results fatally. When attacking the hog it produces swine plague, this being the type of the disease most commonly found among domestic animals, and the one which will be usually most interesting to the agriculturist.

The extreme susceptibility of the rabbit to this particular bacterium has led Pasteur to suggest it as a means of ridding Australia of the rabbit pest, which has in years past become such a serious menace to the agriculturist. The bacillus, being apparently harmless to man, might be, as Pasteur suggested, inoculated into a few rabbits. If such rabbits were allowed to run wild they would spread the infection rapidly among the wild rabbits, and thus perhaps cause the extermination or great reduction in the numbers of these animals. The plan has never been tried and is simply interesting as a suggestion.

Hog Cholera. (**B. suipestifer.**)—The hog cholera is a disease related to the last, although clearly distinct from it, and is one which develops spontaneously in swine only. It is quite common to have the swine plague and the hog cholera together in the same animal. The disease sometimes results in very serious losses. A herd of swine may be attacked by such a violent epidemic that 90 per cent. of the animals succumb to the infection. After the death of the animals the bacilli which produced the disease are found in all of the organs, but especially in the spleen. The disease occurs in an acute form, which runs its course with excessive rapidity, producing death in twenty-four hours; and in a chronic form, which has a slower course, lasting from two to four weeks before finally resulting in the death of the animal. The organism which produces the disease is well known and has been carefully studied. It is a bacillus named *B. suipestifer* (or *B. choleræ suis*), and is very easily cultivated by ordinary methods in the laboratory. It is capable of producing the disease, not only in the swine, but in rabbits, guinea-pigs, mice, and some other animals; but as a spontaneous affection it is found in the hog only.

Glanders. Farcy. Rotzbacillus. (**B. mallei.**)—This disease, well known among agriculturists, occurs not infrequently as a normal infection in the horse and in the ass. It is characterized by the appearance of ulcers in the nasal membranes, by enlarged submaxillary lymphatics, which may turn into open discharging ulcers. Later the lymphatics in the body generally may become tumor-like swellings. Other parts of the body may eventually be affected. The secretions from the various ulcers are found to be decidedly infectious, and it is through these ulcers that the disease is commonly distributed.

It occurs in an acute form and in the chronic form ; the latter, chiefly in the skin, receiving the name of *farcy*, the former, chiefly in the lungs and nasal passages, more commonly known as *glanders*. It occurs spontaneously only in horses and asses, and causes great losses in nearly all localities. It may occur by accidental or artificial infection in many other animals. It occurs occasionally in men who have become accidentally inoculated in the treatment of horses suffering from the disease, and when it does occur in man it is an extremely fatal disease, almost always resulting in death.

The bacillus which produces the disease is well known, and named *B. mallei*. It is a short stationary rod which lends itself readily to bacteriological experiments. It is found to be capable of producing the disease in cats, dogs, rats, field mice, and quite a variety of animals. It is only slightly pathogenic for the sheep and the mice. The pig and the cow seem to be immune from its action.

Symptomatic Anthrax. Black-leg. Quarter-evil. Rauschbrand. (B. anthracis symptomatici.)—This disease, with its variety of names, is extremely common in Europe. It has been rare in the United States, but in recent years is becoming more abundant, being found as an epidemic in certain herds. It is a disease that occurs chiefly among cattle, and is characterized by certain irregular swellings in the subcutaneous tissues and muscles. The swellings are seen especially over the quarters of the animal, and hence the name quarter-evil. The muscles become dark colored, and bloody (hence the name black-leg), and contain large numbers of the bacilli known to cause the disease. It is the cause of considerable trouble to raisers of cattle, being almost universally fatal, although it is not a disease that can be regarded as extremely abundant.

The organism which produces the disease is well known and is named *B. anthracis symptomatici*. It is pathogenic for a large number of animals when artificially inoculated. Swine, dogs, rabbits, fowls, pigeons, guinea-pigs and horses succumb to the disease by inoculation, in addition to cattle, sheep and goats, in which the disease occurs spontaneously. It is most common among cattle as a spontaneous affection, and quite rarely occurs in sheep and goats. In the horse it is never known to occur spontaneously. So far as known, the bacillus is not pathogenic for man, although this has never been demonstrated; but no instance has ever been known of man suffering from the infection, even though every opportunity for such infection has been offered. The disease is, therefore, not regarded as injurious to man. The practice of inoculating animals against the disease by a "preventive culture" is widely adopted in the United States.

In addition to these well-known types of disease of domestic cattle, a few others may be mentioned by name. The **hoof and mouth** disease is one which, though very common in Europe, is rare or almost unknown in the United States. It is a disease which produces very great destruction in Continental Europe, and has been the subject of a large amount of study. Up to the present time no one has succeeded in discovering a microörganism which produces the disease. The general evidence as to the nature of the disease indicates conclusively that it is a germ disease, but bacteriologists have hitherto been unable to discover its cause.

