AGRICULTURAL BACTERIOLOGY

CONN

# AGRICULTURAL
# BACTERIOLOGY

A STUDY OF THE

## RELATION OF GERM LIFE TO THE FARM
WITH
## LABORATORY EXPERIMENTS FOR STUDENTS

MICROORGANISMS OF SOIL, FERTILIZERS, SEWAGE, WATER, DAIRY PRODUCTS, MISCELLANEOUS FARM PRODUCTS
AND
OF DISEASES OF ANIMALS AND PLANTS

BY

H. W. CONN, Ph. D.
*Professor of Biology at Wesleyan University*
AUTHOR OF "THE STORY OF GERM LIFE," "BACTERIA IN MILK AND ITS PRODUCTS," "PRACTICAL DAIRY BACTERIOLOGY," "BACTERIA, YEASTS, AND MOLDS IN THE HOME," ETC.



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# PREFACE TO SECOND EDITION.

Since the publication of the first edition of this work advance along all lines of bacteriology has been very rapid. Scarcely a phase of the relation of bacteria to agriculture has failed to receive substantial contributions. Much new information has been obtained, the relative importance of different subjects has been changed, and in a few points our previous conclusions have been corrected. These changes have been so considerable that in preparing this second edition it has been found necessary to rewrite the whole book in order to bring it up to the times. The subject has grown so large that it is difficult to include within the limits of one volume even the fundamental facts of the rapidly growing science, and many subjects of importance have been treated very briefly.

The growing recognition of the importance of the subject to students of agriculture has caused agricultural schools and colleges to give to it an increasing amount of attention. For this reason this edition has been planned with special reference to its use by classes; and some changes in method of presentation have been adopted in order to make it more useful to students. For the same reason there has been added a somewhat extended set of experiments for elementary laboratory work. These laboratory directions are far from exhaustive and are designed simply to introduce the student to the methods of bacteriological work.

The close relation of the functions of the higher fungi to the functions of bacteria has come to be fully realized to-day and has made it necessary to include more extended references to the higher fungi in this review. The only other important addition in this edition is a considerable extension of the treatment of bacterial diseases of plants that have received so much investigation in the last ten years. With these additions it is hoped that the present edition will be a fair summary of our present information upon the relation of germ life to agriculture.

H. W. C.

MIDDLETOWN, CONN.
*June* 15, 1909.

# PREFACE TO FIRST EDITION.

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, therefore, it will 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 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.

H. W. C.

MIDDLETOWN, CONN.

# TABLE OF CONTENTS.

## PART I.

### General Nature of Microörganisms and Their Activities.


| CHAPTER | PAGE |
|---|---|
| I.—The General Characters of Microörganisms | 1 |
| II.—Fermentation, Putrefaction, and Decay | 22 |


## PART II.

### Bacteria in Soil and Water.


| | |
|---|---|
| III.—Nature's Food Supply. The Carbon Cycle | 37 |
| IV.—Decomposition of Nitrogenous Compounds | 47 |
| V.—Nitrification and Denitrification | 57 |
| VI.—The Manure Heap and Sewage | 69 |
| VII.—Reclaiming Lost Nitrogen | 90 |
| VIII.—Bacteria and Soil Minerals | 111 |
| IX.—Some Practical Lessons from Soil Bacteriology | 118 |
| X.—Bacteria in Water | 127 |


## PART III.

### Bacteria in Dairy Products.


| | |
|---|---|
| XI.—Bacteria in Milk | 137 |
| XII.—Control of the Milk Supply | 165 |
| XIII.—Bacteria in Butter-making | 181 |
| XIV.—Bacteria and other Microörganisms in Cheese-making | 195 |

## PART IV.

### Relation of Microörganisms to Miscellaneous Farm Products.

CHAPTER PAGE

XV.—Alcohol, Vinegar, Sauer Kraut, Tobacco, Silage, Flax . . . 211
XVI.—Preservation of Food Products . . . 235

## PART V.

### Parasitic Bacteria.

XVII.—Resistance Against Parasitic Bacteria . . . 251
XVIII.—Tuberculosis . . . 261
XIX.—Other Germ Diseases . . . 280
XX.—The Parasitic Diseases of Plants . . . 293

## PART VI.

### Appendix.

XXI.—Laboratory Work and Disinfection . . . 307
Index . . . 327

# PART I.

# GENERAL NATURE OF MICROÖRGANISMS AND THEIR ACTIVITIES.

## CHAPTER I.

## THE GENERAL CHARACTERS OF MICROÖRGANISMS.

### MICROÖRGANISMS AND FARM LIFE.

The successful farmer of to-morrow will be the one who most skillfully regulates the growth of microörganisms. Though he may not be conscious of it, much of the work that the farmer is carrying on even now is, as we shall see, really directed toward the control of germ life. In the consideration of the microörganisms related to farm life we are concerned with three different types of plants: **Bacteria, Yeasts** and **Higher Fungi.** Although the latter are plants of considerable size, and hence hardly microörganisms, in many respects they are related to the microscopic bacteria and yeasts, and the functions of all three in farm life are so similar that all must properly be considered together. The molds, being plants of considerable size, have been known a long time, although only recently has their relation to nature's processes been understood. Yeasts were used, under the name of leaven, far back in history; but it was not till 1680 that the Dutch microscopist, Leeuwenhoek, showed with his microscope that yeast consists of minute globules; and it was 150 years later when Schwann and Caignard-Latour proved yeast to be a living plant. Leeuwenhoek was also first to see bacteria, and studied them as early as 1695. 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. He intimated that they might be the cause of disease. But 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.

**Relation to Disease.**—Not until toward the middle of the nineteenth century was it conceived that the microscopic organisms, at first 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 causing *fermentations*. Even before it was known what yeast was, it was recognized as in some way associated with alcoholic fermentation; but not till about 1838 was it clearly proved that yeast plants are the cause of the fermentation of sugar. The development of a knowledge of bacteria followed a little later. One of the first real contributions to a knowledge of their significance was the demonstration in 1840, of the fact that certain microscopic organisms cause blue milk. Thus, at the very beginning of the modern study of bacteria, they were associated with peculiar agricultural phenomena, an interesting fact when we notice that, in the next quarter of a century or more, the chief investigations, and all the interest in them, centered around the question of their agency in producing disease. Bacteria are still suffering in reputation from the fact that, for thirty years, 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 still clings to them. The very word *bacteria*, or *germs*, has become, in the minds of some, almost synonymous with disease. Their relation to the medical profession was soon recognized, and more or less extended courses in bacteriology have rapidly made their appearance in medical schools. Health boards and sanitary officers have recognized that their primary duty is to deal with bacteria; and most of the regulations for the preservation of the public health have been directed toward the destruction or control of these organisms.

As more information has accumulated during the last twenty years or so, it has become evident that microörganisms, including bacteria, do not deserve all the ill repute that they have acquired. It has been learned that there are hundreds and even thousands of kinds of bacteria, and that, while certain species are the cause of disease, others are harmless, some are beneficial in the body, and many perform functions of the highest significance and value. Although the disease side of the bacteria story was the first to be studied, it is only a small part of the subject. Among the many hundred kinds of bacteria known, only a few, less than two score, are as yet definitely known to have any power of causing disease in man. 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 have relations to the phenomena of living things which were wholly unsuspected. Within the last twenty years a larger and larger amount of attention has been directed to the part played in nature by microörganisms which are never parasitic and have no relations to human disease. As a result there has developed a new branch of bacteriology which deals with phenomena wholly separate from disease.

**Relation to Agriculture.**—In particular it has been shown 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 that they are fundamental to the processes of agriculture.

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 relations to disease, even more does the agriculturist need to understand their relations to his industry. These microörganisms play such a fundamental part in the processes of nature that the life phenomena of animals and plants are inextricably bound up in the functions of bacteria, and without them life processes must soon cease. 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; hence, it is a matter of necessity that the agriculturist of the future should have a practical knowledge of the general phases of bacteriology. The solution of the most vital agricultural problems, like that of continued soil fertility, involves bacteriology. From beginning to end the occupations of the farmer are concerned in the attempt to obtain the aid of these microörganisms where they may be of advantage, and to prevent 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 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 would be an impossibility*, for the soil would yield no crops.

The relation of microörganisms to farm life is one of the most recent branches of science. Scarce twenty years have elapsed since the first steps in this direction were taken, and some of our scientists, who are still young, have seen practically the whole development of the subject from its starting-point in the early eighties. With a science as young as this, it is inevitable that many questions remain unsolved. Scientific discovery usually precedes any practical application, and in these early years of the development of agricultural bacteriology we must expect to find the theoretical side of the subject proceeding rapidly, while the application of the facts to farm methods lags behind and is, in many respects, hesitating, tentative, or even unsatisfactory. Nevertheless, the discoveries made have already revolutionized agricultural processes. Changes in agricultural methods, due to bacteriology, have been largely adopted all over the world; but they have generally been adopted by farmers in ignorance 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. Successful agriculture of the future is indissolubly bound up in the problem of the proper handling of microörganisms. 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 at least have a general knowledge of the fundamental factors of bacteriology as they are related to agriculture.

## WHAT ARE MICROÖRGANISMS?

In studying the relation of germ life to the farm we are concerned with a class of phenomena called *fermentation*, *putrefaction*, *decay*, *decomposition*, and the like (see Chapter II). These phenomena are all caused by living bodies that are frequently called *microörganisms.* This term strictly means animals or plants of microscopic size. But this conception of the term is at once too narrow and too broad to cover the organisms we are to study. Some microscopic organisms have no particular relation to the classes of phenomena which we are considering. A great host of microscopic, green water-plants and also many microscopic animals have nothing to do with our subject, though they might properly be called microorganisms. On the other hand, some plants of large size, like molds and toadstools, have a part to play in producing the decomposition of organic structures very similar to that played by bacteria. These cannot properly be called microörganisms, but nevertheless they must be included with the study of bacteria and yeasts, since they perform similar or closely allied functions in nature. A better term to cover the organisms which we must study might be the rather broad term of **Fungi**, for all of the organisms with which we are concerned belong to this general class of plants. But this term is also unsatisfactory, since it fails to convey the idea that the organisms are largely microscopic. To most people the term fungus gives at once the impression of a large plant; and we are chiefly concerned with microscopic forms. We shall, therefore, still use the term microörganism, although some of the plants that we shall refer to are not microscopic. In this discussion we are concerned chiefly with **Bacteria**, secondarily with **Yeasts**, and to a less degree with the **Higher Fungi.**

**The Fungi.**—All of the plants with which we are here concerned belong to the class that botanists call Fungi. There is one characteristic common to all Fungi—*they all lack green coloring matter.* This green coloring material in ordinary plants makes it possible for them to live upon the mineral ingredients in the soil; and green plants only can be thus nourished. The colorless plants are unable to obtain nourishment from the mineral world. The Fungi, since they are all colorless, must live upon food furnished them by other plants or animals. It is this fact that gives them their significance in nature, and explains their important relations to farm life. Fungi are very abundant everywhere, and there are thousands of different kinds. For the purpose of our study, we may recognize three groups:

1. **Higher Fungi.**—Under this general name we will include a large number of colorless plants, mostly of large size. It includes such plants as *molds*, *mushrooms*, *toadstools*, *tree fungi*, and hosts of others less commonly known. Some of them are of great importance in farm life, especially as agents in bringing about the decomposition of vegetable matter, so that it may be incorporated into the soil to be used again; here they play a part secondary only to bacteria. They are of endless variety, and it would be manifestly impossible here to attempt any consideration of their classification. One point concerning them must be understood. In all the higher Fungi with which we are concerned the body of the plant consists of a mass of delicate threads which grow into a dense, usually white mass (Fig. 1). Sometimes the threads are large enough to be seen easily and sometimes they are so delicate that a microscope is required to see the individual threads, though the mass of threads may be of considerable size. The mass of threads grows on the surface or in the substance upon which the plant is feeding. This thread is able

in many cases, by growing, to force its way into the solid mass of hard substances, like wood, and to push itself between the wood fibers. Thus it is a primary agent in effecting the destruction of wood. Such a mass of branching threads is called a **mycelium,** and

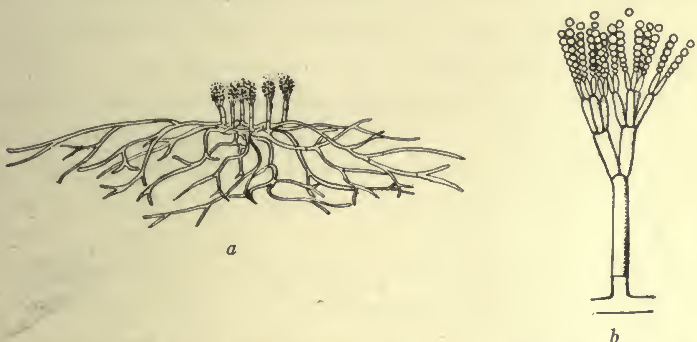


FIG. 1.—One of the higher fungi, the common bread mold, *Penicillium glaucum*. *a*, the whole plant; *b*, one of the spore-bearing branches more highly magnified.

is found in all the Fungi of this class. So far as the mycelium is concerned, most of these plants are much alike. But the different species have many different methods of reproduction, and it is chiefly upon their reproductive bodies that botanists rely to distin-

FIG. 2.—Mucor, a common mold, showing mycelium and spore formation.

FIG. 3.—Aspergillus, a common mold, showing mycelium and spore formation.

guish the different species. After the mycelium has grown for a little time, it commonly sends up into the air small or large branches that produce **spores,** or reproductive bodies. The method of spore production differs sufficiently in the different fungi to make it possible to classify them. Frequently only the spore-producing part of the plant is seen, and it may be the only part known, except to botanists. For example, the toad stool is only the reproducing portion of a fungus; it has a mycelium wholly under ground or buried within the hard mass of the trunk of a tree. It is the mycelium, however, that does the work for which these fungi are responsible, and not the spore-producing part that we see. Figs. 1 to 3 show the general appearance of some of these fungi and their methods of forming spores. With these methods of reproduction and classification we are not concerned in this work, and only such types as are related to our subject will be mentioned later in their proper places.

2. **Saccharomyces** (*Yeasts, Budding Fungi*).—These immensely important plants are all microscopic in size. While varying somewhat, an average size is about 1/4000 of an inch in diameter. They are usually spherical or oval in shape, though sometimes slightly elongated (Fig. 4, *a*). They form no mycelium and cannot force their way into hard substances. Their chief characteristic is their method of reproduction by a process called *budding*. There appears on the side of the yeast cell a minute bud, which continues to increase in size until it becomes as large as the cell from which it has grown. Then the two cells may break apart at once; or each may in turn produce buds before they separate. In either case, two or more cells are produced from the one, and although they may remain attached so as to form irregular masses of several cells, (Fig. 4, *b*), each cell is really complete in itself. Eventually they break apart. This budding takes place rapidly, though not so rapidly as the division of bacteria, which will be mentioned later.

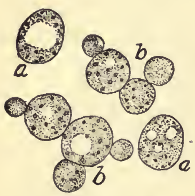


FIG. 4.—Yeast plants, showing method of growth by budding. *a*, single cells; *b*, budding cells.

A second important character of yeasts is the nature of the fermentation they produce. They have an action especially upon sugars, which they break up into *carbonic acid* and *alcohol*. This action makes them play a large part in nature's processes, quite distinct from that of bacteria.

Any further classification of yeasts is quite unnecessary for our purposes.

3. **Schizomycetes** (*Fission Fungi*, or *Bacteria*).—This group comprises the bacteria proper; it is certainly the most abundant of the three, and in some respects it is the most important. It is with the bacteria that we are chiefly concerned in this work. Bacteria have sometimes much the same shape as yeasts. The chief distinction between them is their method of multiplication. Instead of budding they multiply by *fission*. The bacterium elongates a little, and then divides into two equal halves at once (Fig. 5). Hence the name *fission fungi*. Bacteria are also, as a rule, smaller than yeasts, frequently not more than 1/25000 of an inch in diameter. The size would make it possible for 8,000,000,000 to be crowded into a mass no larger than a pinhead; and we can, therefore, easily understand that there may be 100,000,000 in a drop of milk. Occasionally, however, there are larger bacteria and smaller yeast cells. While the size is no sure criterion between the two, when one finds, under the microscope, rather large round or oval plants, he is pretty safe in calling them yeasts, while the smaller ones he may call bacteria. But it is necessary, in some cases, to study the method of reproduction before one can with certainty distinguish yeasts from bacteria.

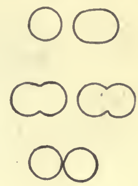

FIG. 5.—Bacteria, showing method of division by fission.

This group of bacteria is of such primary importance to our study that we must learn further facts concerning their classification and characters.

## GENERAL CHARACTERS OF BACTERIA.

**Colonies.**—Bacteria are so minute that they cannot be handled as individuals, but must be treated in masses. One of the primary difficulties in the study of these organisms has been to get masses of bacteria that would be large enough to handle, and yet would contain only one kind of bacteria. Such masses are called **pure cultures,** and it was this difficulty in procuring pure cultures that for a long time prevented the development of the science. Bacteriological study to-day commonly begins with *cultivating* the bacteria, *i.e.*, allowing them to grow in some medium adapted to them until they become abundant enough to be handled in bulk. To prevent the mixing of the different kinds that may be in the material we are studying, they are usually grown in a solid or jelly-like medium, which holds the individuals fast in one spot. As the individuals multiply in this solid medium, they are unable to separate from each other; so they remain in little clusters which in time become large enough to be seen without a microscope. Such clusters are called **colonies,** and figures of some of them are shown in Fig. 6. The shape and appearance of the colonies produced by different kinds of bacteria are often very different, showing, indeed, greater varieties than can be seen in the bacteria themselves with a microscope. As a result the shape and appearance of these colonies are often used to separate the numerous bacteria from each other and to classify them. A colony, when it comes from the multiplication of a single individual bacterium, is made of one kind of bacteria only. This colony may easily be picked out with a sterile needle, and when properly placed in another culture medium it becomes a pure culture. The starting-point in practical bacteriological study is thus the colony rather than the individual bacterium (see Laboratory Work).

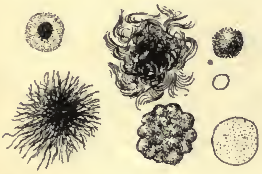

FIG. 6.—Colonies of bacteria.

**Form of Bacteria.**—Bacteria are of three quite different shapes, but are all very simple. 1. *Simple spheres* (see Fig. 7, *a*). Such spherical forms are called **Cocci.** In common microscopical preparations no internal structure can be seen, the bacteria appearing as deeply stained balls. The Cocci, however, differ somewhat in their method of growth, thus enabling the microscopist to distinguish different kinds, as will be mentioned presently. 2. *The rod-formed bacteria* (Fig. 7, *b*). These organisms are longer than they are broad, sometimes only slightly so, but at other times very much longer, forming, indeed, long, slender threads. 3. *The spiral-formed bacteria.* These are either long, coiled spirals, or very short ones, with only a single turn (Fig. 7, *c*). This type is of less importance than the others.

**Motility of Bacteria.**—The next point of distinction among bacteria is based upon their *motility*. Some bacteria are capable of an active swimming motion, others are stationary. The motion is produced by minute, extremely delicate, vibrating hairs, called

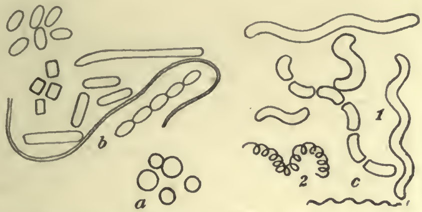

FIG. 7.—General shape of bacteria. *a*, spheres; *b*, rods; *c*, spirals.

**flagella** (Fig. 8). The flagella are so delicate that they cannot often be seen in the living bacteria, and they do not stain by the ordinary method of staining. Therefore, they are never seen in the usual microscopic preparations. They may be seen by special methods, but these are so difficult that the beginner cannot use them satisfactorily. The question of their motility is, however, usually determ-

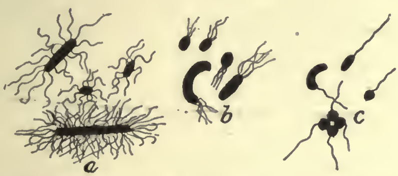

FIG. 8.—Showing bacteria with flagella; *a*, peritrichic; *b*, lophotrichic; *c*, monotrichic.

ined without staining, by the study of the living bacteria (Experiment No. 8). These flagella are differently distributed upon different bacteria. Sometimes there is a single one on the end of a rod (Fig. 8, *c*)—*monotrichic;* sometimes a small tuft at one or both ends of a rod (Fig. 8, *b*)—*lophotrichic;* and sometimes there is a covering of flagella over the whole body of the bacterium (Fig. 4, *a*)—*peritrichic*.

**Classification.**—Based upon the distinctions thus mentioned, the bacteria are divided into groups:

SPHERICAL BACTERIA:

Dividing in *one plain*, so as to form chains (Fig. 9, *a*), **Streptococcus.**

Dividing in *two plains*, and not forming chains (Fig. 9, *b*), **Micrococcus.**

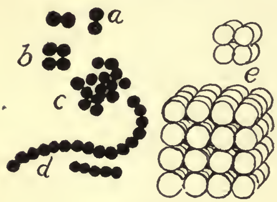


FIG. 9.—Showing different types of cocci. *a*, *b*, and *c*, Micrococci; *d*, Streptococci; *e*, Sarcina.

Dividing in *three plains*, and forming cubical masses (Fig. 9, *c*), **Sarcina.**

ROD-SHAPED BACTERIA:

With flagella and consequently motile (Fig. 8)*, **Bacillus.**

Without flagella and consequently non-motile (Fig. 7, *b*), **Bacterium.**

With a single flagellum, **Pseudomonas.**

SPIRAL BACTERIA (Fig. 3, *a*), **Spirillum.**

The genus BACILLUS is further divided as follows:

Bacilli with only *one flagellum* (Fig. 8, *c*) are named Monotrichic Bacilli, or **Pseudomonas.**

Bacilli with *one flagellum* at each end, **Microsporon.**

Bacilli with a *tuft of flagella* at one end (Fig. 8, *b*), are called **Lophotrichic Bacilli.**

Bacilli with flagella *over the whole body* (Fig. 8, *a*) are called **Peritrichic Bacilli.**

HIGHER BACTERIA (*Cladothrix, Leptothrix, Streptothrix, Actinomyces*) (Fig. 10).

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

*Unfortunately bacteriologists are not agreed to-day in regard to the use of the terms above given. The names *Bacillus* and *Bacterium* are not always used as here stated, and recent classification of the spherical forms recognizes, in addition to the names given, three others, *Diplococcus*, *Metacoccus*, and *Ascococcus*. This variation in nomenclature results in great confusion. In the absence of any well-accepted classification to-day, we shall in this book use the names as above defined.

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. One type of *Streptothrix* is extremely abundant in soil and appears as round, white opaque colonies with an extensive brown halo upon the plates described in Experiment No. 24.

Thus it will be seen that the term *bacteria* applies to the whole group of organisms that multiply by division, the study of which constitutes the study of bacteriology, while the term *Bacterium* refers to a single division of the group, viz.: the non-motile, rod forms. The term *Bacillus* should apply to motile forms only. The names *Bacillus* and *Bacterium* are sometimes confused; for example, the tubercle *bacillus*, according to the above classifiication, is a *Bacterium*, since it is non-motile; and indeed recent study indicates that it belongs to the group of higher fungi; but the name bacillus was given it years before the above distinctions were recognized, and we will still use the common name. Some other bacteria, named twenty years ago, retain their earlier names in some books, but they are slowly having their names brought into harmony with the above distinctions.


FIG. 10.—Actinomyces. *a*, a small colony; *b*, single rods (*Boström*).

The term *Coccus* is applied to any spherical organism of the group bacteria.

This classification gives only what are recognized as the *genera* of bacteria. A further classification of the group into *species* is at the present time in a condition of the greatest confusion. Many hundred varieties 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 hundred described species represent 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 in the way of a proper description and classification of the species of bacteria have hitherto been insurmountable, and at the present time the subject is in such extreme confusion that no one except an expert can understand it. Fortunately this confusion of species is of no importance for our purpose. Agricultural bacteriology is not at present concerned with the problem of the species. All that it 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.

**Multiplication of Bacteria.**—As already mentioned, the primary method of the multiplication of bacteria is by simple division. Bacteria are so minute that it seems strange to assign to them much of a part to play in nature's processes. But their extraordinary power of multiplication gives them unlimited possibilities.

The elongation of a rod and its division into two parts, followed by a repetition of the process, may be extremely rapid. Frequently it does not take 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 that is almost inconceivably great. If a division once an hour 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 there would be a mass sufficient to fill the oceans. This rate is, manifestly, not continued for any great length of time, or the world would be full of them; their growth is checked by lack of food, and still more by the substances they secrete, which act as poisons. But this possibility of reproduction represents an almost unlimited power, constantly curbed by the lack of proper conditions. Bacteria may thus be looked upon as possessing a wonderful possibility of reproduction, a force of inconceivable magnitude, held more or less in check by adverse conditions, but ever ready to exert their influence when the conditions are favorable. Since they are feeding during their growth, they must produce profound changes in the material upon which they feed. 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.

**Production of Spores.**—There is another method of producing new individuals, an understanding of which is necessary to a knowledge of bacteria. This is the production of spores, and is illustrated in Fig. 11, *a–e*. The bacterium there figured consists of a rod. The contents of one of the rods collects itself in a spherical or oval body in the center. This later breaks out of the rod, the rest of the individual then dying and disappearing. The oval body itself is a **spore,** and is capable, when placed under proper conditions, of developing into a new rod, *e*. Inasmuch as only a single spore arises from a single bacterium, it is not a multiplication. Its purpose is not so much to increase the number of individuals as to enable the bacteria to endure adverse conditions without being killed. The ordinary bacteria are likely to be killed by being dried, and will readily succumb to moderate heat, a temperature of 165° F.* being sufficient to kill almost any of them. But these spores are covered with a hard case which enables them to resist the conditions which the active, growing, and multiplying forms cannot resist. They may be completely dried for months, and even years, and still retain their vitality. They may be heated very much hotter than the active forms without injury; indeed, some of these spores may be in boiling water for many minutes —an hour or longer—without having their vitality destroyed, since, if the spores are subsequently cooled, they are capable of germinating and growing into new bacteria. As a result of this it will follow that, while it is very easy to kill ordinary bacteria by heat, it is

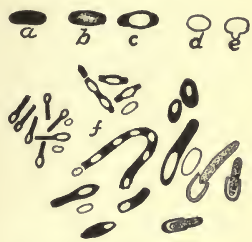


FIG. 11.—Spore formation. *a* to *e*, stages in spore formation and germination.

* Temperatures used in this book always refer to the Fahrenheit scale.

far more difficult to destroy spores. Many species of bacteria produce such spores (Fig. 11, *f*); others do not, and hence some are much more easily killed by heat than others. Milk, for example, contains many kinds of bacteria. By the simple boiling or, indeed, the heating of the milk to a temperature of 160° F., a vast majority of the bacteria are killed; but the few spores that may chance to be in the milk are not thus killed, and subsequently these will be able to develop. If milk contains spore-bearing bacteria, it cannot be sterilized by boiling; and, since it almost always does contain them, boiling is not sufficient to sterilize it. This phenomenon of the high resisting powers of spores must always be borne in mind in all problems of sterilizing.

**Relations to Conditions.**—*Temperature.*—The rate of multiplication of bacteria, yeasts, and molds depends upon the temperature. At freezing they do not grow at all. As the temperature rises above freezing they begin to multiply, and their rate of multiplication increases as the temperature rises, up to a certain point which is the *optimum temperature*. If the temperature rises still higher, the rate declines and finally growth stops. If heated still more, the organisms are killed. The lowest temperature at which they will grow, the *minimum temperature*, varies with different species. Some will grow at a temperature only just above freezing, at 33° F., while, at the other extreme, some will not grow at temperatures lower than 120° to 140° F. The optimum temperature also varies. Some species grow best at moderately low temperatures, 60° or as low as 50° F., while others flourish best at a temperature from 90° to 100° F. When the temperature is above 100°, most bacteria grow less rapidly than when it is a little lower, while at a slightly higher temperature they cease growing. A few species, however, grow best at unexpectedly high temperatures, some having been found flourishing at 140° or even higher. These peculiar bacteria are called *thermophiles*. How they can find conditions in nature warm enough for their growth is a question.

The *death temperature* is a factor of great importance, since it is so closely associated with the matter of sterilization by heat. Most bacteria, when in an active condition, are killed by a temperature of 140° if maintained for half an hour. At this temperature, however, they die slowly; a temperature of 150° destroys them more rapidly still, while a temperature from 170° to 180° is proportionately more effective. A total destruction of bacteria, including their spores, can be brought about only by a temperature above that of boiling water, and this is usually accomplished, in the case of liquids, in a closed chamber where the steam can be generated under considerable pressure. If the steam is allowed to collect in such a chamber at a pressure of 15 pounds, the temperature, then, will be about 240°. This temperature, kept up for half or three-quarters of an hour, destroys even the most resisting spores. Laboratories usually have a small apparatus designed for this purpose, called an autoclav (Fig. 12), and this is used constantly for sterilizing liquids.

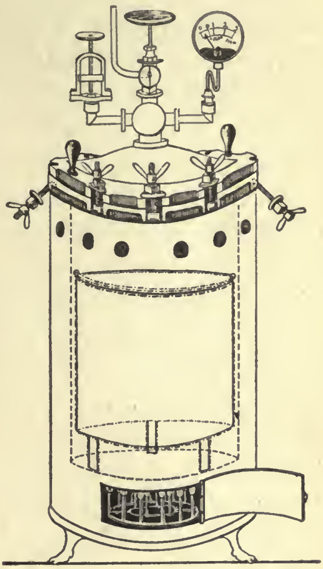

FIG. 12.—An autoclav used in sterilization of liquids under pressure (*Eyr* ).

*Sterilization.*—This is a process closely related to the question of death temperatures. Sterilization is sometimes, to be sure, brought about by adding poisonous chemicals to the material to be sterilized; but more commonly, and almost universally, when we are dealing with food products, sterilization is accomplished by heat. If a material to be sterilized contains only active organisms, it might be accomplished by subjecting it to a moderately low heat, 140° to 150° F. But it is almost always a fact that anything which we wish to sterilize is likely to contain spores, and, since these withstand a higher temperature, no moderate heat will accomplish the purpose. Even boiling is not sufficient to destroy spores, so, to be sure of complete sterilization, a temperature above boiling is necessary. If the object is a solid that can bear heat it is simply heated at about 300° F. for an hour or so. If it is a liquid it is placed in an autoclave (Fig. 12), and heat is applied until there is a steam pressure from 10 to 15 pounds. This produces a temperature sufficient to destroy spores.

Sometimes it is desirable to sterilize liquids that will not stand these high temperatures, as, for instance, gelatin (Experiment No. 12). This can be accomplished by *discontinuous heat.* The material is heated to about 180° F., or more commonly to boiling. It is then cooled and allowed to stand twenty-four hours in a warm place. The heat has destroyed the active bacteria, but has not killed the spores, which, during the twenty-four hours, will germinate and grow into active bacteria. Heat is again applied as before, and this time any active bacteria that may have come from the germination of the spores are killed. The material is allowed to stand another day so that any spores that may have failed to germinate the first day may grow, then heat is applied again. Experience shows that three heatings of this sort will destroy all the organisms and sterilize the liquid. To be successful in this method it is necessary that the interval between the heatings should be long enough for the spores to germinate, but not long enough for the bacteria arising from them to form any more spores. Twenty-four hour intervals have been found to be the best.

*Relation to Cold.*—While heat will destroy all bacteria, cold will not do so. It is practically impossible to destroy the life of bacteria by freezing, at least with any certainty; for no matter how low the degree of temperature used, the life of some of these organisms seems to be totally resistant. Experiments have shown that bacteria cooled to the temperature of liquid air, or even liquid hydrogen, are not all killed, but after being warmed are still able to germinate. Although these extremes of temperature, do not destroy all bacteria, the simple matter of freezing and thawing will kill a great number of them. If water containing a large number of bacteria is frozen and subsequently thawed, the bacteria will be found much reduced in numbers, although they are not by any means all killed. When, therefore, water is contaminated by sewage containing typhoid bacteria, and ice is collected from it for domestic purposes, the typhoid bacteria may still be found alive in the ice. Such ice may still be a source of danger. But it must also be remembered that freezing destroys a very large proportion of these germs, so that the danger from the ice is far less than from the water before it was frozen.

*Relation to Air.*—Nearly all living organisms require air, and it was formerly supposed that nothing could live without it. Certain types of bacteria, however, are able to live without air. Indeed, some species, while they grow readily if they have no contact with air, fail to grow at all when the slightest amount of air is present, growing only in the absence of oxygen. This type of bacteria is spoken of as **anaerobic.** At the other extreme, there is a long list of bacteria which can grow only in the presence of air, failing to grow if they do not have oxygen at their command. This is the type of **aerobic** bacteria. Between the two is an intermediate group capable of growing either in the air or out of contact with it, and these are spoken of as **facultative anaerobic**.

*Relation to Moisture.*—Bacteria will grow only in the presence of considerable quantities of moisture; indeed, they demand more moisture than most organisms. Some of them will hardly grow at all unless there is 30 per cent. of moisture in the material in which they are living, and even then the growth is slow. On the other hand, they flourish most luxuriantly in localities where the water is from 90 to 100 per cent. Hence, as materials dry, bacteria will cease to grow in them, and any substance that can be dried can be thoroughly protected from their action. This explains why dried fish and dried meat, fruits, dried milk, etc., will keep indefinitely. The drying, however, does not actually kill the bacteria, for although they do not grow when the water is extracted from them, they may remain alive for weeks, months, or even years. In other words, it is impossible to depend upon drying as a means of destroying bacteria, for, while many individuals will fail to live, many others do not seem to be injured at all by the drying, and are capable of resuming life again as soon as they find moisture.

Yeasts are much like bacteria in respect to need for water, and will not grow unless the water content is high. But other fungi with which we are concerned, the molds and mushrooms, can get along upon a smaller amount of water. Substances that are too dry to putrefy may be spoiled by molding; hence it is much more difficult to preserve certain kinds of partly dry food from moulding than from decaying. Flour, in a flour barrel, may become musty from the development of molds, but it will hardly show signs of decay or putrefaction unless it becomes actually wet.

**Relation to Food.**—Bacteria and the other fungi feed upon an immense variety of foods. A few of them are able to nourish themselves upon mineral matter, and some can gain their necessary carbon from the small amounts of carbon dioxide in the air, resembling, in this respect, the green plants that are engaged in building up starch and other organic compounds. This class of bacteria is of great theoretical interest and doubtless of much importance in nature. But the vast majority of microörganisms are unable to use mineral foods, requiring, like animals, to be fed with organic food. In other words, they require for their life the same kind of foods that the animal kingdom requires. They can consume proteids, starches, sugars, fats, woody tissue, and, in short, almost anything that is found in the bodies of animals and plants. The different varieties of microörganisms do not all flourish upon the same kind of food. Some seem to be able to live upon a large variety of substances, while others demand particular foods. While almost any kind of proteids will serve for the sustenance of the common putrefactive bacteria, the tubercle bacillus does not flourish well anywhere outside the living body, and, if it is to be cultivated in the laboratory, it demands a very special kind of culture medium. But speaking in broad terms, the three classes of organisms with which we are concerned in our subject, seem to be particularly adapted to different kinds of food. Bacteria have special relations to proteid foods, like lean meat, egg albumen, gluten of wheat, etc., and if substances of this nature are consumed by microörganisms, it is commonly by bacteria. Yeasts, on the other hand, have a special fondness for sugars and, therefore, for starches, which are easily changed into sugars. The larger fungi may feed upon either proteids or sugars, but they have special relations to the woody tissues and celluloses of vegetable structures. This classification of the foods upon which these different forms subsist is by no means exact, for each group may contain members that make use of all kinds of foods; but, generally speaking, the higher molds and mushrooms attack the harder plant tissues, the yeasts attack sugars, while the bacteria are especially concerned in the destruction of proteids.

The food which bacteria consume may be either living or dead when they attack it. In the case of most bacteria the organisms are unable to feed upon the material while it is alive. If bacteria, for example, are placed upon living muscle, they are usually unable to attack it and soon die; but if they are placed upon the same muscle after it is dead, they feed upon it readily and cause it to putrefy. Those organisms which feed upon lifeless bodies include the vast majority of bacteria. There are, however, other species that are capable of living upon the bodies of animals and plants while these are still alive. Inasmuch as they can feed upon living organisms they are liable to produce disease and constitute in general the disease bacteria. Bacteria feeding upon living animals and plants are called **parasites.** Bacteria feeding upon dead animals and plants are called **saprophytes.** Both saprophytes and parasites are of great importance.

# CHAPTER II.

## FERMENTATION, PUTREFACTION, AND DECAY.

### THE NATURE OF THE ACTIVITIES OF MICRO-ORGANISMS.

Everyone at all familiar with nature must realize that there is constantly going on, in earth, water, and air, an uninterrupted series of slow changes. Rocks disintegrate; fruits decay and their juices ferment; vegetables rot; animal bodies putrefy; milk sours; cheese ripens; the soil becomes contaminated by the decaying waste of sewage and then purifies itself; streams become foul and grow clear again; even tree trunks rot and disappear. These and hosts of other kindred phenomena are matters of such every-day occurrence that we scarcely ever stop to think what they mean or how they are brought about. But it is with these phenomena that we are chiefly concerned in the study of germ life on the farm. These changes have one characteristic in common: they are all the result of chemical decomposition. Until recently it has been supposed that they are the result of purely *chemical forces*. The chemical agency of oxidation, especially the so-called slow oxidation, has been supposed to account for most of them.

But it has been proved by modern study that pure chemical forces are not able to produce these phenomena, and that many a process formerly called slow oxidation is not the result of chemical, but rather of *biological forces*. If microörganisms can be kept from them, fruits will not decay, vegetables will not rot, and many other changes will fail to appear. Most of the slow changes referred to are the result of the action of the great class of fungi, foremost among which stand the bacteria and yeasts. The reason why these organisms are so closely associated with phenomena is because they are capable of bringing about profound chemical changes.

From facts already considered it will be evident that microorganisms have properties that certainly fit them for this work. They feed, not upon minerals, as a rule, but upon the organic material in nature; and each kind of organic food, proteid, sugar, starch, wood, etc., is especially subject to the attack of one of the classes of fungi. We have seen also what inconceivable powers of multiplication are possessed by bacteria, and, while the other organisms do not grow so fast, they are all rapid growers. While they are growing and multiplying with such vigor, they are producing profound changes in the chemical nature of the food upon which they are feeding.

## CHEMICAL CHANGES PRODUCED BY MICROORGANISMS.

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 understood, and in regard to the simplest of these our knowledge of the phenomena is yet lacking in many important respects. A few only, bearing directly upon the subject of agriculture, will be explained. They may be grouped under two quite distinct heads.

**Synthetic Processes. Anabolism.**—These consist in the building of complex bodies out of simpler ones. The fundamental importance of synthetical processes to the continuance of life is evident enough. The animal kingdom, in general, demands complex compounds as foods, and cannot live upon the simple compounds found in the air and the soil, like carbonic dioxide, nitrogen, ammonia, etc. ($CO_2$, N, $NH_3$). In order that animals may use the elements existing in nature, some process must build them into complex bodies. 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 free nitrogen store in the atmosphere until it has been built up into some compound like nitric acid. The *constructive or synthetic processes* are thus of fundamental importance to the life processes of both animals and plants. Among the chemical changes which are brought about by bacteria, some are of this synthetic character.

**Analytical Processes. Decomposition: Katabolism.**—The most noticeable action of bacteria is that of decomposition. The great majority of them, just like animals, live upon complex chemical foods, and these compounds are broken to pieces by their action and reduced to simpler molecules. Acting in this way, the fungi 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 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 building of protoplasm is a synthetic process, and is, of course, characteristic of all growing 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, the decomposition activity is, in general, much greater than that of synthesis; they are destructive rather than constructive agents.

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 may be eventually eliminated from the body of the bacteria as **excretions.** Some of the new products arising in a mass of organic material undergoing decomposition by bacteria are thus of the nature of excretions (*e.g.*, *ptomaines*). In other cases the new chemical bodies are apparently produced entirely outside the body of the bacteria, and are not in any sense excreted products. In such cases the microörganisms excrete substances which act upon the outside substances, producing chemical changes in them. The substances thus produced are not excretions, but **by-products.**

## TYPES OF FERMENTATION AND DECAY.

The various processes known as fermentation, decay, putrefaction, etc., are all closely related, and, while an attempt has been made to distinguish between them, no real logical distinctions can be made. They are all *progressive chemical changes taking place under the influence of organic substances which are present in small quantity in the fermenting mass.* They are fundamentally of the nature of chemical decompositions by means of which organic substances are broken down and new substances are formed. They may best be understood by the consideration of three examples illustrating three different types:

**Alcoholic Fermentation.**—When yeast is added to a sugar solution, it grows rapidly and soon changes the sugar into carbon dioxide and alcohol. The sugar is probably taken into the body of the yeast and decomposed into these two products, which are then liberated from the yeast, the gas appearing as bubbles and the alcohol remaining in the solution. The change is sometimes expressed by the chemical equation, $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$. But this equation represents only the end-products and by no means correctly expresses the changes that occur. This fermentation occurs in malt to make beer, in apple juice to make cider, and is the basis of alcoholic industries in general. It also occurs in the raising of bread.

**The Amyolytic Fermentation.**—If a little saliva is mixed with starch and water, there begins at once a conversion of the starch into sugar, and it may continue until all the starch is thus changed. This fermentation is also expressed by a chemical equation, $C_6H_{10}O_5 + H_2O = C_6H_{12}O_6$, though the equation certainly does not represent the change that goes on. Starch is known not to be such a simple molecule as $C_6H_{10}O_5$, but some multiple of that formula, and probably a very high one. Its decomposition into sugar is really a long series of steps, only the final result being partly represented in the equation. This fermentation occurs in food after it is mixed with saliva in the mouth.

**Putrefaction and Decay.**—If any proteid body—meat, eggs, or the like—be left for some time exposed to the air, it will give off unpleasant odors, for it is undergoing putrefaction and decay. These two processes, though frequently considered the same, are slightly different. Both are the result of the chemical decomposition of organic compounds, and the terms are commonly applied only to the decomposition of material that contains proteids. Both result in chemical decompositions which are very complete, and more complex and indefinite than the other two types of fermentations. They are produced by microörganisms, chiefly bacteria, which feed upon the putrefying mass, taking certain atoms out of the organic molecules. These molecules, thus losing some of their atoms, change their chemical nature. The remaining atoms necessarily rearrange themselves to form new compounds which are simpler in structure. The distinction between putrefaction and decay consists in the fact that **decay** is the term applied to decomposition in the presence of oxygen, while **putrefaction** takes place in the absence of oxygen. The former is much more complete than the latter, resulting in the more complete destruction of the substance decomposed.

**Organized and Unorganized Ferments.**—These three examples of fermentation are very different from one another. One seems to break the sugar molecule into two simple portions, carbonic acid and alcohol; the second simply adds a molecule of water to one of starch; while the third results in a complete decomposition of a highly complex proteid into a large number of by-products, both known and unknown. But in some important respects all three agree. Each differs from ordinary chemical processes in several respects and all agree in the following points:

1. They are all closely associated with life processes; *i.e.*, are brought about directly or indirectly by living agents.

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 destroys them so completely that they are unable to renew their action even after cooling. Low temperatures simply check their activity, which they are able to renew if warmed again.

5. Their action is completely stopped by an accumulation of the products of their own activity.

If we ask what is the body producing the action (the ferment), we find that the first and last of the types described differ from the second in one radical point. Whereas the alcoholic fermentation and putrefaction are directly produced by *living germs*, either yeasts or bacteria, the amyolytic fermentation is not produced by a living organism, but by some *non-living substance* secreted from a living being. To explain this a brief account is required of the development of our knowledge of fermentations in general.

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 the fermenting of fruit juices, and the process is one of the most widely-known changes in nature. For a time it was regarded as a putrefying process, the yeasts found in the fermented material being looked upon as an impurity which was separated from the rest. The chemical nature of alcoholic fermentation was determined early in the nineteenth 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 eventually Pasteur and others proved that fermentation is a physiological process, brought about only by the growth of yeast.

For a long time there was no conception of more than one type of fermentation. But even in the days of Schwann it was recognized that there was another type of chemical changes 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 a substance called *diastase* was known which could convert starch into sugar, and in 1836 *pepsin*, causing the digestion of proteids in the stomach, was discovered. Although these processes were realized 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. Whereas alcoholic fermentations, produced by yeast, can be stopped by certain chemicals like glycerine, the other type of fermentation, due to digestive ferments, cannot be stopped by such materials. Moreover, the microscope shows that the second type of ferments does not contain any living bodies like yeast. Hence, while yeast is a living ferment, the digestive ferment cannot be regarded as living. But these latter ferments contain some substances which are very peculiar in their nature. Like living organisms, they are destroyed by high heat, and they act only at a moderate temperature. Unlike most simple chemical changes, these fermentations do not occur at high temperatures, but become impaired and stopped when the temperature rises slightly above 100° F. It has been found possible to isolate from the fermenting material (saliva, gastric juice, etc.) the fermenting body. From the digestive juices a substance can be obtained in the form of a powder which can be preserved indefinitely. It contains no living cells, is not alive, and clearly does not belong to the same class of bodies with the yeast plant. But it will cause the fermentation to take place when added to a fermentable substance.