Hydrophobia or **rabies** may also be mentioned as a disease which is most commonly found in dogs, but occurs also in cats, rabbits, and occasionally even in cattle. The cause of this disease has likewise hitherto escaped the discovery of our naturalists.

Tetanus or **lockjaw** is a disease of rather rare occurrence among domestic animals, but it may sometimes occur if an animal receive a wound by means of some object that has been lying for a long time in the soil. The cause of tetanus is a well-known bacillus (*B. tetanus*), which lives normally in the earth and may get into a wound and produce the well-known and commonly fatal disease.

Abortion.—This troublesome disease sometimes appears in a herd and produces great loss, and endless trouble to the dairyman. Cows attacked by the disease do not carry their calves the full time but drop them early and become useless for the time as milch cows. If the animal is once affected she is likely to have the same trouble the next time she is in calf, and perhaps her usefulness is ended. This trouble has for some time been recognized as contagious and has, in recent years, been demonstrated to be produced by a definite species of bacterium. The bacterium may infect cow after cow, and even the bull may distribute it through a herd of cattle. The best remedy has been found to be thorough disinfection. The calf is to be destroyed, the stable disinfected, genital parts of the cow thoroughly washed with disinfecting solutions and the animal kept from the rest of the herd. A thorough disinfection of this sort will commonly allay the trouble, in time.

It should be mentioned in conclusion that quite a number of diseases known to veterinarians have been named simply from their location. Inflammatory, suppurative and tumor-forming troubles are liable to occur in various parts of the bodies of animals and, in accordance with their location, they have been given such names as *mammitis*, *mastitis*, *garget*, *hoof-rot*, *navel-ill*, etc. As these various troubles are more fully studied they have been found to be extremely different in their nature. Tumors, for example, may be produced by tuberculosis or by symptomatic anthrax, etc. Hence the popular names of such local diseases have no scientific meaning. As bacteriologists have studied these various forms they have found that, in a number of the inflammatory diseases, the troubles are induced by the presence of a certain form of *Streptococcus*, and in accordance with the part of the body where this Streptococcus gains a foothold, we have a disease with varying symptoms. This Streptococcus, for example, is the common cause of mastitis and garget, and some of the other well-known infections of cattle. It does not fall within the purpose of this work to consider these to any extent.

There is another class of diseases found among cattle produced by microörganisms belonging to the higher bacteria (see p. 28). One genus of these higher bacteria, *Actinomyces*, occurs very commonly, not only in cattle, but in a variety of animals, causing inflammation with the appearance of tumors, which may eventually break down into a suppurating form. In cattle this bacterium most commonly finds its entrance through the mouth, and one of its most common locations is in the lower jaw, when it produces a disease called *lumpy jaw*. It is not uncommon on the roof of the mouth when it is known as *malignant tumor*. It frequently attacks the tongue, producing the so-called *wooden tongue*. It is most common in cattle and swine, though it may be given to other animals by artificial inoculation. It is known also to occur in man, though not very frequently. It commonly finds its entrance into the body through cuts and bruises in the skin. The diseases produced by these microörganisms are not uncommon, and cause considerable trouble and loss to the breeders of domestic animals.

Foulbrood of Bees.—The only other bacterial disease of animals which we need mention is that quite common in beehives, known as *foulbrood*. This is a disease which attacks the larvæ while still in their cells, causing them to become sickly, eventually killing them and producing a decomposition of the body of the animal. The hive becomes vile-smelling from the decomposition products, and the whole economy of the hive is interrupted. The bees fail to collect and preserve honey, and the hive is totally ruined for the purpose of honey-making. It is a disease which is extremely widely distributed, being found all over Europe, in America, in Cuba, in Africa and in Australia. It spreads very rapidly under certain circumstances, sometimes a whole district being infected in the course of a single season, so that the industry of the bee-maker is nearly ruined. This disease is produced by a bacillus, named *B. alvei*, that has been known for some years and has been definitely proved to be the cause of the disease in question. It is carried from hive to hive, as now believed, chiefly by the robber bees; that is, by bees who steal honey from other hives. They can readily steal from a hive that has become weakened by the action of the disease, and such hives, of course, would be the very ones from which infection would be acquired. It is also distributed by the custom of selling bees, or sometimes by the custom of selling hives which have been infected by the disease. Remedies against it are not thoroughly efficacious. One is the radical method of destroying the hive, together with all its bees, as soon as the disease makes its appearance. A less radical method is to separate the bees from their larvæ and their honey, keeping them for a day or two without food and then putting them into a new clean hive, destroying or thoroughly disinfecting the old one. This will ordinarily result in the disappearance of the disease. Other more or less successful methods of meeting the disease are by the use of various chemicals.

BACTERIAL DISEASES AMONG PLANTS.

The bacterial diseases of plants have been far less studied than those of animals. They have excited less interest and few bacteriologists have hitherto turned their attention to their study. The whole subject of bacterial plant diseases has been much disputed. In earlier years it has been claimed impossible for plants to have bacterial diseases. Plants are covered by an impervious cuticle through which bacteria cannot normally pass. Moreover, the tissues of the plant are not filled with nutritious fluids as are the tissues of animals, the spaces in the leaves and stem being filled with water, or many of them simply with air. It has been claimed that there is no likelihood that bacteria can live under such conditions and that bacterial plant diseases are, therefore, on *a priori* grounds, improbable or impossible. Even in very recent years this claim has been very vigorously supported, and disputes are still going on, in the pages of bacteriological journals, in regard to the question of the existence of bacterial disease in plants. Almost to the very present day it has been insisted that there is no demonstration that bacteria can produce disease in plants.

Although this claim was legitimately urged a few years ago by conservative scientists, it can no longer be held in the light of recent experiments. In the last few years the evidence for such diseases has accumulated rapidly, and to-day the proof of the existence of bacterial plant diseases stands on identically the same basis as the proof of the bacterial diseases among animals. In quite a number of well-known plant diseases it has been possible, with the greatest of ease, to obtain the necessary steps of proof. First, it has been shown that a certain definite bacterium is present in the plant suffering from the disease in question. Second, it has been found possible to isolate the bacterium and to cultivate it in the laboratory by normal culture methods. Third, it has been possible to inoculate healthy plants with the laboratory cultures of the bacterium in question, with the result of producing inevitably a recurrence of the original disease with all of its typical symptoms ; and in the tissues of the plants thus affected the bacillus is found in great numbers. When these steps of proof have been obtained, as they have in several plant diseases, there is no longer any possible question that these diseases are attributable to the bacteria in question. We must, therefore, look upon bacterial diseases of plants as certainly occurring.

How abundant bacterial plant diseases are can hardly yet be stated, for the subject is one of very recent study. It is possible that they are comparatively few, but those who have studied the subject most insist that bacterial diseases of plants are very numerous, and will probably, in the end, be found to be as numerous as bacterial diseases among animals. At the present time, however, only a few can be regarded as definitely proved, although there is a long list, attributed with more or less reason to bacterial action. The best known and the best attested bacterial diseases of plants may be briefly given and are as follows :

Pear and Apple Blight. Fire Blight of the Pear Twig. Anthrax of the Fruit Tree.—The disease known under these various names is a common one in apple and pear trees. It has been known for over a century. It is characterized by small dead spots on the bark of the tree, which spread somewhat, eventually killing the young twig, and even extending into the stem. The leaves turn a brownish color and may exude a dark fluid. The trees are sometimes ruined, though sometimes the infection is not so serious. The cause of this disease is now known positively to be a bacillus, to which the name *B. amylovorus* has been given. This organism has been carefully isolated from trees showing the disease, has been cultivated, and has been successfully used for inoculation experiments. In the normal growing plant it appears that the bacillus finds its entrance into the plant through the flowers, and feeds upon fluids in the nectary.

The Twig Gall of the Olive.—This is a well-known disease, though one, of course, of little interest in the United States from the fact that we do not here produce olives.(2) The disease is characterized by knots on the branches, from the size of peas to the size of hazelnuts. It is produced, as has been shown through culture and inoculation experiments, by a bacillus named *B. oleæ*. A somewhat similar disease, probably produced by a similar cause, is found on the willow, birch, and pine.

Bulb Rot of Hyacinth.—This disease especially attacks the bulb of this plant, causing it to rot and decay. It is produced by bacteria, as has been demonstrated successfully. One of the common forms is caused by an organism named *B. hyacinthi*, while another type of the disease, with different symptoms, is caused by another organism named *B. hyacinthus septicus*. The diseases attack both the leaves and the bulb, but are especially characterized by a decay of the bulb.

Curcurbit Wilt.—This is one of the best known and most thoroughly attested of bacterial diseases of plants. It is especially common in the tomato and causes a rapid wilting of the plant and its final complete destruction. It has been carefully studied by several bacteriologists and found to be due to a bacillus which has been named *B. tracheiphilus.* (Fig. 40.) The bacillus can be cultivated readily in the laboratory. If healthy plants are inoculated by pricks from needles that have been dipped into the bacteria culture, the inoculation is inevitably followed by the wilting and the browning of the plant, and the extension of the disease with the typical symptoms, the plant tissues becoming filled with bacteria in great quantities.

FIG. 40.