These discoveries led to a sharp separation of ferments 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 were those which contained no organisms and were called **unorganized ferments.** These latter ferments received the name of *enzymes*, which name is now in most common use.

What are enzymes? Over this question there has been not a little discussion. But in spite of it we know very little about them. They seem to be chemical bodies, capable of producing chemical changes in certain substances. But their 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 whatever. In some respects the enzymes resemble living bodies, especially in their relation to heat, and in 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 like glycerine and alcohol, which kill the 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 opposite is true of yeast and bacteria. The distinction between these two classes of fermentations has been kept clearly in mind in the development of our knowledge of fermentation in the last half-century.

## IMPORTANCE OF FERMENTATION IN FARM LIFE.

The value of all these types of fermentation in agriculture is evident from the following list of the most important of them. In the first class may be mentioned:

The *alcoholic* fermentation; the *butyric* fermentation, which produces butyric acid in butter; the *lactic* fermentation, which often causes the souring of milk and various other products, and which is responsible for the ripening of cream; the *acetic* fermentation, which produces acetic acid and forms vinegar; the *proteolytic* or *peptonizing* fermentation, which renders soluble certain insoluble proteids, an example of which is found 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 nitrates or nitrites; the *denitrifying* fermentation, which converts nitrates into nitrites or simpler compounds by depriving them of oxygen. Then there are the phenomena of *putrefaction* and *decay*. which are endless in variety and which lie at the bottom of continued soil fertility.

There is a much longer list of the unorganized ferments, or enzymes, derived from both plants and animals. Some of the most important are the following:

*Diastase*, found in both plants and animals, which changes starch to sugar; *inulase*, which has a similar action upon inulin; *invertase*, *trehalase*, *rafinase*, *melizitase*, *lactase*, which act upon sugars, changing their chemical formulæ or, as the chemist says, inverting them. *Emulsin*, *myrosin*, *erythrozym*, *tannase*, *lotase*, and some others, which act upon chemical substances called glucosids; *pepsin*, *trypsin*, from animal digestive juices, and *galactase* from milk, together with *bromelin*, *papaïn*, and vegetable *trypsin*, from plants, which act upon proteids, causing them to change into simpler compounds. These are called *proteolytic enzymes*. *Lipase* acts upon fats, splitting their chemical molecules; *rennet* acts upon milk, causing it to curdle; *thrombase* is in the blood and is the immediate cause of blood clotting; *cytase* is an important enzyme, acting upon several parts of a plant cell and causing the cell structure to disintegrate; *pectase* causes the formation of vegetables jellies from materials in vegetable cells; *urase* brings about the ammoniacal fermentation of urea. All of the actions above mentioned are the result of chemical decomposition in the fermenting body, generally accompanied by the absorption of water. There is another class of enzymes, called *oxidases*, which cause the fermenting body to absorb oxygen. Among these are, *laccase*, an enzyme concerned in the formation of lacquer varnish from sap; *tyrosinase*, producing colors in fungi; and *oenoxydase*, an enzyme that causes certain diseases in wine. This list could be largely increased by adding other less important enzymes.

The simple enumeration of these lists is sufficient to emphasize their variety, and only a brief examination is needed to show their intimate relation to farm processes. Nearly all of those enumerated have a more or less important relation to farm life, and not a few farm products are quite dependent upon them, for example: vinegar-making, due to the action of both yeasts and bacteria, and cheese making, due to the enzyme, rennet, and likewise to bacteria. When to this list we add the many serious animal and plant diseases caused by germ life, with which the farmer is waging constant warfare, it becomes evident that agriculture and bacteriology must hereafter be closely combined.

Recognizing the great variety of these allied phenomena, it becomes a little uncertain to what the term fermentation should be applied. It originally referred to the alcoholic fermentation, but later it was applied to the changes due to enzymes, and enzymes as well as yeasts were said to be ferments. Frequently it has been applied to any type of sugar fermentation brought about by yeasts or bacteria, by which gas is produced; and when bacteriologists use the term they usually refer simply to this change in sugars. A term with so varied a meaning is of little value, and to-day there is a tendency to give up its use, except in a popular sense to cover such a general list of phenomena.

## IS THERE ANY DISTINCTION BETWEEN ORGANIZED AND UNORGANIZED FERMENTS?

The confusion is rendered still greater by the discovery of a series of facts that lead to the breaking down of the distinction between the organized ferments and the enzymes. It will be noticed that these enzymes all come from living organisms, being secreted by them; pepsin is secreted by the gastric glands, diastase by cells of the grain, etc. The larger part of the enzymes listed above are secreted by certain plants. The power to secrete enzymes is thus quite a common property of plant cells. Indeed, it is becoming evident that many so-called life processes are produced directly by enzymes secreted by animals and plants. Now, the action produced by the enzyme trypsin, secreted by the digestive glands of animals, is very similar, if not identical, with the action produced by certain of the bacteria when growing and acting upon proteid food. It is a natural question to ask if it may not be true that the bacteria secrete an enzyme similar to trypsin, and that their action upon their food is really a digestion due to the enzyme which they secrete. Are not both cases properly called digestion? If we can find such an enzyme in a solution where these bacteria have been growing for a time, it would follow that they must have secreted it and that their action upon the proteid food is due directly to the enzyme. This question will at once broaden into a second one, and we shall be forced to ask whether the action on all organized ferments may not be explained by supposing the living bacteria or yeasts to secrete an enzyme whose direct action is responsible for the fermentative change. If this be the case, the distinction between the organized and unorganized ferments disappears. The so-called organized ferments would then act in exactly the same way as the unorganized, the difference being simply that in the one case the enzyme is secreted by the active cells of larger animals and plants, and in the other by the active cells of bacteria and yeasts.

Now this conclusion is not simply a theoretical one, but it has been demonstrated to be true for at least a considerable portion of the organized fermentations. In the first place, it has been shown that the power of secreting enzymes is a common one among fungi; common molds are known to secrete enzymes of much the same nature as digestive enzymes. They soften up proteid substances, in order, apparently, that they may absorb them. In other words, they "digest" them for their own use. When, in pursuance of this idea, we study carefully the various fermentations at first regarded as belonging to the class of organized ferments, we find, in some cases, that the living bacterial cells do secrete an enzyme that actually produces the chemical change in the fermented body. For example; there is a class of bacteria that has the power of curdling milk without rendering it acid, an action very similar to that of the enzyme *rennin* (rennet), secreted by the stomach glands of calves. But since the curdling of milk by bacteria was produced by living organisms that grow and multiply during the process, it was regarded as one of the class of organized fermentations, and was so identified. But 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 entirely 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 calf. 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.

Many other examples of the same nature might be mentioned. The general processes of putrefaction and decay are produced, it is true, by the destructive agency of microörganisms, but directly, to a great extent at least, by the enzymes secreted by the bacteria. But while many of the organized fermentations are thus explained, some have not been brought so easily into this category, since it has been difficult to prove that they do really produce an enzyme. The longest known fermentation of all, the alcoholic fermentation of sugar by yeast, did not for a long time disclose any enzyme, even though careful search was made for one. But, thinking that perhaps in this case the yeast cell produced the enzyme but did not excrete it, retaining it in its own body, Buchner devised a method of crushing the yeast cell and squeezing out the inclosed juice. Upon doing this he obtained a liquid containing no living matter, but capable of producing the alcoholic fermentation in a normal manner. The liquid evidently contained an enzyme which had thus been pressed out of the yeast cell. This enzyme has been named *zymase*. It would seem from this that the yeast cell is a little chemical laboratory that manufactures an enzyme and then takes inside of itself the sugar which the enzyme ferments, after which the cell ejects the products of fermentation, alcohol, and carbon dioxid.

There are still, however, some fermentations concerning which it has been impossible as yet to prove the formation of an enzyme. The lactic acid bacteria have the power of fermenting milk-sugar and producing lactic acid from it. Careful search has been made, for an enzyme with but partial success. It is very probable that here, too, the enzyme may be produced and that it is not secreted from the bacterial cell. Should this eventually prove to be true, it would apparently reduce all types of fermentation to the one of enzyme action. This would not reduce in the slightest degree the importance of the microörganisms in the matter. It would still be the fact that this large class of chemical changes is brought about by the life activities of living organisms, but we would understand that they perform their action by first secreting enzymes and that the enzymes are the direct agents for bringing about the fermentative changes.

It is desirable to notice also that even if we accept the enzyme conception of fermentations we are no nearer a satisfactory understanding of the real nature of the phenomenon. For over fifty years science has been trying to explain these mysterious changes in fermentable bodies. At one time it was thought that they were purely chemical processes; but this has been disproved by showing that living organisms are necessary to their production. Pasteur thought they were due to "life without oxygen," claiming that the living germ required oxygen for its life, that if it did not find plenty of free oxygen, it would take atoms of this element out of the sugar molecules or other fermentable body, and that the withdrawal of this oxygen caused the molecule to fall to pieces. This theory has also been abandoned. The theory that all living fermenting agents secrete a chemical enzyme appears to stand the test of experiment, but it explains little, for we do not know what enzymes are and we have absolutely no knowledge of how they act. Are they wholly lifeless chemical bodies or are they *semiliving*, as some would say? Whatever they are, they are still as great mysteries as the fermentations they produce have always been. The real nature of the fermenting processes is, in short, quite unknown.

## FERMENTATIONS NOT ALL DUE TO MICRO-ORGANISMS.

One final question needs to be raised. Are all of the many kinds of slow progressive changes, resembling fermentations in a broad sense, due to the action of microörganisms, either by direct action or through the agency of the enzymes they produce? After we have recognized that higher plants may produce enzymes, we see at once that there may be certain fermentative processes in the soil or elsewhere, for which microörganisms are not directly responsible. If a plant produces an enzyme, this body may remain ready for action after the plant which produced it is dead, and fermentative changes may go on in a mass of vegetable tissue for which microörganisms are not responsible. 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 have all the characteristics of true fermentation. In such heaps bacteria are certainly present and the rapidly widening conception of the agency of bacteria in producing fermentations led to the conclusion that they cause all such fermentations. But the growing knowledge of the nature and abundance of enzymes is leading 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 corn during life produced enzymes, these would find their way into the silo and inevitably start fermentations which would, of course, have nothing to do with bacteria.

While, then, fermentations and putrefactions must, in general, be attributed to germ life, we must ever bear in mind that similar or identical phenomena may sometimes be caused by enzymes from a different source.

## THE PURPOSE OF FERMENTATION.

All 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 not only dependent upon fermentations for many minor processes, but it 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 of animal and plant 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. The yeast ferments sugar, and bacteria putrefy proteids for uses of their own. 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 secreting them. If a bacterium or a mold 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 the cheese-maker.

# PART II.

## BACTERIA IN SOIL AND WATER.

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## CHAPTER III.

### NATURE'S FOOD-SUPPLY. THE CARBON CYCLE.

### THE CONTINUATION OF THE FOOD-SUPPLY.

The farmer's primary occupation consists in converting soil, water, and air into human food. This he does through the agency of plants that grow in the soil and furnish the food necessary for his stock, in addition to a part of his own food. So long as plants find in the soil proper conditions for growth, the food-supply will not fail. The problem of keeping up the food-supply of plants thus becomes the one problem of supreme importance.

By far the largest part of the plant food, in weight, comes from the air in the form of carbonic dioxid and water, and these two substances are practically inexhaustible. But, in addition, some foods are obtained from the soil. These last are present in the soil in limited quantities only, and some of them are found only in the upper layers. They are constantly being used by successive generations of plants. This constant use, in the course of centuries, would have quite exhausted the soil were there not some means by which these supplies were replaced. That there is some such means is evident from the fact that plants have continued to grow on the same soil for countless generations, the soil remaining as fertile as ever. Clearly the problem for agriculturists is to find out the factors that have kept up the fertility of virgin soil and to apply them properly to cultivated soil. In this way only can the continued fertility of the soil be assured. While various agencies are concerned in this matter of soil fertility, the agency of microörganisms is certainly one of the largest.

## PLANT FOODS.

The green plants live chiefly upon the following foods:

**Water.**—This material, coming from the rains, is unlimited in amount and need not detain us.

**Carbonic Dioxid.**—This gas ($CO_2$) furnishes the carbon which is the basis of most plant structures, wood, cellulose, starch, sugar, etc. It is present in the air in small percentage only, but is kept fairly constant by processes which we shall consider.

**Nitrates.**—These, which are salts of nitric acid ($HNO_3$), constitute the chief form in which plants obtain their nitrogen. Nitrogen in considerable amount is an absolute necessity for all plant life, and while plants can probably assimilate some nitrogen from ammonia, it is certain that ordinarily they do not obtain much from this source. The higher compounds of nitrogen, like *proteids*, *ures*, or other complex bodies, cannot furnish plants with nitrogen directly, nor, on the other hand, can *nitrites* (salts of nitrous acids, $HNO_2$) or *free nitrogen* in the air supply any nitrogen directly to plants. Practically all the nitrogen must be obtained by the plants in the form of nitrates from the soil, and to keep a constant supply of nitrates in the soil must be the first aim of the farmer.

**Phosphates.**—A small amount of phosphorus is needed by plants and is obtained in the form of the soluble phosphates from the soil. The mineral soil ingredients contain much phosphorus in insoluble compounds, and agencies for rendering these soluble are necessary to soil fertility.

**Potash.**—Some form of potassium salts is necessary. These salts abound in soils, but some agency must be employed to dissolve them.

**Sulphates.**—These salts are also needed in small amounts only.

**Iron Salts.**—Needed in small quantities only.

**Lime** and **magnesia** should also be in the soil for reasons that will be given later, but they are only slightly used by most plants.

There are still other materials used by plants in very minute quantities, but they hardly fall in the scope of our study. All the foods above mentioned are commonly called **inorganic foods,** since they come chiefly from the soil and the air. **Organic foods** on the other hand refer to the more highly organized products, which are the immediate remains of living things, like roots, starches, fats, wood, cellulose and other similar bodies. Our problem, then, is to explain nature's methods of keeping the soil supplies of these various inorganic ingredients from diminishing.

## MICROÖRGANISMS IN THE SOIL.

The upper layers of the soil are exceedingly rich in bacteria, the number varying according to conditions, from a few thousands to many millions per gram. In sandy soil there may be very few, while in soil polluted with organic matter, as in the vicinity of manure heaps, there may be as many as 100,000,000 per gram or even more, 1,600,000,000 per gram having been found in some soils. They rapidly diminish in numbers, as we pass to the lower layers, and at a depth of from four to six feet, they have almost disappeared. Below this they are rarely found, except in places where drainage currents carry them downward. The microörganisms thus found in the soil include bacteria in the greatest abundance, and also quantities of the higher fungi and yeasts. Each of these classes is represented by many varieties, and each has an important share in the complex activities going on in the soil. These functions and the relation of the soil microörganisms to them may best be understood by noticing in succession their relation to the various soil ingredients that constitute plant foods.

## ORIGIN OF SOIL.

The ingredients in the soil may be divided into two classes: 1. The purely mineral matters. 2. The organic ingredients constituting the humus.

**The Mineral Ingredients.**—These come primarily from the rocks that constitute the earth's surface, soil being sometimes described as ground-up rock. The agents that cause the grinding of the rocks are physical, chemical, and biological. The *physical* agencies are chiefly those of freezing and thawing, together with the solvent action of waters. The chief *chemical* agent is direct oxidation by the oxygen of the atmosphere. The physical and chemical agents together produce what has been called the "weathering" of rocks, resulting in their crumbling into fine fragments With these we are not particularly concerned. The *biological* agencies are those of the soil microörganisms. We do not yet know very definitely how great a part they play in this process, but that it is an important part is surely proved. One of the results of their growth is the liberation of carbonic dioxid from decomposing masses. This gas is readily dissolved in the soil water, and water containing carbonic dioxid in solution is able to dissolve a considerable quantity of carbonate of lime. These carbonated waters, therefore, play a great part in the disintegration of limestone, which is one of the prominent factors concerned in the formation of soils. Again, the microörganisms which decompose organic matters in the soil produce a variety of organic acids. Among these are the *lactic*, *butyric*, and *acetic* acids, as well as many others. These acids have a solvent action upon various rock formations, and, by dissolving out certain parts of the rocks, they slowly but surely cause them to crumble. Some of these matters will be considered on later pages in other connections, but we are interested in them here as showing that bacteria are prominently concerned in the disintegrations of rocks which result in the formation of soil.

**The Humus.**—There is a vast difference in the fertility of a sand and a garden soil. Sandy soil may contain all the necessary mineral matters, but it lacks the something needed for plant growth which the garden soil contains. This something is called *humus*, an element rather difficult to define and still more difficult to describe in chemical terms. It is abundant in fertile soil, but scarce or wanting in barren soil. Though its chemical value is too complex to be stated or even known, its origin is easy to understand.

Humus is the remains of life of previous generations. When plants die, their roots, together with their leaves, branches, and fruits, inevitably become incorporated into the soil. Animals, too, leave upon the ground a quantity of excrement and other discharges; and plants likewise probably discharge excretions into the soil. When animals die their bodies also may become mixed with the earth. Thus, practically all kinds of organic matter from animals and plants are being mixed continually with mineral ingredients in the surface layers of the soil. The microörganisms in the soil feed upon these dead materials, causing an extensive series of decompositions and recombinations. To this mass of complex organic bodies undergoing decomposition in the soil has been given the name humus. It will be evident from this explanation of its origin that humus cannot have a definite composition, and that it will hardly be alike in any two soils. It will be composed of different materials to start with, and there will be a variety of different stages of decomposition. We cannot hope to find any definite composition of humus, but we can study the kinds of decomposition and recombinations that are going on in it and that result in making it a suitable food for plants. In this study we must ever keep in mind the fact that dead bodies of animals and plants are not in condition to serve another generation of plants as food. We cannot feed plants upon eggs, or urine, or starches, or sugars. Though containing carbon and nitrogen in abundance, these elements are locked up in them out of the reach of the green plants, and before they can be utilized again they must be freed from their combinations and brought into simpler forms. This is accomplished by the microörganisms in the soil. Our study of these changes may best be centered around the two chemical elements, *carbon* and *nitrogen.*

## THE TRANSFORMATION OF CARBON.

The green plants seize the carbon dioxid ($CO_2$) from the air by means of their leaves and, utilizing the energy of sunlight, build this carbon into higher compounds. *Starch* is formed first, and later other substances—*cellulose*, *wood*, *fats*, *sugar*—are built from the elements found in the starch alone; while, by combining them with some nitrogen, various *proteid* bodies are produced. When once carbon has assumed these forms it is no longer within the reach of another generation of plants. It is locked up and cannot again be utilized until it has once more been reduced to a condition of carbon dioxid.

The compounds thus built up have different destinies. Some of these are eaten by the animal kindgom and, after serving the needs of the animals, are exhaled as $CO_2$ to join the atmosphere again. A large part is not appropriated by animals, but begins at once to undergo destructive changes which bring their ingredients back again to their starting-point. Some of the processes of chemical destruction are comparatively simple. The starches, sugars, and fats are subject to chemical changes which take place 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 burned is united with oxygen and liberated in the form of $CO_2$, the hydrogen being liberated in the form of water. These join the atmosphere, while the minerals remain behind as *ash.* Thus, all forms of combustion in carbonaceous material restore some of the carbon to the atmosphere in the form of $CO_2$, and upon this the plants again feed.

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 be some other means of oxidation. A *slow oxidation* of carbonaceous material occurs in nature at all times, and ordinarily it has been attributed to direct chemical processes. It is quite doubtful, however, if 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 is the direct result of their growth. The various kinds of organisms bring about the gradual destruction of the different types of carbonaceous materials.

**Sugars.**—These are contained in fruits and some vegetables, and as they decay, the sugar commonly undergoes an alcoholic fermentation, produced by the action of yeasts and molds. The fermentation which goes on in a decaying apple is identical with that which occurs in the brewer's vat. The result is the formation of $CO_2$ and alcohol, the carbon dioxid 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 carbon of the sugars, by the agency of yeasts and molds, is restored to the air. **Starches** have nearly the same history, since they are readily converted into sugars by enzymes secreted by the plants, and are then fermented. To a certain extent bacteria also ferment sugars, producing a series of acids.

**Cellulose.**—Cellulose is a material closely related to starch, and is found in the cell walls of all plants. Wood and straw contain it in considerable quantity, while cotton and wood fibers are almost pure cellulose. Swedish filter-paper is one of the purest forms. The material is quite resistant to ordinary forms of decay and is seldom affected by common plant decay. But certain bacteria are able to act upon it so as to ferment it and set free its carbon. Several of these have been isolated and studied. Some of them act in the absence of oxygen (anaerobic), while others act only in its presence (aerobic). The former can carry on their activity in the midst of a manure heap so tightly packed as to exclude air, while the latter will occur in moistened masses of vegetable tissue exposed to the air. These cellulose-fermenting bacteria are abundant everywhere and are constantly at work in the soil, fermenting the hard cellulose parts of the great variety of plant roots, stems, and leaves that accumulate in the soil or in the waters of streams and swamps. When the mass is alkaline in reaction the cellulose may be fermented by bacteria; but when it is acid, as in sour soils, bacteria cannot grow. Under these conditions certain of the molds in the soil may ferment the cellulose. The chemical nature of the fermentation need not concern us, only so far as to notice that, as a result, the carbon is set free, either in the form of carbonic dioxid or marsh gas ($CH_4$), the latter gas becoming readily converted later into carbon dioxid. The total result is the restoration of the carbon to its original condition in the air, where it can be utilized by the next generation of plants. Certain mineral matters are also set free from the cellulose in the form of ash, which adds to the fertility of the soil.

A fermentation of cellulose is believed to occur also in the intestines of herbivorous animals. These animals utilize, to a certain extent, cellulose materials as a food; these undergo a fermentation in the intestines resulting in the formation of certain substances that are assimilated by the animals as food. Cellulose-fermenting bacteria are found in the intestines of such animals in considerable abundance, and it is thought that they play an important part in the ordinary digestion of celluloses. Whether the animal might not be able to digest them without the aid of bacteria has not yet been proved, but it is almost certain that the bacteria do, under ordinary conditions, play an important part in the process. The fermentation begun in the intestines is finally completed in the manure heap, and thus, after a time, the cellulose is completely decomposed and its carbon restored to the atmosphere.

**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 manifest at first, and the hard, woody mass is slowly but surely converted into a soft friable substance, which eventually crumbles into a brownish powder and is incorporated into the soil, contributing to the formation of the humus. This destruction of woody tissue is also brought about by microörganisms, but in this case it is not bacteria that are at first concerned.

The first phenomenon that occurs in such a decaying tree trunk is the growth of larger fungi. Various forms of mushrooms and tree fungi 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 gradually they penetrate the hard wood and, by the chemical excretions they produce, soften this hard, tough substance. Without the growth of such fungi in the wood there would seem to be no way of softening the wood sufficiently for decay. After the wood has been somewhat softened by the fungi, wood-eating ininsects begin their work upon it, using the fungi largely as food. It is probable that bacteria also may assist in this matter, but

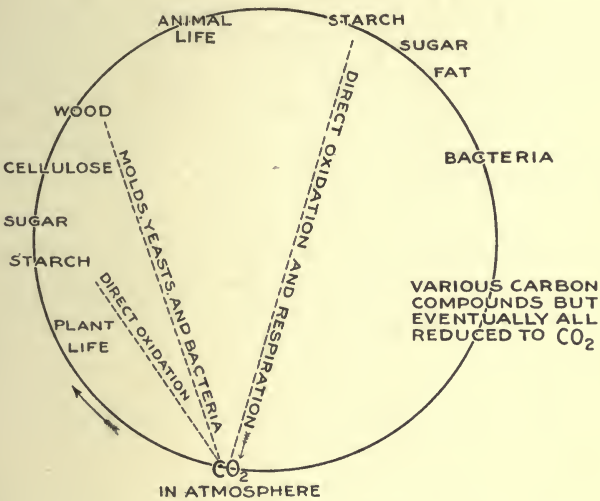


FIG. 13.—The carbon cycle.

the larger fungi are chiefly responsible for the destruction of the woody tissue.* The final result 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 into water, and in their turn enter the atmosphere as water vapor. In this way, by a slow process

*These same processes, so useful in the general changes in nature, are of decided disadvantage when they occur in timber that is desirable to be preserved. The ordinary decay of timber is brought about by the kind of fungi and bacteria above mentioned. Since none of these organisms can grow without water, it follows that well-dried wood will not decay, from which is to be drawn the lesson that the best method of preserving timber is by thorough drying.

of decomposition, wood is converted into simple chemical compounds which join nature's food-supply in the air.

By the means thus indicated the large part of the carbon extracted from the air by plants is restored again to the air in the form in which it first existed. This carbon cycle is represented graphically in the accompanying diagram (Fig. 13).

# CHAPTER IV.

## NITROGEN. DECOMPOSITION OF NITROGENOUS COMPOUNDS.

The nitrogenous foods of plants are next in importance to carbon dioxid and water. Plants cannot grow without nitrogen, and they need it in larger quantity than any other mineral foods. The nitrogenous fertilizers have commonly a more noticeable effect in stimulating crops than other minerals. The amount of material in the world that can serve directly as nitrogen food for plants is decidedly limited, and therefore it is expensive. For these as well as other reasons, the problem of continued soil fertility is more closely bound up with the matter of nitrogen than any other chemical element.

### SOURCES OF NIROGENOUS FOOD.

Plants take their nitrogen from the soil, chiefly in the form of nitrates. While it is true that they can utilize ammonium salts also, under ordinary conditions ammonia furnishes little food directly to the plant, the far larger part being furnished by soil nitrates. The amount of nitrate in any soil is however, very limited, there being only from 0.1 per cent. to 0.2 per cent. in ordinary soils. As crop after crop is grown, the small amount in the soil is gradually used up and must be replaced if the soil is to continue yielding crops. The farmer buys nitrates in the form of commercial fertilizers to replace the amount taken from his soils by his crops. These commercial nitrates, however, are also limited in amount. They are confined to a few deposits of nitrates, chiefly in warm dry regions. The best known come from Chili, whose nitrate mines to-day furnish the greater part of the nitrates for the world. But these beds do not solve the question of continued soil fertility, for they will soon be exhausted and some other source of nitrates must be discovered. Fortunately, we have learned that there are processes in nature by which the soil nitrates are replaced, quite independently of the store of nitrates in Chili or elsewhere, and this replacement is a phenomenon of the life activities of the bacteria and other microörganisms of the soil.

If the nitrates absorbed from the soil are replaced, it must be from one of two sources or from both.

1. *From the Nitrogen in Organic Bodies.*—After plants absorb nitrates from the soil, they build them up into organic compounds, chiefly *proteids*, which subsequently may or may not be utilized as food by animals. Whatever be its history, whether in animals or plants, this proteid material always contains nitrogen; and eventually, by processes which we shall study, this nitrogen may again assume the form of nitrates and restock the soil.

2. *The Nitrogen of the Atmosphere.*—This is an inexhaustible source of supply, if it can be utilized. We shall learn that there are means by which it becomes available for plants.

There seem to be no other possible sources for replacing soil nitrates, and we will therefore consider these two in detail.

## ORGANIC NITROGEN. ITS NATURE.

The nitrates are built up by the plants into a variety of compounds, mostly of the nature of **proteids,** like *gluten* of wheat, *legumen* of peas, and other similar bodies. All plants contain some such compounds which serve a purpose in the life of the plant. While the plant is still alive they remain as proteids without much change. After the death of the plant that produced them the proteids are at the disposal of nature's forces of destruction. Some of the proteids are seized by animals and utilized for their life processes. They are slightly changed inside the animal's body, but are not built into bodies more complex than proteids. The animal forms animal proteids out of them, producing *myosin*, *gelatin*, *chrondin*, and other compounds, none of which are likely to be more complicated than the original proteid of the food. Thus, in the bodies of plants and animals alike the nitrogen reaches a condition allied to proteid. But, while proteids may serve as food for animals and for the great class of colorless plants (fungi) they are quite out of the reach of the green plants, which are the great food producers of nature. Our next problem, then, must be to learn how these proteids are reduced to their original condition of nitrate.

Part of the proteid thus built up into the body of the plant or the animal remains there until the animal or plant dies, and at death it is still a proteid and as complex as ever. In this form it may become incorporated into the soil when the animal or plant dies, or it may become eaten as food and pass through the body of another animal. But much of it will eventually reach the soil while still in the form of proteid.

A second portion of the proteid is used up in the animal's body to furnish energy and heat; it is *metabolized*, as we say. When it is thus used its complex chemical molecule is broken to pieces, and it is reduced to much simpler compounds. But it is 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 $CO_2$. The molecule falls to pieces and various simpler by-products arise; but in the animal's body, practically all of it eventually assumes the form of *urea* ($CON_2H_4$). Though this urea is a nitrogen molecule far simpler than proteid, still it is not simple enough for a plant food. 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. 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. In some animals this urea is secreted as urine by the kidneys, but in others (birds) it is mixed with the *feces;* in all cases it contains the nitrogen which is no longer of any use to the animal world. 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, for all animals, large and small, secrete it or an allied substance, and the total is enormous. What becomes of it all?

Thus the nitrogen of the nitrate absorbed by the plant has reached two quite different conditions. Part of it is still in the highly complex form of proteid, either in the dead body of the animal or the plant. A second part has been partly broken down in its passage through the animal's body, and has reached the condition of urea or some allied body. But in neither condition is it within reach of another generation of green plants. It must be still further broken down before it is available for plants.

## ORGANIC NITROGEN. ITS DECOMPOSITION.

**Decomposition in General.**—This means the breaking to pieces of complex compounds so as to form simpler ones. The term thus defined is a very broad one, and covers a long series of changes, of a purely chemical nature. But more commonly the term has a narrower meaning, and refers to the breaking down of organic products under the influence of microörganisms. This is one of the most important functions of 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; another portion is broken down by yeasts; another by molds and other fungi. But decomposition is chiefly due to a class of bacteria called the **decomposition bacteria.**

But even as thus limited, this term is still a broad one including different species of bacteria and various types of decomposition. Two types are generally recognized, under the names of **decay** and **putrefaction.** These two terms are frequently not very clearly distinguished, being 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.

**Putrefaction.**—This is the name given to a partial decomposition that is far from complete. It is generally produced by bacteria growing in the absence of oxygen, and hence by the *anaerobic* or *facultative anaerobic* bacteria. These break down the proteids, but do not carry the decomposition to its final stages, the final product, thus formed, being still quite complex. Many of them have unpleasant odors and many of them are poisonous.

**Decay.**—This is the type of complete decomposition that takes place in the presence of oxygen. It is produced by *aerobic bacteria*, and results in a very complete disintegration of the decomposing body. The end-products are much simpler than in the case of putrefaction, and the gaseous products arising have little or no odor. $CO_2$,N and $H_2O$ are among these final products, and are all odorless. Putrefaction and decay cannot be sharply separated from each other, the former being in many cases only a step toward the latter. The bad-smelling or poisonous products of putrefaction will, if exposed to the air, undergo further disintegration until the decay is complete. But, though not sharply distinct, the difference above noted is a convenient method of designating the complete decomposition, in the presence of air, from the incomplete decomposition in the absence of air.

Of the many species of bacteria associated with putrefaction and decay, some are likely to be found under one set of conditions and others under different conditions. Some are particularly common in decaying vegetable substances and others in decaying animal tissues, while some are most characteristic in fermenting urea. No attempt need be made here to classify this miscellaneous host of putrefactive organisms. They include *cocci*, *bacilli*, and *spiral* forms as well as *yeasts* and higher *fungi* (Figs. 14, 15). Some of them produce their fermentation only when oxygen is present, while others do so in the absence of oxygen, and 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. These decomposition bacteria occur practically everywhere in nature—in the air, in all bodies of water, and in extreme abundance in the soil. They are so widely distributed and so abundant that they are sure 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, 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 microörganisms, and, as a consequence, they multiply rapidly within the decaying substances, developing vigorously for a time. After they have used up the food, their growth is checked and some of them remain ready to grow again when more organic matter comes within their

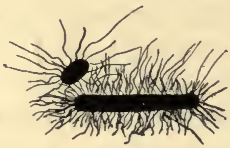

FIG. 14.—*Proteus vulgaris*, a common bacterium of decomposition.

FIG. 15.—Common decomposition bacteria. *B. fluorescens* and *B. subtilis.*

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 ehemists understand them, for they involve problems in physiological and organic chemistry yet unsolved. We know that 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 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 have not 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 putrefying 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 that the end-products are 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 dissolved in the course of time by the rain which falls upon the decaying mass and pass into the soil, perhaps to be drained away in the drainage-water. The insoluble bodies are also 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. A few of these are as follows: Carbon dioxid, hydrogen sulphid, marsh gas, hydrogen, nitrogen, calcium carbonate, propionic acid, valerianic acid, acetic and lactic acids, alcohol, succinic acid, phenol, indol, leucin, tyrosin, skatol, etc.

This list is far from complete. It includes only a few of 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.**—One phase of these decomposition processes must be especially mentioned. After passing through an unknown series of intermediate stages, the nitrogen of the decaying mass assumes, in large part, the condition of *ammonia*. One of the first and easiest substances to undergo this ammoniacal fermentation is urea. Urine is always filled with bacteria, even while in the ducts from the bladder, and among them are several species that cause it to break down to form ammonia

(Fig. 16). Most of these bacteria produce this action through an enzyme that they secrete, named *urase*. Under proper conditions, as much as 97 per cent. of the nitrogen in the urea is converted into ammonia in a space of four days. The ammonia is a volatile product and has, consequently, a tendency to pass off into the air, as may readily be recognized from the odor of ammonia that is frequently perceived around a manure pile. This represents a permanent loss of nitrogen, and should be avoided as much as possible.

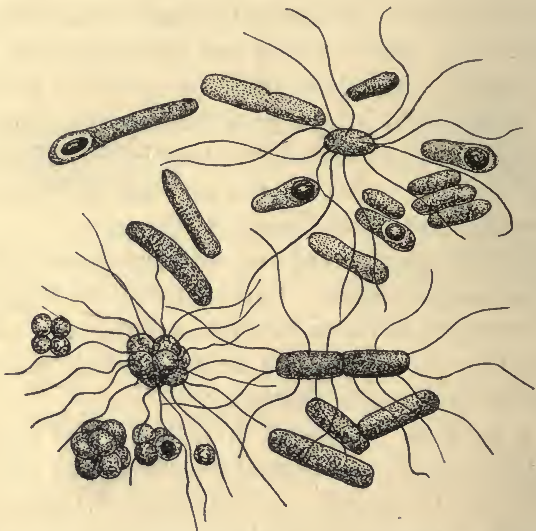

FIG. 16.—Various bacteria causing the ammoniacal fermentation of urea (*Beijerinck*).

The loss is greater when the liquid is concentrated, and consequently less if the urine can be poured upon the soil at once, than if stored in vats or even mixed with solid manure.

Although urea shows the ammoniacal fermentation most readily, other nitrogenous bodies, like proteids, etc., also may give rise to ammonia (Fig. 17), which is, indeed, one of the common end-products of proteid decomposition. The chemical changes that occur in proteid decomposition are complex and not wholly understood. The first step seems to be quite like that taken when they are digested in the digestive tract of animals, for, under the action of the *peptonizing* bacteria, they are converted into *peptone*-like bodies which are simpler than ordinary proteids. These are further reduced into *amido acids*, and the latter finally converted into ammonia, which is quite likely to unite at once with carbonic dioxid, that is also being liberated, to form carbonate of ammonia ($(NH_4)_2CO_3$). The condition in which the nitrogen actually exists in the humus has been a matter of considerable dispute. Some have thought that it remains in the form of proteid in large part, others have concluded that the humus nitrogen is chiefly in the form of amido acids. But it is evident that there is long series of stages between the proteid and the final ammonia compound, and that the nitrogen in any lot of soil may be in any one of these stages. In whatever form it exists in the humus, a certain portion of it is being constantly reduced to the form of ammonia. This portion alone is leading toward a condition where it can again be utilized by plants. The rest, whether in the form of proteid or otherwise, is, for the present, locked up out of the reach of plant life. The humus may thus contain a large amount of nitrogen and still have little of it *available; i.e.*, within the reach of plants.

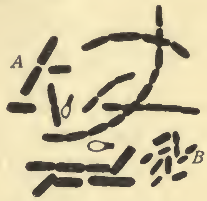

FIG. 17.—Bacteria producing ammoniacal fermentation. *A*, *B*, *mycoides; B*, *B. stutzeri.*

**Self-purification of the Soil.**—The universal occurrence of such a decomposition of organic bodies is no new discovery. It has long been known and its extreme significance is now recognized, since it is the first step necessary to bring the nitrogen locked up in the proteid back again within reach of plants. But its value in producing what has been called the self-purification of the soil, has been only recently appreciated. As we have seen, the final end-products are largely gaseous ($NH_3$,$CO_2$,N, etc.), and these will tend to pass off from the soil into the air. 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, 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 agriculture.

All the types of decomposition which we have mentioned take place in the humus of the soil; sugars, starches, cellulose, woody tissues, proteids, and all other kinds of organic bodies are attacked by microörganisms and eventually thoroughly decomposed. Since this decomposition is the first step in the conversion of the products of one generation of living things toward the condition in which they can again be used, the conditions of the soil should be such as to favor such decomposition. The thorough decay is possible only in the presence of oxygen, and hence a *cultivation* of the soil facilitates decomposition. Hard packed soils are inferior to looser soils for this reason. The presence of large amounts of carbohydrates, sugars, starches, straw, etc., is apt to give rise to acids, and soils containing them may become *sour*. In such soil the nitrogenous decomposition is checked, since decomposition bacteria cannot stand much acid. It is evident, therefore, that in sour soils the addition of lime to neutralize the acid will make it possible for the bacteria to carry on an active decomposition that will soon place its food materials once more within the reach of plant life. The more vigorous the decomposition changes, roughly speaking, the higher the fertility of the soil. Black marsh soil shows the highest amount of decomposition; clay shows less, as a rule, and sandy soil the least. The number of bacteria in any soil is directly proportional to the activity of the decomposition changes going on within it.

# CHAPTER V.

## NITRIFICATION AND DENITRIFICATION.

### NITRIFICATION.

The pulling of the organic nitrogen compounds to pieces does not in itself bring the nitrogen into the best available condition for plants. It is in the form of nitrates that plants most readily absorb nitrogen, and at the end of the decompositions noticed ammonia compounds are formed, but no nitrates. Plants may be able to absorb nitrogen in the form of ammonia salts, but this occurs only to a slight extent, and by far the largest amount is assimilated in the form of nitrates. Consequently, if these decomposition products are to be utilized by plants, they need to be changed from ammonia salts into nitrates. This process has been called **nitrification.**

Nitrification is a process of oxidation. In the oxidation of ammonia compounds to form nitrates there are two separate stages. The first is one by which the ammonia is oxidized into a *nitrite.* A nitrite is a salt of nitrous acid ($HNO_2$), and it contains less oxygen than a nitrate. Nitrites are not plant foods, for, as far as known, ordinary plants never absorb nitrogen in this form. The second change is the addition of another atom of nitrogen to the nitrite, giving a *nitrate* or salt of nitric acid ($HNO_3$), the form in which the nitrogen is most completely available for plants.

Nitrates are really of very great significance in nature. They are readily *soluble* in water, so that they are easily taken up by the soil and absorbed by the roots; thus nitrates feed the whole world of green plants. In addition to this, nitrates form the basis of most explosives. Gunpowder has saltpeter as its basis, and saltpeter is nitrate of potash. Nitroglycerin, too, is made from nitric acid, and practically all the other commonly used explosives are produced from nitrates. Nitrate formation is, then, a matter of the greatest significance. While there are, perhaps, some other methods by which nitric acid can be formed, beyond doubt the nitrate store in the soil has been formed chiefly through the process of nitrification.

**Nitrification in the Soil.**—It is very easy to demonstrate that such nitrification actually takes place in ordinary soil. If we place a quantity of soil in a proper vessel and subject it at intervals to a chemical analysis, it will be found that there is an increase in the amount of nitrates present, after it has remained undisturbed for a few weeks. This fact has been known for nearly a century. The next step in the discoveries was made in 1877, when it was demonstrated that this nitrification is associated with the presence of living matter in the soil. This can be proved by placing two lots of the same soil under such conditions that in the one phenomena of life 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 will show 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.

**Isolation of the Nitrifying Organisms.**—Such experiments repeated many times and verified by numerous observers demonstrated that nitrification is the result of a living process. Inasmuch as such soil contains no plants large enough to be seen, it follows 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. The number of bacteria in the soil is large and 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, that of deoxidizing nitrates, but none of them raised the nitrites into a state of nitric acid. 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 are isolated from such soil by ordinary methods showed no power of nitrification. Evidently the nitrifying bacteria cannot be found by the ordinary bacteriological methods.

The cause of the trouble as well as the secret of successful study was soon learned. In bacteriological studies the common method of isolating bacteria is to get them to grow in culture media made by the bacteriologist. The media commonly used contain a certain amount of organic compounds which serve as food for the bacteria. But experiment soon showed that the presence of the smallest amount of organic matter is directly injurious to the nitrifying bacteria, so that they will not grow at all in ordinary culture media. It was necessary to devise some culture media that contained no organic matter, and as soon as this was done it was possible to isolate from the soil bacteria having the power, under proper conditions, of oxidizing ammonium and nitrite compounds into nitrates. 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, and further study showed 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*. The two steps are not only independent, but they are brought about by two different species of bacteria. One organism has the power of producing nitrite out of ammonia, but can carry the oxidation no farther, failing to produce nitrates. The second species can act upon the nitrites, carrying their oxidation up to the form of nitrates, but it has no power to act upon ammonia. The two together can produce the complete nitrification of both ammonium and nitrite compounds.

**The Nitrifying Bacteria.**—It thus appears that there are two types of nitrifying bacteria. The first converts ammonium compounds into nitrites, and, hence, are called the *nitrite* bacteria (Fig. 18). They have been found in soils of very widely separate localities, and probably live in all soils. Two slightly different varieties have been recognized, both spherical bacteria, and named *Nitrosomonas* and *Nitrosococcus*, names that will probably soon go out of use. They appear to be able to form nitrites from almost any kind of ammonium salt, and, since they are quite universally distributed in all decaying organic matter, as well as in all humus, they will evidently seize the ammonium compounds produced by ammoniacal decomposition, and convert them into nitrites (Fig. 18, A). They are incapable, however, of forming nitrates from any nitrogen compound except ammonium salts, and hence the proteid compounds of decaying bodies cannot be nitrified till they are reduced to the form of ammonia. The second type of nitrifying bacteria is called the *nitrate* bacteria, since they oxidize the nitrites into nitrates. Only a single type of this class has been found, and it was named *Nitrobacter* (Fig. 18, B and C). It is smaller than most nitrite organisms and of a slightly elongated shape. It is also widely distributed, probably in all soils, and is able to convert any kind of nitrite into nitrate. It cannot, however, act upon any nitrogen compounds except nitrites, and hence its action must be preceded by that of the nitrite bacteria.

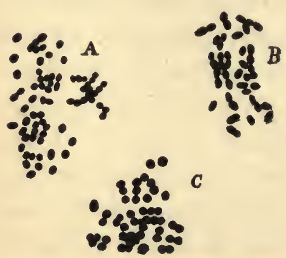

FIG. 18.—Nitrifying bacteria. *A* is a nitrous and *B* and *C* nitric bacteria.

In ordinary soil these two kinds of nitrifiers act together and simultaneously. So closely connected is their action that it is difficult to find any traces of nitrites in the soil, since they are converted into nitrates as rapidly as they are formed. The whole nitrification may be very rapid. If ammonium salts are added to soil, they cannot commonly be found in the drainage-water from the soil, since the nitrification progresses so rapidly that they become completely converted into nitrates before draining away. But though occurring simultaneously, the two steps in the nitrification appear to be distinct and produced by distinct organisms. It has been claimed recently that there are other classes of soil organisms that take these two steps in one, converting ammonia and even organic matter directly into nitrates. If this be true, they represent distinct classes of nitrifiers, but the observations have not yet been sufficiently verified.

## CONDITIONS OF LIFE OF NITRIFYING ORGANISMS.

The importance of the phenomenon of nitrification makes it very desirable to understand thoroughly the conditions under which it may best occur, and, consequently, the means for stimulating or hindering it. The conditions regulating the life of these nitrifiers are, in some respects, peculiar.

**Organic Food.**—In respect to food these nitrifiers are among the most remarkable of all organisms. Not only do they need no organic food, but the presence of organic matter in the solutions, even in small quantity, is directly injurious. The bacteria will grow readily in mineral solutions, but if a small quantity of organic matter is added, the growth stops. In ordinary laboratory solutions a very small amount of organic matter acts like an antiseptic. In the soil, however, the nitrifiers behave differently, and are not checked in their growth by such small quantities of organic matter as serve to check them in laboratory solutions. This injurious action of organic matter is a curious phenomenon. The more highly organized the compound, the more decided its checking action, and thus, the more valuable the material for ordinary kinds of bacteria, the greater its injury upon the nitric bacteria. These bacteria thus grow under conditions detrimental to other bacteria, but 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. Evidently they must obtain their energy from some other source than that which is commonly utilized by animals and plants. That they have a source of energy at command is evident from the fact that they can assimilate $CO_2$ and build it into their own tissues, a process that requires energy. The present belief is that they obtain their energy from the oxidation of the ammonia compounds, a process that apparently can furnish them with all they need. But whatever its source, these nitrifiers are able to live under conditions in which other organisms cannot exist.

Since the nitrifiers are injured by organic matter, it follows that nitrification cannot be expected in highly concentrated decomposing masses. Raw sewage contains so much high organic matter that nitrification does not take place in it, and if it is applied directly to the soil, in considerable quantity, it will effectually prevent the nitrification necessary to render the nitrogen available to plants. In the manure heap, too, nitrification cannot be expected so long as the quantity of organic matter is high.

As the manure rots, however, the organic nitrogens are reduced, until finally nitrification can begin. We have seen that decomposition gives rise to ammonia, usually combining with carbonic acid to form ammonium carbonate. This compound is also injurious to the nitrifiers and, if it becomes too abundant, will stop nitrification until, either by vaporization or by denitrification, or otherwise, it is reduced to an amount not deleterious to nitrification, when the process begins. This checking of the action of nitrifiers by organic matter or ammonia, certainly occurs in laboratory solutions and concentrated compost heaps, but it does not appear to be of much significance in ordinary soil. Nitrification takes place more vigorously in soil than in solutions, and proper testing has shown that the organic matter in ordinary soil does not prevent a vigorous nitrification.

**Moisture.**—The nitrifiers require a moderate amount of moisture. Too dry a soil will not allow of their growth. But, on the other hand, too much moisture is equally detrimental. It has just been stated that they do not grow so readily in laboratory solutions as in soil. It is also a fact that in very wet soil, "water-logged," nitrification is greatly reduced or lacking.

**Reaction.**—The nitrifiers cannot develop in an acid medium and are usually absent from acid soils. Soils may become acid from various causes, one of the chief of which is the production of certain organic acids (*lactic*, *succinic*, *acetic*, *butyric*, etc.), from the bacterial decomposition of carbohydrate material. Large amounts of sugar might give rise to an acid condition, but the more common cause is the decomposition of cellulose and woody substances. In forest land the decay of leaves and branches, as well as other vegetable structures containing cellulose material, usually fills the soil with these acids and, as a result, nitrification has practically ceased in forest soils. The same is true in some open pastures and other soils where such decay is extensive. The value of liming such soils is evident. Lime neutralizes the acids and restores the alkaline condition necessary for the nitrifiers, so that they may resume the activity stopped by the acid. Too much lime, however, defeats its end by making the soil too alkaline.

**Humus.**—That nitrification may take place, it is of course necessary that there be plenty of nitrogenous material to be nitrified. This must be in the form of an ammonium salt which, as we have seen, is the condition reached by the organic nitrogen at the end of its decomposition. The ordinary humus will therefore furnish plenty, but soil deficient in humus will show but little nitrification.

**Temperature.**—Nitrification occurs in the soil under a very wide range of temperature. It goes on at temperatures fully as low as 37° F.; it is most vigorous at about 99°, becomes manifestly checked at 110°, and almost ceases at 122°. From these facts it will be seen that it may continue in the fall until the appearance of frosts and, in many localities where the winter is not too cold, will go on all winter long. For this reason a cultivation of soil in the fall is undesirable, since cultivation, by mixing air with soil, hastens nitrification, and during the winter or late fall there is no growing crop to utilize the nitrates as they are formed. These, therefore, drain away from the soil during the spring and winter, leaving it poorer in the spring than if the cultivation had not taken place. Nitrification is the most vigorous in the summer months, during which season the growing crops are in best condition for absorbing it. This is one of the reasons why a wheat crop is so exhausting to the soil. It grows during the fall and spring, but the ground lies idle in the summer and hence during the season of greatest formation of nitrate, there is no crop growing to prevent the loss by drainage.

**Air.**—Nitrification is a process of oxidation and therefore requires oxygen. The more thoroughly the air is mixed with the soil the more vigorous will be the nitrification. This process, therefore, is more pronounced in sandy loams or mixtures of clay and sand than it is in heavy clay soils. In heavy soils, where the earth particles are very fine, the soils are too poorly aerated to enable the nitrifiers to get a sufficiency of oxygen. From this we learn the very practical lesson that cultivation of the soil stimulates nitrification. Experience and theory both tell that the loosening up of soil during the growth of plants greatly stimulates plant growth, and the primary reason is evidently because this furnishes the necessary oxygen for a vigorous nitrification, thus furnishing the crops with a larger supply of the easily assimilated nitrates. In this fact, too, we find an explanation of the fact that only the upper layers of the soil are fertile since nitrification will go on only in the layers where oxygen readily penetrates. About 65 per cent. of the total nitrification occurs in the upper twelve inches of soil, 30 per cent. more in the layers from twelve to thirty-six inches lower, and little or none below this. Surface soils alone are thus highly fertile.

## EXTENT OF NITRIFICATION.

The production of nitrates in ordinary soil is very vigorous. While in some soils it does not occur at all, from a lack of some of the conditions already mentioned, in other soils nitrates are rapidly formed. In fact, a much larger amount of nitrates is produced in a cultivated soil, ordinarily, than is used by the crops. In some careful tests it has been shown that twice as much nitrate is formed as is used by the crop, the rest being lost to the soil by drainage. This is particularly true when wheat is grown, wheat being an especially exhausting crop. To furnish this amount of nitrate a proper amount of organic nitrogen must be added in the form of manure or otherwise. With plenty of such material as a source, nitrification is very vigorous during all seasons, except when the soil is actually frozen.

**The Unlocking of Soil Nitrogen.**—It happens not infrequently that soil may contain large amounts of nitrogen and yet fail to produce good crops, the plants seeming to be insufficiently supplied with nitrogen in spite of its abundance. These barren soils will not yield good crops unless supplied with a considerable amount of nitrogen as a fertilizer. 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 a considerable quantity of nitrogen. In such a soil the nitrogen is simply locked up in the humus in a form useless to plants. At the end of decomposition, a large part of the nitrogen may be held in a form not available for ordinary vegetation, so that plants growing in such soil will be nitrogen-starved, although growing in the midst of plenty of nitrogen compounds. Such soils might become highly fertile if some agency for unlocking these nitrogenous compounds could free the nitrogen from its stable relations, thus producing compounds of a nature to be assimilated by plants. A nitrification is evidently what is needed to make these soils productive. If a comparatively small amount of manure is added to such soils the results are sometimes surprising in causing an increased fertility far beyond that which might be expected from the small amount of manure itself.

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 there 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 afterward 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 by this excrement the nitrifying bacteria are stimulated, 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. That this is the whole explanation in these cases is by no means sure, but it is certain that the nitrifiers do unlock much nitrogen previously not in an available condition.

## DENITRIFICATION.

There is another group of microörganisms in soil and other decaying masses acting in exactly the reverse direction from the nitrifiers. Whereas nitrification oxidizes ammonia compounds and nitrites, to form nitrates, denitrification takes the oxygen out of nitrates, reducing them to nitrites and ammonia, and may even reduce these to free nitrogen. Nitrification prepares plant foods, but denitrification destroys them. The one process is useful, the the other detrimental, to soils

Three different types of reduction of nitrogen compounds are comprised under this head: 1. The reduction of *nitrates* into *nitrites*. 2. The reduction of *nitrates* to give off *free nitrogen*. 3. The reduction of *nitrites* into *free nitrogen*. The term denitrification is sometimes used to cover all of these types of reduction, and sometimes more particularly to refer only to the reduction of the nitrates and nitrites, so as to liberate *free nitrogen*. In its strict use it should be confined to the latter process.

It is evident that these different types of reduction will have different effects upon soil fertility. Those portions of the nitrogen that are reduced to nitrates or ammonia, under proper conditions may be built up again into nitrates by the nitrifying bacteria. But those that are reduced to a condition of free nitrogen pass off into the air and out of the reach of plant life. This nitrogen, therefore, represents an actual loss to the soil. Denitrification is a process very different from the general type of decomposition which we have described. Decomposition begins with proteids and reduces them to ammonia compounds. Denitrification begins with nitrates and nitrites, and liberates free nitrogen.

**The Denitrifying Bacteria.**—Denitrification is the result of the action of a class of bacteria known as the *denitrifiers*. Very many bacteria have the power of extracting the oxygen from nitrates, reducing them to nitrites, but the list of those that can liberate free nitrogen is shorter. Some of them act in aerobic conditions, and others in anaerobic conditions. The names B. *denitrificans* I and II have been given to two of them, but others have been found with similar properties. They are very widely distributed; they are found not only in soil and water, but in the air and all organic decomposing refuse. They are very abundant in the manure heap, especially if it contains much hay and straw, and they are likely to cause a considerable loss of nitrogenous matter by liberating the nitrogen as free nitrogen gas. Excrement always contains them, but they are more abundant in the excrement of herbivorous animals than of carnivorous animals. These bacteria, in order to grow vigorously, require some carbon-holding food, and they cause the largest amount of denitrification when abundantly supplied with carbohydrates. Sugars, starches, glycerin, or organic acids may furnish this needed carbon, and the cellulose present in hay or straw will also furnish it. Any form of decaying matter that contains great amounts of hay or stubble is especially subject to denitrification. Horse manure, containing as it does large amounts of hay, shows greater losses of nitrogen than the manure of cattle, which contains less carbonaceous material.

The extent of the actual losses caused by these denitrifiers in ordinary farm processes is not fully known. It is certain that in concentrated decomposing solutions the action is vigorous, but it is not so great in less concentrated masses. In the manure heap there is always some loss in this way, and when great quantities of manure are spread over a plot of cultivated ground, denitrification doubtless causes considerable loss. When, however, the manure is applied in limited quantity, so that it is mixed with a considerable amount of soil, the evidence seems to show that the losses are slight if any. In ordinary soil, therefore, denitrification is not a phenomenon of much significance. In concentrated manures, however, especially if they contain much hay, it may be great. One very important lesson is to be drawn from these facts. *Nitrates should never be mixed with manure.* The nitrates will simply be thrown away, since the denitrification in the manure heap will surely reduce most of the nitrate to free nitrogen, thus causing its complete loss. Further, the denitrification is greatest in fresh, concentrated manure, while it diminishes greatly in manure after it has partly decayed. The denitrifiers do not find the partly decomposed organic substance favorable to their life, and do not flourish. Hence, the use of large amounts of partly rotted manure upon a soil is possible without bringing about a nitrogenous loss, while the use of the same amount of fresh manure would be undesirable.

# CHAPTER VI.

## THE MANURE HEAP AND SEWAGE.

### CONTENTS OF THE MANURE HEAP.

The value of the manure heap is recognized by every farmer. So thoroughly is this appreciated that, in some countries, the wealth of the 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, and the extent of his intelligence may likewise be gauged by the care he bestows upon it. We can readily understand its importance when we remember that in this manure heap are going on, in a condensed space, exactly the transformations of food material which we have been considering.

The manure heap is always an extremely complex mixture of organic substances, of nearly every conceivable kind. It contains great quantities of partly broken-down *vegetable tissues*, which have passed through the alimentary canal of the cattle, partly digested. 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 may 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 *proteids*, *albuminoids*, *gelatins*, *fats*, *sugars*, *starches*, and, indeed, nearly all types of organic matter produced by animals or plants, all of which will be in various stages of digestion and decomposition. Lastly, and perhaps most important, it will contain much nitrogen in the form of *urea*, in the liquid manure, which represents the result of the nitrogenous metabolism of animal life. This liquid manure is by far the most valuable part of the manure, since it contains the nitrogen which has been actually metabolized by animals, and which can now be brought back readily into a condition available for plant life. The liquid manure contains three-fourths of the total nitrogen of the whole heap, and four-fifths of the total potash. But farmers frequently fail to realize this, and allow this material to waste by soaking into the ground. In addition to these ingredients manure always contains a large amount of water and an unknown number of species and varieties of bacteria in very great abundance.

In this manure the bacteria find plenty of food and moisture and their growth is rapid. There is a great struggle for existence among them and, in the weeks of fermentation, first one and then another species may gain 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. At first the ordinary *intestinal bacteria* abound; later the *putrefactive bacteria* become most abundant, and finally the *denitrifying* and *nitrifying bacteria* are in the majority. 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.

**Losses from the Manure Pile.**—The result of this bacteria growth is an extensive and profound series of chemical changes by which the manure is profoundly modified. These are partly useful and partly injurious, but, taken as a whole, they are necessary. Most of the material in the manure is in a form not capable of being used by plants, and must be greatly transformed before it is available for vegetation. The transformations are much the same as those we have already considered in the soil, but they take place under different conditions, which somewhat modify them. In our study of the subject it should be borne in mind that the most important feature of manuring is the furnishing of nitrogen to the crops, and the first care should be to protect this material and avoid its loss.

The losses from manure are due to two causes. 1. *Leaching*. A considerable portion of the nitrogen is in a soluble form, including all of that in the liquid manure. From manure heaped upon soft ground, large amounts of this are completely lost by draining away or soaking into the ground. If the manure is left exposed to rains this loss is greatly increased. As a result the ordinary manure heap decreases very much in value during the weeks or months that it is stored in the pile. This part of the loss can be entirely prevented by storing the manure where the liquids will not leach into the soil. 2. *By fermentation.* This subject requires a more extended consideration.

## THE FERMENTATIONS OF MANURE.

**Destructive.**—The first chemical changes which go on are those of general decomposition. An ammoniacal fermentation is universal. The liquid manure is most rapidly decomposed by this fermentation, the substance undergoing in a very few days, sometimes in a few hours, a reduction into ammonia compounds, as already mentioned above. This is completed before the ammoniacal fermentation of the other nitrogen bodies has fairly begun, and suggests that the proper method of handling manure will be to treat the liquid manure separately from the solid portion. Eventually the nitrogenous compounds in the solid manure will also undergo ammoniacal fermentation. The starches, sugars, cellulose and woody tissue undergo a decomposition by which $CO_2$ is set free and various other substances are left. The fats and fatty acids are also decomposed, liberating $CO_2$ with other less known bodies. The decomposition of the proteids liberates sulphur, commonly as $H_2S$, and this may unite with water to form sulphuric acid. The sulphuric acid may combine with the ammonia to form ammonium sulphate, or the ammonia may combine 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 is a misfortune, since it is the nitrogen that the farmer desires to keep.

This loss occurs in several ways. 1. *Liberation of ammonia.* Since the ammonia resulting from decomposition is a gas, it will, to a considerable extent, dissipate itself at once into the atmosphere. Such portions of it as unite with carbon dioxid to form ammonium carbonate are less volatile, but this, too, is partly volatilized. The odor of ammonia common around a manure heap plainly demonstrates this loss. 2. *Denitrification.* If by this term we refer only to the reduction of nitrates so as to set nitrogen free, the process is not very important in a manure heap, since there is present only a little nitrate, none, indeed, at first, when the fermentations are greatest. If nitrates are present, denitrification will cause a loss, and in the later stages of the rotting of manure, after nitrates are formed, this loss might be considerable. But it seems that loss from this cause is not so great as was formerly supposed. 3. *Destruction of ammonia.* There seems to be a direct "burning" of ammonia compounds in the manure heap by which the nitrogen is set free from it as free nitrogen. Little is known concerning this factor at present.

The extent of the nitrogen losses from these sources may be considerable. Various estimates of the amount have been made, and it seems not beyond the mark to say that, in the ordinary conditions on the farm, at least 50 per cent. of the nitrogen is lost to the manure. It is sometimes considerably more than this, 50 per cent. being a fair average. When the farmer remembers the high cost of nitrogen fertilizers he may perhaps realize the very poor economy of allowing this loss to continue. Not all the loss is avoidable, for under the best conditions, perhaps 15 per cent. is lost; but even if this is true, 35 per cent. may be saved by proper care. It is possible for a farmer to know when this loss is becoming excessive by two means: 1. The appearance of a strong *odor* of ammonia tells its own story; and while some such odor may always be expected, a strong odor indicates a too rapid loss. 2. The *heating* of the manure indicates rapid aerobic fermentations and this is always accompanied by a large nitrogen loss. Properly kept manure will not show a great rise in temperature and never a rapid one. The farmer may be confident that a noticeable heating of his manure pile means a large loss. Some manure heats more rapidly than others, that from the horse being especially subject to this destructive fermentation; a fact due partly to the large amount of hay that it contains and partly to its loose and porous nature, which allows a free access of air. It suffers more loss for this reason than most other types of manure—a loss that may be lessened by mixing with it some of the moister, denser cow manure. Liquid manure also is subject to heavy losses, because it so rapidly undergoes the ammonical fermentation.

There are two general methods of controling and reducing these losses. The first is by *chemical* means. Since the ammonia is volatile and a strong base, the addition to the manure of some chemical to conbine with it will produce salts that will be more likely to be retained in the manure. For this purpose quite a list of substances has been recommended. Among them are, *gypsum, burned lime, shell lime, lime-stone, kainit, superphosphates* and *sulphuric acid.* Each of these has a value when properly used, but none of them will wholly prevent nitrogen loss and all are somewhat costly. The losses due to ammonia vaporization may be prevented by these chemicals; but the losses caused by the liberation of free nitrogen cannot be checked by any means short of stopping bacterial growth, and this would check the beneficient as well as the injurious fermentations. On the whole, the result of experience seems at present to be against the use of chemical means of preserving manure.

The second method is *mechanical* and is more efficient. It is based upon the facts already emphasized, viz., that the destructive fermentations take place most vigorously in the presence of a large supply of oxygen and that the volitilization is much more rapid from a partly dry than from a wet mass. Hence manure that is loosely piled loses much more nitrogen than that which is firmly compacted. The practice of firmly compacting manure into conical heaps with smooth sides is best calculated to reduce the losses to a minimum. Experiment has shown that a lot of manure firmly compacted may lose 15 per cent. of its nitrogen during storage, while a similar lot loosely stored loses 35 per cent.; a very striking testimony to the value of compacting. If, further, the manure be

7

stored in cemented pits, that will prevent loss by draining, and if the excess of the liquid manure is caught in special tanks and frequently spread upon the fields before fermentation has progressed far enough to cause much ammoniacal fermentation, the greater part of the ordinary losses may be prevented. In thus storing the manure it should be kept moist by the use of liquid manure, but not allowed to become water soaked. It has recently been shown that the losses from manure may be greatly reduced by spreading fresh manure upon the older manure that has already begun to undergo an active fermentation, probably because the carbonic dioxid evolved in the fermentation of the older portions combines with the ammonia developed from the newer portions, this checking its dissipation. The presence of large amounts of hay in manure makes it difficult to compact and, moreover, furnishes large amounts of fermentable matter that increases nitrogen loss. It is therefore usually unwise to allow much hay or straw to be mixed with solid manure.

Thus the best methods of protecting manure from loss are: 1. The *exclusion of air.* 2. The *regulation of the amount of moisture.* 3. The *separation of the excess of liquid manure* from the solid and its distribution upon the soil at frequent intervals. 4. *Prevention of loss in liquid manure by leaching.* 5. The *presence of some already fermenting manure* to furnish carbonic dioxid to combine with the ammonia as it is produced. 6. The use of some kind of *"litter" to absorb the liquid manure* and prevent its loss.

**Constructive Fermentations.**—In order that the manure may become plant food the end-products of decomposition must be built up into the form of nitrates by nitrification. Nitrification, however, cannot take place in the fresh manure since it contains too large quantities of organic products, which, as we have seen, prevent the growth of nitrifyers.

Exactly when 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. The oxidation of the ammonia salts into nitrites is then brought about by the nitrous bacteria which 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 probably not until decomposition has 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 is more and more filled with nitrates and therefore becomes a better and better food for plants. At last when the process is ended and the manure is fully ripened, enough of nitrogen is converted into nitrates to furnish a most valuable supply of food for vegetation.

**Fresh and Ripened Manure.**—The transformations which we have considered constitute what is called the *ripening*, *rotting*, or *composting* of manure. They are clearly similar to those changes already considered as taking place in the transformations in the humus, but rendered more intense by the concentration of the manure heap. It is evident that manure is of no value to plants until it has undergone these transformations, and equally evident that the transformations may, and some of them do, go on in the soil after the manure is mixed with it as well as in the manure heap. Indeed, they will probably go on better in the soil, and in some important respects it is an advantage to incorporate the manure with the soil while fresh rather than to wait for it to ripen. We have noticed that the loss from decomposition and denitrification is slight when these processes occur in the soil, while they are considerably higher when the ripening occurs in the concentrated manure heap. The loss is especially large from the liquid manure in warm weather, which, if kept in tanks or allowed to accumulate with the manure pile, will undergo a very rapid ammoniacal fermentation resulting in large losses of nitrogen. If, however, it is mixed at once with the soil the ammonia is fixed as fast as formed by the soil ingredients, is soon nitrified, and the loss is largely prevented. There has thus come to be recommended the practice of spreading the manure upon the soil as quickly as convenient, not allowing it to accumulate, and undergo the fermentation that inevitable means loss of nitrogen. Whether this will always be feasible will depend upon conditions on the individual farm; but it is certainly to be highly recommended in all localities where climatic conditions and the exigencies of farm occupations make it possible.

It is to be borne in mind that in using manure as a fertilizer the soil receives advantages that are not derived from mineral fertilizers. Not only does the manure contain a considerable list of substances of value to the crops, present in small quantities only, and not present in mineral fertilizers, but manure contains considerable organic material in a partly decomposed condition that aids in forming a *permanent humus*. The texture of the soil is improved by this so that the final result is a soil superior to that containing only mineral fertilizers. The soil thus treated becomes more tenacious, richer, washes less with rains and is generally to be preferred. Mineral fertilizers, with the exception of nitrates may be mixed with manure. The nitrates, if thus mixed, would be lost by denitrification.

## 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.

Saltpeter plantations have gone out of existence since the introduction of Chilian saltpeter. It is probable that the nitrate beds of Chili are the remains of some old inland arms of the sea where great growths of seaweeds accumulated which, after the drying of the inland sea, were converted into nitrates by the processes of decomposition and nitrification due to bacterial action.

Nitrates are 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 unites with oxygen and produces nitric acid. The acid combines with the calcium present in the brick-work 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 be readily prevented by sprinkling the walls with a strong solution of some powerful antiseptic, such as formalin or corrosive sublimate.

## 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 in 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. Indeed, any abundant vegetable substances may thus be heaped into a mass, and, if moistened sufficiently by rains, the bacteria will be sure to work within it, gradually transforming the nitrogens, and converting them finally 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 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.

## SEWAGE AND ITS TREATMENT.

**Composition of Sewage.**—By sewage we ordinarily 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 no longer useful; also large quantities of both animal and vegetable foods which have passed through the alimentary canals of men and animals unassimilated. It contains a large amount of urea which has come from the animal metabolism; and also woody matter, cellulose, fat, starch, 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 quantities of water, it contributes to the sewage. The sewage thus contains exactly the same sort of material as that 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.

## TREATMENT OF CITY SEWAGE.

As cities have grown, the matter of disposing of their sewage becomes more and more difficult. In small communities the digging of cess-pools is satisfactory; but as larger numbers of people congregate together, this method becomes objectionable, and finally impossible. The treatment of city sewage has become a problem involving the ingenuity of the expert sanitary engineer. It cannot be said that any wholly satisfactory method has yet been devised for handling this difficult problem.

In the first place it is evident that sewage contains material that is very valuable if it can be used upon the soil. The large amounts of nitrogen in the urea alone from a large city would be worth millions of dollars yearly if it could be utilized. The nitrogen was taken originally from the soil by the crops, and the continued fertility of the soil is dependent upon its being in some way replaced. It requires no argument to show the wastefulness of throwing this valuable material away without attempting to utilize it. In China careful attention is paid to prevent the loss of such material, and as a result the soil remains fertile; while in our country a constantly decreasing fertility has followed the practice of wasting it. The only methods yet devised of utilizing city sewage on a large scale is by what is called sewage farming.

**Sewage Farming.**—This method of disposing of sewage has been established in the last thirty years as a means of at once disposing of and utilizing the sewage of large cities. These farms, necessarily located as near as possible to the city, receive its sewage and distribute it over the fields by conduits, thus furnishing the crops at the same time with nourishment and water. Upon such soils crops are raised, mostly garden crops, since these are sure of a ready market in the city. This plan of utilizing sewage has been very vigorously urged, and many such farms have been organized in England, in continental Europe, and some in this country. Enormous sewage farms are cultivated near the cities of Paris and Berlin, the latter city having thousands of acres under cultivation. In some of the arid western sections of United States, where water is especially valuable, sewage farming has also become very profitable.

There can be no doubt that this method of disposing of sewage is, theoretically, the proper one. It has two distinct advantages: 1. *Economic*. It puts back into the soil the great quantities of nitrogen and other materials taken from it by the crops. 2. *Sani-*

*tary*. The sewage ingredients, after being incorporated into the soil, undergo the various types of bacterial decompositions which have been described in recent chapters. The organic compounds are decomposed by bacterial action, a part of the resulting decomposition products going into the air as gas, and a part being built up, in the soil, into nitrates, to feed the growing crops. The offensive waste material is thus disposed of by being converted into inoffensive and useful products. Sewage farms thus prevent the sewage contamination of streams, harbors, and seaside resorts. This in itself is sufficient to make this method of disposal a desirable one.

But the appearance of certain practical difficulties has prevented a wide development of this system. It is, of course, impossible to expect farmers to adopt this system unless it is profitable, and, unfortunately, it frequently proves that such sewage farms are run at a loss instead of a gain. To be sure, in some places very favorable returns have been yielded, and largely increased crops have been reported. But in other places unfavorable reports have been made, and at the present time sewage farming is not increasing. Since the objections to it are purely practical, they may in time be overcome. The chief objections are as follows: 1. It is unhealthful to irrigate garden crops with a sewage that is sure to contain disease germs. This objection applies chiefly to vegetable products which are eaten without cooking, like lettuce, celery, etc. 2. Land near the large cities is usually too valuable to be used for farming processes. If the farm is at some distance from the city the expense of carrying the sewage to it becomes so great as to make the undertaking a losing instead of a profitable one. 3. Only a fairly porous and partly sandy soil can absorb the quantity of sewage necessary for the disposal of the product. In order that the soil may absorb it, the sewage must be decomposed and nitrified by bacteria. If this nitrification does not occur, the soil becomes clogged with the sewage products. Hence, if the soil is heavy and contains much clay it cannot be used for sewage farming. While sewage farms are successful and profitable in the sandy soils around Berlin, they are not possible in many another locality where the soil is of a different texture. 4. The extreme dilution of the sewage makes it impractical, or even impossible, to use it in some localities. The soil can absorb only a certain amount of water without becoming too wet for the raising of crops. In regions where rains are common, all the water is furnished by rain that can be handled by the soil, unless it is very sandy, so that the addition of much more water would spoil the crops. In many localities, especially in the United States, the sewage from a city is very dilute. We are very extravagant in the use of water, and a given quantity of American sewage contains much less fertilizing material than the same amount of German sewage. American sewage cannot be valued at more than one cent a ton, and it is impossible for any but the most sandy soils to absorb to advantage such great quantities of water, for the minute quantity of nourishing matter it contains.

These are some of the reasons why sewage farming is not generally successful, and there is little to hope for along these lines until some new methods can be devised for making the fertilizing ingredients of sewage more easily available and more profitable. It must be recognized, however, that this end is to be desired, since surely there ought to be some method of saving the enormous loss that comes from waste sewage. It can at present be profitably undertaken only in dry regions, or where a sandy soil can absorb large amounts of water.

**Sewage Disposed of as a Waste Product.**—For the reasons just given it will be understood why sewage has come to be regarded as a waste product, to be disposed of as inexpensively, but as efficiently as possible, the desire being to destroy it and not utilize it.

*Chemical Treatment.*—The first method used was to treat the sewage with chemicals that partly purify it. This is an expensive method, troublesome and unsatisfactory, and although used for a few years, it has been replaced quite generally by the bacterial method of treatment, which is the one most commonly adopted to-day by communities that need to find some method of sewage disposal.

*The Bacterial Treatment of Sewage.*—This method is based upon exactly the same bacterial activities that we have been considering in recent chapters, the sewage being treated in a manner that hastens the decomposition power of the bacteria so that they will rapidly destroy the organic products in the sewage. The method has not been devised by any one person, but has been the result of observations and experiments of several, extending over many years, and finally crystallized into practical results.

The bacterial treatment of sewage depends upon the destructive action of the decomposition and putrefactive bacteria. Putrefactive bacteria decompose 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 matters 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, 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. 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 free from putrescible matter.

To bring about this result two different methods of treatment have been adopted, sometimes used together and sometimes separately, each of which has several modifications.

*The Septic Tank.*—This is a method of making use of the *anaerobic* bacteria which decompose products rapidly, but incompletely. The septic tank 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 a slow uniform passage of the sewage 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 twenty-four to forty-eight hours.

During this slow flow through the tank bacterial action is vigorous, and it is chiefly the anaerobic bacteria that develop, since the closed tank allows little oxygen to enter. Furthermore, a heavy scum usually grows on the surface that prevents the excess of oxygen. In these anaerobic conditions, therefore, decomposition proceeds rapidly, the organic bodies becoming partly broken down. Gas is evolved in quantity and bubbles up through the liquid to find exit from the tank by special vents. The gases represent the partial destruction of the organic matters in the sewage, and as fast as they are evolved the organic ingredients in the sewage disappear and the sewage becomes clearer. When it leaves the outlet, after flowing through the tank, it is much purer than when it entered, and may then be discharged into streams without greatly contaminating them, if the process has been efficient. The evolved gases are only partly oxidized and are sometimes collected and burned into the final condition or $CO_2$, etc. The fermentation, being of the nature of putrefaction, gives rise to unpleasant odors, since the gases contain various compounds of sulphur and phosphorus that are only partly oxidized.

*The Filter Bed and Contact Bed.*—These two methods, though differing in detail, are identical in principle, and both are designed to stimulate the activities of the aerobic bacteria. In the *filter* beds the sewage is received upon great open beds, the bottoms of which are made of masses of coke, broken stone, clinkers, sand, etc., arranged in layers of different degrees of fineness, the finest at the top. Through these the sewage filters and appears below, greatly purified. It was at first supposed that the process was a mechanical filtering through the sand, but it is now known that the mechanical filtering has little to do with it. The *contact* beds are similar, large, open beds, filled with coarse coke, clinkers, or other material, but not arranged for filtering. The sewage is conducted upon these beds, allowed to remain there for a few hours, and then withdrawn to be replaced by more sewage. Although no filtering takes place, this sewage is purified by its sojourn in the contact bed.

In both cases the primary action is that of *aerobic* bacteria, aided, doubtless, by direct chemical activities of the oxygen of the air. The bacteria rapidly cause the decomposition of the organic products, and the decomposition is more complete than in the septic tank, so that simple gases, like $CO_2$ and N are evolved. The gases are no longer oxidizable. The action on the sewage is made more complete and efficient if the sewage be first passed through the septic tank and then over a contact or filter bed.

*Sprinkling, Trickling, or Percolating Filters.*—This represents a third slightly different method of bacterial purification of sewage. A mass of stones or some other favorable material, broken into fragments not less than half an inch in diameter, is spread in a layer several feet thick. The sewage is then sprayed or sprinkled upon this mass, and it slowly trickles through the rock layer. The broken rock fragments are so coarse that there is no filtering action, but, as the sewage slowly trickles downward, it is acted upon by chemical and bacterial agents, so that it flows out below quite changed in its nature. Sewage treated in this way does not look clear after treatment, but the organic products in it have undergone a change that makes them non-putrescible, and they will not undergo any further putrefactive changes. This method of treating sewage is recent and as yet not fully understood.

*Effect on Organic Products.*—As a result of these two types of decomposition the various organic bodies in the sewage are very largely destroyed by processes similar to those that occur in the manure heap. Various gases are liberated ($HN_3$, 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 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.

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.

*Effect on Bacteria.*—It might be supposed that the bacterial treatment would 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 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 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 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, but its efficiency varies widely in different instances.

Whether these methods of treating sewage destroy its dangerous nature as well as its offensiveness is not easy to answer. The danger in sewage comes primarily from the disease bacteria it may contain, foremost among which is the typhoid bacillus. The bacterial treatment greatly reduces the number of bacteria, but does not by any means eliminate them. Does it eliminate the disease germs? So far as evidence goes to-day it seems that the typhoid bacillus is eliminated by the treatment, and the effluent from such beds fails to show typhoid bacteria, even when they have been purposely put in the sewage. Bacteriologists who have had confidence in the efficacy of the purification have not hesitated to drink freely of the water from such a sewage filter bed. It is certain, therefore, that the treatment greatly improves the healthfulness of the sewage. But that it removes all danger from it cannot be positively stated.

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. We have learned that there are efficient forces in nature for bringing back from the atmosphere the nitrogen dissipated from the soil, and 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 VII), it is no serious matter if some of the nitrogen is thrown away.

## TREATMENT OF FARM SEWAGE.

Upon the ordinary farm the sewage problem is rarely of any importance, because of the small amount of material. The wastes which form the sewage in the city are kept separate on the farm and are not all treated alike. Part goes to the manure or compost heap, and later is returned to the soil with the manure. Part goes to the privy vault and is handled like manure; while still another part drains from the sink and is generally allowed to waste itself on the ground. A considerable portion of city sewage, like the refuse from factories, etc., has no counterpart on the farm.

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 manure, 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. The contents of the privy vault should never be left in position where it can possibly pollute the water of either brook or well. Precaution ns should also be taken to prevent its distribution around the farm by means of soiled boots or tools which have been used in handling it. There is much more likelihood of finding pathogenic bacteria in *human* excrement than in that of domestic cattle, and 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 slight, and it can be allowed to run out on the soil where, generally, it is rapidly absorbed and decomposed without

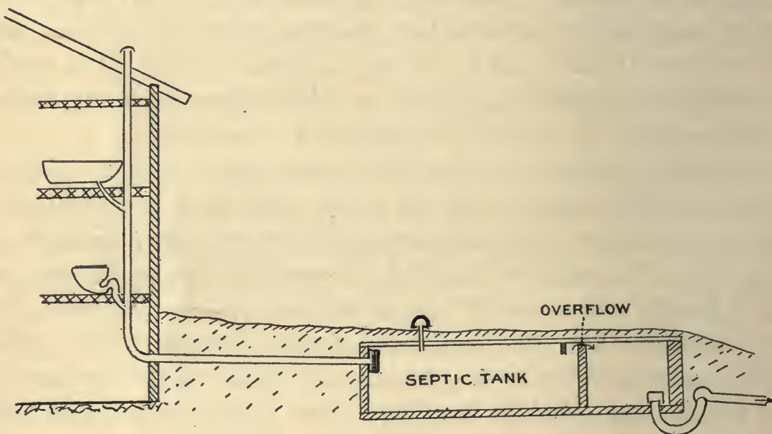


FIG. 19.—Diagram showing the method of applying the septic tank to a farm house.

any undue pollution. The organic matter undergoes the same type of decomposition as that to which all organic bodies are subjected under the influence of bacteria, and becomes eventually converted into plant food and incorporated into soil. The drainage which comes from the large dairy or creamery may be too much to be disposed of by such a simple manner. In this case some means must be adopted for its disposal. The problem thus presenting itself 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.

The time is coming, and, in some places, has arrived, when it is necessary to find a plan for disposing of the sewage on the ordinary farm in some other way than by emptying it into a stream.

It is very easy for the farm to make use of the principles of a septic tank in caring for and even utilizing its sewage. Fig. 19 shows diagrammatically a means of accomplishing it efficiently and at comparatively small expense. The diluted sewage from the house is conducted to a tank sunk in the ground at any convenient distance. The tank should be of such a size that it will hold the entire sewage for twenty-four hours. If each person uses twenty gallons of water

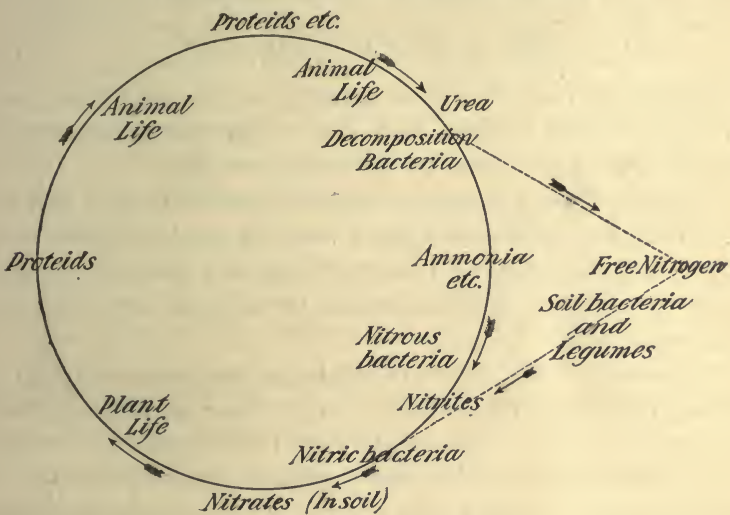


FIG. 20.—The nitrogen cycle.

per day, the tank for a household of ten should be three feet deep, two feet wide and six feet long. It must be covered so as to exclude air and light, and the sewage must flow slowly and quietly through the tank, thus making it a *septic tank*. The discharge from the tank is best received into a second tank from which it can be conducted to a stream or upon the garden for fertilizing and irrigating it.

# CHAPTER VII.

## RECLAIMING LOST NITROGEN.

### THE LOSS OF NITROGEN.

In spite of these transformations of nitrogenous compounds, and partly because of them, there is a constant loss of nitrates from the soil. This loss is from the following sources:

1. *Sewage.*—Any animal or vegetable material that falls into the streams will, unless dissipated into the air, be carried to the ocean. Much nitrogen is brought to the city where it is used as human food and, as sewage, carried to the river and perhaps to the ocean, where it is lost.

2. *Drainage.*—The rains are constantly percolating through the soil and carrying away dissolved material. Since nitrates are soluble, large amounts are thus carried off to the rivers which drain the land.

3. *Decomposition and Denitrification.*—These processes, by reducing organic nitrogen to ammonia and by reducing nitrates to free nitrogen, cause large losses. These gases of course pass from the soil to join the atmosphere. Such processes are going on wherever organic matter is found—in the river, the soil, the manure heap, sewage, and elsewhere.

4. *Direct Chemical Decomposition.*—A considerable portion of the earth's fixed nitrogen is dissipated by direct chemical processes. Explosions of gunpowder or other nitrate explosives liberate free nitrogen, which goes at once to join the nitrogen of the atmosphere.

Through all these channels the soil is being constantly deprived of its nitrogen. Eventually it all reaches the atmosphere, for whether it enters the ocean or dissipates itself in the streams, soil, or elsewhere, by means of decomposition it finally allows the nitrogen to pass of as a free gas to join nature's inexhaustible supply in the air. It is quite necessary for the continuance of soil fertility that this lost material should be restored.

Our farm lands slowly become incapable of supporting the crops demanded of them. This loss of fertility in 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 have depended mostly upon commercial fertilizers, which commonly contain nitrogen in the form of nitrates. Of such fertilizers there is a small supply in the world, chiefly in South America, and as they are brought from long distances they are sold at high prices. But the few large deposits of nitrates in the world 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 learned in the last few years the significance of nitrogen for agriculture.

The continuation of agriculture depends upon the existence of some means of reclaiming the nitrogen from 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 a possibility 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 the soil could have obtained from the air a stock of nitrogen to replace that lost by the processes already indicated.

Where did the nitrates come from that are now in the soil? Soil is made of crumbled rock which did not originally contain nitrates; it certainly must have obtained them from some source. The various bacteria we have been studying only *transform* nitrogen compounds; they do not make a new supply. The nitrogen in the air would be an inexhaustible source if it were only available; but the bacteria we have considered have no power of obtaining this nitrogen. They can transform nitrogen compounds, but they cannot fix or gather nitrogen from the air. It might naturally be supposed that ordinary plants could obtain nitrogen from the air as they do $CO_2$. But the most careful testing has shown that when such plants are growing under ordinary conditions they cannot assimilate any nitrogen from the air, but must depend upon the compounds in the soil. Free nitrogen is of no use to them, only nitrogen compounds. Some other source of soil nitrates must be sought.

**The Ammonia Theory.**—For a time it was held that the ammonia in the air was the source from which plants obtained nitrogen, and that it was carried into the soil by the rains. When this supply was found to be insufficient to account for soil nitrates, it was claimed that plants could absorb ammonia directly from the air through their leaves. But this theory failed to stand the test of experiment, and was finally abandoned.

**Fixation of Nitrates in Soil.**—It was next shown that, under proper conditions, ordinary soil will increase its stock of nitrates, independently of visible vegetation. 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 may be 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 may actually be a larger amount of total nitrogen in the soil than there was at the start. If, then, this total nitrogen has been increased, it must have been derived in some way from the atmosphere.

## NITROGEN-GATHERING OR NITROGEN-FIXING BACTERIA.

It was soon demonstrated that nitrogen fixation in the soil is not due to purely chemical processes, but rather to the growth of microorganisms. 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.

Such facts plainly pointed toward bacteria or allied organisms as the real agents for fixing nitrogen from the air, and this suggestion once made was quickly followed by the isolation of the bacteria concerned. It is now a demonstrated fact that the power of *gathering* atmospheric nitrogen and *fixing* it in the soil belongs to bacteria, and during the last fifteen years much study has been devoted to the microörganisms concerned. It appears that there are two general types of such nitrogen fixations associated with different classes of bacteria: 1. Nitrogen fixation by bacteria alone (*non-symbiotic*). 2. Nitrogen fixation by bacteria in connection with legumes (*symbiotic*).

### NON-SYMBIOTIC FIXTURES.

These are soil bacteria that are able to produce an increase of nitrogen in ordinary soil without the aid of other organisms. Of these there are two types, the first acting anaerobically, and the second aerobically. 1. The first one that was found with this power was isolated from soil and named *Clostridium pasteurianum* (Fig. 21). It is an anaerobic bacterium, and in culture media will not grow in the presence of oxygen. In the soil, however, it is often associated with a second bacterium that is aerobic, the latter absorbing the oxygen so that the anaerobic form can grow. Ordinarily the nitrogen fixation in the soil is due to these two growing together, but the *Clostridium* alone is able to assimilate nitrogen if kept in an oxygen-free atmosphere. In its growth the bacterium consumes some of the organic material in the humus, and from this source obtains the necessary energy for its action. The organism is widely distributed, having been isolated by several bacteriologists from different soils. Practically nothing is known as to its activity in soil under ordinary conditions. 2. In 1901 it was proved that the soil also contains bacteria of the aerobic type that can fix nitrogen.

FIG. 21.—*Closterium pasteurianum;* an anaerobic nitrogen fixer (*Winogradski*).

FIG. 22.—*Azotobacter agilis*, an aerobic nitrogen fixer (*Beyerinck.*)

FIG. 23.—*B. danicus*, an aerobic nitrogen fixer (*Loh. and West*).

Two different varieties of these were first isolated, and to them was given the general name of *Azotobacter* (Fig. 22). Several other varieties have been found later (Fig. 23). They are considerably more vigorous than the aerobic type, and fix a considerably larger amount of nitrogen—two or three times as much. In order to develop efficiently they must be supplied with a considerable quantity of organic food, and in ordinary soil the humus furnishes this food. By the energy they obtain from this source they gather from the air an extra quantity of nitrogen. These nitrogen fixers are very susceptible to the presence of the smallest amount of acid, and fail to fix nitrogen entirely if the soil is even slightly acid. The use of a little lime to neutralize the acidity may thus frequently start an active nitrogen fixation in a soil in which it did not previously occur, and hence greatly increase its productiveness. It has been shown also that this class of nitrogen fixers, though able to grow alone, will perform their functions best when growing with certain other soil organisms. They grow well with other fungi and with some algæ, organisms generally found associated with them in the soil.

*Other Nitrogen Fixers.*—It has been claimed that other organisms are capable of fixing atmospheric nitrogen. Some species of *molds* have been placed in this class, and certain species of *algæ*, as well as a considerable list of other kinds of bacteria. Indeed, the power of fixing nitrogen has been said by some to be a fairly common property of bacteria. Concerning this subject little is known at present, but it is quite likely that the list of nitrogen-fixing organisms will be considerably extended in the next few years.

**Nitrogen Fixation in the Soil.**—As yet little is known concerning the actual efficiency of these bacteria in the soil, although they are certainly very active. Soils do gain nitrogen and continue to do so for periods of years. A long series of tests has shown that crops can be removed from some soils year after year with no diminution in the amount of nitrogen that may be found there. In these soils no legumes have been grown, and hence it would seem that the supply of nitrogen must have come from the supply which the bacteria gather from the air. The general belief to-day is that this method of fixation of nitrogen is of very great significance in all soils, and plays a much larger part in the maintenance of the soil nitrates than we formerly supposed. It is probable that the bacteria do not fix the nitrogen in a form immediately available to plants. They probably build it into some more highly complex compound, very likely of a proteid nature, which is incorporated in their own bodies. Later the processes of decomposition and nitrification act upon these compounds and eventually convert them into available nitrates. Of this series of changes, however, little is yet known.

## SYMBIOTIC BACTERIA AND LEGUMINOUS. PLANTS.

**The Value of Legumes.**—It has been known for a long time that leguminous plants in some way enrich the soil, even the Romans having commented upon the fact. The idea was revived in the eighteenth century and has been more or less fully realized by farmers since that time. To what this soil-enriching function is due has not been understood till within the last thirty years. It is now known to be due to the fact that the legumes increase the nitrogen present. As already noticed, experimental evidence indicates that ordinary plants are unable to assimilate 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 1883-4 that this conclusion did not hold in regard to the great family of *legumes*. It was 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 was contradictory to the belief accepted at the time, and although vigorously disputed, was soon found to be strictly correct. Mnay 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.