B. tracheiphilus. **Causing the curcurbit wilt. The two lower figures are more highly magnified and show flagella. (*Smith.*)**

Black Rot of Cabbages.—This disease attacks not only the cabbage, but other cruciferous plants, such as the rutabaga, turnip and cauliflower. It is characterized by a blackening of the leaves, which extends until eventually it may ruin the plant. It is quite common in America and produces not a little loss from our gardens. It has also recently been shown to be abundant throughout Europe. The cause of the disease is a bacterium called *Bacillus* (or *Pseudomonas*) *campestris*.

This bacillus has been most carefully isolated, cultivated and studied. When used for inoculation it is found to produce the typical disease in the plant. The inoculation experiments are so numerous and definite as to leave no doubt as to the bacterial nature of the disease.

Brown Rot of Potatoes, Tomatoes and Eggplant.—The rot of potatoes is a disease which produces an immense amount of devastation all over potato-raising countries. Not all kinds of the potato rot are caused by bacteria. Some of the widely distributed types are produced by certain species of higher fungi; indeed, until recently it has been supposed that all potato rots are fungus diseases. Without doubt, however, some forms of this general trouble are bacterial in their origin. The so-called *brown rot* is widely distributed and produces in some localities a very great loss to the agriculturist. It is characterized by the plants wilting and turning brown, and this is followed by the invasion of the tubers by the disease. The tubers become affected and, in the course of time, more or less decayed in their interior, and perhaps absolutely ruined. The wide distribution of the disease and the great losses which it sometimes involves, make it one of especial interest to agriculture. The cause of this disease has been found to be a bacillus which has been named *B. solanacearum.* This bacterium has been isolated, cultivated and used for inoculation experiments, producing the disease in the plants without fail.

In addition to these diseases, a few others, which are as yet less thoroughly studied, may be simply mentioned by name. The following diseases are with more or less certainty caused by bacteria:

The water spot of beans, produced by *B. phaseolus;* the *Bacteriosis* of beets, produced by *B. betæ;* the *sorghum blight*, producing red and black spots on the leaves of the sorghum and believed to be produced by a bacillus called *B. sorghi;*

the *bacteriosis of carnations*, *black disease of maize*, *gall of the pine*, *canker of the ash*, *canker of the ivy*, *lilac disease*, *black disease of mulberry*, *red coloration of wheat*, and some forms of *potato scab* have also been claimed to be produced by bacteria. The evidence is conclusive in regard to the first three. In regard to the others the evidence is not quite conclusive, and it may be that some of them have an entirely different explanation. However that may be, the first ten mentioned have been so conclusively demonstrated to be caused by bacteria, that we cannot longer question the causal connection of the bacteria and the diseases. Bacteria are thus of very great significance in producing plant diseases and will in the future, doubtless, be of far more interest to agriculture than they have been in the past.

CHAPTER XVII.

DISINFECTION.

Bacteria are both useful and detrimental. Taking together the facts mentioned in the previous pages it is evident that their usefulness outweighs the injury they do. Still it is clear that they sometimes occur where they are not wanted and produce mischief. It becomes, therefore, a matter of especial importance to the farmer, not only to learn how to stimulate the growth of the friendly species, but how to check the growth of the hostile forms, and if possible how to destroy them entirely. A brief consideration of the methods of disinfection, adopted under various conditions, is necessary before closing our consideration of bacteria in relation to agriculture.

The purpose of all kinds of disinfection is to treat material infested with bacteria in such a way as either to destroy the bacteria present, or to prevent their further active growth. These two objects are, however, somewhat different from each other. Manifestly the prevention of active growth is more easily accomplished than the actual destruction of bacteria. By the term *germicide* is meant some substance or method of treatment which actually kills all bacteria, while the term *antiseptic* refers to what will check their growth, and thus destroy their power to do injury, although not actually killing them. Nearly all agents which will act as germicides when applied in large quantity, will act simply as antiseptics when used in lesser quantity. We look therefore for antiseptics and germicides along similar lines.

In considering how we may guard against the injurious action of bacteria which may have infested our houses, barns, clothing, food, etc., it is best to divide the subject into two divisions: (1) We must learn what agents are efficacious in destroying bacteria. (2) We must learn how these agents may be practically used. It is manifest that not all germicides can be used under all conditions. A violent poison may be a good germicide, but it cannot be used for preserving food. The question of the practical application is of fully as much significance as the actual germicidal properties of different agents.

The methods of destroying bacterial life are both physical and chemical, each of which has its uses under different conditions.