**Root Tubercles.**—The next 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. 24). 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 can easily be found on nearly any legume growing luxuriantly in the soil, if the roots are carefully dug from the soil in such a way as to prevent the nodules from being destroyed, and if the soil is carefully washed away. They were at first 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 is, however, evident that if they are of the nature of a disease, they do the plants no injury, for the plants developing these tubercles are as luxurious as those without them. Indeed, as soon as the nitrogen-fixing power of legumes was demonstrated, it became evident that the fixation of nitrogen was associated with the formation of tubercles. Only such plants as develop tubercles are able to increase the amount of nitrogen in their tissues, and the amount of nitrogen fixation is roughly proportional to the development of tubercles. Plants without tubercles show no increase; those with a moderate number, a slight increase; and those with abundant tubercles grow luxuriently and show a larger increase in nitrogen.

FIG. 24.—Tubercles on the roots of the Soy bean.

These facts led to experiments in regard to their formation of tubercles. The tubercles will not form upon the roots of legumes grown in sterilized soil. Under these circumstances the plants develop no tubercles, fix no nitrogen and, unless fed with nitrogenous food, make 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 moistened 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 show two interesting stages of growth. They sprout readily and for a short time grow vigorously; then the vigorous growth ceases and the plant seems 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 seed, and consequently all that was within reach. Control plants, grown in similar soil and watered with pure water, never recover from this stage, but those that were watered with the soil infusions, after a few days of such nitrogen hunger, recover, begin once more a vigorous growth and eventually produce large-sized plants with a good yield. Upon examining the roots of the plants they are found to have developed tubercles, while the control plants, watered with sterilized pure water, do not develop tubercles. These facts of course indicate that in the soil infusion some agencies are present which stimulate the development of tubercles and the consequent fixation of nitrogen, and that the power of absorbing atmospheric nitrogen enables the plant to recover from the nitrogen-hunger stage.

**The Tubercle Bacteria.**—These facts naturally suggest that bacteria, or other microörganisms, are the cause of the tubercles. Microscopic study of the tubercles shows a somewhat perplexing structure. The tubercle is the result of the excessive growth of the cells of the root, but they are filled with peculiar bodies. During the early growth of the tubercle, long, thread-like sacs appear, which force their way through the cells (Fig. 25). These filaments seem to be hollow tubes which contain smaller bodies, somewhat like bacteria. As the legume increases in size these bacteria-like bodies undergo a transformation in shape, growing larger and branching somewhat, so as to form structures like those shown in Fig. 26. These are called bacterioids, and they are characteristic of the tubercles of legumes. The next step was, naturally, to isolate these bodies and study them by bacteriological methods. It is easy to isolate from the tubercles bacteria that will grow in culture media, and these organisms were named *B. radicicola.*

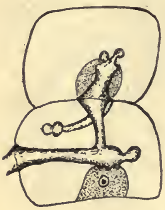

FIG. 25.—Showing the thread-like pouches in the tubercles. The figure represents two cells with the sacs penetrating them (*Stefan*).

Experiments with the bacteria thus isolated have been extensive and, on the whole, satisfactory, though occasionally they have been conflicting. It has been proved many times that tubercles can be produced upon legumes by the cultures thus obtained. Legumes have been grown in sterilized soil and watered with bacterial infusion from these cultures: the usual result has been the growth of abundant tubercles and the fixation of nitrogen. Some striking experiments have been 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 the soil. By dipping the tip of a needle into cultures of the microörganisms and then pricking the rootlets of young legumes at various points, the development of tubercles will almost inevitably follow such slight wounds. In favorable experiments the tubercles appear in six days after the inoculation and always at the point of inoculation. These facts proved that the cultures are concerned in the development of the tubercles.

The study of the organisms themselves and of their relation to the legume tubercle has proved somewhat puzzling. The organisms isolated are ordinary bacteria, *B. radicicola*, and in laboratory

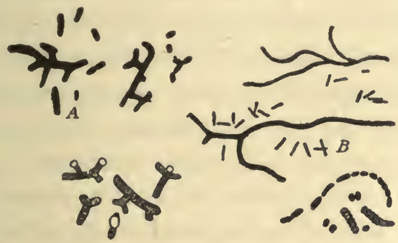


FIG. 26.—Showing the bacterioids found in root-tubercles.

culture media they resemble other bacteria, occasionally producing the peculiar bacterioid forms. Usually there is nothing in their growth in the laboratory culture media to suggest that they may produce the peculiar bodies found in the tubercles. When such cultures are inoculated into the roots of legumes the results are not always successful, sometimes no tubercles following. But, as a rule, the inoculation is followed by the growth of the tubercle, the development of the curious tube-like filaments growing among the cells, and there is the subsequent appearance of the bacterioids in the filament. The appearance of the pouch-like threads and the bacterioids has been a puzzle that has not yet been wholly explained.

It is evident that the tubercle bacteria must exist in the soil. But in spite of careful search no bacteria have yet been isolated from the soil which have the power of producing tubercles when inoculated into legumes. This, together with the irregularity of results obtained in trying to use the cultures of *B. radicicola*, has led to suspicions that the actual bacterium that produces the tubercle may not be the *B. radicicola* which has been isolated, but some other, which has escaped observation and which is frequently attached to *B. radicicola*. Indeed, DeRossi recently claims to have found the *B. radicicola* associated with what he thinks is a second bacterium that has quite different properties. The latter is much more like the bacteria forms that appear in the young tubercle, and shows a tendency to form bacterioids in culture media. According to DeRossi, it always produces the tubercles when inoculated into the root tissue of legumes. These bacteria do not grow well in culture media, not becoming visible for about two weeks, and have been overlooked in previous experiments since they are hidden by the vigorously growing *B. radicicola* with which they are closely associated. DeRossi thinks this a new organism and the cause of the tubercle rather than the species ordinarily accepted as the cause. It is doubtful whether this is anything different from *B. radicicola*. But whichever result is reached, it remains equally true that the tubercles are the result of the action of bacteria that enter the root tissues, and stimulate the root cells to excessive growth, although, perhaps, *B. radicicola* is not the real exciting cause. This conclusion of DeRossi, if true, would in a measure explain the irregularity of results obtained by the use of what were previously supposed to be pure cultures of the tubercle organism (see page 107).

**The Production of Tubercles by the Bacteria.**—Just how the bacteria produce the tubercle is not known. *Tubercles*, *galls*, or *tumors* are not infrequently produced in plants by bacteria and molds, these constituting one of the well-known types of plant diseases. Apparently these legume tubercles are produced in somewhat the same way, only instead of injuring the plant they benefit it. It appears too that the plant offers some resistance to the entrance of the bacteria into its root, and, when well nourished, is able to prevent their entrance. When there is plenty of nitrogen food in the soil, the plant grows vigorously, so that this resistance may be sufficient to prevent the formation of tubercles. When, however, the nitrogen food is scanty, the plant is weaker and cannot resist the entrance of the bacteria. The growing pea, when it enters the nitrogen-starved stage (see page 97) becomes weakened and the tubercle organism readily penetrates its roots. Thus it happens that these tubercles form only upon plants that grow in soils somewhat deficient in nitrogen, and thus under exactly the conditions where nitrogen assimilation is needed. Whenever the bacteria do succeed in entering the root they stimulate the plant to excessive growth which results in the formation of tubercles, and they themselves become transformed into the bacterioids.

**Assimilation of Nitrogen.**—Just how the nitrogen is assimilated is also uncertain. It is possible that the legume thus stimulated can absorb the nitrogen directly from the air. A second possibility is that the bacteria assimilate the nitrogen, and that later the legume utilizes this extra supply that has been absorbed. It is impossible to decide between these views, at present, although, considering that some bacteria are known to possess this power of nitrogen fixing while green plants do not have such power, the probability is in favor of the latter view. However this may be, it is certain that the legumes obtain from the growth of the tubercles an extra supply of nitrogen which is derived from the air.

## ARE THERE DIFFERENT SPECIES OF TUBERCLE BACTERIA?

It is practically certain that nearly all soils contain bacteria capable of living in symbiosis with leguminous plants. Nearly all soils, except extremely sandy soils that support little or no vegetation, will support leguminous plants and develop tubercles on their roots. One can hardly 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 are the bacteria all of the same species? A very large number of species of legumes with their tubercles can grow in most if not all soils. Are the bacteria that form tubercles upon the clover the same as those that form them upon the pea, or is there a different species of bacteria for the different species of legume? It would not seem probable that there could be in the soil a different variety of bacteria for every variety of legume, but rather that one kind of bacteria can grow in many legumes. But the facts are not quite so simple as this. 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 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. Afterward these particular plots of land were capable of producing large luxuriant crops of the soy bean, with abundant root tubercles and a large fixation of atmospheric nitrogen. Evidently Connecticut soil does not contain the bacteria adapted for producing the tubercles in the soy bean, although those which produce tubercles on the pea and the clover are abundant enough.

Similar experiments have been repeated elsewhere until it has become evident that the root tubercle bacteria are not all alike. Varieties adapted to one species of legume may be unable to produce tubercles upon a second species; in some cases one type of bacteria may be able to grow in the roots of several allied legumes but not in others. For example, the tubercle organism of sweet clover will do well with alfalfa. All of these facts have suggested that there are different types of leguminous bacteria, each adapted to different species of legumes.

To what extent this conclusion is true it is by no means easy to determine. It is certainly true that some 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 it is of importance to know 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 they assume slightly different physiological relations. They can accommodate themselves to growth in one or another legume, and having become especially adapted to one species, they do not so readily develop in the root of a second species, but, allowed to develop in the soil in which the latter plants are growing, they adapt themselves in time to the new plant. In other words, experiments indicate that there is probably only one 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. The matter of practical importance is that a soil may support one species of legume luxuriantly, with abundant tubercle production, while a second species will not flourish upon it because of lack of tubercle bacteria properly adapted to the second species.

## THE UTILIZATION OF THE NITROGEN-FIXING POWERS OF LEGUMES.

Although there are still unsettled questions concerning the nature of the tubercles, the power possessed by legumes of fixing nitrogen through their aid is of the utmost importance. The legumes are the most practical means within reach for restoring to the soil the nitrogen dissipated into the air, and it becomes a matter of great significance to agriculture to determine the best practical method of making use of this power. 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 the 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; but so much has been learned that already our agriculturists are making use of the knowledge to a great extent, 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 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. In selecting the legume the individual must take into consideration all the facts within his reach. Some

species 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. In clay soils *red* and *yellow clover*, *lupin*, *seradilla*, *horse beans*, and *vetches* are successfully grown. Which of the varieties 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 may increase its nitrogen store it is evidently necessary for tubercles 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 insure this result may sometimes require a little experimenting and observation. Some species of legume find in a certain soil the tubercle organism adapted to them, while other species of legume may not find the proper organisms in the same soil. The soy bean is a most excellent crop for nitrogen gathering 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.

Further, a legume, which, during the first season produces only a small number of tubercles, may succeed better the second year than the first and may 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.

*Soil Inoculations.*—Experience has shown that it is not always possible to get a good growth of the desired legume, because of the failure to obtain a proper quantity of tubercles. That this is due to the lack of the right variety of bacteria in the soil seems certain, and has led to the practice of inoculating the soil. The first method of doing this is to obtain soil from some locality where the legume is known to produce a goodly numbers of tubercles and then either to

FIG. 27.—Two snap-bean plants, growing in coal ashes, one with and one without inoculation (*Ferguson*).

mix this soil with that of the field to be planted, or to make a soil infusion to be used for soaking the seeds or for watering the young plants. The results of this procedure are, in the main, satisfactory, for generally the production of tubercles is thus stimulated, and much increased crops produced (Fig. 27). In many instances of this kind it has been found possible to cultivate a legume in soils in which it would not previously grow, by simply inoculating the new soil with soil where the legume has previously grown. Alfalfa, for example, has been successfully started by this means in many places in the eastern part of this country where it would not previously grow. There seems no doubt that the phenomenon is simply one of inoculating the soil with the proper bacteria.

But soil inoculations with legume earth are troublesome. Soil is bulky and a considerable quantity is needed. To obtain a sufficient amount involves expensive freight charges and the carting of heavy loads. Soil inoculations may also distribute plant diseases and troublesome weeds. If tubercles are produced by bacteria it ought to be possible to obtain the results by inoculating with pure cultures of bacteria. It should be possible to cultivate the bacteria in a laboratory and then to distribute to the farmers the cultures of the organisms. If this could be done it would be a far simpler matter than the use of soil itself. The first attempt to furnish such a culture resulted in an article called *Nitragin*, which was brought out in Germany. This product was eagerly tried by experimenters and practical farmers; but, although in some cases it seemed to give favorable results, the success attending its use was so uncertain that it fell into disrepute. Later, various improvements were introduced into the methods of making and distributing the cultures, and a new product, called *New Nitragin*, has been put forward which gives somewhat better results. This has been tried quite extensively in the last three years and the results have been much more positive than in the earlier attempts. Meantime other attempts toward the same end were made in this country. In the laboratories of the Department of Agriculture extensive experiments were carried out, seeming to show the possibility of increasing the nitrogen-fixing powers of these bacteria by cultivating them in solutions that are poor in nitrogen. After continued experiments in this line, cultures of the tubercle-producing organisms were sent out for testing from the department. These too proved unsatisfactory. They were first sent out upon absorbent cotton, but the bacteria did not live long on the cotton fibers and the farmers were likely to receive cotton containing only dead bacteria. Then they were sent out in a liquid or upon agar but their use has never been very great.

It thus appears that the use of pure cultures of tubercle organisms for soil inoculation has hitherto not been very successful. But this does not by any means indicate that the methods will not soon be so improved that they will be practical. The plan is logically a proper one, and if it be found possible to develop the tubercle bacteria in sufficient quantity and to distribute them in a living condition, these soil inoculations may become of great value to the agricultural industry.

The reasons for failure thus far are varied. The difficulties of keeping the cultures pure, of distributing them to the farmers in a still vigorous condition, and of finding some device by which the farmers can successfully inoculate legumes with cultures have been regarded as the primary obstacles. Moreover, some soils are already stocked with proper tubercle bacteria so that the addition of more would be superfluous. If, however, the claims of DeRossi are correct and the tubercles are caused not by the *B. radicicola*, but by another much more slowly growing organism, the irregularities in these results are readily explained, and it will be necessary to proceed along a different line in developing cultures of the organism that really produces the tubercles. At the present time, therefore, the pure cultures of the tubercle organisms that have been put on the market for soil inoculation are not reliable, although we may confidently expect that the methods will be so improved in the future as to make them of practical value. Meantime, soil inoculations continue to be made with legume earth from lands where the desired legumes are growing vigorously; and this method of soil inoculation has proved of much practical value in developing a vigorous growth of legumes and a consequent increased fixation of atmospheric nitrogen.

3. **Utilization of the Nitrogen.**—The next problem is how such a store of nitrogen, fixed in the soil, may best be 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 legume to grow vigorously for a time, and then in plowing the whole crop 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 based upon the facts already noticed.

When these crops are thus plowed into the soil they are brought at once within reach of the soil bacteria.

The bacteria seize hold of the proteid products in the plants, as well as the cellulose and other organic substances, and cause their rapid decomposition. After this process is finished the nitrifying bacteria in the soil oxidize the ammonia left after the decomposition ceases and convert it into nitrate. Thus, after a few weeks, a considerable portion of the nitrogen material which was fixed in the legume has been converted into nitrate, available for plant life. These 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 has been converted into an available form by the soil bacteria.

A second method of utilizing the nitrogen is by converting it into manure. The crop of legumes is reaped and fed to animals, the roots and stubble only being plowed into the soil. The portion fed to the animals is later returned to the soil as manure. Part of the nitrogenous material is thus metabolized by the animal body to urea, and part passes into the feces unassimilated, while part remains in the roots and soil. But it is all 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, most of it is returned to the soil in a form available for plant life. This method of utilizing the nitrogen is certainly the best economy, since it has a double advantage: The nitrogen is used twice, once as a food for the stock and a second time as a food for the crops in the form of manure.

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 by denitrification which liberates 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.

# CHAPTER VIII.

## BACTERIA AND SOIL MINERALS.

### MINERALS NECESSARY FOR PLANTS.

Although the different minerals in the soil are needed by plants in smaller quantities than the nitrogenous foods, still they are quite as necessary, and vegetation cannot be supported without them. They come primarily from the rocks that form the earth's crust. In these rocks there is practically an unlimited supply of the necessary minerals, but they must be rendered available as plant food. Most of these rocks contain their minerals in an insoluble condition and, in order to be absorbed by vegetation, they must be dissolved in the soil waters. Although this subject has not been studied so thoroughly as the transformation of nitrogen, still it is known that chiefly through the agencies of the soil microörganisms the minerals are brought into solution.

### LIME AND MAGNESIA.

These two minerals may be considered together since they are closely allied and their relations are the same. The importance of lime to soil has long been recognized and our previous study has shown one of its most important uses. We have learned how necessary is the activity of the soil bacteria in the transformation of plant foods, and how, as a rule, bacteria cannot grow in the presence of the slightest acid reaction. But general processes of decomposition are constantly giving rise to acids, so that the soils tend to become more and more acid. As the acidity increases the bacterial action declines and fertility correspondingly diminishes. The addition of lime to such soils is necessary, therefore, to reduce the acidity. Other needs there may be for lime, but the primary one is to keep the bacterial activities in the soil at a high state of activity.

But there are also constant losses of lime from the soil. A small quantity is carried away by the crops taken from the land, but a far larger quantity is lost to the soil by drainage. The soluble lime salts are dissolved by the soil waters and pass off with the drainage. Very large amounts are thus removed so that a more or less frequent liming is necessary to maintain in the soil a quantity sufficient to keep the proper condition for bacterial action. Different soils show wide differences in the amount of lime needed. Soils containing limestone rock have an abundant natural supply, while soils without limestone need to be furnished with it in varying amounts. The lime thus drained away is a permanent loss, for it finds its way into the ocean whence it is not easily returned to the soil. But this loss is not serious, since limestone rocks are practically unlimited and there need be no lack in the supply of available lime. Lime is rendered available chiefly, if not wholly, through the action of bacteria. Limestone consists mainly of carbonate of lime which is only very slightly soluble in water, and cannot be utilized directly, for this reason. But water containing carbonic dioxid in solution readily dissolves the carbonate of lime. We have seen that by the constant decomposition processes going on in the soil, carbon dioxid gas is being set free from the decomposing organic compounds, such as proteids, sugar, cellulose, etc. This gas is taken up by the water, which is then able to dissolve the limestone. The greater the extent of the bacterial action, the greater will be the amount of carbon dioxid eliminated, and the amount of lime brought into solution; the more effectually also will the soil be maintained in proper condition for bacterial growth. Hence, as the amount of lime in the soil increases, the bacterial action will become greater, more lime will be dissolved, and consequently more will be lost by drainage.

In this way the limestones on the earth's crust are being dissolved and carried away. The extent to which this is possible is indicated by the huge limestone caves whose great spaces show how the limestone has been dissolved by waters which held carbon dioxid in solution. All such dissolved lime finds its way to the ocean where it supplies marine animals and plants with the lime for their shells, and where it is also laid down in the deposits of lime material that may, in later ages, form new limestone rocks. But, aside from this future possibility, the bacterial agencies of the earth's surface are constantly dissolving the limestones and adding them to the soil to be subsequently carried away by drainage.

In recent years calcium cyanid has become much used as a fertilizer. It furnishes lime and results in distinct nitrogen gains to the soil. In the utilization of this material bacteria are necessary to convert it into ammonium salts before it can be assimilated by plants.

## PHOSPHORUS.

Vegetation needs only very small amounts of phosphorus, but these small amounts are requisite to the production of good crops, as has been many times appreciated by the farmer who finds decidedly increased crops following the application of phosphate fertilizers to the soil. There are many substances containing phosphorus which may be used to supply the amount needed by the soil. They are: 1. *Mineral compounds*, of which the chief are *ground phosphate rock* (floats), *superphosphates* and a by-product of steel manufacture called *Thomas slag*. 2. *Organic compounds*. A considerable quantity of phosphorus is contained in the *humus*, likewise in *bone*, which is used as a fertilizer chiefly for its phosphorus. The solid part of *barnyard manure* contains phosphorus, and a variety of other sources, are also utilized—*ground fish*, *tankage*, *castor pomace*, and the like. The phosphorus in some of these substances is readily soluble in water, and this must always be the case before it can be utilized by plants.

Apparently the solution of the phosphates is dependent upon bacterial action. It is easy to understand how the phosphorus from organic sources is rendered available through the agency of the soil organisms. As these bacteria decompose the various organic products in the soil, the phosphorus contained in them is set free from its combinations. Bone, for example, is vigorously attacked by the bacteria, and is in time completely disintegrated, the phosphorus being, of course, freed from its relations. The entire series of changes through which it passes is not yet known. Part of the phosphorus finally assumes an insoluble condition, but a part is dissolved in the soil water and becomes available as plant food. This solvent action of the bacteria is attributable to the acids that are produced. As we have already seen, the decomposition of organic products always gives rise to certain organic acids and these are capable of dissolving phosphorous compounds that would be insoluble in water alone. The solvent action resulting from bacterial decomposition is not wholly the result of the acids, for by some means yet unknown the phosphorous compounds may be dissolved even when no acid is produced. They are not, however, dissolved in sterile soil; therefore the availability of the phosphorus is due to bacterial activities. Such a formation of soluble phosphorus from decaying organic compounds is going on constantly in the humus, and in soils rich in humus the process furnishes phosphorus in sufficient quantity for vegetation.

Sometimes, however, more phosphorus is needed, and it may be supplied by minerals. The rock phosphates are rendered available in much the same manner as the organic phosphates. The phosphorous compounds in the rock are very slightly, if at all, soluble in water. In ordinary soil small, but sufficient quantities are dissolved through the agency of the soil bacteria. Hence also the acids produced by decomposition are important agents in dissolving the rock which, though not soluble in water, is soluble in acids. It is a well-known fact that these phosphates are made more available as a fertilizer by being composted for a time in manure, a fact clearly explained by the solvent action of the acids produced by decomposition, as well as by other functions of the bacteria not yet understood. They are also made more effective when plowed into the ground with the plants used for green-manuring, this condition giving rise to rapid bacterial action, resulting in a decomposition which aids in rendering the phosphorous compounds available. Thomas slag is also dissolved by similar activities. In short, while bacteria do not furnish phosphorus, they are the active agents in rendering available the phosphorus from both organic and mineral sources.

## POTASH.

The relation of potash in the soil is almost exactly the same as that of phosphorus. It comes primarily from the rocks where it exists largely in the form of silicate of potassium. This is an insoluble salt, and soils may contain it in large quantity and still suffer from lack of available potash. It is rendered available in very much the same way as in the case of phosphorus, largely through the action of the decomposition produced by the soil bacteria.

## SULPHUR.

Sulphur is one of the ingredients of protein, and, therefore, is necessary to plant life. Ordinary plants obtain it only in the form of sulphate, which they absorb from the soil. But microörganisms are concerned in the transformations by which the soil is properly stocked with the sulphates. The transformations show at least two different steps:

1. *Sulphur is set free* from its combinations. 2. *Sulphur is recombined* into sulphuric acid that unites with mineral matter to form sulphates.

**Liberation of Sulphur as $H_2S$.**—All proteid matter contains sulphur, and when its decomposition takes place through the agency of bacteria the sulphur is liberated in the form of *hydrogen bisulphid* ($H_2S$) which vile-smelling gas may usually be detected around decomposing proteid. This same gas is liberated from the decomposition of sulphate of lime that is carried in drainage waters to the ocean. Several kinds of bacteria have been found capable of liberating $H_2S$ from such deposits. In certain parts of the world large deposits of such sulphites (gypsum) have accumulated and are constantly acted on by bacteria which liberate $H_2S$, producing the "curative muds" of the Black Sea and other localities. Such muds are saturated with hydrogen bisulphid gas. This reduction of sulphates is, in a way, comparable to denitrification since it is the result of deoxidation and since it also destroys substances that are already plant foods. Some species of bacteria appear able to attack pure sulphur, causing it to combine with hydrogen as $H_2S$. From these several sources much sulphur is liberated into the air in the form of $H_2S$, the liberation in all cases being due to the action of bacteria, different classes acting on different compounds containing sulphur.

**Recombination of Sulphur Compounds.**—The recombination of the $H_2S$ to form *sulphuric acid* and then *sulphates* is brought about in some cases apparently by purely chemical forces, since the gas will easily combine with oxygen if the conditions are right. But a large part of it enters new combinations through the agency of microörganisms. There is a group of bacteria that consume $H_2S$, oxidizing the gas within their bodies and utilizing the energy thus liberated for their own life energy. They are as dependent upon the presence of $H_2S$ as ordinary plants are dependent upon $CO_2$. In the presence of this gas they flourish, and as they oxidize the gas the sulphur is set free from its combination with hydrogen and separated as *pure sulphur*. The sulphur appears then within the bodies of the bacteria as minute reddish dots (Fig. 28). The bacteria that can perform this function seem to be of two types, one type belonging to the higher fungi (see page 12) and the other being true bacteria. The latter are sometimes called the "red bacteria" because of the color produced by the sulphur grains within them. These bacteria may continue thus to liberate the sulphur and in waters where $H_2S$ is abundant large quantities of pure sulphur may be deposited. These are the so-called sulphur springs around which deposits of sulphur may be found. As long as the gas is abundant the bacteria flourish; but if the gas disappears they appear to use up the sulphur in their own bodies, after which they die. In some way the sulphur in their bodies is in the end converted into sulphuric acid, which then combines with any lime that may be present to form sulphate of lime. Very little is known as to when or how the sulphur in the cell walls of these sulphur bacteria gets converted into sulphuric acid, or whether it is a purely chemical or a biological phenomenon. But although the whole

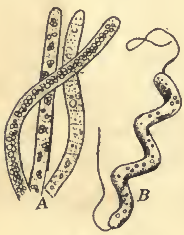


FIG. 28.—Sulphur bacteria. *A*, Beggiatoa; *B*, Ophidomonas. Both show sulphur masses in the rods (*Winogradsky*).

process is not fully understood, it is evident that microörganisms have a close relationship to the transformations of sulphur in the waters and soils. They liberate it from its combination in proteid, they oxidize the liberated gas into sulphur and finally into the form of sulphuric acid which soon forms a sulphate. It is also claimed that some kinds of bacteria can oxidize *sulphites* into *sulphates*. Microörganisms are thus responsible for the constant metamorphosis of sulphur compounds that keeps the soil properly supplied with this element. Of their activities in ordinary cultivated soil we know little or nothing.

## IRON.

A small quantity of iron is needed by plants, and a group of bacteria, called iron bacteria, has been supposed to have some relation to a circulation of iron nature, somewhat similar to that of sulphur. These bacteria are commonly seen covered with a deposit of hydroxid of iron, giving them a reddish-brown color. It has been thought that they used iron in their activities much as sulphur uses iron. But the most recent work indicates that this is probably an error, and makes the agency of bacteria doubtful. At all events, nothing reliable is known to-day upon the subject, although it is not impossible that here, too, the bacteria of the soil, and especially of waters, may be of some significance.

# CHAPTER IX.

## SOME PRACTICAL LESSONS FROM SOIL BACTERIOLOGY.

The close dependence of soil fertility upon the action of microörganisms is manifest, and it is evident that farm processes should be such as to stimulate desired bacterial action and check these activities that are detrimental. Practical methods of doing this have been only partly devised and there are still many problems for the future concerning the method of treating soil. Nevertheless, the knowledge of bacterial action has already taught some definite and useful lessons. The uncertainty still attached to certain phases of the subject may be illustrated by a recently discovered fact that the *sterilizing* of certain unfertile soils will decidedly increase their fertility. This has been proved definitely, but the meaning of the fact is still obscure. Bacterial action is positively needed in the soil, and it is rather surprising that sterilizing soil will increase its fertility. It has been suggested that the treatment kills injurious bacteria, giving the beneficial species that subsequently get in a better opportunity for growth. It has been suggested likewise that the sterilization kills all animals and plants that may be in the soil, thus giving the bacteria that subsequently get into the soil, or that may have resisted the sterilization, an extra amount of organic matter to decompose and to reconvert, by nitrification, to nitrate. The fact of this beneficial influence of sterilization is undoubted, although its explanation is uncertain; and the phenomenon is here mentioned as an illustration of the gaps still existing in our knowledge of soil bacteriology. But in spite of it all, some definite conclusion as well as practical lessons can already be drawn.*

*Reference should be made here to the conception concerning soil fertility held by some, notably those connected with the Bureau of Soils of the Department of Agriculture, that the primary trouble in "worn-out soils" is not lack of sufficient plant food, but the presence of poisonous excretions that prevent the growth of plants. It is claimed that each crop

## SOIL INOCULATIONS.

The need of bacterial action in the soil naturally suggests the question whether the necessary activities may not be brought about or increased by inoculating the soil with the desired bacteria, just as a brewer inoculates his malt with yeasts. This plan has been tried extensively by at least two different methods.

**Nitrogen Fixers, Alinit, Nitrifying Bacteria.**—Alinit is a material placed on the market and widely used for a time. It was said to be made from a pure culture of the bacteria that can fix free nitrogen in the soil. Careful testing, however, failed to show any favorable results from the use of alinit, and so it has been abandoned. Other nitrogen fixers have also been tested as pure cultures inoculated into soil, with like failure. The attempt to simulate nitrification by the use of cultures of nitrifiers has likewise failed.

**Tubercle Bacteria of Legumes.—Nitragin.**—As already noticed, this commercial product, supposed to contain the bacteria-producing root tubercles, was found to be a failure, although claims are still made of the success attending the use of the *New Nitragin* and of some of the other cultures of similar organisms. While these may be found practical and while it would seem as if this method would be likely, in the future, to become successful, at the present time the results are so uncertain that a decision as to the value of such inoculation for the developing of legume tubercles must be held in abeyance. Inoculations with *legume earth*, however, have proved to be of great value, and whenever it is desired to cultivate a legume in a soil where it does not readily grow, this inoculative

excretes into the soil certain substances that serve as poisons to another similar crop on the same soil. It is said that practically all soils at all times contain a sufficiency of food, but that the accumulation of these excretions after a time renders the soil incapable of supporting a satisfactory crop. The value of fertilizers is not to give food, but to neutralize these excretions, and that a proper rotation of crops will serve just as well, since the excretions from one kind of plant, while injurious to the same plant, will not injure a different kind of plant. Such a conception would largely revolutionize the methods of treating the soil, since, if accepted, it would lead to the abandonment of any attempt to feed the crops and would replace such methods by those designed simply to remove the poisonous excretions. This theory as to soil fertility would bring into greater prominence the agencies of soil bacteria, but it is very vigorously disputed and certainly has not reached a position where it warrants an acceptance.

method should always be tried. Soil from some locality where the legume grows luxuriantly should be imported and mixed with the field which is to be planted with the legume. But one should be careful that the inoculating soil does not bring troublesome weeds or plant diseases. In using either the earth inoculations or pure cultures, it must be borne in mind that if a soil is already well stocked with the tubercle bacteria, inoculations are not likely to do any good; but in soils where a new legume is to be grown or where the legume to be planted does not flourish, soil inoculation may be of decided advantage.

**Soil Inoculation with Manure.**—Manure is added to the soil primarily as a means of furnishing plant food; but it has become evident that the inoculation of the soil with the immense amount of bacteria in the manure is in itself of extreme value, sometimes, as in pasture soil, of more value than the actual food substances in the manure. The use of manure upon the soil must, therefore, be looked upon as one of the very useful methods of inoculating the soil with the bacteria needed to carry out the soil transformations.

## CONTROL AND STIMULATION OF SOIL BACTERIA.

It has become more and more evident, as information has accumulated, that a vigorous activity of the bacteria found in the soil is needed to carry out the various transformations of plant foods. These bacteria are commonly abundant enough; and sometimes they find the conditions so favorable that they grow rapidly, producing vigorous actions; but at other times the conditions in the soil are unfavorable, and they are held in check. What is chiefly needed, then, in the treatment of soil is, not the inoculation of more bacteria, but a modification of the soil conditions so as to favor the growth of those already there. While this is a complicated subject and one that will require different treatment in different cases, a few general principles may be formulated.

**Acidity.**—Most bacteria, and practically all the useful bacteria, are very sensitive to the presence of acid, failing to grow at all in an acid medium. If the soil is but slightly acid, bacterial agencies are checked, while the activities of molds and larger fungi are increased.

In the soils of forests, for example, the fungi and molds grow luxuriantly, but bacterial action is comparatively slight. While the higher fungi are valuable agents in bringing about the decomposition of certain organic bodies, and are therefore useful, they cannot perform the final transformations by which the soil ingredients become available as plant foods. These transformations, especially nitrification, require bacterial growth. Hence, it follows that one of the first necessities of proper bacterial activity is an alkaline reaction in the soil. In some localities this matter cares for itself. If the soil contains lime in any form, the solution of lime by the carbonated water, resulting from the carbonic dioxid of decomposition, will keep the soil properly alkaline. Decomposition in itself will also produce an alkaline condition, since the ammonia resulting from ammoniacal fermentation will neutralize the acids. If, therefore, a vigorous decomposition of organic matter is going on, little attention need be given to the matter of acidity. But some soils are acid from one cause or another, and proper bacterial activities cannot be expected here without the correction of this acidity. This is most easily done by the addition of lime, either in the form of limestone, plaster, ground shells, or some other common substance. The restoration of alkaline reaction will be followed by a stimulation of bacterial activities and an increased fertility.

**Aeration.**—The soil bacteria are aerobic and anaerobic, and both types are sometimes useful and sometimes detrimental. Speaking broadly, however, the aerobic processes in the soil are the more desirable. Anaerobic decomposition is incomplete, and gives rise to many undesirable products, while aerobic decomposition is complete and hence a more useful process. Nitrification, too, can go on only in the presence of oxygen, and is stimulated by a quantity of this gas. The value of a frequent stirring or cultivating of the soil, which introduces air into it, is, therefore, evident. The simple stirring of the soil, to bring oxygen into close contact with its bacteria, may be of as much value as an application of manure. In some soils, indeed, it is more valuable than manure, since there may be plenty of organic products in the soil which only need transforming in order to be available for plants. All desirable processes which are likely to occur in the soil are benefited by aeration. In especially rich collections of organic refuse, however, like the manure heap, aeration will cause large losses through denitrification, and hence the manure should be closely packed to exclude air. This may be true also in some instances of intensive gardening, where large amounts of manure are applied to the soil as top dressing. But in ordinary soil, aeration from frequent stirring stimulates desirable bacterial activities.

**Manures Better than Commercial Fertilizers.**—This perfectly evident conclusion is of so much importance as to deserve special emphasis. The reasons for the conclusion are several. Manure adds bacteria as well as chemical food. It helps to keep a proper alkalinity in the soil. It adds various ingredients that help to form humus in the soil, resulting in a better texture and more lasting good. Manure adds to the soil in small amounts various useful materials, which are not present in commercial fertilizers. For these cogent reasons manure fertilizers are to be preferred, as a rule, to chemical fertilizers. From all these facts may be drawn the practical lesson that a properly kept farm should keep plenty of live stock and, instead of selling its manure, should use it freely on the soil.

## FALLOWING.

It is an old idea that the soil, after yielding several crops, needs a rest, an idea that goes back as far as the Romans. From this arose the plan of occasionally allowing the soil to remain without a crop for a season or for part of a season. This plan has practically gone out of use in all ordinary soils, for careful study shows that a detriment rather than a benefit results from such *fallowing*. Under certain conditions fallowing may be an advantage. There is a smaller loss of water from fallow land than from land with growing crops, due partly to the increased evaporation from the stirred soil and partly to the fact that crops draw quantities of water from the soil, to be evaporated from the growing leaves. In climates where water is scarce and must be conserved, fallowing may result in advantage. It is also claimed that fallowing may enable the soil to dispose of the poisonous secretion from plants that would injure a second crop growing on the same soil. But, apart from these facts, fallowing results in a loss to the soil. In the first place, fallowing adds nothing to the soil, while a crop, especially a legume, may do so. Moreover, during the fallow season the bacterial activities in the soil continue, converting the material in the humus into nitrates and other soluble substances, which are then available plant foods. If a crop is growing in the soil these will be absorbed by the crop and utilized. If, however, the land is fallow, there is nothing to utilize these products as they are formed, and they will be, in a measure, lost; for they will be dissolved in the soil waters and drained away from the soil into the general system of brooks and streams. If, in the meantime, nothing of any value is added to the soil, at the end of the fallowing it will actually be poorer than at the beginning, except in the matter of water. Its store of humus will be partly converted into available plant food and lost, while nothing takes its place. For these reasons the practice of fallowing has been almost wholly given up, except for special soils. Indeed, it is a growing custom not to allow the soil to remain fallow at all, not even during the season of the year when the main crop is not growing; but to sow it with a *cover crop*, which will catch and hold the plant foods that are constantly being made available. Nitrification, as we have seen, goes on at all seasons when the soil is not actually frozen, and considerable losses of nitrogen will result from the leaching of these nitrates away from the soil at the seasons when it is not covered with a crop. The loss may be largely retained by a quickly growing cover crop. Such cover crops plowed into the soil will benefit it, and the next main crop will be improved; but a fallow season leads to direct loss.

## GREEN MANURING.

The use of cover crops, just mentioned, is closely related to the practice of green manuring, but the latter has an additional purpose besides all the advantages of a cover crop. It not only prevents the loss of available plant foods by drainage, but it also adds a considerable quantity of food to the soil. It not only serves as a catch crop to hold the nitrates that may form during the season when the main crop is not growing, but it may add to the total nitrogen of the soil. The general plan of green manuring is to grow upon the soil some leguminous crop that increases the nitrogen content of the soil, and, after proper growth, to plow the whole crop into the soil. The addition of this large amount of organic matter to the soil will stimulate the bacterial activities of decomposition. The roots, stems, leaves, and fruits of the crop undergo a decomposition and subsequent nitrification, resulting finally in the formation of nitrates which can be utilized by the next crop grown on the same soil.

When adopting the plan of green manuring the first thing is to select the crop that is to be so utilized. Manifestly, from what has been learned, this should be some one of the legumes, since this family of plants alone assimilates nitrogen from the air, at least in any considerable quantity. Other plants have been used for the purpose, but while they are valuable in supplying some organic material which helps maintain the store of humus, they are far inferior to legumes which, in addition to all the other advantages, add usable nitrogen in quantity. Attention should be given to the nature of the soil, and to the kind of legume that will best flourish in the soil; the legume must produce plenty of root tubercles, otherwise the chief value of the green manuring is lost. Green manuring is of particular value in sandy, loose soils, where the humus is scanty, and where the texture of the soil facilitates losses by draining. In such soils so rapid is the draining that it is sometimes difficult to get fertilizers to remain in the soil long enough for their proper assimilation by the plant. The use of legumes, plowed under to furnish a mass of decaying vegetation, greatly improves the texture of the soil and will, in time, give them a fair humus content. By this means very unpromising sandy soils can be reclaimed to a fair condition of fertility. The legumes found to be best adapted to such sandy soils are the cow pea, the *soy bean*, the *velvet bean* and the *crimson clover*.

With clay soils, on the other hand, green manuring must be handled somewhat differently. The density of the soil reduces the ordinary losses by drainage, so that there is less need of the green manuring. The legumes found most useful on such lands are *lupins*, *seradella*, *yellow*, *red* and *crimson clover*, *field peas*, *horse beans* and *vetches*. Because of the density of the soil, decomposition does not progress so freely; hence care must be taken not to overdo the treatment by plowing in too much of the green plant.

The extent of the utilization of the legume after growing, depends upon the completeness of the decomposition and the eventual nitrification of the material that is plowed under. It is possible to plow under such a large quantity of vegetable matter that it will not properly decay, either because the density of the soil prevents sufficient aeration, or because too much acid forms, checking bacterial activities. No value accrues from green manuring unless thorough decomposition occurs. For this reason it is generally best to plow in the leguminous crop before it has fully matured, for then it has assimilated most of its nitrogen but has not become too bulky for proper decay in the soil. Frequently it is best to reap the crop and feed it to cattle, plowing in only the roots and stubble, this giving all the organic matter that can be readily decomposed in the soil. If, subsequently, the manure from the cattle that eat the crop is added as a dressing, the greatest possible use will have been made of the leguminous crop.

The actual value of such green manuring has been demonstrated many times. Sandy soils have been brought under fair cultivation, and depleted farms have been reclaimed to cultivation by the skillful use of this nitrogen-fixing power of legumes, aided by the tubercle bacteria. A constant increase in nitrogen can be brought about thus till the quantity is sufficient for large crops. In one extended series of experiments and observations it was found possible to increase the amount of nitrogen in a soil from .02 per cent. at the start, to .17 per cent. at the end of about twenty-five years, equivalent to 5,000 pounds of nitrogen per acre. In another test the plowing in of a crop of the velvet beans stubble upon a soil subsequently planted with oats, increased the yield of oats from seven to thirty-eight bushels per acre; and in this case the velvet bean crop was reaped and utilized, only the roots and stubble being necessary for the increased yield.

The great lesson to be drawn from this subject is that, by means of the nitrogen-fixing power of the legumes, aided by bacteria in their roots, the farmer has a practical means of maintaining the nitrogen content of his soil at a proper degree for high fertility. There is *no need of purchasing nitrate fertilizers.* The money may be better spent on phosphorus and potash. The cultivation of legumes seems to be the secret of the continuance of agriculture, and if the farmer will only learn the principles and acquire the habit of alternating legumes with his other crops, he may maintain indefinitely a high fertility in his soil, in spite of long-continued cultivation. When in addition to this we remember the fact that the soil minerals are being constantly dissolved in the soil waters, it becomes evident that the farmer is by no means as dependent upon artificial fertilizers as has been supposed.

# CHAPTER X.

## BACTERIA IN WATER.

This subject is of great importance in the relation it bears to the water supplies of cities, and most of its important phases concern only the city water-supply. So far as relates to farm life the subject has interest in two directions: 1. *The purity of the drinking-water.* 2. *The pollution of streams.*

## ABUNDANCE OF BACTERIA IN WATER.

All surface waters contain bacteria. We shall find them whenever we examine the water of the ocean, the brook, the pool or the reservoir. Even rain water contains them, doubtless washed from the air, and the same is true of snow and hail.

The number of bacteria in water is not exactly what would be expected in accordance with our ideas of pure water. The water in the running brook is commonly thought of as purer than that of the stagnant pond. But this is certainly not true; the brook contains more bacteria than the pond, and the supply streams always contain more bacteria than the water of the lake or reservoir. The reason for this is evident. The brooks form the drainage system of the country. The rains wash the whole surface of the land, and all 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. In the lake many of them soon die; others settle to the bottom; the water in the reservoir rapidly becomes purified, and it always contains fewer bacteria than the water brought into it by its supply streams.

The number of bacteria in a body of water will depend upon the extent of the contamination which it receives from sources of active bacterial growth. The actual number 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.

## I. THE PURITY OF DRINKING-WATERS.

In determining the purity of drinking-water we are not so much concerned with the number of bacteria it contains as with the kinds. Very large numbers may be present and yet the water may be perfectly wholesome, while, on the other hand, with only a small number present the water may be deadly. As we shall see in a later chapter, the bacteria in milk may be reckoned by the millions per c.c., and yet the milk may be perfectly healthful; and at the same time bacteriologists regard with suspicion water that contains them only in thousands. The reason for this difference is simple. When milk contains these large numbers they are almost sure to be harmless types; but if water contains even a few thousands, the typhoid bacillus is likely to be among them. It is impossible to condemn any sample of water simply from the number of bacteria which it contains; still the number serves as a useful measure of purity for the following reason: Water that is fairly pure and contains only the bacteria liable to come from ordinary sources seldom contains more than a few hundreds of bacteria per c.c.; it is only water that is receiving contamination from sewage or some other source of decaying filth that contains large numbers of bacteria. Hence, the finding of large numbers of bacteria in a water-supply suggests sewage contamination and the water at once becomes suspicious.

### SEWAGE CONTAMINATION.

Water may receive hosts of bacteria from various sources, but the one great and almost only source of real danger is from sewage contamination. Most of the types of bacteria found in nature and in natural water are perfectly harmless, so that it makes little difference whether they are abundant or few in the water we drink. But this is not true of the types likely to be found in sewage. Sewage contains every form of human excretions; and since it is by means of the excretions that the pathogenic bacteria find exit from the diseased patient, it will thus be easily understood that sewage-contaminated water is likely to contain bacteria which are pathogenic for man. Such water is therefore always dangerous, a fact abundantly proved by the great prevalence of water-borne diseases in cities whose water-supply is contaminated with sewage. When the bacteria in water are in the thousands per c.c., 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 kinds of disease actually distributed by water is very small. Only one of our common diseases is known to be frequently distributed by water. This is typhoid fever, in regard to which the evidence is abundant and conclusive. This evidence need not be given here, but it is sufficient to demonstrate that typhoid fever is very commonly acquired from 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.

Other water-borne diseases are of less importance. *Asiatic cholera* is distributed by water, but this is, at least in this country, of no significance. Certain forms of *dysentery* are probably distributed by water, but little is known of this matter as yet. No other diseases are known to be thus distributed.