I. PHYSICAL AGENTS.

Heat.—Heat and light are the only physical agents which are of value in destroying bacterial life. No living things can withstand high heat, and heat is therefore one of the best disinfectants. The only question is, how high a heat bacteria can withstand, and whether a sufficient heat may be applied for the object to be disinfected. All *active* bacteria are killed by a moderately high heat, most forms of actively growing bacteria being destroyed by a temperature of about 140° F. A few can live and actually grow at a temperature closely approximating this, but such bacteria are unusually rare, and do not commonly enter into the question of disinfection. But while such moderate temperature destroys most active forms, it is rarely of any use as a disinfectant, since large numbers of bacteria form resisting spores which can endure a much higher heat. The spores of bacteria commonly resist the temperature of boiling water for a short time, and some species resist this temperature for an hour or more.

The temperature which will destroy bacteria differs much according to the method of application. *Dry heat* is far less efficacious than moist heat. Bacteria spores, when dry, are known to resist a temperature of 280° F. for some hours.

While this is extreme it is a universal fact that dry heat is very inefficient in destroying bacteria. Hence it is difficult to destroy the bacteria in dust or upon the dry walls of rooms, by simple heat.

Moist heat destroys much more quickly. While some bacteria will resist, for a time, a temperature somewhat above boiling, most of them are rapidly destroyed in boiling water. A temperature of a few degrees above boiling, obtained by steam heated under pressure, is sufficient to destroy all life, even the most resisting spores. The exact temperatures vary widely for different bacteria, but a temperature of 235° F., continued for fifteen minutes, is sufficient to destroy all bacterial life. It must be remembered that such high temperatures are not needed for destroying most bacteria, and, with the exception of the anthrax and tetanus bacillus, all of the bacteria which commonly cause *diseases* among man and our domestic animals, are destroyed by a temperature of boiling, and commonly at a temperature much lower.

This is a matter of great practical importance. It teaches that it is not necessary to use such high temperatures as obtained by superheated steam, in order to disinfect material infected with the bacteria of common diseases. Cholera, for example, is killed by a temperature of 156° F., if continued for half an hour, and the same is true of typhoid fever and diphtheria. Tuberculosis is, probably, at least rendered harmless by a temperature of 140° F., if continued for half an hour under proper conditions.

Sunlight.—Sunlight is a very effectual germicide. Bacteria exposed to direct sunlight are killed in a few hours, and even diffused daylight has a marked injurious effect upon their vitality. The brighter the light the more efficacious its action. While sunlight is thus an exceptionally good germicide, its practical value in disinfection is not very great because of the difficulty of its application. To disinfect an object by sunlight the light must shine directly upon the bacteria. Consequently, articles of infected clothing can thus be disinfected only when they are very thin, so that the bacteria are wholly on the surface. Such a condition is, of course, unusual and hence the direct value of sunlight in disinfecting clothing is not very great. At the same time it must be remembered that light is hostile to bacteria, and dark rooms or stables will offer better chances for their growth than light rooms. Any article which can be exposed to direct sunlight may thus be readily disinfected upon its surface by a few hours' exposure.

Cold.—The application of cold is apparently useless to destroy bacteria. Bacteria resist for hours, and even for days, the extremely low temperatures of liquid air. If the action of such cold be maintained for a long period it does, in the end, have a considerable germicidal power. Bacteria frozen in ice will remain alive and active many weeks, but after a time they die. Recent experiments have shown, for example, that typhoid bacilli, frozen in ice, remain alive for several weeks, but eventually begin to die and, when the ice has been preserved for three months, it contains practically no live typhoid bacilli. This is a matter of great practical importance in connection with the use of ice harvested in the winter from waters liable to contamination with typhoid bacilli. But while long-continued freezing may in the end destroy bacterial life, the use of cold is never adopted as a practical disinfectant.

2. CHEMICAL AGENTS.

The most common methods of disinfection are by the use of chemicals. A large number of substances have been used for the purpose, but we can consider only those which have proved to be the most practical and efficient.

Corrosive Sublimate.—This is one of the most efficient germicides known, and its small cost has given it very wide use. When in a water solution of 1–500 it is a germicide. As an antiseptic much weaker solutions are efficient. The most common strength for its use is 1–1,000, and in this strength it is so efficient that it rapidly destroys the septic powers of all bacteria. This strength is used for washing the hands, when they need to be specially clean for surgical operations, and this strength may be used for washing floors or walls of infected rooms. The objections to its use are two. It is intensely poisonous to man, and the greatest care must be exercised in handling it. It is unsafe to leave it upon the walls of stables that are liable to be licked by cattle, and, indeed, after its use a thorough washing should always follow. Secondly, it has a violent corrosive action upon metals, and cannot be used for disinfecting anything which is made of iron or steel. This limits its use in many cases. But, nevertheless, it is one of the best and most widely used of all chemical disinfectants. A solution of about the proper strength (1–1,000) may be made by dissolving one-quarter ounce of the sublimate in two gallons of water. Such a solution can be freely used for washing anything that does not contain metals, and should be followed by thorough washing. A more effective solution is made as follows: 1 part corrosive sublimate (15 grains), 2 parts common salt (30 grains), 1,000 parts water (1 quart).