**Detection of Sewage Contamination.**—Sewage contamination is a rapidly growing danger. As population increases, the amount of sewage also increases, and it becomes more and more difficult to dispose of it so as to prevent its contaminating the sources of drinking-water. Many a stream formerly used for drinking purposes has had to be abandoned because it has become so polluted with sewage as to be no longer safe. It is thus a matter of prime necessity to find some delicate means of determining whether water is sewage contaminated; for while sometimes the contamination is so great as to be evident, in most cases, especially in wells, it cannot be detected by ordinary examinations. The *chemical analysis* of water gives no sure indication, and the determination of the *number of bacteria* alone is only suggestive. It chances, however, that there is a species of bacterium called *B. coli*, that is a common inhabitant of the human intestine, but is rarely found free in nature or inhabiting pure waters. This *B. coli* is so abundant in feces that it is practically sure to be found in all sewage-contaminated waters, while it is not found, at least to any great extent, in water free from sewage contamination. Since this bacillus is fairly easy to recognize by bacteriological methods, it is not difficult to determine whether or not it is present in a sample of water. Hence this bacterium becomes a test for sewage pollution. A sample of water showing the presence of *B. coli* is almost surely contaminated by sewage, while water free from it is not thus polluted. The report from a bacteriologist that *B. coli* is found means, then, that the water is unsafe, since sewage contamination may at any time infest it with typhoid germs. While *B. coli* itself is harmless, its presence indicates the certainty of danger.

### PURITY OF DRINKING-WATER FROM DIFFERENT SOURCES.

Recognizing sewage contamination as the great source of danger in drinking-water, we may classify waters as pure or safe in proportion to their freedom from such contamination.

**Water from Streams.**—Ordinary streams are the most likely to be sewage contaminated. They constitute the drainage system of the land, receiving sewage from towns, villages, and cities. The amount of sewage, and hence the extent of the danger, depends upon the number of people contributing to produce it and upon the size of the stream. The only safe position to hold, however, is that *all streams upon whose banks are human habitations are polluted and unsafe for drinking.* The question of the purification of such water will be noticed later.

**Wells.**—Next to running streams, wells are the most dangerous source of drinking-water. The extent of the danger depends upon the location of a well and its depth. In very deep wells bacteria have a chance to be filtered out of the water as it passes through the soil before it reaches the well, so that, if care be taken to prevent contamination at the surface, the water is safe. This is always true of artesian wells. But in the shallow well the chance of dangerous contamination is great. The most common, as well as the most dangerous contamination of well-water, comes 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 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 contaminate the vault, and may soon infect the well. The infection may be from water percolating through the soil or from surface currents in time of rains, washing the contaminated water into the mouth of the well. 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. Nearly three hundred typhoid epidemics 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 most critical. 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 one 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.

Since the water in the well is likely to become contaminated with typhoid bacteria, if excreta are thrown upon the ground or are placed in a vault in the vicinity of the well which is used for drinking or dairy purposes, especial care should be taken that no surface rivulets in time of rain should run toward the well. If they do, contamination of the water by surface *drainage* is almost certain.

**Cisterns.**—Cisterns, to hold rain-water caught from the roofs of houses, have frequently been used as a source of water and are, to a certain extent, so used to-day, particularly in localities where the natural waters of the soil are very hard. These cisterns are just as dangerous as wells; sometimes more so. They are generally placed where it is almost sure that they will become contaminated in some way, and actual examination of such cisterns usually shows the *B. coli* present, indicating sewage contamination. Instances are also known where they have been the means of distributing typhoid fever.

**Stored Water in Reservoirs or Lakes.**—These constitute a far better source for drinking-water, and under ordinary circumstances are perfectly safe. Even the water of a contaminated stream will become free from dangerous disease germs when it has been stored for a few weeks. This is partly because the bacteria sink to the bottom, and are not likely to get into the water mains; but it is chiefly because the disease germs cannot live very long in water. Typhoid germs cannot live more than six weeks (usually not so long) in ordinary water, and if it be stored so long before it is used, it will be free from this danger, even though at first it was sewage contaminated. The stored water of reservoirs thus constitutes the best large supply of water. It may be something of a surprise to be told that stored water is purer and safer than running water, but study and experience have shown this to be positively the case.

**Springs.**—These are thoroughly reliable sources of drinking-water if they are properly guarded. The water comes from underground and has filtered through the soil for unknown distances. There may be cases, it is true, where the filtering is through only a thin layer of soil, insufficient to purify. But if such cases exist, they are very unusual, and examination shows spring-water to be free from disease germs, unless carelessly contaminated after the water leaves the soil. The spring should be classed with the artesian well in this respect, and is the best source of water that can be obtained.

**Filtered Water.**—The rapidly extending contamination of waters by sewage and the growing demand for water have led to development of methods of filtering such contaminated water in large quantities. This is done by passing it through layers of sand which are constructed in such a way as to remove most of the bacteria. These filters are in wide use to-day by cities that have to depend upon a contaminated supply. The bacteria are not wholly removed from the water, but so nearly that practically all dangers disappear. Experience has shown that the use of filters very greatly reduces the amount of typhoid fever in cities dependent upon a contaminated water-supply. It is also found that the purified water improves the general health of the community, quite apart from the decrease in typhoid fever.

**Ice.**—Ice, though not thought of as water, in summer months is put into drinking-water to cool it. The ice melts and whatever bacteria are in it are liberated and swallowed with the water. It has been a belief that freezing purifies water, so that many have been perfectly willing to use ice from ponds whose water they would not drink. It is a very wide practice to cut the year's ice supply from sewage-contaminated streams, and from places where no one would think of drinking the water: *e.g.*, from the Hudson River, below Albany. It has become a matter of great importance to know whether freezing does purify such ice and render it safe. The subject has been most carefully investigated, with the following conclusion. Ice does in a measure purify itself in freezing, but not wholly. If typhoid bacilli are in the water, they may be found in the loose snow ice at the top of the frozen layer, but there are very few, if any, in the clear ice below. After the ice has been stored for a while the typhoid bacilli become less and less abundant, and after a few weeks they practically disappear. Even after months of freezing, however, a few may sometimes be found, so that no ice from contaminated water can be guaranteed as absolutely free from them even after six months' storage. But the number that resists this storage is so extremely small that the ice is as pure as filtered water. No cases of typhoid fever have been definitely traced to such a source, though one or two doubtful cases have been so attributed. In general, then, it appears that stored ice is safe, provided it is free from snow ice and has been stored at least two or three months.

## II. THE CONTAMINATION AND PURIFICATION OF STREAMS.

The sewage contamination of streams has been increasing year by year, until many a stream that was clear and limpid thirty years ago is now a vile collection of filth. Until some other means of disposing of sewage is generally adopted, this pollution must continue to increase. There has been a widely held belief that running water purifies itself, and that these streams rapidly become free from their pollution. This is partly correct and partly erroneous. A stream does not purify itself by running, but there is always a tendency for water to become pure, and in time sewage contamination quite disappears from water, whether running or stagnant. The best studied example of this is in the Chicago Drainage Canal. Recently the city of Chicago converted the Illinois River into a drainage canal for the great 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 some 300 miles. It empties a few miles above the point where St. Louis takes its water-supply, and naturally it excited considerable alarm in the latter city. A careful examination of the bacteria in the river shows that there is a constant decrease in numbers as the distance from Chicago is increased, and when it finally empties into the Mississippi, all of the bacterial contamination from Chicago has disappeared. In this flow the river has purified itself of sewage bacteria. In other examples when the polution is less a flow of even ten miles largely purifies the water.

Evidently the phenomenon is practically identical with the bacterial purification of sewage, modified by the different conditions. The following factors have been advanced as explaining it:

The dilution of the water by tributary streams. This doubtless accounts, in part, for the decrease in number of bacteria 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.

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 streams. But its action in muddy streams must be very slight.

Other living organisms in the water have a deleterious action. Microscopic animals certainly destroy great quantities of bacteria, actually feeding upon them, and they may be one of the efficient means of the self-purification of streams.

It is well known that bacteria are generally heavier than water and that they will slowly sink to the bottom. In slowly flowing streams sedimentation probably plays an important part.

The food in the water is of course used up either by bacteria or some other organisms, and finally becomes insufficient to support bacteria life.

Although these factors do not wholly explain the purification of streams, it is certain that sewage-polluted streams are in time freed from most of their bacteria. Commonly, however, such streams continue to receive contamination all the way to their mouth, and *never again become fit for drinking purposes*, unless the water is subsequently purified by filtering or otherwise.

# PART III.

## BACTERIA IN DAIRY PRODUCTS.

## CHAPTER XI.

### BACTERIA IN MILK.

In no phase of farm life has bacteriology made such profound changes as in dairy methods, changes so great as to amount almost to a revolution. Many dairy methods of twenty-five years ago have been abandoned and many new ones adopted, chiefly through the discoveries of bacteriologists.

### BACTERIA IN MILK WHEN SECRETED.

Milk, when secreted from the mammary gland of a healthy cow, is generally, if not always, free from bacteria. It has been no easy matter to demonstrate this fact, since there are bacteria in the milk before it leaves the udder. But a sufficient number of careful experiments have shown that these really come from the outside, entering the udder through the milk ducts, and that they do not come from the milk glands.

If the cow is not in perfect health her milk may not be free from bacteria. When a cow is suffering from generalized tuberculosis, or when she has this disease localized in the udder, her milk, when secreted, is sure to contain bacteria. Indeed, any udder infection due to bacteria, 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. Milk from a cow suffering from udder trouble is no longer pure milk. It may contain *tuberculosis bacilli*, or, in cases of inflamed udders, it is likely to contain *pus*, together with considerable quantities of chain-forming *streptococci.* These should not be present in good milk, and there is reason for believing that they are the cause of certain illnesses in man.

Confining our attention for the present to milk from healthy animals, we notice that, if we could keep bacteria out of the milk, none of the ordinary changes, not even the souring which is so nearly universal in normal milk, would take place. Indeed, milk which is free from bacteria will remain visibly unchanged for an indefinite time. It is not, however, absolutely free from subsequent chemical changes, since there is present in the milk an enzyme which produces slow changes. This enzyme, called *galactase,* is secreted by the milk gland with the milk, and may thus be said to be part of the milk. It can slowly convert the casein of the milk into soluble proteids. Its action is very slow, however, and seemingly of no significance, except in the ripening of cheese. At all events, none of the ordinary fermentations appearing in milk are attributed to this galactase or to any other part of the milk itself, but are all due to microörganisms. We may, therefore, take as a starting-point these two highly important facts: 1. Milk from healthy cows will, if it could be kept free from bacteria, show none of the ordinary milk fermentations. 2. All of these fermentations are due to microörganisms that get into the milk after the milk is secreted from the mammary gland.

## SOURCES OF MILK BACTERIA.

Recognizing that milk is germ free when secreted from the milk gland, 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, it may contain bacteria to the extent of many thousands per c.c. But this is frequently and, indeed, commonly the case. The number of bacteria in freshly drawn milk varies greatly with the conditions existing in the dairy. There may be only a few hundreds in each c.c., or, under exceptional conditions, a smaller number still; but it is much more likely that the milk, by the time it has been removed from the stall, contains many thousands of bacteria.

Since all the troublesome changes which occur in milk and make it such a difficult product to handle, are due to the action of bacteria upon the milk, it is to the interest of the dairyman, the milk distributor, and the consumer to have as few bacteria as possible. Therefore, it is 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 precautions in the production and caring for the milk that will materially reduce their number. A slight attention given at the right point will produce better results than a much greater attention unintelligently applied.

**The Cow.**—The first source of milk bacteria is the cow. Although the healthy 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 ducts ready to be washed into the milking pail with the first jet of milk. At the close of the milking enough milk is left in the ducts to furnish food for bacteria, which may get in through the external opening; and between the milkings, at the warm temperature of the cow's body, these bacteria multiply (Fig. 29). Bacteria are thus always abundant near the opening of the teat, although the inner parts of the duct contain smaller numbers. They are sure to contaminate the first jets of milk drawn, so that this first lot, 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 may be actually sterile when it leaves the duct. While this is not always the case, the last milk is always purer than the first.

From these facts it follows that milk is sure to contain bacteria by the time it reaches the mouth of the milk duct. While, by the use of special precautions, small amounts of milk can be drawn so carefully as to avoid all bacteria, this is an impossible procedure in dairying, and the dairyman must recognize that there is no practical means by which he can obtain sterile miik. Indeed, it would avail but little if he could, for it would be contaminated almost at once from other sources.

The exterior of the cow is an even more prolific source than the milk ducts. Her skin, even when kept in fairly good condition, is never very clean, and will always hold more or less dirt and dust laden with bacteria. The cow in many poorly kept dairies is rarely,

FIG. 29.—A cow's udder cut across and showing the milk ducts.

if ever, cleaned; her flanks, tail, and skin become covered with a coating of manure, until the amount of filth thus attached to the animal is surprisingly great. All of this filth is laden with bacteria, and during the milking process numerous particles of it are constantly shed from her body by the movements of her flanks, by the switching of her tail, and by the rubbing of her skin by the milker. Since the milk-pails are generally widely opened, they receive a large amount of this filth, which consists of almost every conceivable kind of material. Besides excrement there are insect wings, grass, straws, hairs, and many other small particles, all bacteria laden.

**Milk-vessels.**—The next prolific sources of bacteria are the milk-pails and other dairy vessels, in which the bacteria remain alive from one milking-time to the next. On an ordinary farm these vessels are rarely, if ever, washed bacteriologically clean; for washing in hot water with subsequent drying in the sun is wholly insufficient to remove the bacteria. They are sure to remain in the vessels, clinging in 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 in the next lot of milk drawn. The ordinary farm has no really effective means of washing milk-vessels. Even live steam, as ordinarilly used, a few seconds on each pail, will not do it completely. 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. So far as numbers are concerned, those in the milk-vessels probably form the largest source of bacterial contamination.

**The Air.**—Other sources furnish bacteria to a less extent. Some doubtless come from the air. In earlier years it was thought that this was a great source of contamination, but now we know that the air bacteria are ordinarily of little importance, although sometimes they may be a source of trouble. Fresh, out-of-door air does not contain many bacteria, and if milking could take place in the open air, this source of contamination would be almost excluded. In a close barn, however, conditions are very different. The motions of the crowded cattle dislodge bacteria from their skins. Hay, dirt, cobwebs, soiled bedding and other dry dust-producing materials are allowed to accumulate, and particles from any of these sources are likely to be dislodged and float for a time in the air. The general manner of feeding the animals is even a larger source of contamination. If dry hay or other dry food is thrown down in front of the cattle, a large amount of dust will arise and spread through the air of the stable. Such dust is crowded with bacteria, many of which are alive and will settle into the milk-pail during milking. The common practice of keeping cattle in the same room where they are milked is thus very productive of a large source of bacterial contamination.

**The Milker.**—Of late years it has become evident that the bacteria coming from the milker or other persons in the dairy are among the most serious. This is not so much because of the number of bacteria that may enter the milk from this source, but because of their types. In ordinary dairies the milker rarely makes any special toilet before milking, but is liable to perform this task in old, soiled clothing, with no attempt at cleaning his hands and face. Under these circumstances, while, so far as concerns numbers, he is not so great a source of bacteria as the cow, some of these organisms are sure to fall from his hands or clothes into the milk-vessels, especially if he adopts the filthy habit of *wet milking*. The number of bacteria from such a source is, probably, not great, and does not add materially to the bacterial content. But in one respect these bacteria assume a more important significance. The bacteria which produce diseases in one animal do not necessarily produce diseases in other animals. Those which produce diseases in cattle, with some exceptions (tuberculosis), do not usually have the same effect on man. But it is evident that any disease germs that may be present in one man are just the kind that can develop in any other human being. Therefore, bacteria contamination from human sources is more dangerous to other human beings than any infection from animals. For this reason the bacteria which enter the milk from the milker are liable to be more dangerous than those which come from any other source.

## TYPES OF BACTERIA FOUND IN MILK.

Many different types of bacteria get into milk from these various sources. Some of them are useful, some are of no particular significance, some are troublesome to the dairyman though not distinctly harmful, while some are decidedly injurious either to the dairy products or to man. A knowledge of these types is of primal importance to an understanding of their relations to dairying. The more important types are given in the following pages. For clearness and convenience we may divide them into three groups: 1. *Normal milk bacteria.* 2. *Abnormal milk bacteria.* 3. *Disease bacteria.* The first two concern dairy problems only, while the last concerns the relation of milk to the public health.

### 1. NORMAL MILK BACTERIA.

Under this head we refer to types of organisms that are practically always present in milk and cannot be avoided by any ordinary means. They do not, of course, belong to the milk, but they are so widely distributed in barns and dairies that practically they cannot be avoided. There are very many different kinds among them, several scores at least having been described in milk from various localities. But they may be conveniently grouped and studied under three heads:

**Lactic Acid Bacteria.**—The most common fermentation of milk is its souring, a phenomenon so universal that it has been supposed to be a change belonging to milk itself. But it is now known to be produced always by the growth of bacteria. These organisms transform the milk sugar into lactic acid, a change that is sometimes expressed by the formula $\underset{\text{(Sugar)}}{C_6H_{12}O_6} = \underset{\text{(Lactic acid)}}{2C_3H_6O_3}$; but this equation fails to express the real nature of the change that occurs, which is much more complex. The fundamental phenomenon, however, is that the milk is made sour by the formation of lactic acid out of milk sugar. This is first seen in the appearance of a sour taste and later in the curdling. Milk contains its casein in a state of partial solution, but if the milk is made sufficiently acid the casein can no longer remain in solution and is precipitated. The precipitation of casein is the curdling of milk, and it occurs when we add to it any kind of acid. In the normal souring of milk, when the acid reaches 0.7 per cent. to 0.9 per cent. the milk curdles.

*The Souring of Milk during Thunder Storms.*—The only natural agent that causes souring is the growth of microörganisms. There is, however, a wide-spread belief that thunder storms will sour and curdle milk. This belief rests upon a mistaken interpretation of observed facts. It is certainly true that milk is frequently found sour after a thunder storm, and the natural interpretation is that the electricity of the storm has produced the souring. A careful study of the phenomenon has shown that this inference is incorrect. Electricity, in the form of a current or electric sparks, has no power to sour milk; and, further, if milk is kept properly cooled, the thunder storm has no effect upon it. Moreover, if milk has been deprived of bacteria, it will keep indefinitely, remaining 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 thunder and lightning have no direct effect upon the souring of milk.

What, then, brings about the frequent souring of milk during thunder storms and the wide-spread belief that thunder is the cause? The answer seems to be the simple one, 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 that 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, frequently in the afternoon. 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 may be 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.

*Varieties of Lactic Acid Bacteria.*—A very large number of apparently different kinds of acid-forming bacteria have been obtained from milk. The different varieties all agree in producing lactic acid, but differ in some other slight points, recognized by bacteriologists. To what extent these many varieties should be combined so as to make a small number of groups, and to what extent they should be kept separate, is a matter over which there is as yet no agreement. It is known that the same bacterium can show differences under different conditions. The power of a bacterium to curdle milk may be increased by proper laboratory methods, and when we find that, of these numerous described types, some differ from others only in the rapidity with which they curdle milk, we naturally infer that the different results are brought about by the same bacterium growing under slightly different conditions. Those who have given the most attention to the subject are convinced that the lactic acid-forming bacteria that have been described must be reduced to a few types, though no one yet ventures to say how few.

Among them are three well-marked types, quite radically distinct from each other, and each playing an important part in the dairy. Two of them are the dairyman's friends, while the other is always his foe.

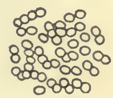

FIG. 30.—*Bacterium acidi lactici* (*Streptococcus lacticus*).

I. *Bacterium acidi lactici*, *Streptococcus lacticus.*—These two names are applied to the same organism. The first name was originally given to it when it was described as a short rod (Fig. 30). Recently it has been claimed that it is not a rod, but a coccus, and with this conception the second name has been given as the only correct one. Which of these two names is more correctly applied has not yet been settled. But whatever its name and microscopic appearance, it is a quite well-known organism, with a distinctive action on milk. This type of lactic acid bacterium grows better when not in free contact with the air. It grows better under the surfaces of media than on the surface, failing to make any visible growth on the surface of potato and scarcely any on agar culture slants (see page 313). In milk, however, it grows

13

with great rapidity, soon turning it acid. The rapidity of the acid production is variable with different cultures. It is so rapid in some cases that, if the specimen be placed at body heat, it will curdle in six hours. With other cultures, the curdling under similar circumstances would not occur for three days; with some cultures curdling never occurs. All specimens of milk, however, become acid, although not always sufficiently to precipitate the casein. Between these extremes every conceivable grade may be found in cultures that are, in other respects, identical; and they represent, doubtless, one type, differing in its power of producing acid.

To this type belongs the largest number of bacteria known to cause the souring of milk. Most of the butter starters and cheese starters (see page 189) belong to this general class. But the name represents a type rather than any single organism. In other words, *B. acidi lactici* represents a group of closely allied varieties. If we are asked whether it represents a species or a collection of species, we must answer that no one knows what is meant by the term species among bacteria. The term species, whatever its significance among higher animals and plants, seems to have no meaning among bacteria. It is impossible, therefore, to say whether *Bact. lactis acidi* is a single species or a group of species; and we may be content simply to recognize under this name the group of lactic acid bacteria which most commonly cause milk souring and which comprise varieties that, while agreeing in most respects, have slightly differing characters.

The type of milk curdling produced by this organism is quite easily recognized. The milk becomes strongly acid, and turns into a hard curd, without any trace of gas bubbles, and without the separation of whey: it has a clean, sharp taste, and no odor (Fig. 31, *b*). This type of curdling has been recognized as a desirable one by the dairyman, since it is most favorable for dairy processes and is consistent with the production of the best grades of butter and cheese. This organism grows readily at temperatures from 60° to 100° F., growing more rapidly at higher temperatures. At a temperature of about 70° it grows with great rapidity, and at this temperature it seems to be more vigorous than any other bacterium ordinarily found in milk. For this reason, as we shall presently see, milk kept at 70° becomes, in a short time, almost completely filled with this species of bacterium at the expense of all the others that might originally have been there. This type of organism is a friend to the dairyman, unless he be a milk man who wants to deliver his milk sweet.

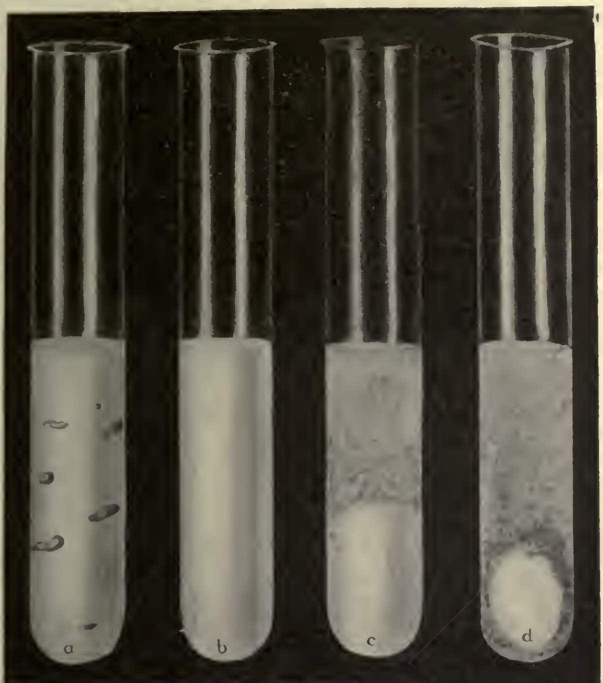


FIG. 31.—Showing the action of different types of bacteria upon milk. *a*, the aerobic type (*Bact. aerogenes*); *b*, the anaerobic type (*Bact. acidi lactici*); *c* and *d*, the peptonizing type, with the curd in different stages of digestion.

II. *Bacterium aerogenes.*—This type of lactic acid organism is not so abundant in milk as the first, although it may be found more or less abundant in most samples of milk. Microscopically, it is practically indistinguishable from the first variety, but it differs in two pronounced points (Fig. 32). The first is that it grows luxuriantly in contact with the air or oxygen, while the first variety does not. The aerogenes type, therefore, grows abundantly on the surface of culture media, like potato or agar. The second and more noticeable point is the fact that it produces a fermentation of milk-sugar, giving rise to a quantity of *gas*. When inoculated into milk it causes a souring which is rapidly followed by curdling, the rapidity of the curdling varying in different specimens. The curd which is produced differs very much from that of the first type of lactic acid bacteria. It is always more or less filled with gas bubbles, and when care is taken to obtain a typical curd, it appears crowded with holes, which represent the bubbles of gas foimed by the organism (Fig. 31). The whey commonly separates in a short time from the curd, and the final appearance is strikingly different from that of the curdled milk produced by the first type.

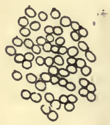

FIG. 32.—*Bact. aerogenes.*

The production of gas is the cause of the ruin of vast quantities of cheese. If milk, when it is made into cheese, contains a considerable quantity of these bacteria, instead of the more common type, the bacteria grow and develop gas, the cheese becomes filled with the bubbles, swelling more and more, until it finally results in what is known as *swelled cheese* (Fig. 33). At the same time that this swelling occurs, the flavor of the cheese becomes unsatisfactory, so that the swelled cheese may be practically worthless. These organisms have been the cause of the loss of enormous amounts of money to cheese makers. In butter-making they are not so disastrous, but here, too, their presence is undesirable, for they sometimes produce unpleasant flavors in the creams, resulting in an inferior grade of butter. This type of organism, therefore, is decidedly the dairyman's foe.

Several varieties of bacteria belong to this general type of gas-producing organisms. Among them is *B. coli*, which is very similar to *B. aerogenes*, except that it is motile. This, an inhabitant of the intestine (see page 130), is very commonly found in milk.

III. *Bacillus Bulgarious.*—A third radically different type of acid bacterium is one that has recently come into prominence in various forms of beverages composed of soured milk. In certain parts of

Europe sour milk is commonly used as a beverage, and has been highly recommended as a healthful drink. It is claimed that the lactic acid, present in abundance in these sour milks, has a very beneficial action in the intestine, preventing the growth of the common putrefactive germs, and serving in general as a corrective for various intestinal disturbances. It is not ordinary sour milk that is

FIG. 33.—Curds from two lots of milk soured by a gas-forming and a non-gas-forming bacterium.

especially recommended for this purpose, but a special form, found most common in Bulgaria, and used very widely as a drink in that country. Such milk contains a variety of lactic acid bacterium very different from the two above described, and deserving to be called a distinct type. It is in the form of a long, large rod (Fig. 34), which frequently forms long chains. It differs from the more common type in producing a much larger quantity of lactic acid. The ordinary sour milk organisms produce from 1.2 per cent. to 1.5 per cent. of lactic acid, and then cease to grow; but this Bulgarian type produces as much as 3.0 per cent. of acid, double the amount produced by the common type. This type is very vigorous and when growing in milk will soon destroy other bacteria. Quite a number of commercial products containing this organism are now on the market, and are used somewhat widely in making a fermented milk.

FIG. 34.—*B. Bulgaricus.*

Though originally found in Bulgaria, bacteria that agree with it in all essential respects have been found elsewhere. It has been found in this country as well as in Europe, but thus far on grain rather than in milk. Several of the fermented milks found in different countries appear to contain representations of this type of lactic acid organism.

**Peptonizing and Rennet-forming Bacteria.**—Occasionally a dairyman is puzzled by a somewhat unusual phenomenon: his milk curdles, but remains sweet. This is apt to occur in the fall or spring when the food of the cattle is being changed, and is due to a class of bacteria that secrete enzymes. The bacteria in question really secrete two enzymes, one of which is similar to *rennet*, secreted by the stomach of a calf, and the other is similar to *trypsin*, secreted by the pancreatic gland of man and other animals. Hence these bacteria secrete two enzymes that have actions essentially like those of digestvie fluids.

When this class of bacteria grow in milk, both of these enzymes act upon it. The rennet enzyme shows its effect first, and causes the milk to curdle; but since no acid is produced by these organisms this curd is not sour. The curd is also softer than that produced by the lactic acid bacteria. The phenomenon is sometimes called *sweet curdling.* After a short time, usually two or more days, the second enzyme begins to show its effects. This, acting like a digestive fluid, changes the nature of the casein from an insoluble to a soluble condition, and as fast as this occurs the curd is dissolved in the liquid of the milk. The curd thus disappears, as the casein is dissolved, and, finally, the whole curd may be dissolved so that the milk becomes liquid again (Fig. 31, *c*, *d*). But it is a totally different product from the original milk, since it no longer contains casein, but only the digested and dissolved products of casein. This softening and dissolving of the curd is so much like the digestion that goes on in the intestine of animals, that it has commonly been called *digestion*. The bacteria that produce it are also sometimes called the *peptonizing* bacteria, since they produce peptones among the soluble products that come from the digested casein.

Still another term is applied to these bacteria. One of the common culture media used in bacteriological work is solidified

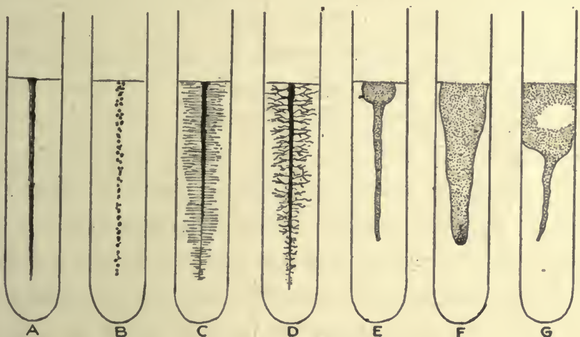


FIG. 35.—Gelatin stab cultures. *e, f,* and *g*, show liquefaction; *a*, filiform; *b*, beaded; *c*, villous; *d*, arborescent; *e*, napiform; *f*, infundibuliform; *g*, stratiform.

with gelatin. Now, many kinds of bacteria cause the solidified jelly to become liquid (Fig. 35 E. F. G.). The liquefaction of the gelatin is due to the same enzyme that causes the digestion of the milk curd. Hence, we find that the bacteria that liquefy gelatin commonly have the power of dissolving milk curd. The term *liquefiers* is applied to them, since they liquefy both curdled milk and gelatin. This type of bacteria is very abundant, and in the number of kinds exceeds the number of lactic acid bacteria. They are found profusely in the dairy, especially in the filth that gets into the milk. Practically every sample of fresh milk will contain them in greater or less numbers. But it is very doubtful whether this type of bacteria is of much or of any significance in ordinary dairying. Although the varieties may be numerous and their number may be great in fresh milk, 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 enzyme 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, 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, as in cases of sweet curdling. This class of bacteria plays an important part in the changes which may take place in so-called sterilized milk, which has been heated to a temperature of boiling water. Such milk still contains a considerable number of spore-bearing bacteria that resist this temperature. The milk does not sour, inasmuch as all lactic acid bacteria are killed, since they never produce spores. The class of enzyme-forming bacteria, however, are very commonly spore bearers, and resist the temperature of boiling water. Milk which has been boiled, therefore, not infrequently undergoes changes which affect its taste and its chemical nature, due to the class of bacteria here considered. Occasionally they are of significance in cheese-making. During the long ripening of cheese they have a better chance to grow than in milk. Whether they have much influence upon hard cheeses seems doubtful, but in the ripening of soft cheeses they sometimes produce very bad results, causing much loss to the cheese-makers. While, therefore, they are of little importance to the one who handles milk, they play a considerable part in the making of cheese.

This class of liquefying bacteria usually produces no acid; but there is a small group of the same class that differs from the others in producing both a digesting enzyme and an acid. They are sometimes called *acid liquefiers*. It has been thought that they play a part in the ripening of cheese, but this is by no means certain and in general they are of little significance.

**Neutral Types.**—Among the normal milk bacteria there are many that appear to have no noticeable action on milk. They produce no acid and, apparently, no enzyme. When they grow in milk they produce no noticeable effect upon it. So far as we can see they are of no significance in dairying. Nor do we have any reason for believing that they have any pathogenic effect upon persons drinking the milk. They are, therefore, simply classed as neutral types, and need not here be further considered.

## ABNORMAL MILK BACTERIA.

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 nearly always contain bacteria of the peptonizing class, the following kinds of bacteria are not commonly 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, one which may be prevented. While souring of milk cannot be prevented by any practical means, because of the universal distribution of lactic acid bacteria, these types of troublesome infections may be prevented if sufficient care is taken in regard to cleanliness, and they may be checked if the dairyman simply learns whence the contamination arises. For these reasons, in practical dairying, it is a matter of special importance to understand their sources.

**Slimy Milk.**—Slimy milk is not an uncommon trouble in the dairy. It is sometimes produced by a diseased condition of the cow, slimy milk being a common characteristic of *garget.* In such cases the milk is slimy when drawn. Such milk is certainly not fit to drink.

In other cases the milk is not slimy when drawn, but appears like normal milk. After a few hours, at about the time when milk would usually sour, instead of becoming acid in the normal way, it becomes viscid, and finally it may be so slimy 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. In some countries, indeed, such slimy milk is a favorite beverage; but in this country most people, not wishing to drink slime, 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 follow, such infections may generally be traced to some common source of distribution. For example, a central creamery, receiving such slimy milk from some patron, may distribute the trouble over the whole patronizing district by returning to the farmers the milk vessels not properly sterilized.

FIG. 36.—*B. lactis viscosus;* the common cause of slimy milk (*Ward*).

The cause of this sliminess is the growth of bacteria. Several different kinds of bacteria have been discovered with this property. The best known of them, and probably the most common, is one that has been named *B. lactis viscosus* (Fig. 36). This has been found to be the cause of the trouble in Europe, and a similar if not the identical organism has been found in America. It appears to be a very vigorous organism, and, when once present, will grow so rapidly as to make the milk slimy in spite of the action of the ordinary acid-forming bacteria that may be present.

To understand the sources from which this troublesome organism is derived may be a matter of great importance to a dairyman. Three sources have thus far been detected: 1. Sometimes it may come from water used in washing the milk cans or, more likely, from the water in which the cans have been standing to cool the milk. 2. It may come from the udder of the cow; perhaps a single cow in a herd being thus infected and her milk contaminating that of the whole dairy. 3. Slimy milk bacteria have been found in the dust of the air in dairies. If, therefore, a dairy is troubled with slimy milk, the dairyman should look first to his water-supply, especially if the milk has been cooled by standing in cans in the water. Then he may turn his attention to the food of the cows to see if he has any special lot of hay or other food that holds the troublesome organisms. This may be tested by changing the food for a time. Lastly, he will do well to keep the milk of the different cows separate for a few days, to see if the trouble can be traced to any particular cow. Having once found the source, the remedy is simple: either by applying some method of disinfection at the source of infection, or seeing that infected water does not come in contact with the milk cans, or removing the milk of the cow that is at fault, or changing the food.

**Bitter Milk.**—Next to slimy milk, perhaps bitter milk offers the most trouble to the dairyman. Three quite different sources of bitter milk can be distinguished: 1. *The cow.* She may give bitter milk because of improper food, such as lupines, which will impart a bitter taste. Bitter milk is also quite common in a late stage of lactation. These types 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. 2. *Microörganisms.* In such cases the bitterness is a matter of slow development. The milk, when drawn, tastes as usual, and the bitterness appears after standing a few hours, increasing in intensity until, in a short time, it is at its maximum. In these instances the bitterness is produced by microorganisms which grow in the milk. Two or three varieties of bacteria have been described that have this power, and have been the cause of a troublesome bitter fermentation in milk (Fig. 37, *a*). The source of the trouble has been traced, in one case, to organisms in the udders of a single cow in a herd. Bitter milk is not of very common occurrence. In cheeses the development of a bitter taste is much more common, doubtless because, during the ripening, the bacteria have a longer time to develop their bitter products. A bitter taste in cheese has been in some cases traced to bacteria, and in one extended series of troubles, which affected the cheese-making

Fig. 37.—Organisms producing bitter milk. *a*, A coccus; *b*, a yeast *Torula amari* (*Harrison*).

over an extensive territory, the cause was found to be due to a yeast that infected the factory and the utensils used in cheese-making (Fig. 37, *b*). The remedy in such cases lies in a thorough disinfection of the cans, vats, etc. 3. *Microörganisms in boiled milk.* It may frequently happen that boiled milk will become bitter. The boiling destroys the acid-forming bacteria, but leaves alive some of the spore-producing organisms. These may subsequently develop and produce bitter products. This type of bitter milk is of little significance, however, since the practice of keeping milk after it is boiled has almost disappeared.

**Fermentations Changing the Color of Milk.**—The first bacterial fermentation of milk clearly described was that of *blue milk*, noticed and studied over sixty years ago. This trouble has, therefore, an historic interest because of its connection with the early development of bacteriology. It has little practical interest, however, since it is of very rare occurrence. It is caused by a well-known bacterium named *B. cyanogenes* (Fig. 38) that has been found in this country as well as in Europe. When the organism is inoculated into milk it produces no visible effect until the milk is two or three days old. Then blue patches appear in it that extend as the milk sours, until the whole becomes of a sky-blue color. As a dairy infection it is very unusual, and no well-marked case of such blue milk infecting a dairy has apparently been reported from this country.

FIG. 38.—The organism producing blue milk. *B. cyanogenes.*

Other fermentations producing pigments are also reported by bacteriologists. *Red milk* has occasionally been mentioned. Milk may sometimes be red when it is drawn from the cow because of the presence of blood, due to some trouble in the udder. But there are also types of red milk developing slowly, and due to the growth of bacteria. *B. prodigiosus*, *B. erythrogenes*, and *B. lacto rubifaciens* are three species that have been described as having this power. None of them is of practical importance in the dairy. Red spots in cheeses do sometimes result from the growth of bacteria, but red milk is the rarest of occurrences. In addition to these we sometimes hear of *yellow milk*, *orange milk*, *green milk*, *amber-colored* *milk*, and *black milk*. By carefully selecting the varieties of bacteria, and inoculating them into tubes of sterile milk there may be produced samples of milk, each showing different colors, all the colors of the rainbow being thus obtained. All of these phenomena do certainly occur in the bacteriological laboratory, and all are produced by the growth of different species of microörganisms. But they are usually procured by inoculating sterile milk with particular kinds of bacteria and allowing them to act on the milk for many days. They are not ordinarily dairy phenomena, and will hardly ever be likely to appear as dairy infections. They are of scientific rather than of practical interest.

**Miscellaneous Faults.**—There is a considerable list of troubles appearing occasionally in milk that are due to the growth of unusual bacteria. Some of these are the following: *Premature curdling*, the milk curdling too quickly and without souring; *failure to curdle* at all, even after several days; *bad tastes* such as *turnip taste*, *rancid taste*, *putrid taste; difficulty in churning; bad tasting sour milk; yeasty smell; soapy consistency*. These faults are all unusual and in all cases the growth of unusual bacteria is the cause. The remedy is always the same, more care in cleanliness and more thorough sterilization of the milk-vessels. Any sample of milk in which lactic acid bacteria fail to develop normally will be sure to show some trouble due to the growth of bacteria that happen to be present and whose rapid growth is not prevented by the acid-forming bacteria. Sometimes such troubles may be remedied by the addition to the milk of a culture of ordinary lactic acid bacteria.

**Alcoholic Fermentation of Milk.**—Most sugar solutions will readily undergo an alcoholic fermentation, but milk sugar does not easily make this change. It may be converted into lactic acid, but not readily into carbon dioxid and alcohol. Hence an alcoholic fermentation of milk is not a normal phenomenon, although it may be produced by the addition of a little cane-sugar to the milk. The possibility of making milk undergo an alcoholic fermentation by the addition of yeasts is made use of in the manufacture of *kummys*. This beverage was originally prepared by the Arabs from mare's milk, which will normally undergo an alcoholic fermentation; but now an imitation product is widely made and used, prepared from cow's milk. A small quantity of sugar is added to milk and some common baker's yeast. An alcoholic fermentation soon begins, and the fermented product is kummys. Various modifications of this general process are adopted by different makers, for kummys has become a commercial article.

In addition to these there are several other types of beverages made from milk in common use among different nations, in the production of which alcohol is formed. One, known as *kefir*, has long been used in the Caucasus mountains. The fermentation

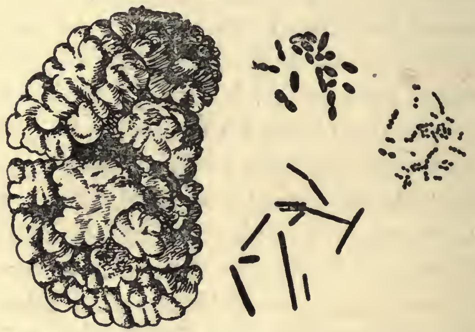

FIG. 39.—A large-sized kefir grain and the three species of bacteria of which it is composed (*Freudenreich*).

is brought about by adding to the milk what are known as *kefir grains* (Fig. 39). 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 from the fermented milk 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. In Egypt the people use a fermented milk called *leben*. Another, called *mazoon*, is common in Armenia. The Turks have one they call *yoghourt*, and in Sardinia still another is found with the name of *goiddu*. In all these cases the beverage is prepared by the use of ferments that the people keep on hand, whose original sources are unknown. These ferments, so far as they have been studied, prove to be based upon the combined action of yeasts and bacteria. Very likely the bacteria change the milk-sugar into a fermentable form and at the same time sour the product. The yeast is probably responsible in all cases for the alcoholic fermentation proper, although in some the milk souring by the bacteria is the primary feature, while the action of the yeasts is secondary and is not regarded by some as at all essential to the product. In several of these products the type of lactic acid organism mentioned under the name of *B. bulgaricus* is present. The beverages are generally regarded as more digestible than ordinary milk.

## GROWTH OF BACTERIA IN MILK.

The number of bacteria that may be in any sample of milk is, in the first place, dependent upon the number and variety that get into the milk during and after the milking. But the original contamination is only a small factor in determining their number at any subsequent time. Milk furnishes excellent food for bacteria, and when drawn from the cow it is warm. Hence a rapid multiplication of bacteria begins; but although the milk furnishes such an excellent medium for them, they do not begin to multiply at once. For a few hours their number remains the same or even decreases. There seems to be something in fresh milk that injures them. Whatever this may be, its influence ceases after a few hours. This power of checking bacteria growth is sometimes called the *germicidal power* of milk, and it lasts from three to twenty-four hours, according to temperature, being less at higher temperatures. After it has passed, the bacteria begin to increase rapidly and the number present at any later period is more dependent upon the extent of their multiplication than upon the original contamination. The rate of multiplication of all bacteria depends upon temperature. The majority of milk bacteria grow best at temperatures between 60° and 100° F., and, generally speaking, they grow more rapidly at the higher temperatures. The effect of temperature is shown by the following example:

| | | |
|---|---|---|
| Fresh milk contained . . . . . . . . . . . . . | 6,525 bacteria per c.c. | |
| After 25 hours at 50° the same milk contained, | 6,425 bacteria | " |
| After 25 hours at 70° the same milk contained, | 6,275,000 bacteria | " |

In this example it is seen that for twenty-five hours the bacteria in milk kept at 50° did not multiply at all, while in that kept at 70° they multiplied one thousand fold. It is not common to find such a striking difference, but in all cases there is a very marked contrast.

## PROTECTIVE ACTION OF LACTIC ACID BACTERIA.

If ordinary proteids, like eggs or meat, are left undisturbed to the action of bacteria, they will putrefy. Milk also contains a proteid, casein, which is just as liable to putrefaction as other proteids. But under ordinary conditions it does not undergo this unpleasant change. Milk sours, but rarely putrefies. The reason for this is found in the power of the lactic acid bacteria to restrain the growth of other species. Almost from the start, the lactic acid bacteria in milk grow more rapidly than the other types, and as they become more abundant, they prevent the other kinds from growing; they thus effectually restrain the growth of the putrefactive bacteria, so that milk that has begun to sour will not putrefy. This is really a very useful function, for, whereas *soured milk is wholesome, putrefied milk is not wholesome,* and the lactic acid bacteria thus protect the milk from a decomposition which would be far worse than souring. It has also come to be a recognized fact that many of the troublesome faults in milk may be remedied by using a culture of lactic acid bacteria. In cases of bitter milk, of premature curdling, and of other miscellaneous troubles, due to undesired bacteria, a remedy is found by putting into the milk a culture of a vigorous lactic acid bacterium that will grow rapidly and prevent the undesirable bacteria from developing sufficiently to cause trouble. The use of this principle in butter- and cheese-making has become very widely extended.

It is this restraining action of the lactic acid bacteria that explains the generally recognized fact that sour milk, or butter-milk, is not only a wholesome, but a very useful beverage. It seems a little strange that these products, containing as they do, bacteria reckoned by the hundreds of millions per c.c., should be recommended as beverages for infants and invalids. A glassful of well soured milk will certainly contain 100,000,000,000 bacteria, and yet it is a wholesome as well as a refreshing beverage. The explanation however, is simple enough. These myriads of bacteria are practically all of the type of lactic acid bacteria, which are not only harmless in themselves, but which prevent the growth of various kinds of putrefactive germs that might produce trouble in the intestine. The presence of a goodly number of lactic acid bacteria, therefore, may prevent the growth of certain types of intestinal putrefaction that would otherwise cause trouble. The farmer's belief that butter-milk and sour milk are healthful drinks, which seemed hardly credible for a while when the immense numbers of bacteria contained in them were first recognized, appears, after all, to be well founded on scientific fact. The use of such milk is becoming recommended very widely, and already there are on the market commercial preparations of lactic bacteria to be used in preparing such milks. These preparations, as a rule, contain the particular form of lactic bacteria mentioned on page 148 which has the characteristic of being more vigorous, and making milk more acid than the ordinary lactic acid bacteria, and, therefore, having in even greater degree this power of preventing the growth of other more mischievous organisms. The healthful properties ascribed to the alcoholic beverages mentioned on page 158 are probably due to the presence of the beneficent lactic acid bacteria.

## DISEASE GERMS IN MILK.

It has long been recognized that milk may be a distributer of disease. This general statement is disquieting, but the knowledge is of little use unless it can be made more definite. The subject can be made more intelligible if we notice what kind of diseases are thus distributed and how the dangers arise. There are four definite diseases known to be distributed in this way, and, in addition, a less definite type of intestinal trouble.

*Tuberculosis.*—This subject will be considered in a separate chapter.