Carbolic Acid.—This material is much less powerful in its action than corrosive sublimate. It is a germicide when dissolved in water in proportion of 1 to 20, and acts as an efficient antiseptic when in solution of 1 to 400. It is not so violent a poison as corrosive sublimate, and may be used in much stronger solution. A solution of 1 to 40, or even 1 to 20, may be freely used for washing the hands, although stronger solutions are apt to produce a burning of the skin. It owes its wide use partly to the fact that it was the first antiseptic carefully studied, and because it was used in surgery long before others were discovered. It is very efficient and, as ordinarily used, is harmless. One of the reasons for its popularity is the fact that it possesses a distinct odor, since most people who do not understand the matter have a feeling that a disinfectant having a strong odor must be efficient. Carbolic acid acts as a *deodorant* and an *antiseptic* at the same time, and has consequently been popular.

Chloride of Lime.—This is one of the cheapest and, at the same time, one of the best disinfectants. It has been widely used. It may be applied dry if the material to be disinfected contains some moisture. But, since it acts only in the presence of moisture, it may be best used in a water solution. A solution of 1 pint to 25 of water (1 pound to 6 gallons of water) is most commonly used, and this is very efficient for disinfecting walls, floors and furniture that can be washed by it. It owes its efficiency as a disinfectant to the chlorine gas which is liberated from it. Common *slacked lime*, which is very commonly used on the farm as a whitewash, is of far less value as a disinfectant. It liberates no chlorine and does not act like chloride of lime. Its cheapness makes it possible to use it in large quantities, and it can be used under conditions where other disinfectants are impractical. But its disinfecting power is small.

Sulphur.—The fumes of burning sulphur have been widely used for disinfecting rooms, inasmuch as they are most easy of application. In a room which it is desired to disinfect there are always many cracks and crannies which cannot be easily washed with disinfecting solutions, and into which the fumes of burning sulphur readily penetrate. It is so easy to shut up a room and burn a quantity of sulphur in it, that the use of sulphur has been very popular and widely extended. Unfortunately its efficiency is not so great as at one time believed. When the room to be disinfected is tightly closed and a sufficient quantity of sulphur is burned in it, the fumes of sulphur do destroy most of the bacteria, except the very resisting spores. But under ordinary conditions it does not kill all the germs of the common diseases. Its use, of course, must be limited to spaces that are so enclosed as to be capable of being tightly closed, and upon the farm, therefore, it can be of value only in disinfecting sick rooms. It should be stated that the practical use of sulphur seems to be more satisfactory than its theoretical value would imply. Although we know that it does not destroy *all* bacteria, it is nevertheless true that its use by health boards in many places appears to be very satisfactory, and rooms thus treated rarely give evidence later of being improperly disinfected.

The manifest desirability of some disinfectant which is in the form of a vapor has led in recent years to the wide employment of a new disinfectant, *formalin*. This is a watery solution of *formaldehyde gas*. The gas acts as a violent poison upon bacteria. Solutions of 1 to 10,000 are sufficient to destroy the vitality of resisting bacteria immersed in the solution. The material looks like water, and may be used for washing, thus disinfecting any clothing which can be immersed in water. As a material for washing clothing it is extremely efficient, and is as harmless in its action on delicate fabrics as pure water. As a general wash for rooms or stables it is hardly practical, since the gas given off from it is very disagreeable and oppressive to the eyes. But the use of formalin gas has recently been very widely recommended. Special forms of apparatus are made which produce this gas in large amount, and the gas made in the machine may be conducted by rubber tubing into a room to be disinfected. A simpler method of disinfection can hardly be conceived than to close a room tightly, and to run this tube, connected with the machine, through the keyhole, and then start the manufacture of the formaldehyde gas. These machines have been brought to a very high state of efficiency and have been rapidly adopted by health boards in recent years. But just as in the case of the use of sulphur fumes the efficiency of the vapor as a means of disinfection is questionable. Bacteriologists are very doubtful whether formaldehyde gas when so used is sufficient to insure the thorough disinfection of a room.

Many other chemicals have been used for disinfection, but those mentioned are in the widest use and the most practical from their cheapness and ease of application.

APPLICATION OF DISINFECTANTS.

In the study of the practical application of disinfectants there are two points to be determined: (1) Where the disinfectant should be applied; (2) the proper disinfectant to be used.