*Typhoid Fever.*—Typhoid fever is produced by a well-known bacterium primarily inhabiting the human intestine (Fig. 40). Inasmuch as the cow is not subject to typhoid fever, milk, when freshly drawn, will never contain 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 sources of these secondary contaminations are: 1. Direct contact with persons who have or are recovering from the disease. It is well known that patients may, after recovery from this disease, carry around the living bacilli for a long time; "bacillus carriers" they are called. In other cases the patient may be so slightly sick with the disease as to keep about his work, having what is called "walking typhoid." If people from either of these classes are employed in the dairy, they will be pretty sure to infect with typhoid fever germs whatever dairy utensils they handle. 2. Patients who are sick enough to be confined in bed eliminate large numbers of bacilli in their excretion, and this, together with clothing soiled by it, may be carelessly handled by some one who is employed in the dairy. The chance of milk infection from such persons is, then, very great, and no one who has anything to do with the care of a typhoid fever patient should be allowed to have any contact with the dairy. 3. Infected water is a common source of contamination. This does not mean that the milk is necessarily watered; but milk may become infected by simply allowing the cans to stand in impure water while they are cooling or by rinsing the cans in such water after they have been washed. There are also other secondary sources. That the danger from these sources is real and not imaginary, may be judged from the fact that already at least three hundred typhoid epidemics have been traced to milk.

Fig. 40.—The typhoid bacillus.

*Scarlet Fever and Diphtheria.*—There is positive evidence that these two diseases may be distributed by milk and that some epidemics are attributable to the milk-supply. 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 the cow with more or less certainty, and it is beyond doubt also that the milk may become infected with the cause of this disease by secondary contamination. The farmer should, therefore, take precautions to prevent any person from working in the dairy who is recovering from scarlet fever.

FIG. 41.—The diphtheria bacterium.

Diphtheria is produced by a well-known bacillus (Fig. 41). Here again there seems some doubt whether cows have the disease. 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.

*Diarrheal Diseases.*—Besides the diseases mentioned, milk is responsible for a portion of those obscure diseases characterized by diarrheal troubles, which are especially prevalent in warm weather. Among these are *cholera infantum*, which is responsible for the death of so many children, and *summer complaint*, which is less serious. These troubles are not yet so well understood as the others we have mentioned. They do not appear to be caused by any single specific bacterium, but are probably due to the excessive multiplication of a number of certain kinds of bacteria in the milk. That they are due to milk bacteria is proved by the facts that (1) they occur most frequently at the seasons of the year when milk bacteria are most numerous; (2) they are more prevalent among infants fed upon cow's milk than among breast-fed children. What kinds 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 due to putrefactive bacteria, which are the bacteria of filth. Anything which increases the amount of filth in the milk will have a tendency to increase the amount of such troubles, and any advance in cleanliness will have an influence in the opposite direction.

# CHAPTER XII.

## CONTROL OF THE MILK-SUPPLY.

A better regulation of the milk-supply is emphatically needed, and this need has become more and more evident as the facts enumerated in the last chapter have been gradually disclosed. It would enable the dairyman to avoid the many troubles due to undesirable organisms and would be to the public at large a means of protection from the illnesses due to milk. In consequence of this need, a series of regulations and suggestions have arisen looking toward the improvement in the quality of milk We may best consider these under three heads: 1. Dairy pro' lems. 2. Transportation problems. 3. Public control.

### I. DAIRY PROBLEMS.

Manifestly the first place demanding attention in the attempt to reduce the possible evils resulting from undue bacterial contamination is the dairy. The primary lesson to be learned here is the need of **cleanliness.** But there are several subordinate divisions of this general subject.

**The Cow.**—The health of the cow is a matter of such great importance that it hardly needs to be said that no sickly cow should be allowed to contribute to the milk-supply. All tuberculous cows, in particular, should be excluded, or their milk used only after pasteurization. Every dairyman should be on the watch for udder troubles, and if any signs of hardness, of inflammation, or of running sores appear on the udders, or if the animal gives bloody milk, she should at once be excluded from the milk-producing herd until completely recovered. The cow should also be kept clean. Fortunately, there has been a decided change in this respect, and at the present time cattle in dairies are not infrequently groomed and brushed, and are sometimes kept in as cleanly a condition as ordinary horses. The habits of the cow, especially when closely confined in the stall, inevitably result in a large amount of manure adhering to the animal's flanks, tail, and udders, and unless this is removed by curry comb and brush, and by washing if necessary, the character of the milk is sure to suffer.

**The Stables.**—It is much better to have stables on high ground, where there is ready drainage, than on low ground. Both air and light are necessary in stables, for the best results. Each cow should have three to four square feet of window surface, and 400 to 450 cubic feet of air space. While the animals are in the yard, as they should be daily, the stables should be thoroughly aired.

The cleanliness of the stable is a matter of utmost importance. The habits of the cow and the nature of the manure are such as render a high state of cleanliness very difficult. But the dairyman should understand that all accumulation of manure or other filth is a direct detriment to the quality of the milk. The removal of the manure from the stalls should be as frequent as possible, never less than twice a day. The manure, when removed, should be taken as far as possible from the barn, and should never be heaped outside, close by the barn nor be allowed to accumulate in the cellar. By far the best method is to distribute it daily upon the fields, where it may serve as a fertilizer. Attention should be given to the dust, cobwebs and hay that may be clinging to the ceiling of the barn, for all such are traps for accumulating dirt, as well as sources of bacteria, thus aiding in the contaminating of the milk. Plastered or sheathed walls and ceilings are very much to be preferred to a rough finish. The bedding of the cattle is a matter of some importance also; and clean shavings appear, on the whole, the best for this purpose. A coat of whitewash should be applied with a spray pump at least once a year.

**Personnel.**—Special attention should be given the persons employed on a dairy farm. The milking clothes should be made of washable materials. Some dairies insist that this clothing must be sterilized each day. A thorough washing and drying of the hands should precede the milking. All these measures are necessary, since the persons employed in the dairy are more likely to be a source of danger than anything else. *No one should be allowed to handle any milk, to wash the milk cans, or to have anything whatsoever to do with the milking utensils if he is suffering from or recovering from any contagious disease.* Nor, indeed, should any farm furnish milk to the public if there is a case of typhoid, scarlet fever, or diphtheria among its employees, unless a health inspector pronounces the sanitary conditions satisfactory.

**The Milking-room.**—It is quite customary to milk cows in the ordinary cow stalls. Some of the better dairies have adopted the plan of having a separate milking-room, and find beneficial results in the character of the milk. It is certainly preferable to using the ordinary cow barn as a milking-room.

**The Milk-vessels.**—Perhaps the most important factor for reducing bacterial contamination is the proper cleaning of all milk-vessels. This refers to milk pails, strainers, coolers, separators, milk cans, glass bottles, etc., used in the dairy. The cleaning of such utensils is no easy task, and after the most thorough washing and scrubbing many bacteria will still be left in the cracks and clinging to the milk-vessels, ready to feed and multiply in the next lot of milk. All milk-vessels should be of metal, and if the coating of tin is worn off they should be discarded, for they cannot be kept clean. They should not be allowed to dry before washing, for dried milk is difficult to remove. They should first be soaked in warm water to loosen the milk; then washed thoroughly in hot water, containing, preferably, soap or sal-soda, and thoroughly scrubbed; after this they should receive a second rinsing in hot water. Such a cleaning is not, however, sufficient to sterilize them. Hence, no creamery should depend upon the farmer to wash milk cans. Where a supply of steam is to be had a sterilization should follow the washing. Washing with hot water is better than with cold, washing with sal-soda is better than simple washing, but sterilizing is best of all. Each dairyman should adopt as thorough a cleaning as parcticable.

**The Milking.**—Moistening the udder with a damp cloth or sponge just before milking prevents the fall of much of the dirt into the milking pail. The precaution is a simple one, costs nothing and really has a surprising result in decreasing the number of bacteria that get into the pail.

**Covered Milk-pail.**—The old fashioned pail had a flaring top, the purpose of which was to make the milking as easy as possible; but, incidentally, it resulted in exposing the milk to much contamination by dirt and bacteria. Various devices for protecting the milk from such exposure by the use of covered milk-pails are now used. There is quite a variety among them, but they all have the general plan of decreasing the size of the opening of the milk-vessels, so as to expose less surface for the entrance of dirt, and they also have in the opening some kind of a cloth strainer for catching the larger particles of dirt, thus keeping them from the milk (Fig. 42). This is one of the easiest, cheapest and most efficient means for improving the character of the milk.

FIG. 42.—A milk pail with a special cover designed to keep out the dust which falls into the pail during milking.

*Milking Machines.*—A still more recent means of reducing contamination is by milking machines. These consist of rubber tubes ending in special cups for attachment to the teats of the cow, and connected at the other end with large cans that can be sterilized. The cans are connected with a system of vacuum tubes, and at the point where the rubber tubes are attached to the can there is a mechanical device by which the vacuum is made to draw the milk through the tubes intermittently, thus imitating natural milking. It would seem that such a plan, which carries milk directly from the teat to the sterilized can, would be almost ideal, and would practically remove all dirt contamination. Where these machines have been intelligently used they have been found efficient in producing a very clean quality of milk. But the long rubber tubes are by no means easy to keep clean, and when they are used by careless employees, the bacteria become very abundant inside the tubes and the other parts of the somewhat complicated machine. In other words, it requires very great care to clean and sterilize these milking machines in order to produce even as good results as are obtained by hand milking. But if care be taken to sterilize thoroughly all of the apparatus, better and more reliable milk can be obtained by the use of the milking machine.

*Rejecting Fore Milk.*—For reasons already indicated, the first milk drawn at each milking will contain more bacteria than the rest. The practice of rejecting the fore milk, either allowing it to waste upon the floor, or collecting it in a separate dish, is, no doubt, an advantage, but the extent of the advantage has been overdrawn. The extra number of bacteria obtained in a pail of milk from the entrance of the fore milk, is very small compared with the larger number that enter the milk from other sources.

*Value of Trained Dairymen.*—Apparatus without a proper man to use it is valueless. It makes no difference how many rules may be drawn concerning the dairy, how complicated the apparatus becomes, or how careful may be the directions given to the employees, it is quite impossible to expect satisfactory results without properly educated and trained assistants. An untrained man will succeed in getting only bad results, even with the best of apparatus. The employees in our dairies at present are, in many cases, without any proper training. They do not know the character of the product they are producing; they do not know the dangers to which it is subject; they do not understand the universal presence of bacteria; they do not understand, in general, the problems that are concerned with their business. They are quite likely to believe the whole subject of bacteria in milk to be foolishness and not worth their attention. Under these circumstances, no matter how many directions are given or how much instruction there may be, satisfactory results will never be obtained.

**Cooling.**—The importance of cooling the milk, and cooling it immediately, cannot be overstated. When milk is drawn from the animal, it is at a temperature to stimulate the growth of bacteria to their utmost. It is true that, for a while, because of the germicidal property of milk (see page 159), the bacteria do not grow; but this condition lasts only a short time, after which, if the milk is warm,

15

they begin to develop with great rapidity. But if the milk is at once reduced to a low temperature, the bacteria that have found their way into the milk will not grow very rapidly. These facts are so simple as hardly to require statement; but, unfortunately, many a dairyman, although he may theoretically understand them, fails to appreciate their importance. The essential point to be emphasized is the necessity of immediately cooling the milk to a temperature as low as 40° F. if possible. It is just as necessary to cool clean milk as it is to cool dirty milk. Unless it is done, the cleanest milk will soon contain as many bacteria as the dirtiest milk.

**Straining and Filtering.**—The long-continued practice of straining the milk through a metal strainer or through cloth has in its favor the fact that it will remove the larger particles of dirt; but it does not remove the bacteria, for they will pass through any strainer. Sand filters have also been used by some dairy companies, and these are more efficient than simple straining. But these filters are not of very much value and they are not widely used. Centrifugal force is sometimes used for cleaning the milk, and is fully as efficient as sand filtering. All of these means, while effective in removing the large particles of dirt, are practically of no value in removing the bacteria, which show as high numbers after such treatment as before.

## II. TRANSPORTATION PROBLEMS.

Under this head will be included not only methods of treating milk during transportation, but also of preparing it for preservation during the transportation or until it is consumed. Milk, as a rule, receives no preparation for transportation, except that of cooling and placing in clean cans. Then, if rapidly shipped and kept cool, it should remain good until some time after it has reached the consumer. But the rapidity of bacteria growth, especially in hot weather, makes it difficult to transport milk, in good condition, for very long distances. Consequently, careful search has been made for some method of treating milk so as to preserve it.

**The Use of Preservatives.**—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 methods are illegal, and are unhesitatingly to be condemned.

**The Use of Heat.**—A more legitimate method of obtaining the same result is by the use of heat. All bacteria are destroyed by heat and therefore, by this simple means, it is, possible, 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*.

1. **Sterilization.**—This means the use of heat sufficient to detroy *all* bacteria at once. It is perfectly possible to do this, but since milk always contains spore-bearing bacteria, sterilization requires a high temperature for the purpose. A temperature of boiling will not destroy the spores, so 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 a procedure inevitably makes the milk rather expensive. But milk thus prepared is supposed to be *germ-free*, and, consequently, should keep indefinitely. Unfortunately, even these temperatures do not always destroy all the spores, for some samples of milk thus treated have subsequently undergone fermentative changes, due to the germination of the spores that are left alive. 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. Sterilized milk, if it does retain a single spore, will be in time, 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 has disappeared.

The term sterilization is sometimes applied to the simple *boiling* of milk. This was recommended by physicians long before its real significance was understood and has been very widely used in all civilized countries. Its ease of application explains the reason for its popularity. It is only necessary to place the milk upon the stove and allow it to come to a boil, and the end is reached. In some 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 distributing diseases by the destruction of 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.

But several practical objections have arisen:

1. The bacteria spores are not destroyed, and such milk, if kept, will surely undergo a fermentation. But this is of little importance if the boiled milk is to be used at once.

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 ivalid 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 reduce the amount used.

3. Milk treated to a temperature as high as boiling becomes somewhat less easy of digestion and assimilation. The heat pro-

duces several important changes which result in its being less easily handled by the digestive organs. The difference is not very great, and a healthy individual is able to digest such milk well enough; but delicate children and invalids are not so well nourished upon boiled milk as upon raw milk.

4. Boiling the 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.

The practice of boiling milk in order to "sterilize" it is widely adopted in private families, but the objections urged against it have led to its being less and less recommended. In its place has come an extended use of the second method of treating milk by heat.

2. **Pasteurization.**—This method, originally devised by Pasteur for treating wine, consists in heating the milk to a moderate temperature only, and then rapidly cooling it. The temperatures chosen have varied. Sometimes a temperature as low as 140° F. is adopted, and continued for from twenty minutes to half an hour or more; sometimes 150° to 160° is used for about ten minutes, and sometimes as high a temperature as 180° is used, the milk being just brought to this point and then cooled at once. Any of these methods is called pasteurization.

Such temperatures are manifestly not sufficient to sterilize the milk, since they are even less efficient than boiling. But in pasteurization no attempt is made to sterilize it, but simply (1) to destroy the large majority of bacteria and (2) to destroy the disease germs that are liable to be in the milk, and therefore to render it safe for drinking. A low temperature is chosen in order to avoid the chemical changes that are produced by boiling and that show themselves in the boiled taste. These changes begin to appear at about 156°, and any temperature below this scarcely changes the milk at all, while higher temperatures will bring them about. For this reason the lower temperatures are better. But will these moderate temperatures accomplish the desired ends?

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 the lactic acid bacteria, which are the cause of the souring of milk, produce no spores, and consequently they are largely killed by such moderate heat. Hence the total number of bacteria in milk is immensely reduced, and the milk has its keeping quality much increased. Milk thus treated will frequently remain good two days longer than similar milk not pasteurized.

Will such temperatures destroy disease bacteria? Of the diseases mentioned above as liable to distribution by means of 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 the low heat (140° F. for one-half hour); the same is probably true of scarlet fever. The tuberculosis bacillus, however, will withstand higher heat without injury, and hence, in order to be sure of destroying these organisms, it has been thought necessary to heat the milk to temperature of 185° F. At this temperature the cooked taste and the chemical changes begin to appear. The present conclusion, the result of the most recent and careful experimenting, is happily a satisfactory one. If milk is heated in such a manner as to avoid the formation of a scum on its surface, at a temperature no higher than 140° F., but continued for half an hour, the virulence of the tubercle bacillus will be so much reduced that milk containing these bacilli will be rendered harmless. This temperature is considerably below that at which the chemical changes in the milk take place. Milk may thus be 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.

The value of pasteurization is becoming rapidly recognized, and this method of treatment is being widely adopted. The advantages 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 quite true of the typical diseases mentioned. Whether it similarly removes the danger of diarrheal 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. Several forms of apparatus have been devised that accomplish the end rapidly and upon large quantities of milk. Of these, there are two general types. In one a large quantity of milk is heated to the desired temperature and maintained at this temperature as long as desired, after which it is cooled. These are called *discontinuous pasteurizers*. In the other type the milk is passed through the apparatus in a constant stream, being heated and cooled while it passes through. In these machines the milk is sometimes only just brought to the desired temperature, and cooled at once; and in all cases the extent of the heating is dependent upon the rapidity of the stream flowing through. These are called *continuous pasteurizers*. Generally speaking, this type is apt to be less efficient than the discontinuous pasteurizers, and are more subject to irregularity. Either type is efficient *if properly managed*, but carelessness and haste on the part of the employees may render either kind unreliable and inefficient.

In the last few years the plan of pasteurizing the milk on a large scale has come to be frequently adopted. It is done in creameries in connection with butter-making, and in some of our large cities for the treatment of the general milk-supply. In the pasteurization of the public milk-supply the purpose has not been, primarily, to protect the public, but to keep the milk from souring. Milk distributers have found it difficult to furnish milk that will keep without preservatives, but have learned that the application of heat enables them to do so. For this reason pasteurization has become adopted by some large milk companies.

Pasteurization is sometimes applied to cream, to enhance its keeping and enable it to find a market. The cream keeps well, but loses some of its consistency. It appears thinner than before treatment, and will not whip so well as ordinary cream. Its consistency may be restored by adding a little of a material called *viscogen.* This is made by adding a strong solution of cane-sugar to freshly slacked lime, and allowing the mixture to stand until the upper part of the mixture is clear. This clear liquid is poured off and added to the cream in the proportion of one part to one hundred or one hundred and fifty parts of cream. This restores the consistency to the cream, but, since it is an addition of a foreign substance, its use is illegal.

In our larger cities a considerable part of the milk on the market is pasteurized; sometimes it is sold as pasteurized milk and sometimes it is not so labeled.

**Preparations of Milk.**—The microörganisms that spoil milk will not grow in it if the water is removed, and several methods have been devised for producing a form of milk that will keep, all of which are based upon the removal of the water. Condensed milk is the oldest and has a wide use. It consists of ordinary milk evaporated to about one-third of its original bulk, to which is commonly added a large amount of sugar. The sugar prevents the growth of bacteria, and this condensed milk, put up in cans, keeps well. In some forms of condensed milk the sugar is not added, but the product is preserved by sterilizing by heat. When subsequently diluted with water, condensed milk does not exactly replace the fresh article, because of the added sugar in the one type and the effect of sterilization in the other. A product known as *concentrated milk* has recently been placed on the market. In this case the milk is first skimmed and then subjected to a heat of 140° F. till enough water is evaporated to bring the milk to about one-fifth of its original bulk. Then the cream, which has also been subjected to the same heat, is replaced, making a product one-fourth its former weight. This milk has been pasteurized by the heat used in evaporation and is consequently free from disease bacteria. When the original amount of water is replaced it is indistinguishable from fresh milk. Concentrated milk will keep for several days without spoiling, and can be much more easily handled than ordinary milk. In still other preparations the water is almost wholly removed, producing milk powders, several different brands being on the market. They are prepared by various means, but in all the water is dried away from the milk, leaving a form that can be converted into a powder. Since they contain little water they will keep almost indefinitely. They have great use for special purposes, but are not a satisfactory substitute for fresh milk since they do not readily dissolve again when water is added to them.

**Transportation.**—In the transportation of milk to market three factors are to be borne in mind: 1. *Cleanliness.* This means that only thoroughly sterilized cans should be used to hold the milk, and that they should be completely closed, so as to avoid contamination from without. The necessity for a complete sterilization of the milk cans cannot be exaggerated. 2. *Temperature.* If the milk is to be delivered in a good condition, it must be kept cold during transportation. This is accomplished fairly well by the ice car. 3. *Rapidity.* The more quickly milk can be delivered to the customer, the better the result. But milk kept cold, below 45° F., may be delivered from 24 to 36 hours old and be in better condition than milk fresh from the farm, only five or six hours old, which has not been properly cooled. For this reason it not infrequently happens that milk brought in a milk cart, directly from the farm only a few miles distant from the consumer, is of poorer quality, so far as numbers of bacteria are concerned, than milk that has been brought long distances in a well iced milk car, though it may be 36 or even 48 hours old. As an actual fact, milk furnished small communities near the source of the supply is frequently of poorer quality and, on the whole, less reliable than that furnished the larger cities.

## III. PUBLIC PROBLEMS.

Although this subject primarily concerns the regulation of the milk-supply after it reaches the city, certain aspects of it are intimately associated with farm life. City authorities are every year extending their control more and more directly to the farm. The public is making certain demands regarding the milk-supply that must be acceded to by the milk producer.

**Freedom from Disease Germs.**—This demand needs no argument. To meet it the only plan within sight at present is the insistence that only healthy cows shall be used in the production of milk; that no milk shall be distributed for drinking purposes from cows having any kind of udder disease; that no person suffering from or recovering from a contagious disease, or having direct contact with others thus suffering shall be employed in the dairy or handle the milk in any way; that the milk shall not be watered, and that in washing the milk-vessels no water shall be used that is in the slightest degree open to suspicion of sewage contamination. In addition to these demands it must be insisted that precautions be taken for excluding stable filth from the milk, and that the milk be cooled at once to prevent undue growth of bacteria.

**Milk Standards.**—A legal standard set for the chemical composition of market milk is nearly everywhere adopted and does not concern our immediate subject. A few cities have set a standard as to the number of bacteria that will be allowed in milk offered for sale. Boston has a standard of 500,000 per c.c. This has as yet been done in only a few places and it is still uncertain whether such standards can be enforced or are of much value. The milk producer needs only to remember that, to reach these standards, he must use care in the dairy to insure cleanliness along lines already pointed out. Special grades of milk are becoming more or less common in various localities. *Sanitary dairies* of exceptionally high character have been conducted with more or less success. In these every possible precaution is adopted to produce milk under ideal conditions. Only tested and inspected cows are used, and numerous devices are carried on to protect the milk from all possible suspicion of filth contamination. The milk from these dairies is certainly superior to the ordinary milk, but the production is so expensive that it must be sold at a high price, and this has interfered with the commercial success of some of these enterprises. What is known as *certified milk* has, in recent years, come into some prominence. This is milk produced in dairies that are under the inspection of a certifying board. This board, usually composed, in part at least, of physicians, keeps a constant oversight of the milk from certain dairies and over the methods of its production. If they find that the milk comes up to the somewhat high standard that they set, and if they are convinced that proper methods are used in its production, this board gives to that dairy the right to use its certificates. It is not so expensive to produce milk under these conditions as to carry out the many precautions adopted by the sanitary dairies. Some extra care is needed, but it is within the reach of almost any well kept farm to produce certified milk. This milk brings a higher price than ordinary milk, but it is more reliable because more care has been required to produce it. Neither sanitary milk nor certified milk forms anything more than a very small portion of the milk-supply of our cities.

**Dairy Inspection.**—During recent years the practice of inspecting dairies has sprung up. This was started first by some of the milk-supply companies of the large cities, because they wished to protect their supply for commercial purposes. Some of them began, at least a dozen years ago, to send inspectors periodically among the dairies which furnished them with milk. Within a few years it has been realized that a public dairy inspection of this sort would be of great value in improving the general milk-supply and in furnishing the public with better milk. Such a public dairy inspection has been begun in some sections around the larger cities. The inspectors visit the farms, note all methods employed, condemn the faulty ones and make suggestions as to improvement. The inspection is for the advantage of the consumer and producer, and the dairyman should welcome rather than resent such visits and helpful suggestions.

The inspectors give attention to the following points: 1.

General cleanliness in the dairy. 2. The condition of the cows. 3. The source of the dairy water. 4. The condition of the barn. 5. The method of disposing of the manure. 6. The method of milking. 7. The condition of the milkers. 8. The treatment of the milk after milking. 9. The method of washing and sterilizing all dairy utensils. 10. The bottling of the milk. 11. The method and care of transportation. The farmer should be prepared to meet the inspector upon all these points.

While it is expensive to produce sanitary milk, and while the same is true, though to a less extent, of the production of certified milk, it is possible greatly to improve the character of the milk without any material increase in expense. The purpose of dairy inspectors is to show how the milk may be improved; and the dairyman should remember that by the use of such simple precautions as cleaning the cow, moistening the udder before milking, using a covered milking pail, and more thoroughly sterilized milk-vessels, the character of the milk will be greatly improved. It is gratifying to know that there has been a decided improvement in the quality of milk furnished our cities in recent years.

# CHAPTER XIII.

## BACTERIA IN BUTTER AND OLEOMARGARINE.

### BACTERIA IN BUTTER-MAKING.

In the making of butter, bacteria are the dairyman's allies. 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 *ripening*, or, in Europe, more commonly called *souring*. In butter-making, the cream is not usually churned immediately after it is separated from the milk, but it is allowed to lie in a moderately warm vat for a period of twelve to twenty-four hours or even longer, that it may ripen. 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 most butter is made from ripened cream.

#### CREAM-RIPENING.

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. On ordinary farms, the cream is left to take care of itself, and is thus 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 handled daily, has put a new aspect upon the problem. The ripening will no longer care for itself, but must be carefully attended to by the butter-maker. The necessity for some accurate means of controlling the ripening has become more and more apparent with each step toward the concentration of 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.

**The Purposes of Cream-ripening.**—These are as follows: 1. Ripening the cream makes it churn more easily and increases the yield of butter. This is true, at all events, for gravity cream; it is less significant, and perhaps not true, for separator cream. 2. Butter made from properly ripened cream is thought to keep better. 3. By far the most important purpose in cream-ripening is the production in the butter of a desirable *flavor* and *aroma*. Butter made from unripened cream lacks the peculiar flavor of high-grade butter, since this is the result of the ripening. If the ripening is not satisfactory, the flavor and aroma of the butter are sure to be inferior.

The importance of this factor in butter-making for our creameries is very great. The market price of butter depends largely upon the flavor. 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 frequently add one-third or one-half to the price which could be obtained for poorly flavored butter or for butter without flavor. Hence, the success or failure of a creamery business depends, in large measure, upon the ripening. A creamery which fails to ripen its cream properly fails to obtain a desirable flavor. Hence, it obtains 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 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 ripening of cream is a phenomenon of bacteria growth. The many bacteria in the cream find it an excellent medium for food, and if kept at a fairly warm temperature during the ripening period, their development is rapid. For the twelve to twenty-four hours of ripening, the bacteria multiply, and, by the time the cream is ripened and ready to be churned, they are present in prodigious numbers. Analyses of ripened cream have disclosed the fact that, whereas in the sweet cream bacteria may be from 2,000,000 to 3,000,000 per c.c., in the same cream when ready to churn there may be about 500,000,000 per c.c. The numbers at the time of ripening, however, vary widely, being sometimes as low as 200,000,000, or even lower, and sometimes as high as 2,000,000,000 per c.c.

The growth of bacteria in the cream produces chemical changes which considerably modify its nature. The lactic acid bacteria always develop lactic acid, and the cream becomes sour; but there are other changes as well. We do not yet know what all these changes are or to what extent they contribute to the ripening phenomenon. That the other changes have something to do with the production of the flavor in butter is evident from the fact that a butter flavor cannot be produced in the cream by adding lactic acid to it, and if the ripening were wholly the result of souring, the addition of lactic acid should produce the same results as normal ripening.

**Growth of Bacteria During the Ripening.**—At the outset cream contains many kinds of bacteria, and the composite cream of a creamery has more kinds than that of a private dairy. The cream is commonly kept between 60° and 70°, at which temperature many bacteria develop rapidly, but not all kinds with equal vigor. During the first few hours there is a general increase in the number of nearly all the kinds of bacteria originally present in the cream, so that, after six or eight hours, there are higher numbers of all species of bacteria than were found at first. During this time, however, the lactic acid bacteria, especially of the *Bact. acidi lactici* type, increase more rapidly than the others. In the very fresh cream this species may have been comparatively small in numbers, forming not more than 1 or 2 per cent. of the whole, but the percentage rises rapidly. After several hours, the time varying with different specimens, the acid bacteria constitute a large proportion of the whole. From this time, after they form perhaps 50 per cent. of all the bacteria present, the other species begin to be seriously affected by the acid produced. The acid-forming germs still continue to increase in numbers, while the others cease to grow so rapidly, soon begin to diminish, and finally may largely or wholly disappear. The result of this is that, during the last stages of the ripening, there may be present in the cream nothing but acid bacteria, which sour the cream and produce the final changes in the ripening.

Thus it will be seen that the ripening of cream may be divided into two stages. In the first the growth of the miscellaneous species of bacteria continues, and all types may become more or less abundant. In the second the acid-forming germs gradually force the others into the background and finally crowd them out entirely. Both of these stages doubtless contribute to the final product. Without the proper lactic organisms it is impossible to get the proper flavored butter. But butter made from pasteurized cream and ripened by pure cultures of lactic acid bacteria does not develop so much flavor as that in which the original bacteria are allowed to grow with the acid germs. Hence, it is probable that the development of the miscellaneous bacteria in the first phase of the ripening has not a little to do with the final butter flavors.

**The Effect of Different Species of Bacteria.**—The buttermaker thus needs bacteria, but he must have the right kind. When cream is collected for a large creamery from many sources there are sure to be in it quantities of different varieties of bacteria, each patron contributing his quota. Each species may be expected to have its effect upon the cream during the ripening, and the resulting butter will show this effect. Actual study has proved that different species of bacteria, when allowed to grow in the ripening cream, produce very different types of butter. Some produce *bitter butter*, others *tainted butter*, others *insipid butter*, and others a *strong odor*, almost like that of putrefaction. Some species produce a *tallowy* *butter*, others a *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.

Since the bacteria are so varied in their action, it may be a matter of surprise that cream-ripening, if left to itself, so commonly results favorably. The primary reason for this is the superior vigor of the lactic acid bacteria. Since, in the ordinary bacterial growth in cream, the lactic bacteria finally get the upper hand and grow at the expense of all the others, it ordinarily happens that the ripening produces a good flavor, and a satisfactory butter is obtained. Unfortunately, however, the favorable species of lactic bacteria do not always get the upper hand in the cream-ripening. Sometimes large numbers of other bacteria are present in the cream, just as vigorous and just as capable of rapid growth as the desirable lactic acid germs. In such cases the unusual bacteria may develop abundantly and produce a variety of uncommon changes in the cream, with the result of giving an undesirable flavor to the butter. Such a phenomenon explains the occasional appearance of bad-tasting butter. The fact that such improper ripening does sometimes occur clearly points to the need of some control over the ripening, especially in creameries where a uniformly good product is necessary for financial success.

## CONTROL OF CREAM-RIPENING.

The butter-maker has no control over the kinds of bacteria that get into his cream, and a creamery must take cream filled with whatever bacteria chance to be most common in the dairy furnishing it. But though he cannot control this factor, he can, more or less satisfactorily, regulate the growth of the bacteria.

**Temperature of Ripening.**—At a temperature of from 65° to 70° the favorable lactic acid bacteria get the upper hand of other species more readily than at either a higher or a lower temperature. At temperatures above or below this, different species, mostly unfavorable, are more likely to gain the upper hand. Hence, by keeping the temperature at about 65°, the undue development of mischievous bacteria is more likely to be prevented.

**Duration of Ripening.**—The butter-maker can stop the ripening at any point, for, after the cream is churned into butter, the bacteria growth ceases. The necessary duration of the ripening will vary, however, with the conditions. Sometimes cream, when brought to a creamery, is already sour and has, therefore, become ripened even before the butter-maker receives it. In other cases, especially in winter, it will not only be sweet, but will contain small numbers of bacteria and require a much longer ripening. Moreover, milk produced under good dairy conditions, clean and fairly free from bacteria, will ordinarily require longer ripening than milk produced under less favorable conditions and containing already great numbers of bacteria. The length of time will vary also with the temperature, being, of course, longer at lower temperatures. To determine when the cream is sufficiently ripened, the butter-maker has two methods. One is the general appearance to his eye and taste, and the other is the degree of acidity. The latter factor is determined by the methods described on page 309, and the ripening is generally continued until the acidity is 0.5 to 0.65 per cent.

## THE USE OF STARTERS.

By far the most important change in the methods of cream-ripening is in the wide and almost universal introduction of *starters*. Twenty-five years ago it was sometimes customary to add a starter to cream in cold weather simply for the purpose of starting the ripening; but to-day almost all good creameries use starters, not so much for starting, as for regulating the ripening.

Prof. Storch, of Copenhagen, first conceived the possibility of furnishing to butter-makers cultures of the proper species of bacteria, which they might add to their cream for the purpose of ripening, somewhat as yeast is used in brewing. This experimenter not only conceived the method, but put it into practical operation in Denmark. His method consisted 1. in pasteurizing the cream at about 165° F., for the purpose of destroying most of the bacteria that might be present, and 2. in adding to it a pure culture of bacteria, whose value in producing a good flavor had been determined by experiment. This method is, of course, logically 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 introduced by the adding of the pure culture. Professor Storch was soon followed by other experimenters and the method adopted in Copenhagen was 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.

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 were not in condition to pasteurize their cream. In 1895 a slight change was made in the process. In order to bring the subject more widely to the attention of dairymen, a method was suggested of using the cultures without previously pasteurizing the cream. This seemed illogical, since the cream is already filled with bacteria, and the addition of a new culture could hardly be supposed to give entirely satisfactory results. But when we remember how a vigorous lot of lactic acid bacteria can overcome other species, the method does not appear so illogical after all. With this change our butter-makers were willing to try pure cultures and in a short time American butter-makers learned of their meaning and began to experiment with them widely. The result of the dozen or so years of experience has been to show the extreme value of starters as a means of controlling the ripening, until to-day starters of some kind are almost universally used in all good creameries and dairies.

**Preparation of Starters.**—While starters are very widely used to-day, they are not always pure cultures. Two quite different methods of preparing them are in use.

*Natural Starters.*—A natural starter is nothing more than some normally soured milk. In order to obtain it it is only necessary to select several quarts of good milk and place it in a clean, sterilized pail or can, covered to keep out the dust, and keep it in a temperature of from 65° to 70°. After one or two days the milk should show signs of souring; when it has become decidedly sour, but not yet curdled, it is to be used as a starter. It requires some skill on the part of the butter-maker to know whether the starter thus obtained is of the best character and whether it should be used or thrown away and another obtained. Starters made in this way are not sure to be uniform, inasmuch as the different samples of milk may contain different types of bacteria, and experience is needed on the part of the butter-maker to know whether the starter is satisfactory.

*Starters from Commercial Cultures.*—Commercial starters are now a well-known article, and several different brands may be purchased. In all cases they are prepared by bacteriologists and consist of a culture of bacteria—usually a pure culture, though not always—that have been found by experiment to produce favorable results in the ripening. These starters as purchased are sometimes in the form of a powder, sometimes in the form of a liquid, but in all cases contain too small a quantity to add directly to the cream that is to be ripened. The quantity of bacteria must, therefore, be increased before using by a process called *building up*. The procedure is as follows:

A quart of skim milk, whole milk, or cream is placed in a glass jar and sterilized, either by boiling or, better, by pasteurizing at 180° for half an hour, stirring frequently to insure uniform heating. The milk is then cooled, and when it has reached a temperature of 80° the commercial culture, from a *freshly opened* package, is thoroughly stirred in; the whole is covered to keep out the dust and placed at a temperature of about 65°. When the milk has become quite sour, but before it is curdled, it is ready to use as a starter. If a larger amount of starter is needed, this first starter is placed in a large can of pasteurized milk and allowed to grow in it at 65° until the whole becomes soured. By this means any desired amount can be prepared.

The starter thus prepared is added to the cream in varying proportions, the larger the amount the quicker the ripening. Sometimes one part of the starter to ten parts of cream is used; in other cases a smaller amount is used and sometimes more. After the ripened cream is ready to churn, a certain quantity of it is removed, placed in a clean can, and set aside to serve as a starter for the next day's churning. In this way some starter is reserved each day, to be used in the cream collected that day; and thus the original starter is carried on from churning to churning. After some days, however, it is necessary to resort once more to a pure culture, built up in the same way.

There is not very much to choose between natural starters and commercial cultures. Natural starters cost nothing except the trouble of making them, but, on the other hand, they are not uniform, and not always to be depended upon. Commercial cultures cost a small sum, but they are rather more uniform than natural starters. It has been claimed that the flavor of butter from cream ripened with a natural starter is higher than that ripened with a pure culture. This is easy to understand. A good starter should sour cream promptly; should thrive at 60° to 72°; should coagulate milk and cream into a homogeneous mixture, and should produce an agreeable aromatic taste. No single bacterium known has all these characteristics, but a mixture, such as a natural starter, may have them. On the other hand, if a creamery notices the development of "off tastes" in the butter, the best method of removing them is by the use of a commercial pure culture. Both kinds of starters thus have their advantages.

## THE USE OF STARTERS.

**In Pasteurized Cream.**—If the cream is first pasteurized so as to destroy most of the bacteria present, the added starter will have a free chance to grow. The pasteurizing of cream is simple and not very expensive, and it produces a medium largely free from bacteria. The use of starters in pasteurized cream has become practically universal in Denmark and some of the other countries of Northern Europe. There are two reasons for this: 1. A higher and more uniform grade of butter can be obtained in this way. 2. The prevalence of tuberculosis has brought about the enactment of a law requiring all milk that goes through the creamery to be pasteurized in order to destroy the tuberculosis germs. For this reason Denmark butter is always made from pasteurized cream, and this makes it necessary to use an artificial starter, since pasteurized cream will not ripen of itself. The pasteurization destroys practically all of the acid bacteria, and, as we have learned, when the acid bacteria are absent the putrefying bacteria are quite sure to develop. Hence, pasteurized milk requires an acid starter to insure a proper ripening.

**In Unpasteurized Cream.**—By this method the starter is simply added to the ordinary cream. The use of starters in this way is open to a theoretical objection. The cream already contains bacteria in large numbers and, ordinarily, in considerable variety. These would themselves produce the ripening of cream, even without any starter. The effect of the starter added to the cream already filled with bacteria will, evidently, not always be uniform. It might produce little or no effect, or, if the starter is added in considerable quantity, it might overcome the effect of the smaller number of bacteria originally in the cream. In practice it is found that the use of starters does have this latter effect, and in most cases, there is a noticeable improvement in butter made from cream thus ripened. The results, however, are not absolutely uniform, and even with the use of a large amount of starter it will sometimes happen that the bacteria present in the cream will have more influence than those of the starter, and the butter will suffer.

The use of cultures in unpasteurized cream was first begun in the United States and has been more widely adopted here than anywhere else. Butter made from unpasteurized cream is not so uniform as that made from pasteurized cream, but the butter made in this way is, at least to the American taste, superior to butter made with pasteurization, due probably to the fact that pasteurization prevents the growth of miscellaneous bacteria that ordinarily occurs before the lactic bacteria develop. Pasteurized cream butter is somewhat milder in flavor than that made from unpasteurized cream, and the American market demands a flavor somewhat stronger than that which is popular in Europe. Hence, to the American taste, up to the present time, the butter from pasteurized cream is not superior to that made from unpasteurized cream.

THE GENERAL VALUE OF STARTERS.

The fact that starters, with or without pasteurization, have become almost universally used among the better class of creameries is in itself sufficient proof that they are of practical value. Their advantage lies in four directions: 1. They enable the butter-maker to handle his cream 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. 2. The use of starters has produced a greater uniformity in the grade of butter. The butter-maker can depend more certainly upon producing butter of a high grade, month after month, than he can without starters. There is a general belief also among those who have tested the butter in countries where starters are widely used, that there is an improvement in the average quality of the butter, as well as in its uniformity. 3. 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. But the fact that all good dairies now use them is sufficient testimony to their value in improving the general quality of the butter. 4. They are the best means of remedying butter "faults." Every creamery has experiences of deterioration in the flavor of the butter without any visible cause. Such troubles are known to be due commonly to the growth of unusual and undesirable bacteria in the cream. When they are discovered, the sterilizing of the dairy utensils and the use of a large quantity of a vigorous starter will generally remedy the trouble at once. Moreover, the constant use of a starter goes a long way toward preventing these "faults."

It is doubtful whether the use of starters produces butter of a character superior to the best butter made without them. Indeed, some think that it is not quite equal to the best butter made without starters. But the uniformly high grade of culture butter is admitted, and the greater satisfaction in being able to control the process has caused the wide adoption of starters among butter-makers.

## BACTERIA IN BUTTER.

Although bacteria continue to grow during the ripening period, their growth is practically stopped by churning and butter-making. Many of them are removed with the butter-milk; 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, though, 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. As a result of this and the compact condition of the butter, together with its small amount of water, the bacteria do not find butter a favorable medium for growth, and they begin to diminish in numbers. A very few hours' time shows a great reduction in numbers, and this continues until, after a few weeks, the butter contains comparatively few. 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, 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.

| Two hours old. | One day old. | Four days old. | Thirty days old. |
|---|---|---|---|
| 54,000,000 | 26,000,000 | 2,000,000 | 300,000. |

It is well known that, if butter is not used immediately, certain changes occur in it 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, totally ruined for use. The cause of this rancidity has been difficult to determine, apparently because a variety of factors contribute to it. It is probably due, in part, to chemical fermentation, produced by enzymes in the milk, and in part to the growth of bacteria. The rancidity is much more likely to occur if the butter is exposed to the light, and it develops more readily in warm than in cold temperatures. At temperatures below freezing rancidity does not occur. If butter is, therefore, kept cool and in large masses, it may be held for a long time without the appearance of any very noticeably strong flavor. In the end, however, the rancidity is sure to appear. To what extent bacteria are concerned in this change we do not yet know, although most investigators have concluded that they are prominently concerned in the phenomenon. Rancidity may certainly be looked upon as a fermentation change, and the only method the dairyman has of controlling it is by cool temperatures, by packing the butter in large masses, by paraffining the butter tubs, and by keeping it from the light. It may be delayed by pasteurizing the cream and by using pasteurized water for washing, facts that show its close relation to bacteria. Fortunately, it is a matter of no very great importance, because butter can be kept without difficulty for some months, and it is almost always possible to market it before it has spoiled.

## BACTERIA IN OLEOMARGARINE PRODUCTS.

The materials out of which oleomargarine is made are chiefly stearin, lard, cottonseed oil, and other oils. These are warmed to the melting-point, are thoroughly mixed, and then drawn off into cold brine, which chills the oils into a hard mass. 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. In order to make the product the more resemble butter, it has been customary to color it; but this practice is now largely prevented by a prohibitive tax on colored oleomargarine. The bacteria normally present in oleo products are commonly much less numerous than in butter, and the oleomargarine is, on the whole, less likely to distribute infectious diseases than ordinary butter, inasmuch as the chance for contamination is less.

But, although the oleo products thus made resemble butter in appearance, they do not resemble it in taste, and the 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 obtain them from a similar source. 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. After the milk has properly soured, or ripened, it is placed in the mixing vat with a quantity of melted oils, generally in the proportion of about one part of milk to four parts of the oils. When this mixture is hardened by the cold brine, the milk is held with 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 clear that this flavor is due to exactly the same factors as those which produce the 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, he has not been satisfied to depend upon such a method, but has come to use, more and more largely, pure cultures of bacteria in order to introduce greater regularity in the process. In some oleo factories, indeed, so fully aware have the makers been 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 employed bacteriologists to keep constant guard over these factors in the oleo-making.

# CHAPTER XIV.

## BACTERIA AND OTHER MICROORGANISMS IN CHEESE.

### CHEESE-RIPENING.

Cheese consists primarily of the casein and fat of milk, collected first as a curd and then allowed to undergo a series of chemical changes called *ripening*. Ordinarily the casein is precipitated from the milk by rennet, although it is done in some types of cheese by simple souring. Then the curd is separated more or less from the whey, and pressed into definite shape. The whey removes most of the milk-sugar, and the cheese retains about two-thirds of the food material in the milk, and since it is in a very dense form, it is one of the most nutritious of our foods. The popularity of cheese as a food depends rather upon its flavor than its food value and the flavor develops during the ripening. Cheese-ripening is a very complex phenomenon and one as yet only partly understood. This is due partly to the intricacy of the subject and partly also to the fact that there are very many different kinds of cheeses, and the ripening of the different types is not by any means the same. Although there are some hundred varieties of cheese, they may be arranged fairly well into two groups: 1. The **hard cheeses,** and 2. The **soft cheeses.** The ripening of the hard cheeses is very different from that of the soft, and the ripening of the different types of soft cheese varies greatly one from the other. Each kind of cheese must, therefore, be studied as a special problem.