1. The determination of the places where the disinfectant should be applied is the most important factor connected with the whole subject. Unless the disinfection is applied at the proper place, there is likely to be a vast amount of misdirected effort. If, for example, a dairyman is troubled with slimy milk due to bacteria *in the water* in which he sets his cans, and in attempting to get rid of it fails to notice this *source* of trouble, he is likely to spend a large amount of money and labor in disinfecting stalls and dairy, and possibly cows, without producing any result; while a little labor properly directed toward the dairy *water* supply, would produce a satisfactory result with far less trouble. The same thing will apply everywhere. A small amount of effort properly directed will be successful, whereas a large expenditure of effort misdirected will be useless. To determine the place where disinfectants should be applied is the most difficult problem in disinfection. No general rules can be given as a guide, but each case must be determined by the intelligence of the individual who attempts disinfection. It is, of course, impossible to state the *source* of infection in many instances which may come up for treatment. Although no general rules that can be given, there is one fact which every one who attempts any

method of disinfection should remember. In case of contagious diseases the *excretions* eliminated from the infected animal are practically certain to contain the infectious organisms. Hence disinfection in such cases must be primarily directed towards destroying such excretions, and in preventing them from being distributed from animal to animal by any possible means. One cannot go far wrong, in his application of disinfectants, if he makes his primary effort that of destroying the bacteria possibly present in the excretions from suffering animals.

2. The next problem is the selection of a disinfectant. It is manifest that different disinfectants must be used under different conditions. The comparatively harmless formalin can be used where the poisonous corrosive sublimate would be out of the question. For example, formalin has been used for the preservation of milk, one teaspoonful being sufficient to increase greatly the keeping qualities of ten gallons of milk; when used in this quantity formalin does not produce any noticeable results, at least at first, upon the person drinking the milk. The use of the other disinfectants, corrosive sublimate or carbolic acid, would be manifestly impossible in these cases. But on the other hand, formalin is a material extremely irritating to the nose, and very irritating to mucous membranes and raw surfaces generally. Formalin, therefore, though less poisonous than carbolic acid, cannot be used in surgery, and cannot be used in any place where the gas becomes abundant enough to be irritating. For every case, then, the disinfectant must be chosen which is most applicable.

A very few suggestions for disinfection may be given as likely to be of practical value to the agriculturist.

The Person.—When it is desired to disinfect the person, either of man or of animals, the common disinfectant used is corrosive sublimate in proportion of 1 to 1,000, or carbolic acid in proportion of 1 to 20. These may be used freely upon the skin without danger. To disinfect mucous membrane of the raw surfaces of wounds these cannot be used in quite so great strength. For disinfecting wound surfaces carbolic acid must not be used stronger than 1 to 40, and corrosive is not used at all. A better disinfectant in such cases is *boracic acid* in 10% solution. This may be used in the most delicate places, even in the eye, and acts very satisfactorily.

In the disinfection of cattle or other animals, exactly the same rules apply. The weaker carbolic solution, or the boracic acid, must be used for disinfecting delicate portions or the body, such as the *teats*, or *genital parts*.

Clothing, etc.—No one method can be given for the disinfection of clothing, but one must be guided by the nature of the material to be disinfected. The following general rules may be serviceable in this respect.

1. *Burn* everything which is not of great value. This is a sure method of disinfection and the only satisfactory one.

2. *Boil* all clothing which will not be injured by the process, or if boiling is impractical, subject the clothing to steam. For this purpose the material must be placed in a large closed box, and a current of superheated steam passed into the box. Such a method of disinfection is quite effective and may frequently be applied to carpets and curtains, which cannot be boiled. Of course the ordinary farmer will have no convenience for such a method of treatment.

3. *Formalin Gas.*—Clothing too valuable to be destroyed, and which cannot be treated by steaming, may be disinfected by formalin gas. This of course cannot be done by the agriculturist, inasmuch as it requires special apparatus. The best method of accomplishing it is to hang the clothing in a room which is then filled with the formalin gas by the method described later. A simple soaking of clothing in water containing 1 part of formalin to 25,000 of water is efficient.

4. *Sunlight.*—Sunlight cannot be depended upon as a dis-

infectant for heavy clothing because it does not penetrate much beyond the surface. Thin articles of clothing may be effectually disinfected by exposing them to bright sunlight for a few days, and even heavier articles, by long exposure to sunlight and air, will in time be satisfactorily disinfected.

Thick, heavy articles of clothing or bedding cannot be properly disinfected by any means at our disposal. There seems to be no satisfactory way of disinfecting mattresses, and, in cases of exceptionally contagious diseases, the only proper method of treatment of such articles is their complete destruction by burning.