**Chemical and Physical Changes.**—During the ripening, the cheese, which is at first rather hard, tough and elastic, gradually becomes softer. The extent of this softening depends largely upon the amount of water present, and, in the soft cheese, with their large amount of water, a slimy and almost liquid consistency may finally be reached. This softening is due to a change in the chemical nature of the casein by which it changes from an insoluble into more or less soluble products. The changes in the casein during the ripening are, in general, similar to those that occur when the casein is digested under the action of digestive juices, so that they are frequently spoken of as a *digestion of the curd.* During the ripening the insoluble casein is converted into a series of simpler chemical bodies, *peptones*, *proteoses*, etc., and as these become partly dissolved the hard texture of the cheese becomes softer. Not only are the changes similar to those of digestion, but they are produced by enzymes similar to, though probably not identical with, the enzymes in the digestive juices. The ripening of a cheese is thus a predigestion which renders the cheese more easily digested when eaten.

The enzymes that produce this ripening of the cheese come from three quite different sources. One enzyme with this power of digesting curd is in the original milk itself. This is the *galactase* already mentioned. Another is added to the milk with the rennet, for rennet is made from the stomach of a mammal, and will always contain some of the pepsin from the stomach. The latter is an enzyme with strong digestive power and is sure to be present in some quantity in the rennet, and hence in the cheese after the addition of rennet. These two enzymes doubtless continue to act upon the casein during the ripening, and are responsible for a certain portion of the digestive changes that are taking place. But there is also a third source of enzyme that, in some cheeses, is more important than the others. As already noticed, certain microörganisms have the power of secreting enzymes, and some of them, growing in or on the ripening cheese, develop enzymes which contribute largely to the ripening. In some of the soft cheeses this is certainly the chief source of the enzymes.

**Flavors.**—The production of flavors is of no less importance than the chemical digestion of the cheese. At the present time, however, there is a very profound ignorance concerning the real source and cause of cheese flavors. They are without doubt the products of decomposition. They appear in the cheese only toward the end of the ripening process, and are regarded generally as due to the end-products of decomposition. The peptones and proteoses that result from the digestion of the caseins do not themselves have any of these peculiar cheese flavors; but toward the end of the ripening some of the material seems to be still further broken down into the simpler end-products which show the flavors characteristic of cheese.

The relation of microörganisms to the ripening of hard and soft cheeses is so different that they must be considered separately.

## THE HARD CHEESES.

These include many varieties, the best known of which are the Cheddar cheeses (the most common in America), the Swiss cheeses, and the Edam cheeses of Holland. In all these cheeses the water is pressed out of the curd as completely as possible so that the resulting cheese is very dense. In spite of its dense consistency, a development of bacteria goes on for many days during the ripening. Since cheese is made from milk, it must necessarily contain from the outset many kinds of bacteria, and among them there is a considerable percentage of lactic acid organisms. For a number of days, sometimes for several weeks, these bacteria increase in number. After this they decrease until, when fully ripened, they are very few, compared to their numbers at certain stages of the ripening. A single example will illustrate. Fresh cheese contained 6,600,000 per gram, when four days old, 51,000,000 per gram, and when four months old, 1,000,000 per gram.

It is the lactic acid bacteria that are most persistent, and while in the early stages of the ripening other types are abundant, in the fully ripened cheese, or even the half ripened cheese, there are usually none left except the lactic acid bacteria. While there are variations in the bacteria in different kinds of cheese and in different specimens of the same variety, the above represents the general history of their growth, and in all cases it appears that the lactic acid organisms are finally found alone.

It must be confessed that we do not yet know very much about the part bacteria play in the ripening of the hard cheeses. That they are necessary to the ripening is proved by the fact that cheeses do not ripen normally when they are ripened in chloroform vapor, which prevents bacteria growth, but allows the enzyme action to continue as usual. Although some of the digestive changes go on as usual in these cheeses, the ripening does not become complete, and the cheeses never develop either the same final chemical character or the flavors of cheeses in which the bacteria have had an opportunity for growth.

**Chemical Action of Bacteria.**—When cheese-ripening was first studied, it was believed to be primarily due to the action of bacteria. We have already seen that certain kinds of bacteria have the power of changing casein to peptone—the liquefying type—and this change in the cheese-ripening was at first supposed to be due to the growth of these peptonizing bacteria. But later it became evident that the liquefying bacteria are not common in cheeses, especially in the better grades. If present at the beginning they rapidly decrease in numbers, until they almost or entirely disappear, a fact which forced the conclusion that they cannot contribute materially to the ripening of cheeses. More recently, it has been claimed that certain "acid liquefiers"—*i.e.*, peptonizing bacteria that at the same time produce acid—are intimately connected with the ripening. But there does not yet appear to be much evidence for this.

These facts led to a suggestion that the ripening is due really to the lactic acid bacteria. These do not liquefy gelatin and do not ordinarily have any power of changing casein to peptone. They produce lactic acid which curdles the milk, after which they apparently cease to act upon it at all. Hence, it would not seem that they could digest cheese. But if the acid which they produce is neutralized by the presence of some alkaline, like carbonate of soda, the bacteria continue to grow, and eventually produce the peptonization of the casein. Moreover, the grade of the cheeses is very closely dependent upon the growth of lactic bacteria, and cheese from which lactic acid bacteria are excluded by aseptic milking will not ripen normally, while they would do so if the acid germs were present. All of these facts together led to the conclusion that it is this peptonizing power of the lactic acid bacteria, under certain conditions, which is responsible for the chemical changes that take place in the ripening cheese. This conclusion, however, has not been very generally accepted; for while the lactic acid bacteria, under these conditions, do produce a certain amount of peptonization of the casein, the action is extremely slow and not very complete; and it has not seemed to most students that the phenomenon in question is sufficiently explained by this slow action of the lactic acid bacteria.

**Flavor Production by Bacteria.**—Apparently the *flavors* must be due to bacterial action. Cheeses ripened in chloroform vapor, which allows the enzymes to act, but prevents bacteria from growing, though they ripen, do not develop flavors and these must be due to some other cause than enzyme action. That they are the end-product of chemical decomposition seems to be extremely probable. In many cases they are associated with ammonia; and ammonia, as is well known, is one of the final products of proteid destruction. The only known agency that commonly produces the complete destruction of proteids is bacterial, and, while the matter has never been put to any satisfactory test, the most probable explanation seems to be that these cheese flavors are the result of bacterial decomposition.

Against this view, however, has been urged the fact that in the well ripened cheeses hardly any bacteria, except lactic acid organisms, are present, and that this class of bacteria does not, so far as is known, have any power of producing cheese flavors. Some bacteria, if they grow in proper abundance in milk, will in time develop well-known cheese flavors; but these organisms have not been found in old, strongly flavored cheeses. Whether they have anything to do with the production of cheese flavors is, therefore, uncertain. It has been suggested that the flavor of cheeses may be due to the bacteria which grow in them during the first few days. Liquefying bacteria are found during this early period, and before the miscellaneous bacteria disappear, as they do later, some of these liquefiers may secrete from their bodies substances, possibly enzymes, that continue their action in the cheese, slowly, but for a long time. Although the bacteria that produce them soon die, the chemical ferments which they have produced may continue their activity until they finally produce the new products that give the flavor.

One fact appears to be certain amid much that is still unsettled. The lactic acid organisms certainly play an important part in the process; at least in the ripening of the Cheddar cheeses and probably the other hard cheeses as well. The lactic acid developed in the early ripening cheese is necessary to the digestive changes that occur, for the acid combines with the casein, a preliminary step in the ripening. While their total action is not yet understood, it is certain that they have a necessary part in the cheese-ripening.

As a result of these facts, cheese-makers have, in recent years, learned that the use of *lactic acid starters* is decidedly advantageous. This practice enables the cheese-maker to control much more accurately the ripening, and to reduce the number of failures. The reason why the inoculation of a lactic starter tends to reduce the failures in cheese-making can easily be understood from the facts already presented. The lactic acid bacteria have the power of checking the growth of other germs, and even of destroying them altogether. When, therefore, the milk has a large quantity of lactic bacteria developing rapidly in the curd, the other bacteria, which might under different circumstances produce putrefaction, are prevented from increasing. In the making of cheeses this protecting action of the lactic bacteria becomes very important, and is, indeed, the secret of good cheese. The cheese remains for weeks, or even months, in a moist condition, and there is opportunity all this time for the growth of bacteria. If a proper lactic organism is present at the outset, the cheese will be protected from the various putrefactive types that would otherwise surely injure it. Their presence in sufficient quantity is responsible for many of the defects to be noticed later.

For these reasons, then, the cheese industry is learning the prime importance of a strong lactic fermentation in milk that is to be converted into cheese, and in order to bring this about it is rapidly adopting the method of using starters. Cheese starters are essentially identical with those used in butter-making, and they are used in much the same way. *Home starters* are frequently made and inoculated into the milk, and the use of *commercial starters* is also rapidly growing. It is interesting to find that the types of lactic bacteria that are useful in butter-making are not always satisfactory in cheese-making. Bacteria that give a fine flavor and aroma to butter may produce a bitter taste with ruinous results when used in cheese-making. The use of starters in the cheese industry seems to be firmly established at the present time and is practically sure to extend, for it is one of the methods of safe-guarding the cheese against undesirable fermentations.

**"Faults" in Hard Cheese.**—The value of a cheese is wholly dependent upon the success of the ripening. A great loss is entailed upon cheese-makers by an imperfect ripening, resulting from a variety of defects called *faults*. These are commonly due to the growth of certain kinds of microörganisms which do not grow in normal cheeses. The injury resulting to the cheese may be only sufficient to make the cheese a little "off" in flavor but still passable, or it may be so great as utterly to ruin the cheese and make it a total loss. Some of these faults have been traced to their sources and will be considered under the following heads:

*Swelled Cheese.*—This is, perhaps, the most common fault. It is due to the development of a considerable quantity of gas which fills the curd full of holes and causes it to swell and lose its shape. Sometimes the holes are extremely numerous and small, and sometimes they are fewer but of larger size. In any case they are undesirable. Even good cheeses are apt to show some gas holes, but so few as to do no special injury. Sometimes the gas is so abundant as to cause the cheese to burst, in which case it is completely ruined. Between these extremes are all kinds of intermediate grades. The development of the gas is accompanied by an unusual fermentation and an unpleasant taste and smell. The cause of the trouble is the development of gas-producing bacteria. Several different species are known which have this power of developing gas in great quantities in the ripening cheese (Fig. 43). Most of them, perhaps all, belong to the type which has been referred to

FIG. 43.—A bacillus causing swelled cheese (*B. Shafferi*).

in Chapter XI as *Bact. aerogenes* type. As pointed out in that chapter, the different varieties of this type vary much in the amount of gas they produce; sometimes the quantity is very slight, sometimes it is extraordinarily large; and it is easy to understand how different strains and different quantities of bacteria will produce-grades of gasiness in cheeses.

*Bitter Cheese.*—The development of a bitter taste is one of the common troubles of cheese-makers. Sometimes this defect will involve the whole output of a cheese factory and cause heavy losses for a considerable period. Two different causes have been determined upon as responsible for the trouble. In one extended series of losses thus resulting, the cause was found to be a *yeast* (*Torula amari* Fig. 37) that obtained access to the cans and vats and continued for a long time to make trouble. The difficulty disappeared with the thorough cleaning and sterilizing of all articles in the dairies and factories. In another series of bitter cheeses the trouble was found to be in one of the *liquefying bacteria*. This class of organisms is nearly always present in milk, and though the growth of the lactic bacteria usually crowds them out, sometimes, either because of their extra abundance and vigor or because of lack of sufficiently vigorous acid organisms, they are not overgrown by the lactic acid bacteria, but continue to multiply until they develop bitter flavors that injure or spoil the cheese. This cause of bitterness has been detected in both the hard and the soft cheeses.

*Putrid Cheese.*—Sometimes soft spots appear upon the surface of a cheese. They may become larger, eating their way into the cheese, and producing a more or less slimy appearance. The trouble is undoubtedly due to the growth of putrefying bacteria, but not much is known about the matter at present.

*Fruity or Sweet Cheese.*—This is a phenomenon which occurs sometimes over widely extended districts and detracts from the character of the cheese without always ruining it. It is characterized by a peculiar sweet taste, which, although not unpleasant, spoils the flavor of the cheese and thus injures the sale of the product. This trouble has been found to be due to a yeast which gets into the milk.

*Rusty Spot.*—This is characterized by rusty, red spots on the outside and, indeed, not infrequently throughout the whole cheese. The cheese loses its value and may in the end become quite ruined if the trouble develops sufficiently. The cause is a bacterium, *B. rudensis*.

Many other "faults" may be recognized as interfering with the normal ripening. *Black spots* and *blue spots* are sometimes noticed and a variety of "off" flavors that cannot be described. In regard to all of these troubles the cheese-maker has the serious disadvantage that they cannot be discovered until the ripening has become partially completed, and then it is too late to apply any remedy. The method of meeting them must be by *prevention rather than cure*, and after an improper ripening has begun, practically nothing can be done to stop it. By cleanliness, by frequent sterilization of vats, and by the use of vigorous lactic acid starters much can be done to prevent these troubles, but there seems to be no remedy after the improper ripening has begun. A vigorous lactic acid starter will go far toward preventing gassy cheese, and the slimy whey used in the Edam cheese prevents it in that particular brand. A regulation of temperature during ripening will also aid, since the gassy organisms grow best at higher temperatures, while at a lower, about 60°, the common lactic acid bacteria are the more vigorous. When to these two suggestions of vigorous starters and cool temperatures we add great care in keeping all milk-vessels clean, and in sterilizing frequently by steam, we have included practically the only methods in use of much significance in guarding against the various cheese faults.

## SOFT CHEESES.

The essential difference between a hard cheese and a soft cheese is that the latter has a very much higher percentage of moisture. To bring about this condition, the method of manufacture is designed to retain the whey in the curd. After the milk is curdled the curd is sometimes dipped out directly into forms provided with holes in their sides, through which the whey drains naturally without the application of any pressure. In other cases the curd is cut in the vat, but the curd and whey together are dipped into forms for draining. As a result, there is produced a cheese which contains a much greater amount of water than is allowed to remain in the hard cheeses.

This large amount of water produces a ripening of a totally different character from that which occurs in the hard cheeses. The process of ripening is different, the agents that bring it about are different, and the final results are very different from those in the hard cheeses. Moreover, the ripening is liable to greater variations in the soft cheeses than in the hard cheeses, and it is more difficult to control, because of the presence of so much moisture. Bacteria, yeasts, and molds all find a suitable medium for growth in the wet curd of the soft green cheese, and unless the progress of the ripening is exactly right, the cheese-maker may expect the development of kinds of microörganisms that are unfavorable to his product and that will spoil his cheeses. Soft cheeses are much less uniform in character than hard cheeses. They differ very greatly in texture and flavor, and are subject to various defects that injure or ruin them. They are, in short, more difficult to make with success than the hard cheeses, largely, if not wholly, because the water they contain offers such a favorable medium for the growth of bacteria and other microörganisms.

The ripening of several of these cheeses has been carefully studied by bacteriologists. A brief description of the phenomena in three of these will illustrate the principles concerned The three described represent three types of soft cheeses.

*Camembert Cheese.*—This, together with *Brie cheese* and some others, represents a type in which the ripening is due partly to bacteria in the curd and partly to molds growing on the surface of the cheese, but not penetrating below the surface. The cheeses are small, about four inches in diameter and one inch thick. The soft curd is dipped out with the whey into forms and allowed to drain by its own weight. The cheese, with its large moisture content, is then allowed to ripen in a damp room where the temperature is low. Sometimes two rooms of different temperatures are used.

The ripening occupies from five to eight weeks, and a series of changes takes place in the cheese, of which the following is a summary:

The first phenomenon that occurs is the souring of the curd, which is brought about by the *Bact. lactic acid* type of organism. These bacteria grow in the milk previous to the addition of the rennet, although the milk is not allowed to become very sour before curdling. But they continue to grow during the curdling and for a day or two after the cheese is made. If by any chance the lactic bacteria fail to develop vigorously, a ruined cheese is sure to result.

The second step in the ripening is the appearance on the surface of the cheese of a species of mold, which has been named *Penicillium camemberti* (Fig. 44). This mold appears in from two to four days and is at first of a pure white color; later, when it begins to produce spores, it becomes a steel gray, but never a deep blue like the common mold. It is a species of mold that apparently does not occur in America, but is very common in Europe in those sections where Camembert cheese is made. Its absence from America is the chief reason why this country has been unable to make Camembert cheese. Where successful cheese of this type has been produced, it has been by importing and inoculating this type of mold into the American cheese factories. This white mold grows on the surface of the cheese, but does not penetrate below the surface. After about two weeks it reaches its limit of growth, forms spores, and dries down to a somewhat thin crust. The growth of this mold, together with other organisms, neutralizes the acid of the curd at the surface of the cheese and renders it slightly alkaline.

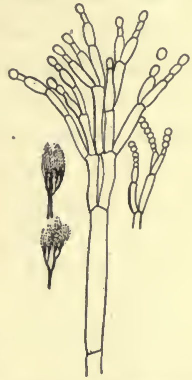

FIG. 44.—*Penicillium camembertii*, the mold ripening camembert cheese (*Thom*).

In the meantime the mold has secreted an enzyme which has the power of digesting the curd. As fast as the acidity of the curd is reduced and the enzyme secreted, the latter acts upon the curd, changing it from a hard consistency to a soft texture. At first the cheese is a hard, solid curd from surface to center; but as this enzyme acts beneath the mold there is formed a thin layer of soft material. This layer grows deeper and deeper as it encroaches upon the curd. The enzyme produces a profound change in the casein, converting it first into peptones and similar bodies; later these break down into simpler bodies, or *end-products*, among which ammonia may always be detected. These latter end-products give the flavor, and appear to be produced by bacteria rather than by the action of the enzymes secreted by the mold. During the ripening the cheese will be found to have a core of a sour, acid curd in the center, surrounded by a layer of soft, digested material. The cheese ripens thus, from the surface inward, and is not completely ripened until the soft layer reaches the center.

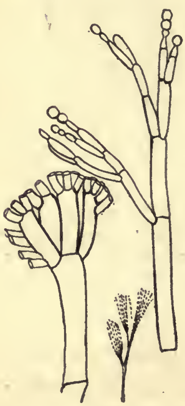

FIG. 45.—*Penicillium roquefortii*, the mold ripening Roquefort cheese (*Thom*).

The flavors are not due to the enzyme digestion, but to the end-products of decomposition. In the case of this cheese, as in the hard cheeses, no positive knowledge is at hand as to the exact source of the flavor. That it is not due to the mold alone is certain, from the fact that the softened cheese may be nearly tasteless, if a pure culture of mold has completed the ripening. The peculiar Camembert flavor is, beyond doubt, associated with some of the microorganisms growing in or on the cheese, but at present no more is known about the matter.

*Roquefort Cheese.*—This represents a type of cheese that, like Camembert, is ripened by both bacteria and molds. Closely allied to it are the *Stilton* and *Gorgonzola* cheeses. The mold is a blue instead of a white one, and it grows through the cheese and not alone on its surface. To bring about the growth in the center of the cheese special means are devised in its manufacture. The cheese-maker begins by cultivating the necessary mold on bread. After the mold on the bread has produced a great quantity of spores, the mass is dried and ground into powder (Fig. 45). After curdling the milk with rennet in the usual way and draining the curd, it is placed in a form in a thin layer, and over the top of the layer is strewn a quantity of powdered, moldy bread with its thousands of spores. Over this is placed another layer of curd with more mold spores; then a third layer of curd over all. The mold is thus planted within the cheese. The whole is then pressed by moderate pressure in a form. After a few days the cheese becomes hard enough to be removed from the form and is next placed upon a machine which punches it full of holes by means of small needles. The purpose of this is to allow air to enter into the center of the cheese, thus furnishing the molds in the center with the air they need for growth. The cheese is then put into the ripening room where the molds develop, growing primarily within the cheese. As the molds grow they develop a peculiarly peppery, piquant taste, which is characteristic of the Roquefort cheese. Just before the cheese is fully ripe it tastes bitter; but this taste disappears as the final flavor develops. A good Roquefort cheese is only possible when there is a luxuriant growth of these molds within the cheese, no surface growth being allowed.

The successful manufacture of the Roquefort cheese in the United States is yet to come. There seems to be no reason why a cheese cannot be made in this country which, if ripened by the Roquefort mold, will have the Roquefort flavor; but it is not likely that a real Roquefort can ever be made in America because, the typical Roquefort is made of sheep's milk, and it is doubtful if Americans will ever be content to raise sheep and milk them. Stilton and Gorgonzola cheeses are, however, made from cow's milk and ripened by the same mold that is found in the Roquefort. These cheeses can certainly be made in this country. Stilton has already been made in Canada, and there is no reason why its manufacture cannot be undertaken and developed in the United States.

*The Limburger Cheese.*—This represents a type of cheese in which molds play no part in the ripening, but bacteria are the primary and perhaps the sole agents.

After being drained in a mold until firm enough to be handled the cheeses are placed in a ripening cellar. Every few days they are removed from the shelves and rubbed over with some liquid, water being commonly used, although vinegar is sometimes put into the water. The surface is thus kept constantly moist. Because of this constant moisture on the surface of the cheese, molds cannot grow upon it, for they need a damp, not a wet surface; but a quantity of bacteria grow instead. These ripen the cheese, doubtless by the secretion of chemical ferments, although the process has not as yet been fully studied. The resulting cheese develops very high flavors, closely resembling those of decay, and the cheeses rapidly putrefy when they become old. If they are marketed at the right stage the flavors are not strong enough to be disagreeable, and many persons are very fond of them. The Limburger type of cheese includes Backstein and some others.

## PRACTICAL RESULTS.

The practical application of bacteriology to cheese-making is just in its infancy, and it is quite impossible to determine the extent of its development in the future. As already pointed out, cheese-makers, in the last few years, have been using pure cultures of lactic acid bacteria as starters to insure a more complete and more uniform souring of the curd. This practice is rapidly growing. The lactic starters have two purposes. First, the growth of the acid organisms checks the growth of other bacteria that would be likely to spoil the cheese, and this check seems to be quite necessary for the proper ripening and for the preventing of *faults*. Second, the formation of lactic acid appears to be a needed step in the chemical changes that constitute the ripening. Hence, the use of a good starter of acid organisms has a reasonable foundation, and we may confidently assume that the practice of using starters will increase. In the making of the Edam cheese of Holland, the practice of using *slimy whey* to aid the ripening of the cheese has become very extended. The slimy whey is a culture of bacteria in whey, and is, therefore, simply another method of using a bacterial starter to control the cheese-ripening. It hastens the ripening and makes it more uniform, but it does not improve the cheese. In the manufacture of Roquefort cheese, mold cultures are intentionally added to bring about the proper ripening, and in recent years pure cultures of molds have been applied in the manufacture of cheeses of the Camembert type. In these cheeses, too, lactic acid starters are very commonly used. From these few instances it is evident that the practical application of bacteriology to cheese-making has already begun, and it is almost certain that a large field is open for the future in this direction.

# PART IV.

## RELATION OF MICROÖRGANISMS TO MISCELLANEOUS FARM PRODUCTS.

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## CHAPTER XV.

### ALCOHOL, VINEGAR, SAUER KRAUT, TOBACCO, SILAGE, FLAX.

Although the problems of soil fertility and dairying offer the largest field for the application of bacteriology to farm life, there are many other problems of minor importance where microörganisms play a part. Most of these concern food products, either their preparation or preservation, although some have no relation to foods.

In all temperate and cold climates it is necessary to preserve food for the winter season. This applies equally to the farmer's own food and to that of his cattle. There is a difficulty in preserving some kinds of food because of the readiness with which putrefactive bacteria cause their disintegration and decay. Bacteria will feed upon almost any kind of organic matter, provided there is plenty of moisture at hand; but some of the foods, like most grains, have such a small amount of water in them that bacteria are unable to grow, and there is little difficulty in preserving these for an indefinite length of time. Nature herself, at the end of the growing season, extracts the water from the seeds, leaving the comparatively dry

FIG. 46.—Showing nature's method of preserving seeds by drying. The lower figures are dried and the upper are fresh seeds.

mass to remain over the period of rest until the growing time comes again (Fig. 47). Other kinds of food contain a large amount of water, and the farmer must find some means of protecting such food from bacterial action. This is accomplished in a variety of ways, but may best be considered under two heads: 1. The agency of microörganisms 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 methods adopted for preserving the material furnish it with flavors or other characters which distinctly add to its value.

## THE ALCOHOLIC FERMENTATION.

The fermentation of sugar into alcohol and carbonic acid is due to yeast. Among the various aspects of farm life there are quite a number based upon this type of fermentation. We have seen that yeasts are especially related to sugars, and that any product which contains much sugar is more likely to undergo alcoholic fermentation than putrefaction. Yeasts, when dry, may remain alive for a long time and float around in the air. The air at all times and in almost all places is, therefore, sure to contain these living yeast plants, ready to begin to grow and produce a fermentation whenever they fall into a sugar solution. These air yeasts are sometimes called *wild yeasts* in distinction from the cultivated yeasts that are now articles of commerce. But whether from the air or from a package of commercial yeast, the organisms are essentially the same and their action the same.

The chief products of our farms which are liable to direct alcoholic fermentation are the fruit juices. Alcoholic fermentation is also the foundation of the gigantic distillery and brewery industries, which make use of grains and other farm products. But these hardly belong to our immediate subject. There are, however, a few forms of fruit-juice fermentations more or less common on our farms.

**Wines.**—The name wine is given to the fermented juice of fruits. The most common fruit used for this is the grape, whose juice is rich in sugar and easily pressed from a mass of the fruit, as a clear liquid. Upon the skin of the grape there are sure to collect, during its growth, a variety of microörganisms, mostly from the air, and among them will be enough yeasts to start a fermentation of the sugars as soon as the juice is extracted from the grape. The grape juice, therefore, needs no yeast added to it to start a fermentation, since the wild yeasts are sufficient to give all the inoculation necessary. In the making of wines the usual method is simply to press out the juice from the grape and then to allow a spontaneous fermentation to occur. Occasionally the practice of adding yeast to the juices, in order to hasten or control the fermentation, has been recommended. This method, which has made a complete revolution in the brewery industries, has not, as yet, been very extensively applied to wine-making. The knowledge that there are many kinds of yeasts with different values in fermenting, certainly suggests that, in wine-making, an improvement may be anticipated by this use of pure cultures. The use of pure cultures in wine-making is becoming more common, and where they have been used an improved product or a better control has been claimed.

Some farms, where grapes are raised in abundance, prepare for market an unfermented grape juice which is essentially wine that has not been allowed to ferment. The expressed juice is sterilized by a temperature of about 170°, which is sufficient to destroy the yeast cells and to prevent fermentation, if the juice be subsequently kept from further contamination by being bottled. The principle concerned is simple, but there are various practical difficulties in the way that make it difficult to produce a good quality of grape juice.

The term wine usually refers to the fermented juice of the grape. But in sections of the country where grapes are not extensively grown other fruit juices are used. Wines are made from the juice of *blackberries*, *currants*, *raspberries*, *elder berries*, etc. In the making of wine from these fruits, since the juice is not very sweet, sugar is commonly added in amounts varying with the sweetness of the fruit and depending also on whether a sweet or sour wine is desired. The mixture is then generally left to ferment spontaneously under the influence of the wild yeasts that are abundant enough to produce a vigorous action. Yeast is sometimes added. As a rule, the fermentation is allowed to continue as long as it will, after which the wine is bottled and thus preserved for use.

**Cider.**—This is nothing but apple wine, and is made in large quantities in sections of the country where apples are abundant. The expressed apple juice is seldom treated at all, but left to ferment spontaneously. The amount of sugar in apple juice is small, and the completely fermented product contains a proportionately small amount of alcohol. *Sweet cider* is a name given to the product while it is still fermenting; it contains but a small amount of alcohol, but is filled with the carbon dioxid gas that is produced by the fermentation. *Hard cider* is the name applied after the fermentation is nearly or quite over, when the evolution of $CO_2$ has ceased and the alcohol is at its maximum. In the making of cider, as in most other fermentations, great improvements have been made in recent years by the application of the discoveries of bacteriologists. The use of pure cultures of yeasts, in the place of spontaneous fermentation, makes the product of a better character and the fermentation more uniform. Numerous other improvements have been made in the details, so that this product, formerly made on the farm in a haphazard fashion, without care and with little or no knowledge of the processes, is now made on a larger scale in special cider factories, resulting in a cider of a much higher quality.

**Yeasts in Bread Raising.**—The most common use of yeast is in the raising of bread. All nations and all peoples have been accustomed to make bread from the flour of different grains. The earliest method was simply to stir the flour in water and bake the mixture into a hard, *unleavened* bread. The next step was to allow the dough to stand for a number of hours in a warm place until it became somewhat swollen by the gas formed within it, and then to bake it. The gas made the dough porous and resulted in a bread filled with holes, easier to masticate, of better flavor, and more easily digested. This was *leavened* bread. The next step was to take a little of the leavened dough and mix it with the next lot of fresh dough, to hastenen the leavening, a process that was simply inoculating the dough with the yeast organisms in the leaven. The next step, taken a long time afterward, was to discover that it is the yeast in the leaven which produces the raising of the bread, and then to separate the yeast from other undesired materials in the leaven, and use it in pure cultures. This finally gave the yeast that has been used for half a century or more. The use of leaven has not altogether disappeared, but yeast is quicker and more reliable.

The action of the yeast in bread-raising is very simple. The dough contains a considerable quantity of starch and also a small quantity of *diastase*, an enzyme capable of converting starch into sugar. The yeast acts upon the sugar thus produced and forms from it alcohol and carbon dioxid. The latter, being a gas, forms bubbles in the dough, causing it to swell and become lighter. When subsequently baked, these gas bubbles leave their traces in the numerous holes that one finds in raised bread.

In the raising of bread the practice of depending on the wild yeasts of the air has long since disappeared and yeast cultures are now almost universally used. These commercial yeasts have been chosen from the considerable variety of yeasts known, and are, of course, the ones that have been found to produce the best results in bread-raising. They are not the same varieties as those found best for brewing. The yeast is cultivated in large quantity and then put up in a convenient form for distribution, sometimes dried, in which condition it will keep alive for weeks, and sometimes compressed into a moist cake, *compressed yeast*, in which condition it will keep only a few days.

*Bacterial Impurities in Bread-raising.*—The yeast cakes are never pure yeast, but may contain undesired bacteria, which then get into the dough and sometimes produce trouble. Occasionally, too, such bacteria may get into the dough from other sources than yeast, such as dirty water or dirty utensils in the kitchen. During the raising, lactic acid bacteria always grow, and they seem to be necessary in order to prevent the growth of other species of more troublesome organisms. Sometimes such bacteria grow too vigorously and may cause trouble. At least two different *faults* in bread-making are known to be caused by undue growth of bacteria. The first of these is *sour bread,* due to the growth in the dough of acid-forming bacteria. This occurs most commonly when the raising is allowed to continue too long or at too high a temperature. The bacteria in question will not produce trouble if fresh yeast be used and the raising be completed in less than eight hours' time. A second fault, due to bacteria, is *slimy bread.* This is of rare occurrence, though sometimes it will infect a bakery and continue day after day. This bread, when fresh, appears normal; but after a few hours it becomes slimy, so that, when broken, it appears as if filled with cobwebs. The trouble is due to bacteria, the source of which may be either the utensils, the flour, or the yeast (Fig. 47). The remedy is in cleaning and sterilizing all baking dishes. If this does not remove the trouble it is well to change the brand of flour or get a fresh supply of yeast. The trouble may also be relieved by keeping the bread cold so as to prevent bacteria growth. The addition of a little lactic acid to the dough, or the use of equal parts of sour whey and water in mixing the dough, is also recommended as a remedy, based upon the fact that lactic acid bacteria will check the growth of other organisms.

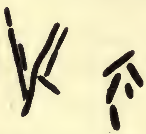

FIG. 47.—The bacteria that cause slimy bread.

## VINEGAR-MAKING.

Vinegar is used both as a direct condiment to give relish to foods and as a preservative, as in the manufacture of pickles. It is made on a large scale in vinegar factories and on a small scale on farms. It is always made from some weak alcoholic solution, like cider, weak wine, or beer, each locality using as a source the alcoholic solution most easily obtained. The essential part of the process is the chemical union of the alcohol with oxygen from the air, by which it is converted into acetic acid. Such a simple oxidation can be brought about by a purely chemical process. As long ago as 1721, Davy discovered that platinum-black, or finely divided platinum, when mixed with alcohol, causes an active union with oxygen to take place, which results in the production of acetic acid.

When vinegar is formed in the usual way, a brownish gelatinous mass, called "mother of vinegar" is formed, and for a time it was believed that this mother of vinegar acted like the platinum-black, "condensing" the oxygen, and thus causing a chemical union. But the error of this conclusion was later shown. 1. The ordinary vinegar fermentation is stopped by the accumulation of 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 in vinegar is most abundant at about 95° F., diminishing rapidly at higher temperatures, and may in itself produce so much heat as actually to ignite the alcohol. 3. Later the production of acetic acid by the growth of pure cultures of certain bacteria showed vinegar-making to be a true fermentation.

**The Vinegar Organism.**—The mother of vinegar is a soft, semi-solid mass, commonly forming a scum on the surface of the fermenting alcohol. Vinegar is not formed if this material be lacking, 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 1812, without having any suspicion that the skin was the cause of the acetic acid. Later, Kützing, showed that this skin was made of numerous minute, living organisms, and positively asserted that they were the cause of the acetic fermentation. But the chemist Liebig checked the advance of discovery by his own theories of fermentation, which regarded the whole class of phenomena as chemical processes.

It was eventually Pasteur who demonstrated that the process is really a fermentation due to the activity of the microörganisms in the mother of vinegar. An examination of this material shows it to be made of a mass of bacteria. Pasteur used for them the name *Mycoderma aceti*, but he did not study them sufficiently to show what they were, although he demonstrated their relations to vinegar-making. Hansen later proved they were bacteria, and showed that in the different samples of "mother" there are several varieties.

Henneberg still later increased our knowledge of the bacteria, so that we now know that there are at least fifteen varieties of these vinegar organisms, differing from each other in various respects, such as their optimum temperatures, the amount of acid they will produce, etc. *Bact. aceti*, *Bact. pasteurianum*, *Bact. kützingianum*, *Bact. xylinoides*, *Bact. orleanense*, and *Bact. xylinum* are names applied to the most important types.

All of these acetic bacteria have certain very characteristic points which they share in common, thus forming a group by themselves. They all exist in three different forms, shown in Fig. 48.

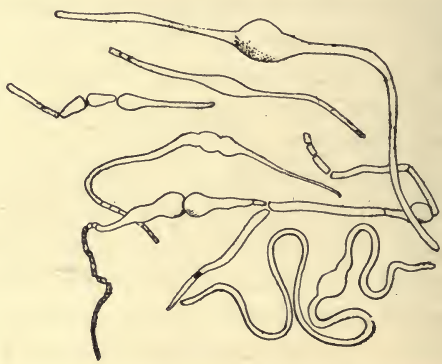

FIG. 48.—Acetic acid bacteria, showing long rods and rounded swollen centers.

They may form chains of short rods, looking like ordinary bacilli. At high temperatures they grow out in long slender threads, sometimes very long, without any traces of divisions. These threads may subsequently break up into short elements. At low temperatures they have a peculiar habit of forming long threads with rounded swollen centers. When these threads break up into short forms only the thick, swollen centers remain undivided. This character is such a peculiar one that it places these acetic bacteria in a class by themselves. These different varieties are distinguished not only by slight differences in structure, but also by some important differences in relation to condition, and in their power of forming acetic acid. They vary 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 conditions *B. pasteurianum* will produce 0.08 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 as high as 42° F., whereas *B. pasteurianum* at the same temperature will hardly multiply, and produces no fermentation. Some of the species produce the 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.

**Methods of Vinegar-making.**—The farmer's method of vinegar-making is simply to allow cider to remain in barrels for a sufficient number of months to turn into vinegar. The result of this method is variable, sometimes very good and sometimes very poor vinegar resulting. Chance is relied upon to insure the presence of the proper vinegar organisms, although to make more sure of a good result, barrels are sometimes taken that have previously been used for the same purpose, and that are, therefore, more likely to contain the desired bacteria.

But vinegar-making, like other farm processes formerly carried out by each farm independently, is becoming largely localized in factories, where it can be conducted on a large scale and can be more carefully watched. In these factories two methods are used, differing radically from each other, and while different factories have varying details in the process, the methods employed are modifications of these two.

*The Orleans Process.*—Oaken casks are used, each new cask being first steamed and then impregnated with 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. This mixture is kept at about 70° F. for a week or so, when a little more wine is added, supplemented in another week by 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 some six or eight years continuously, but eventually it becomes so filled with tartar and mother of vinegar that it must be cleansed.

Shortly after the process of vinegar-making is started a skin of the vinegar organisms grows on the surface, soon covering the whole. Its action causes the alcohol to unite with oxygen from the air, thus producing acetic acid. The bacteria in this method grow chiefly on the surface of the liquid, and they develop 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 manufacture of vinegar. This loss, though sometimes considerable, is generally not great, for the accumulation of acetic acid will soon stop the growth of the organisms. Different species of the organisms can endure different amounts, but when the acetic acid has reached 14 per cent. the bacteria are never able to produce any more.

*The Quick Process.*—A second process of vinegar-making, known as the "quick process," does not, at first sight, appear to be caused by microörganisms. This process consists simply in an intimate mixture of alcohol with air by means of shavings. A mass of shavings is placed in tall vessels and thoroughly moistened with an alcoholic solution. Then the whole is inoculated with a little warm vinegar followed by alcohol. The vinegar thus added starts the process, and in a few hours new vinegar is produced. Alcohol is now added at the top, slowly but continuously, and it percolates through the shavings and appears at the bottom as vinegar. Such a process seems at first to be more like a chemical phenomenon than a fermentation induced by microörganisms. But it 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, it has been proved that, if the growth of the fungi is prevented in such a mixture, no vinegar is formed. The shavings simply furnish a large surface for the spreading out of the organisms. Hence, the quick vinegar process is also dependent upon the presence and active growth of the vinegar plants.

**Use of Pure Cultures in Vinegar-making.**—The different types of vinegar-making organisms vary much, requiring quite different conditions of temperature for their best work. In the processes of manufacture hitherto used considerable losses have resulted from the fact that the acetic acid formed is destroyed by the further action of the bacteria. The fermentation is apt to be irregular and, even under the same conditions, does not always produce equally desirable results. The reasons for the irregularities are to be found in the fact that, in different factories, sometimes even in the same factory at different times, many varieties of the vinegar organisms are found. Moreover, several of these organisms are almost sure to be found together in any mass of natural "mother." It is impossible to devise conditions that will be most favorable for all of the organisms, and hence, with a "mother" composed of many varieties, there is sure to be some loss. Theoretically, it would seem that vinegar-making could be improved by the use of pure cultures of these organisms. With a pure culture it would be possible to make the conditions such as to produce the best results with the variety in question, and thus prevent the losses that come from one variety destroying the product of another variety. The use of pure cultures has revolutionized brewing, has greatly improved the methods of butter-making, and is rapidly changing the process of cheese-making. Bacteriologists feel confident that the same principal can be applied to vinegar-making.

Hitherto there has been but little application of pure cultures to vinegar-making. To obtain absolutely pure cultures is difficult, and to keep them pure from subsequent contamination is more difficult still. Vinegar-makers have not thought the advantages derived from such methods sufficient to pay for the labor and trouble of applying them. They have, to a considerable extent, adopted rougher methods of obtaining the vinegar organism in quantity and in a comparatively pure condition. A little white spirit vinegar is filtered through fine bolting cloth to remove the vinegar eels. This is mixed with a little alcohol of 90 per cent. volume, and some white wine which has been previously boiled, filtered and cooled. This mixture is placed in shallow dishes and covered with glass plates. The vinegar organism appears in a few hours as a thin scum which ordinarily will be pure, or nearly so. If the scum does not show any white spots (molds) it is gently lowered 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.

The farmer who simply lays aside his few barrels of cider or other alcoholic solution that it may be converted into vinegar will not be troubled or especially interested in the matter of pure cultures. A little loss is nothing to him, while the preparing and preserving of pure cultures is 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 Preservation of Vinegar.**—Vinegar is apt to deteriorate by standing. It loses some of its acidity, falls off in flavor, and may become muddy and slimy. All these various troubles are caused by the continued growth of the microörganisms in the vinegar. In such vinegar may be found various growing bacteria, and very commonly, especially in cider vinegar, may be found vinegar eels in abundance. These latter are little *worms* that get into the vinegar from some source and find it a favorable locality for growth and multiplication, They probably injure the quality of the vinegar, although, so far as is known, they are harmless to one who may consume the vinegar. Their presence is, however, undesirable. All these troubles increase as the amount of acid decreases, for the miscellaneous bacteria and the vinegar eels cannot grow if the acidity is high, but they will grow when this acidity decreases. The reduction in acidity is commonly due to the action of bacteria and usually to the very kind of bacteria that originally produced it; for these organisms, after producing the acid, may cause a further oxidation which destroys it. Hence, it has been suggested that pasteurization of the vinegar for the purpose of destroying the bacteria and vinegar eels will enhance its keeping property. Whether there is any practical value in this procedure has not yet been determined by experience.

## SAUER KRAUT.

This is a food used so widely in Europe and coming to be so popular in this country that it may well be regarded as one of the most important of the minor farm products. It is made of cabbage, slightly fermented and prevented from decay by lactic acid bacteria (Fig. 49). The cabbage leaves, after being washed, are shredded and packed in casks under pressure, to remove most of the moisture. In these casks a fermentation soon starts, which is two-fold. Yeasts that are present produce an alcoholic fermentation, evolving gas that causes the whole mass to foam and froth. At the same time, the lactic acid bacteria, the same species apparently that sour milk, develop rapidly and cause the mass to sour. The bacteria growth is primarily in the juice squeezed out of the cabbage tissue, and not in the solid matter. As the mass becomes sour from the acid, the growth of all bacteria is checked. By this means the ordinary putrefactive organisms are prevented from producing the decay of the vegetable mass, as they would otherwise do. When properly soured, sauer kraut has a flavor that makes it a relish. It may be ready for consumption in two weeks, but it is usually kept much

FIG. 49.—The bacteria and yeasts that ferment sauer kraut.

longer than this. So long as it remains properly acid it will keep, and if kept cold will remain in this condition for many months. Eventually a scum appears on its surface. This scum proves to be made of microörganisms, chiefly a species called *Oidium lactis*, a very common species around farms. These organisms, growing in the scum, gradually absorb and destroy the lactic acid and, as they do so, the sauer kraut becomes less acid and is finally alkaline. After this has occurred the putrefactive bacteria that are sure to be present have an opportunity to grow, and the sauer kraut begins to decay, so that it rapidly spoils. This product is, thus, one that is at first prepared and preserved by certain kinds of microörganisms, but is eventually ruined by the growth of other species.

Certain other vegetables are prepared in a similar way. *Soured beans* are prepared in certain countries, and the souring of cucumbers to make *dill-pickles* is, apparently, an identical process. Soured beets and asparagus are also articles of diet. Bacteria similar to those found in sauer kraut are concerned both in the souring of these products and in the subsequent neutralization of the acid preparatory to the final spoiling of the product.

## THE CURING OF TOBACCO.

Tobacco is a product whose value is almost wholly dependent upon the success of its curing and its final preparation for market. The green plant, as taken from the field, is in itself valueless, and many a crop is injured or perhaps ruined in the curing. The relation of the curing to microörganisms is not yet settled, but since the curing is undoubtedly a fermentation, it properly belongs to our subject.

When the leaves are fully grown the crop is reaped and hung up in a shed or barn to undergo a partial drying. After the drying process has reached a desired stage the leaves are ready for the *fermentation* proper, the process upon which the value of the product largely depends. There are two quite different methods of bringing about this fermentation. In the first method the leaves are left hanging a long time, and are eventually packed closely in boxes weighing several hundreds of pounds each. These boxes are then left to take care of themselves. They are generally packed in the cool weather of fall 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; but after a number of months the boxes are opened to determine the success of the fermentation, and the crop is sold at a price depending upon the character of the product.

The second method of fermentation, adopted chiefly in warmer climates, keeps the whole process under close observation and is, in this respect, undoubtedly superior. The leaves, after drying, are piled upon each other, not too tightly, and a great heap is made, sometimes three feet high, sometimes more. For the proper fermentation of this heap there should be a warm, moist atmosphere, such as is found in tropical and semi-tropical climates. Within a short time the temperature of these masses begins to rise, sometimes as high as ten degrees in a day. When the temperature reaches a point between 125° and 130° F., the piles are opened and the leaves are heaped up again in other similar piles, care being 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. This second method is quicker and, in some respects, better than the first method. The fermentations do not always end here, however. The manufacturer commonly allows the tobacco to undergo a second fermentation, called "sweating," which brings the leaf into a better condition for use.

The primary fermentation is clearly the essential process of 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 nicotin, 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 the texture of the leaf, a change in color (the final color brown) and a change in flavor. These numerous changes, while quite varied in nature, are mainly due to oxidation.

**The Cause of Tobacco Fermentation.**—Three different theories have been held and, to a certain extent, are still held, concerning the cause of the fermentation. While not one of them explains all the facts, all three may, in a measure, be correct.

*The Chemical Theory.*—This assumes that the free oxygen of the air acts directly upon the cells of the tobacco leaves, although it is admitted that microörganisms are necessary to raise the temperature sufficiently to make the oxidation possible.

*The Bacterial Theory.*—This assumes that the fermentation is the result of bacterial growth. Bacteria are found upon the leaves of fermenting tobacco, and several distinct species have been isolated and carefully studied. Some of them are well-known species, but others are peculiar to tobacco. Some have been named *B. tobacci I, II, III, IV*, and *V*. It has been claimed that these have a causal relation to the fermentation of the tobacco, and experiments have been carried out to test it. Tobacco leaves have been sterilized and it is found that they will not undergo fermentation. If, however, they are inoculated with some of these bacteria, they will undergo a fermentation, although the result does not show a good flavor or aroma, which fact suggests that, though bacteria are concerned in the process, there are other factors.