The Stable.—The disinfection of the stable is not infrequently demanded of the agriculturist. Such disinfection is difficult because of the roughness of the lumber with which the stable is made. A satisfactory method of disinfection is as follows: First remove all dirt from all surfaces in the stable. This must be done *thoroughly* or the disinfection will not be complete. Water must be used freely to moisten up the dry filth that has accumulated in various parts of the stable. The removal of the dirt is thus facilitated, and the cleansing must be thorough. After such cleaning, the whole stable should be washed with a solution of corrosive sublimate, above given (1–1,000). This may be done by simply washing with a broom, or better, by spraying, provided a proper spraying apparatus be at hand. It must be remembered, however, that corrosive sublimate corrodes metals badly, and no metal spraying apparatus can be used. After the thorough wetting down of all surfaces of the stable by the disinfectant, the stable must again be washed with water to remove the disinfectant. Instead of corrosive sublimate, a strong solution of chloride of lime may be used in the same way in washing the walls and floors. Such a disinfection, if thorough, will usually be found satisfactory.

The Dairy.—The disinfection of the dairy must follow along essentially the same lines as the stable. Everything must be first cleaned as thoroughly as possible, and then all woodwork may be washed with corrosive sublimate, or better, with a three- to five-per-cent. solution of carbolic acid. These solutions must *not* be used for washing the vessels which contain milk. For cleaning these vessels nothing but boiling hot water or steam are legitimate. After the disinfection of all parts, the whole must be washed with water. If the dairy is capable of being closed tightly, the disinfection mentioned may be followed by the use of sulphur or formalin, as described below.

Other localities inhabited by animals. To disinfect the *barnyard* in which cattle are allowed to roam, is practically an impossibility, and the same thing is true of the *pig pen.* The amount of moist material accumulated in these localities is so great as to make disinfection impractical by any means yet devised. We must make the same statement in regard to pastures where infected cattle are allowed to roam. To disinfect a pasture is an impossibility; it must be left to the action of sunlight and rains, and these will, in the course of time, commonly produce the disinfection.

The Sick Room.—In every person's household it becomes occasionally necessary to disinfect the room in which persons suffering from contagious diseases have been confined. To discover a practical and satisfactory method of such disinfection has been very difficult, but the following procedure is the best that has as yet been devised. Carpets, curtains and bedding, must be removed and treated as above mentioned. All surfaces of the room, including floors, walls and surfaces of furniture, must be washed with a solution of corrosive sublimate, 1–1000. This disinfectant should be used freely, and especially should be allowed to run in all cracks and crevices that there may be around the room. If the corrosive sublimate can be thus thoroughly used, the room is satisfactorily disinfected;

but it frequently happens that there are places in the room which the disinfection cannot reach, and for this purpose it is desirable to use some gaseous disinfectant which will reach every part of the room. The gas most commonly used is the fumes of sulphur, which may be advantageously applied as follows: The room is thoroughly closed, all cracks being sealed up, even to the keyholes around the doors. Then into a metal vessel is placed some ordinary sulphur, 5 pounds being used for every thousand feet of space to be disinfected. The vessel containing the sulphur is placed in the middle of the room, preferably in a tub containing an inch or two of water; a little alcohol is poured over the sulphur, the sulphur ignited, and the room then completely closed by shutting the door. The sulphur will burn, filling the air with sulphur fumes. The room must be kept closed for 24 hours, after which it may be opened to allow the fumes to disappear. Rooms disinfected in this way are found in practice to be quite thoroughly disinfected, although experimental evidence indicates that sulphur fumes do not perfectly destroy all pathogenic bacteria.

The uncertainty of sulphur has led in recent years to the use of *formalin gas* for disinfecting rooms. The method used is to close the room, sealing all cracks, as in the case of sulphur disinfection, and then to conduct into the room, frequently through the keyhole by means of a rubber tube, a quantity of formalin gas, generated by a special apparatus devised for this purpose. This apparatus is at the present time to be found frequently in the hands of Health Boards, and will be used by them. No private individual will be likely to have such apparatus, and consequently no further directions concerning its use need here be given.

Excreta.—No lesson is more useful to learn than the absolute necessity of taking care of *all excretions* from men or animals suffering with a bacterial disease. These excretions will commonly contain the specific bacteria of the disease, and will be the most efficient means of distributing the diseases. It is to the destruction of such secretions that disinfection must be primarily directed. Sometimes such disinfection offers no difficulty. The feces or urine may be readily disinfected by *chloride of lime* or even, in the case of animal excrement, by common *slacked lime*, used in large quantity. Precautions must be taken to prevent these excretions from being carried around upon the boots of attendants, and especial care must be used to prevent their obtaining access to any source of water supply. Where the excretions are from the skin, as in the case of scarlet fever or small-pox, the difficulty of disinfection is great and practically insurmountable. Much may be done, even in these cases, by bathing the skin, or by keeping it moistened with glycerine.

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INDEX.