*The Enzyme Theory.*—The recognized importance of enzymes in fermentation in general, has naturally raised the question of their relation to tobacco-curing. Enzymes are known to be produced by plants, and would be expected in the tobacco leaves. Indeed, they are found there readily enough, and among them are certain enzymes called *ozydases*, *peroxydases*, and *catalases*, which have the power, under different conditions, of producing an oxidation of other substances. Are not these, rather than bacteria, the cause of tobacco fermentation, which is chemically an oxidation? Arguments for this view are found in the following facts:

1. 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. 2. The fermentation will go on in the presence of corrosive sublimate that prevents bacteria growth. 3. While bacteria may be found upon the leaves of the fermenting tobacco they are generally found only in small quantities, too few to account for the fermentation which is producing a rise in temperature of 10° per day. Moreover, 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. 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 growth.

On the other hand, these enzymes are found in abundance on the leaves, and they are capable of producing an oxidation, such as occurs during the fermentation. The conclusion that the enzymes from the tobacco leaves are active agents in the curing seems indisputable.

But while these facts suggest that enzymes may play the chief part in the fermentation, they by no means exclude the action of bacteria. 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 has in its own home. Cuban tobacco is now raised in the United States, but its flavor is inferior to that raised in Cuba.

We have already noticed that in the ripening of cheese, though 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 containing 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. On such petuned leaves bacteria are abundant. But there is no evidence at hand to indicate whether these bacteria have anything to do with the production of flavor. It is certainly not impossible nor 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.

Of course the suggestion that the flavor of tobacco may be improved by the use of artificial *pure cultures* in the fermentation is a natural one. The acknowledged relations of bacteria to the flavors of butter, cheese, and other products naturally suggest an attempt to improve the flavors of tobacco by bacterial inoculation. Several experimenters have been trying this plan for years, with what success it is hardly possible to say. The manifest financial importance of such a process, could it be made successful, has inclined experimenters to keep their work secret. While it has several times been claimed that by the use of proper bacterial cultures Havana flavors can be obtained in tobacco fermented elsewhere, these claims have not yet been substantiated by any public demonstration. They are still made by some who insist that they have actually been successful in this line of experimenting and that they have made Havana flavors from common tobacco by bacterial inoculations.

**Diseases of Tobacco.**—Whether or not microörganisms play a part in the normal ripening, it is certain that they sometimes injure the crop and produce abnormal fermentation. The presence of too much moisture on the leaves is likely to be followed by the growth of mischievous microörganisms. Molds are the most common injurious organisms to appear under these conditions, but bacteria may also develop and produce disastrous results. From the time the tobacco begins to grow 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 plant, and slight changes in moisture or temperature are almost sure to bring about troubles of some kind that injure or ruin the crop. Of these troubles some are produced by molds or special fungi, and some by bacteria. The consideration of these various troubles, whether bacterial or of a different nature, concerns only the person interested in raising tobacco and is of no special interest to the agriculturist in general. We shall not, therefore, further consider them in this work.

## SILAGE.

In the silo the agriculturist has devised a method of utilizing certain food products of which the soil yields large crops, but which contain so much water that they lose their value in great measure when dried. The silo not only enables him to preserve such food, but it impregnates it with new flavors which, in some respects, enhance its value, for it makes a product especially relished by cattle.

**Preparation.**—In the preparation of silage the material to be used, most commonly corn not fully ripe, is cut into moderately small pieces and packed away firmly as a solid mass, in a tall, air-tight compartment. Sometimes the silo is filled quickly, and sometimes more slowly, and the rapidity should depend upon the rapidity of fermentation. 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 there is little or none around the sides or bottom, so that only the superficial layers are affected by it.

**Fermentation.**—After the packing important and profound changes take place in the silage. The first phenomenon to be noticed is a rapid rise in temperature, the *primary fermentation.*

The extent of this rise is dependent upon the amount of oxygen present and the readiness with which the heat is radiated. The temperature should not rise above 150° F., and, to give the best result, it should be much lower. The proper production of silage, however, 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.

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. This is a *secondary fermentation.* of a different type. It develops a certain amount of acid, its chemical nature is altered, and it develops a new flavor and aroma which should be distinctly aromatic, without any 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 chiefly a loss of carbohydrates, although there is also an appreciable loss of albuminoids. The loss is largely parallel to the amount of oxygen that finds its way into the silo, being very slight if the oxygen of the air be thoroughly excluded. Perfect exclusion of air is, then, 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 become decayed and ruined, but the central mass itself is not affected. After the feeding from a silo is commenced its contents 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 Causes of Ensilage Fermentation.**—Three different factors have been suggested as causes for the fermentations inside the silo. These are: 1. The *action of bacteria.* 2. *Respiratory* *changes* in the plant tissues, and 3. The *action of enzymes*. The probability is that, as in the other cases where there has been a similar dispute, all three processes are concerned.

*Respiratory Changes*.—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 dioxid. In this respiration carbohydrate bodies are used, with some albuminoids as well, and 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, and recognized by botanists for a long time. To these respiratory changes is due part of the fermentation in 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 dioxid. These changes are thought to be fully sufficient to explain the first changes in the silage, with the initial heating and evolution of gas.

*Fermentations Due to Enzymes*.—As already noticed, living plant tissues secrete a variety of enzymes with varying powers of acting upon carbohydrates and albuminoids. Such enzymes are present in the corn stalks and fruit, and when these are packed in the silo, the enzymes are of course stowed away with them. As the mass is warmed up under the action of the respiratory process it is inevitable that the enzymes will begin their action, and that the fermentations occurring during the next few weeks will be affected by these enzyme activities. It has as yet been impossible to say to what extent the enzyme action is concerned in the phenomenon. Certainly they must have much to do with the result.

The respiratory processes and the action of the enzymes together are capable of producing silage of ordinary type without the aid of bacteria or other living agencies. Silage can be made in experimental jars in which chloroform vapor prevents the growth of bacteria, although it allows the enzyme action to continue as usual. It goes through a fermentation that is fairly typical, a fact that shows that the essential phenomena of ensiling may be wholly the result of these two sets of activities.

*The Action of Microörganisms.*—It was first thought that the fermentation of silage was a bacterial action wholly, but further study showed the fallacy of this conception. The original fermentation, by which there is a rapid rise in temperature, cannot be the result of bacterial growth, since it is too rapid and the temperature rises too high. There is no evidence to suggest that any bacteria can produce a rise as high as 150°, a temperature that destroys the life of most organisms. If the rise were due to bacteria it would be rather slow, rising only as the bacteria had the opportunity to develop, while on the contrary it is very rapid. Finally the possibility of making silage in a jar filled with chloroform vapor shows that bacteria are not necessary for the phenomenon.

But this does not by any means exclude the agency of microorganisms in the ordinary formation of silage. Bacteria are certainly present and, in some cases, they are present in great numbers. Some bacteriologists have not been able to find them so very abundantly, but this seems to be due to the fact that they did not use favorable media in studying them, for when a medium is used that is adapted to the silage bacteria, they are found in abundance. Certainly, if they are present and develop during the fermentation, they must have some effect upon the silage. One effect they certainly seem to have. The silage turns acid during the ensiling, and this acidity appears here, as in other cases which we have noticed, to be a means of preventing subsequent putrefactive changes. Without doubt this acidity may be attributed in part, if not wholly, to acid-forming bacteria growing in the silo.

Further, there develop in the silage certain prominent flavors which contribute largely to its value, and the source of these flavors is as yet unknown. Aromatic flavors such as are found in silage do not come from respiratory processes, nor do enzymes develop such flavors, so far as is known. There are some who think, however, that silage flavors are really due to enzyme action. But considering the fact that enzymes do not commonly produce any such flavors while bacteria do, and also that bacteria certainly grow in the silage after the first fermentation is over, it seems on the whole more likely that the flavors must be attributed to bacterial action.

As a summary, then, it appears that silage involves three distinct processes, each of which is capable of producing a profound modification of the material in the silo. Probably all three may be concerned, in different degrees, at different times. The various lots of silage do not always ferment alike, even under seemingly identical conditions; and very possibly these three different processes are concerned, in varying degrees, in the different lots of silage. The subject is complicated and probably so variable that we cannot at present say, with any degree of accuracy, just what is the usual course of events in this fermentation. A large amount of study remains to be done on this subject, and doubtless, when the matter is properly studied, so that it is better understood, great improvements can be made in the process.

It is perhaps fitting to say that silage forms a good food for cattle, although some dairy companies refuse to accept milk from silage-fed cows. The reason that this kind of fodder has an effect on the milk is probably due to the dirt and filth that get into the milk from the silage food after milking, rather than to the silage that the animals have actually eaten. If the milk were kept clean and all the dairy processes carried out in a proper manner, it is doubtful whether any trace of silage feeding would show in the milk. But considering the carelessness in the ordinary dairy and the dirt that commonly gets into milk, it may follow that the effect of silage in the stable will show in the milk.

## SOUR FODDER.

This is a food for cattle made out of the waste from beet sugar manufactories, and other waste material. Slices of beet roots, after the sugar is extracted, steamed potatoes, corn stalks, and various other vegetable substances, may serve as its basis. This material is packed in trenches in the ground, pressed by heavy weights, and left to ferment. It undergoes a fermentation that converts it into an acceptable food for cattle. Its value lies in the fact that it is a means of utilizing what would otherwise be a waste product. It is of little significance in this country.

## THE RETTING OF FLAX AND HEMP.

**Linen** is made from the long tough fibers that are found beneath the bark of the flax plant. In the plant they are firmly bound together, and with the wood and bark make a solid mass, glued together by a substance called *pectin*. To remove these fibers so that they may be woven into linen, this pectin must be disposed of in some way. The method by which this has been accomplished from time immemorial is by "retting." The flax is tied up in bundles and immersed in the water of a stream or in vats. Here it remains until the water bacteria have pretty thoroughly rotted or "retted" it. By the decomposing action of these bacteria, the pectin is dissolved and the fibers in the flax stem are loosened from their connection with the other parts of the plant. A little combing over properly constructed teeth separates the fibers from each other, and gives the desired product for spinning and weaving. The separation of the flax fibers has practically always been done in this way. The bacteria concerned have been isolated from the retting flax and obtained in pure cultures, and it is found that they are able to produce the result when inoculated upon flax in pure culture. Hitherto no substitute for this bacterial action has been found that will satisfactorily replace the natural retting. It is quite possible that some chemical means may be found that will replace the bacterial process. Indeed, certain secret processes are now in use that are based upon chemical methods and are claimed to give uniform results in a much shorter time than the ordinary retting. How soon these may replace the agency of bacteria in the linen industries cannot be predicted.

**Hemp** is prepared from the hemp plant by a means essentially similar to the retting of flax.

## CHAPTER XVI.

# THE PRESERVATION OF FOOD PRODUCTS.

### THE SPOILING OF FOOD.

Practically all the materials raised on the farm as food for man or animals may serve also as food for microörganisms. If any of the various fungi attack foods, their presence is shortly made manifest by signs of decomposition. The food, we say, begins to *spoil*. Any one of our food products that contains sufficient water is sure to be attacked sooner or later by some of the various types of microörganisms, especially by the three classes we have recognized.

**By Bacteria.**—These are particularly adapted for feeding upon proteid matter, but they are not fond of sugar. Hence we find them especially concerned in the spoiling of proteid foods. Meats, milk, eggs, wheat, and other cereals, all contain unusually large quantities of proteids and are liable to putrefaction, whenever they are moist enough; and their putrefaction will practically always be found to be due to bacteria. In all attempts to preserve these substances it is to be remembered that we are dealing with bacteria, some of which are liable to form spores and for that reason are difficult to destroy. Pure sugar solutions, on the other hand, will not undergo a bacterial fermentation, although impure sugars may do so.

**By Yeasts.**—Although sugars are not attacked by bacteria, they are the favorite food of yeasts, which destroy them by setting up an alcoholic fermentation. If the sugar is in considerable abundance, it serves as a partial protection against bacterial growth, although it favors yeast activities. From this it follows that fruit juices in particular are subject to yeast fermentation, but are not specially liable to bacterial action. All such substances as *fruit* *juices, jellies, jams,* in short, anything preserved by sugar, are liable to yeast action. Therefore, in their preservation, it is to be borne in mind that we are dealing chiefly with yeasts which are much more easily killed than bacteria. Sterilization of these products is much easier than sterilization of proteid foods.

**By Molds and Higher Fungi.**—Although these are less important agents in the spoiling of foods than bacteria, they are important in several directions. Many kinds of food—bread, cheese, etc.—will support a mold growth if kept rather moist. Molds grow chiefly on the surface, but when they become luxuriant they cause the material to become "musty" and to develop unusual as well as unpleasant flavors. Almost any food might in time be completely spoiled by molds, but usually the bacteria and yeasts act more rapidly than the molds, so that mold action is secondary. In the decay of wood and timber it is the higher fungi that play the chief part (bracket fungi and other tree fungi). They force their mycelia into the trunk of the solid tree, softening it and beginning the process of decay. The common molds are the primary cause of the decay of fruit, for they force their mycelia through breaks in the skin of the fruit and then through the whole fruit. While yeasts and bacteria may sometimes be concerned in the rotting of fruit the molds are almost universally the cause of this phenomenon.

## PRESERVATION OF FOODS.

The extremely varied nature of farm products has made it necessary to find many different methods of preservation, since what is well adapted for one may be useless for another. The method of preserving wheat, for example, is not adapted for preserving milk or fruit. There are several fundamental methods in use, each of which has numerous modifications.

**Protection from Microörganisms.**—If it were possible to prevent bacteria, yeasts, and molds from gaining access to food materials, the food could be preserved indefinitely. But these organisms or their spores are so abundant everywhere that this is impossible, except by hermetical sealing. Some foods, however, are thus protected. Fruits have a certain amount of protection against the molds that cause their decay, since their uninjured skins resist their entrance. The smooth hard skin of many fruits is impervious to the mycelium of the mold, though it can readily force its way in through a bruise or crack into the softer substance within. Hence the bruised apple decays quickly. Wiping the skin of fruit clean and dry will protect it for a long time from decay. The wiping cleans off most of the mold spores that may be on the skin, and the drying of the skin leaves no moisture in which the few spores left can germinate. If moisture condenses on the skin, as when the fruit is taken from a cold room into a warm one, decay is sure to follow, since this moisture starts the germination of the spores into a mycelium and the latter is pretty sure to find some place in the skin through which it can pass. Once inside the skin, it grows rapidly through the soft pulp and the fruit is soon spoiled. The preservation of fruit is, thus, a matter of keeping it dry, at a low temperature, and with an unbroken skin. Even the wrapping of fruit in paper materially aids in its keeping, since the paper absorbs the moisture that collects on the skin.

## PRESERVATION BY DRYING.

The simplest means of preventing the growth of bacteria in food products is by drying. 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 other is so effective.

**Grains.**—In the preservation of the valuable cereal products nature herself adopts this 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 dried grains not only refuse to germinate unless moistened with water, but bacteria are utterly unable to grow within them. 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 flesh of animals contains so much water that it undergoes decay at very short notice. 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 was 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 by 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 this country.

The same end is very commonly reached on the farm by artificial drying, accompanied by *salting* and *smoking*. In the preparation of *smoked hams*, *bacon*, or other flesh, bacterial growth is prevented, partly by the drying 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 is 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.

It is well to remember that drying and smoking do not kill the animal parasites that may be in the meat, like trichina or tape-worms.

**Fruit.**—The drying of apples, squashes, pumpkins, and other vegetables 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 readily yield to this method of treatment. In fruit prepared in this way the water is not all removed, 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 in the preparation of hay. The fresh grass contains so much moisture that it could not be preserved in masses without undergoing extensive decomposition, and to obviate this the farmer resorts to the simple plan of drying out some of the water. But this phenomenon of drying is not always as simple as it looks, and sometimes 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 great deal of rain.

*Curing of Hay by Self-fermentation.*—In countries where rains are frequent and sunshine rare, the sun's rays cannot be depended upon for the curing of hay, so a different principle is used. If the moist grass is heaped in piles and allowed to stand for a few days, there appears a marked rise in temperature. This continues rapidly, the rate and the temperature reached depending upon the conditions: the denser the packing, the higher the temperature. Commonly the rise is not above 160°, although under some conditions it goes above this, even to the point of spontaneous combustion. This latter phenomenon is of rare occurrence, however, although probably it is the cause of the spontaneous combustion that occurs occasionally in a barn when the hay is packed away in too moist a condition.

The cause of such self-heating has not been definitely settled. It is evident that the phenomenon has a decided resemblance to the fermentation of tobacco and also to that of silage. Three possible causes may be concerned: 1. The respiratory changes in the still living cells of the grass. 2. The action of enzymes from the grass. 3. The growth of microörganisms. Experimental tests have not yet settled positively the relation between these possibilities. Sterilized hay will not undergo this heating, while the same sterilized hay, if inoculated with certain species of microörganisms (*Oidium*), will show a rise in temperature apparently identical with the self-heating. This would clearly indicate that microörganisms may be prominently concerned in the process. But the sterilizing kills the plant cells so that the respiratory changes are stopped, and also destroys most of the enzymes present. Hence the fact that sterilized hay will not thus heat is no proof that microörganisms alone are concerned in the phenomenon. Further, it is extremely improbable that bacteria or molds could develop heat sufficient to kill themselves, still less sufficient to cause a spontaneous combustion. No experiment with organisms has given a heat higher than 160° as the result of their action. Hence, the extreme heat must be due to other causes. Probably this fermentation, like many another, is of a mixed nature. The moist grass still contains some living cells that for a time remain alive and carry on respiratory processes; the enzymes in the grass probably also start some chemical action and, lastly, the microörganisms on the grass, by their growth, add to the fermentative changes going on in the hay. As a result of all three, the temperature rises.

This self-heating is utilized in some countries to cure the hay. 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 firmly packed, 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. The temperature frequently rises as high as 160° F., but not 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 on 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, if the heating is too great, it may 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. This loss of material is one of the objections to this method of curing hay. It is known as **brown hay**.

Sometimes a slightly different method is used. The freshly mown grass is piled in heaps from 10 to 13 feet high, the mass being trodden down as tightly as possible to prevent the admission of air. The temperature in these heaps rises rapidly, and is tested by a thermometer. When it rises to about 158° F., which occurs generally in from 48 to 60 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. Hay thus prepared is called **burnt hay,** and develops an aromatic odor which ordinary sun-dried hay does not possess.

Lastly, it should be noticed that ordinary sun-dried hay will sometimes, especially if stored in too moist a condition, undergo a similar heating in the mow. The hay may be considerably injured by such heating, so that it will lose some of its nutriment. Sometimes the heat is sufficient to cause an actual ignition of the hay.

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

## PRESERVATION BY COLD.

All the common species of bacteria grow more slowly as the temperature is lowered, and 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 may be 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, although it is claimed that some molds can grow at temperatures below freezing. It must be remembered, however, that the low temperatures do not kill the bacteria, but simply delay their action, and as soon as such food products are warmed, the bacteria immediately begin to grow. Indeed, food spoils very rapidly when taken from cold storage.

Where such low temperatures are not feasible, a moderate degree of cold may check bacterial growth and delay putrefaction. An ice chest usually maintains a temperature below 50°, and this is very efficient in helping preserve food. A cool cellar answers the same purpose, as well as the common plan of placing milk and other foods in cold water to delay the spoiling. Fruits are particularly benefited by these cool dry temperatures, and a cellar which has a fairly uniform and low temperature is of great value on the farm in helping to preserve fruits and vegetables that would otherwise soon spoil. The value of ice as an aid in keeping all sorts of perishable material is too fully understood to require further notice.

## PRESERVATION BY THE USE OF CHEMICALS.

Many chemical substances are destructive to bacteria, and foods may frequently be protected 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. The number of substances that can be used without hesitation is not very great.

The more powerful antiseptics, like *carbolic acid* and *corrosive sublimate*, are, of course, out of the question. Certain milder ones, *borax*, *boracic acid*, *salicylic acid*, *formalin* and *benzoic acid*, are more or less extensively used. There are on the market various commercial preservatives, *Preservaline*, *Anti-fermentine*, *Freezine*, etc. These several articles have different compositions, but all are wholly or in part made up of the substances named, most of them being either borax or formalin. They are undoubtedly efficient in preventing putrefaction and decay, for they are antiseptics, and if used in sufficient quantity will stop bacteria growth. They have been widely used in meats and in milk.

But the question arises whether they are not injurious to health. Each is injurious to man if taken in sufficient quantity. Are they, then, objectionable in the small quantities used in preserving food? This question has led to much experimenting, especially by the U. S. Department of Agriculture. The general result of these extensive experiments is to show that these preservatives, when used in small amounts day after day, are injurious, although this conclusion is still disputed. Hence, the general conclusion is that the preservatives in question are to be condemned. They are certainly illegitimate, and, since the same results can be reached in another way, there is no excuse for their use in any ordinary food products. They are especially to be condemned in milk.

### NON-POISONOUS PRESERVATIVES.

**Salt.**—Salt is not an antiseptic in any proper sense and it does not destroy bacteria. But it may be a preservative and when much of it is present in a solution, it has a decidedly repressing action upon bacterial growth, and may stop the ordinary putrefactive changes. When used in the preservation of butter and fish, it also has the advantage of imparting a relish to the product. It is 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* is pork preserved in strong salt brine, and *corned beef* and *corned bacon* are preserved in much the same way. *Ham* is partly preserved by salt, and fish wholly so. *Butter* and *cheese* both have their keeping qualities increased by salt. But salt used in this way does not kill the bacteria, and any flesh that contains injurious organisms, bacteria or others, is not rendered wholesome by salting.

**Sugar.**—A moderate amount of sugar checks most bacterial growth, and a large amount even stops yeast growth. Sugar, therefore, is widely used as a preservative. Since it is in itself a good food there can be no objection to its use as a preservative, although it always changes the taste and nature of the product. *Condensed milk* may contain 30 to 40 per cent. of sugar. *Jellies, preserves, jams, marmalades* are all fruits prepared in various ways and mixed with more or less sugar as a preservative. *Raisins, figs*, and *prunes* are whole fruits partly dried and preserved by the drying and the large percentage of sugar contained in them. There are practical difficulties in the way of using sugar with some foods, but with others it has its value.

**Vinegar.**—Acetic acid is another legitimate food preservative, and is extensively used in the manufacture of *pickles*. The acid gives a sharp taste to the pickles and also largely prevents the growth of the common putrefying organisms. The vinegar is frequently mixed with spices, both for the purpose of adding flavor and of aiding in the preservation. Vinegar pickles will not keep indefinitely, for after a time a scum grows over the surface. This is made of microorganisms which gradually weaken the strength of the vinegar until the final decay of the pickles is only a matter of time, unless precaution is taken to prevent the deterioration of the vinegar.

**Spices.**—Many common household spices are more or less efficient as antiseptics and tend to delay putrefaction. In some kinds of pickles spices are used, and mince meat, sausages, and highly spiced fruit cakes are preserved chiefly by the spices they contain.

## PRESERVATION BY CANNING.

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, a Paris confectioner in 1810, and consisted first in boiling the material to be preserved and subsequently sealing it hermetically. 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 in order to kill all living organisms and then, after hermetically sealing, watching to see if life developed in the sterilizing 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 general method adopted in canning is well known. Microorganisms are so abundant everywhere that every bit of food is certain to be infested with them. The first step taken must be the *killing* of all the organisms that may be adhering to the food to be preserved. This is done by heat, the material being commonly placed first in the receptacles in which it is to be finally sealed. The amount of heat necessary varies much with the nature of the material. If it is of a proteid nature, like meat, beans, peas, or corn, it is likely to contain spore-forming bacteria, and will require high and prolonged heating. This is especially true of corn, peas, and beans, since these materials contain such resisting spores that it was once thought that they could not be successfully canned. But modern methods of applying heat above the boiling temperature have made it possible to sterilize thoroughly even these resisting substances, and their canning is now perfectly feasible. To-day temperatures of 230° to 250° are commonly used for such materials. In canning fruit, as a rule, such high heat is not needed, since fruits are more often spoiled by yeasts and molds than by spore-bearing bacteria, and these organisms are easily killed by simple boiling, or by even less heat.

The second step is the *sealing*. This consists simply in closing the vessel containing the sterilized mass in such a way as absolutely to exclude the air and with it all microörganisms. It is sometimes done in tin cans, when a small hole is left in each can so that the air and steam may escape during the sterilizing, after which process the hole is sealed by a drop of solder. Sometimes glass jars are used, and these are sealed by covers pressed forcibly down upon a soft rubber ring. The principle is the same in either case. The sterilized food thus removed from any possible means of contamination will keep indefinitely.

The development of the canning industry does not belong to our immediate subject, but certain facts connected with the matter 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 aid 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 straw-

FIG. 50.—Three species of bacteria causing the spoiling of canned corn (*Prescott* and *Underwood*).

berries as abundantly as he pleases, and whatever he cannot find an immediate market for 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 the process of canning is one of the means of making a special product upon a farm yield proper returns. Canning makes possible an intensive farming, undreamed of a few years ago.

The present condition of the canning industry has been reached only after years of experience, accompanied with many failures and losses. Whole shipments have sometimes been ruined by "swelling," which means that the cans swell out from the force of the putrefying gases forming within. The failure to appreciate the difficulty of killing bacteria spores has caused great losses of canned corn, peas, and beans, as well as tomatoes. It must be recognized that, for successful canning, every spore must be killed; for if a single one be left alive in the middle of the can, the product is sure to spoil (Fig. 50). The slowness with which heat will pass to the middle of the can was not recognized until many losses had resulted from insufficient heating. But the failures proved instructive, and after bacteriologists studied the different problems presented by the attempts to can food stuffs, the questions were answered one by one, and successful rules were devised for the canning of any food products subject to such methods. To-day failures are rare and are all attributable to carelessness in the process. So thoroughly has this subject been mastered that to-day any food that can stand heat may be perfectly preserved by boiling and canning. Of course the flavors are commonly changed by the process, and few of them appear like the fresh material. But usually the flavors are less changed than by any other method of preservation, and canned goods are vastly superior to the dried foods with which our grandfathers were forced to be content in winter. Canning is especially adapted to foods containing a good deal of water, and hence is of especial use among foods that cannot be well preserved by drying. Fruits are well adapted to canning, but ill adapted to drying, and are ruined by salting.

## 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 might be 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 commonly 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 avoiding them completely. 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 bacteria, and a body which is practically sure to undergo decay in the course of time. There are fewer bacteria in winter eggs than in those laid in summer, and the cleaner the nest the less the bacterial contamination.

It is, however, possible, by certain devices, to delay or prevent the growth of bacteria in the egg. The bacteria found in eggs do not develop at low temperatures and the eggs may be kept almost indefinitely at a temperature of 34°. But since cold storage is not always available, other methods must usually be adopted. One of the simplest and best is by the use of *water glass*, a material made of sodium and potassium silicate. This may be purchased cheaply in the form of a thick syrup. It is then mixed with nine parts of water and placed in clean stone jars. The eggs are placed in the mixture and the whole set aside in a cool place. If the temperature is not allowed to rise above 60° the eggs may be kept from decay for a long time by this method, many weeks and even months elapsing before they will decay. In preserving eggs in this way it is important to know that April eggs will keep better than May eggs and these better than June eggs, while eggs laid in the hot months of the summer are less easily preserved, a fact probably due to a greater bacterial contamination during the warmer months. From this it will follow that the June storage eggs should be used first and the April preserved eggs last.

Eggs thus preserved will keep from decay, but they will lose their fresh taste. Indeed, this fresh flavor disappears in a very few days and there is no way known by which it can be retained for very long. But after the fresh taste is gone the eggs will remain without further change for a long time and be usable.

## 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. One source of trouble experienced in sugar-making consists in the appearance, at various stages of manufacture, of jelly-like masses which may become very abundant and troublesome. This has long been known and has been studied by bacteriologists for many years, with the result of proving that it is 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 section 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 microörganism, when growing out of contact with sugar, develops no slime. A second trouble is in the loss of sugar by *inversion*. This occurs in unrefined sugar during storage or transportation, and is due to a bacterium that has been isolated and tested.

# PART V.

## CHAPTER XVII.

## PARASITIC BACTERIA.

### RESISTANCE AGAINST PARASITIC BACTERIA.

We have learned that microörganisms may be both useful and harmful. If they grow where they are wanted, they are useful; but if they grow where they are not wanted, they produce many undesirable effects. They spoil foods by causing their putrefaction, they destroy vinegar by consuming the acetic acid. In wines the growth of mischievous microörganisms causes a variety of bad results that are sometimes spoken of as "diseases of wine," and we also hear of "diseases of beer." But there is no good reason for the use of the term here any more than in speaking of the diseases of butter and cheese, when unusual bacteria cause them to ripen abnormally.

In most of the examples thus far studied the material upon which the microörganisms grow has been supposed to be lifeless, the bacteria existing as *saprophytes*. There remains the study of these organisms when growing upon the living tissues of animals, thus living the life of *parasites*. In the latter case they may do injury to the animal or plant upon which they live, thus becoming *pathogenic*, or *disease germs*.

### HOW MICROÖRGANISMS PRODUCE DISEASE.

When they multiply inside the body, microörganisms show very different habits. Sometimes they become distributed over the whole body, located at no particular spot (*blood poisoning*), while in other cases they may be definitely localized at some one place (*diphtheria*). Between such extremes there are many intermediate types. Whenever the microörganisms multiply in the body they produce chemical changes, just as they do elsewhere. New chemical bodies are secreted by them and among these, in the case of disease germs, there are some that are poisonous in their nature. Such substances are called **toxins.** Wherever they are produced they are liable to be absorbed by the blood, and the body may thus be directly poisoned by them. If the bacteria are in the blood itself, this poisoning is easy to understand, but localized diseases are similarly explained. Diphtheria, for example, is produced by bacteria growing on the inside surface in the throat. The bacteria themselves do not enter the body, but, growing in the throat, they develop very powerful toxins, and these are absorbed into the blood, producing a general poisoning of the whole body. All disease germs produce poisonous materials which are absorbed by the body, and these cause the direct injury characteristic of the various diseases.

## RESISTANCE AGAINST MICROÖRGANISMS.

A very large majority of microörganisms are quite unable to live within the bodies of living animals or plants, and therefore are not parasitic. If common putrefactive bacteria be inoculated into the blood of a living cow or into her flesh, they will speedily die without multiplying, disappearing in a very short time. If these same bacteria are inoculated into the same animal after it is dead, they will grow with rapidity, quickly causing the flesh to putrefy. Why is there this difference? The complete answer to this question is one for which bacteriologists have long been searching, but have as yet only partly found. A partial answer is that the living tissues contain substances that are injurious to the bacteria. What these substances are, how they act, why they disappear after death, and numerous other questions concerning them are among the most important of the problems before bacteriologists to-day. With these complicated questions, however, we are not concerned in this work.

It is evident enough that some kinds of microörganisms can overcome this resistance, otherwise there would be no parasites. These, capable of living and multiplying in the body, may produce injury and are the disease germs. Fortunately, the number that can thus live is small. Many hundred kinds of bacteria have been discovered, carefully studied, and described in bacteriological literature. We have no idea how many varieties exist in nature, but there are certainly hundreds, and perhaps thousands. Of these only little more than a score are known that can produce disease in man and animals, and a somewhat larger number that can produce disease in plants. A few yeasts occasionally produce similar troubles. Quite a large number of molds, as parasites, give rise to disease in plants, and a very few cause trouble in animals. The great host of bacteria and other fungi live upon dead matter, and cannot live as parasites. They may spoil foods, and destroy wines, beer, butter, cheese, and other valuable substances, but they cannot produce disease, since they are not able to overcome the resistance offered by the living tissue.

The body has a resisting power against all kinds of microorganisms, disease germs as well as the non-parasitic species, although, in the case of the former, it is insufficient to prevent their invasion. 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 having the power of absolutely resisting certain bacteria, when we call them **immune**. Man is immune against hog cholera, while the hog is not. It also varies with the individual, some members of a species having the resisting power highly developed, while others yield easily to invasion. This we speak of as **individual resistance**. The resistance varies also with the vigor of the germs. Some epidemics of measles, for example, are mild and some severe, and a person's resistance against an attack is partly dependent upon the vigor of the germs.

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 microörganisms, 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 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 resisting them. We say his "blood is in a 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 healthy 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. 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 first years of the study of bacteriology there was a very general tendency, in the attempt to avoid diseases, to place the whole emphasis upon the methods of avoiding bacteria. If a disease is caused by a bacterium, what more natural method could be suggested for avoiding it than to avoid the bacterium? In accordance with this idea there developed 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.

**Increasing of Individual Resistance.**—Recently there has been a manifest reaction against this one-sided attitude. While the importance of preventing the distribution of bacteria is still acknowledged, there is to-day a growing recognition of another side to the question. The strengthening of personal vigor is of no less importance—many believe it is of more importance—than the preventing 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. Personal resistance of the individual will enable him to repel many an attack of disease bacteria, even if he has been directly exposed to them, while a weakened resisting power may 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; 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 attacks of some of the disease germs. Manifestly, then, the best means of elevating the healthfulness of the race is to raise the resisting power, at the same time doing our utmost to destroy pathogenic bacteria.

These facts are equally true, whether we are dealing with animals or with man. It is of more importance for the farmer to understand them when 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. When a lot of pigs are attacked by that very fatal disease, hog cholera, some of them 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, 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 vitality, and the animal is much more likely to succumb.

Tuberculosis, for example, is much more prevalent among cattle that are kept stabled most of the time, than among those that spend a considerable portion of the time in the open air. This may be due, in part, to the fact that stabled cattle have a greater chance of acquiring the contagion, since they 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 they are put under similar conditions. In localities where the animals run out of doors all the time the disease is rare. The more closely they are housed the greater the tendency to this disease, and it is practically certain that this greater tendency is not because they are so much more likely to be infected, but because of the depressing influence which such a restricted life has upon the vitality of the animals, reducing their 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 which have been bred for the purpose of producing great quantities of milk are rather more likely to yield to the 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. 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 close contact of animal with animal. That the latter phenomenon is also a factor is, of course, evident.

The conditions of life among domestic animals are, to a very large degree, under immediate and perfect control. We can regulate the amount of outdoor life they have, their activity, their food, their drink, and many other factors upon which their physical vigor depends. We may keep the cow housed so that she has little air; we may give her highly stimulating food with practically no chance to use her muscles; or we can make quite a different animal of her by changing her life and food. We can control the conditions of life among animals far better than we can, or will, those of our own life. In the conditions of civilized life each individual demands his personal freedom in regard to matters regulating his own affairs, 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 force people to adopt them. But the farmer has absolute control over the life conditions of his stock. He can regulate their life as suits him, and he can, if he will, work out among cattle 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 to greater liability to disease.

The adoption of precautions for preventing the distribution of 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.

## ACQUIRED RESISTANCE.

Some species have a perfect resistance to the diseases that other species will take, a condition called *race immunity*. Some individuals will resist a disease that others of the same species cannot resist, and this is *individual immunity*. An individual may also develop a resistance to a disease which he did not at first possess. This is *acquired immunity.* It has long been known that if a person has one attack of certain diseases and recovers, he is, for a time at least, protected from a second attack of the same disease. It is not common, for example, to have scarlet fever twice, and the same is true of a number of other diseases. This acquired immunity is, however, quite variable. In some cases it is almost a perfect protection for life, or at least for many years. With other diseases it is weaker, affording only a partial protection and lasting only a few months or perhaps only a few weeks. The question of what causes this acquired immunity is closely akin to what causes race or individual resistance. Doubtless the two are closely related and are probably attributable to the same general cause. For our purpose it is only necessary to know that recovery from one of these diseases leaves the individual with his body filled with substances capable of resisting the kind of bacteria that produced the disease. As long as these resisting substances are present the individual will have immunity.

It is somewhat surprising that recovery from a mild attack of one of these diseases gives as much immunity as recovery from a severe attack. Hence, with this principle in mind, the question has arisen whether it may not be possible to give an individual a mild case of some of the more dangerous diseases in order to give him power to resist the more severe and perhaps fatal types. This was first done in the case of smallpox, which has for a century been fought upon this principle, since the *vaccination* pustule seems to be essentially a mild type of smallpox. Hence, when a person is vaccinated, he is given a mild form of smallpox, and this guards him from a more severe attack. That vaccination is a protection against smallpox is pretty generally admitted to-day, although some deny its power.

But whatever be the facts in regard to smallpox, there is no doubt at all in regard to the successful application of this principle to other diseases. Pasteur was the first to attempt an application of this principle to a disease other than smallpox. He was at the time working upon a serious disease of cattle—*anthrax;* one that is practically always fatal. He argued that if he could find means for producing a mild type of the disease, he might protect the herds from the severe and fatal infection. But to induce a mild attack was not easy. It would do no good to inoculate an animal with a small number of the bacilli which produce the disease, for these, by multiplying, would soon become so numerous as to bring about a severe attack of the disease. It was evident that this end could be reached only by weakening the power of the disease-producing 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 obtained cultures that 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 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 them all, inoculated the entire 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 from the inoculation.

A more beneficial discovery has hardly ever been made. From the date of Pasteur's experiment a constant succession of bacteriologists has been trying to apply the same principle elsewhere. We cannot here attempt to follow the development of the work, but can only state that practical results of the utmost value have been obtained. It has not been found possible to use just the same method in other diseases that Pasteur used, but by a modification of it, or by others that have come from it, it has been found possible to withdraw the terrors from some of the most dreaded diseases.

The human diseases *diphtheria*, *lock-jaw*, *bubonic plague*, *cholera*, and *hydrophobia* have either been mastered or at least mitigated by discoveries that have come from the study which Pasteur started; while among animals at least two diseases are controlled by preventive inoculation—*anthrax* and *black leg*. Some success also has attended similar methods with tuberculosis.

## MICROÖRGANISMS THE CAUSE OF DISEASES.

The studies of the last twenty-five years have demonstrated that the majority of diseases in animals and plants are produced by the growth of parasites of some kind, and mostly by what may be called microörganisms. Bacteria, yeasts, and molds are all concerned and, in addition, some diseases are produced by microscopic animals. The subject of germ diseases has become one of very wide range and cannot be considered to any great length in this work. The discussion of such diseases among animals belongs to veterinary medicine, and of those among plants to botany. In our general consideration of microörganisms as related to agriculture, we can review only the important principles involved, and give a brief survey of the more important diseases.

# CHAPTER XVIII.

## TUBERCULOSIS.

Of all germ diseases there is none so widely distributed as tuberculosis. Not only is it of great significance from the standpoint of human health, but it is the one disease of domestic animals which demands almost universal attention and interest. Tuberculosis among cattle forms one of the most serious problems of agriculture.

**Cause of the Disease.**—The organism which produces this disease was first described by Koch in an epoch-making monograph published in 1882. 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 forms of tuberculosis. The organism itself appears commonly in the form of a short, slender rod (Fig. 51). Although commonly called *Bacillus tuberculosis*, it cannot properly be called a Bacillus, since it possesses no flagella.* (see page 12). Although this organism does not form spores, it has a considerable resistance against heating and even drying. It may be dried, and yet remain alive for months, without losing its power of


FIG. 51.—Tuberculosis bacillus. *a*, in a bit of animal tissue; *b*, showing irregularities resembling spores; *c*, typical appearance of the bacilli from ordinary cultures.

* Recent studies have shown that the organism may show branching which is not the case with any true bacteria. It has been suggested that it should be placed in a special family named Myxobacteriæ. It will doubtless retain the name B. tuberculosis.

growth. It will withstand the heat of 140° for fifteen minutes or more, and under some conditions a considerable higher heat. A few minutes' heating at 175° will, however, kill it. Unlike many bacteria the tuberculosis organism is quite limited as to the conditions under which it can grow, and an understanding of these conditions is of the greatest importance in comprehending the problems of its distribution. The temperature limits within which its development is possible are quite narrow. It grows best at a temperature between 96° and 105° F., but it will grow more slowly at a temperature as low as 84° F. Below this it will not multiply at all. At first it was supposed that it would not grow in any artificial medium which could be prepared in the laboratory. In his original experiments Koch was obliged to use coagulated blood serum as a culture medium. It is now found that it can live and flourish in a variety of culture media, provided a certain amount of glycerin be 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 anmial. But here, too, bacteriologists have changed their views, for the tubercle bacillus will grow 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 the bodies of animals, we may look to various places in nature as a source of infection, but if it demands for its existence conditions of the living body, we may look to animals alone for its source. Now, although it can grow under conditions quite different from those of the living body, nevertheless, so far as our present knowledge goes, the tubercle organism does not grow outside the bodies of animals under any normal conditions. It does 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 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 animals. While it can flourish in the artificial media of the laboratory, when kept at special temperatures, it does not flourish in nature, outside the bodies of animals upon which it lives as a parasite.

**Animals Subject to the Disease.**—Besides living in man the organism can flourish in the bodies of *cattle*, *hogs*, *dogs*, *cats*, *monkeys*, *rabbits*, *guinea-pigs*, and some other animals. In all these it produces very similar symptoms, differing slightly, of course, 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 frequently regarded as a different type of the organism.

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 in man it receives different names; *consumption*, *scrofula*, *lupus*, *hip disease*, *nephritis* are some of its common names.

**Resistance Against Tuberculosis.**—Although this organism can attack almost any part of the body, it is also certain that the body has a strong resisting power against it. It by no means follows that a person will take the disease because some of the bacilli find entrance into his body. On the contrary, as a general rule, they are soon overcome by the body resistance. Careful study has shown that most people, by the time they have reached twenty-five years of age, have not only been exposed to the disease, but have had mild attacks, from which they have completely recovered. By building up a proper physical vigor, an individual may successfully combat these parasites. Plenty of wholesome, but not too rich food, exercise, life out of doors as much as possible, sleeping in rooms with windows open in winter as well as in summer, and deep breathing exercises, by means of which the lungs are filled with fresh air, are the means by which such resistance can be developed and maintained. All of these conditions are usually within the reach of everyone, so that there is no reason why a person who will, cannot develop a high resistance against this dreaded disease.

## ARE BOVINE AND HUMAN TUBERCULOSIS THE SAME?

Apart from its relation to the human being, the farmer is most naturally interested in this disease, since it attacks his cattle. Bovine tuberculosis is one of the most serious dangers, and threatens the continuance of dairying.

The significance of the question whether human and bovine tuberculosis are identical is self-evident. If the two are the same, it will follow that the disease may pass from animals to man; if they are not identical such transmission is impossible. For some fifteen years after the cause of the disease was discovered, no question was raised as to their identity. Both diseases are produced by bacteria that appear identical, and that they were the same was taken for granted. In 1900, however, Prof. Koch raised the question whether they were not distinct, and gave experiments to show that the human bacillus does not produce the severe bovine tuberculosis when inoculated into cattle. The question caused intense interest and much discussion, and in spite of many experiments designed to settle the matter, there is still some dispute. A fair summary of the facts as they appear to the majority of bacteriologists to-day is as follows:

Both bovine and human tuberculosis are caused by a bacterium that has great similarity in the two animals. But there are slight differences between them, both in microscopic appearance and in methods of growth, sufficient to make it necessary to recognize them as somewhat different types. When inoculated into animals, the organism from the bovine source proves to be more virulent than the one from the human source. The human bacillus, when inoculated into cattle, generally produces only a slight trouble, while the bovine bacillus is apt to bring about a progressive case of the disease of very serious character. What effect the bovine bacillus has when inoculated into man cannot yet be told from direct experiment, but there appear to be a number of tolerably sure cases of accidental inoculation of human beings with the bovine bacillus that have been followed by a development of the disease. The general conclusion is that, although the two are slightly different, each may produce the disease in the other animal, and that the disease is, therefore, transmissible from animals to man. While the conclusion is still doubted by Prof. Koch, it is accepted by most other bacteriologists. Whether the bovine bacillus is more virulent for man than is the human bacillus, as it is for other animals, is by no means settled. Furthermore, it is pretty generally agreed that human tuberculosis comes more often from human sources than from cattle.

## BOVINE TUBERCULOSIS.

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 be located in the udder; and in these cases the milk of the animal becomes a source of danger. Fortunately, the percentage of cases of udder disease is comparatively small. In cattle it rarely attacks the bones, joints, or muscles.

## METHODS OF DISTRIBUTION.

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. If the disease is located only 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 one animal to another. But if it be located in the lungs the bacilli will be discharged into the air passages and pass through the trachea into the mouth. They will then 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, since the cow does not expectorate, she does swallow the secretions from her mouth. The tubercle bacilli will thus be carried to the stomach, and through the intestine, from whence 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, even in 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 may be 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 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 may become mixed with her food which is commonly thrown on the floor in front of her; or the particles may 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 come in contact with every healthy animal kept in the same barn.

Once distributed from infected animals, the bacilli may find entrance into the healthy animals, by a variety of channels. Some find entrance to the lungs, either by the dust particles or by the bacilli-laden moisture drops from coughing animals, which are breathed by healthy animals. The bacilli which find their way 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. These two means of entrance are doubtless responsible for most cases of bovine tuberculosis, and it is very easy to understand 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. It is said not to occur in Africa, and until recently it has been absent from China and Japan, having lately been introduced with imported cattle. In the western part of the United States, among the cattle living 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.

It is practically impossible to state the percentage of cattle suffering from tuberculosis. 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. or even lower in some places, 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 other whole herds are entirely exempt. In the eastern States the percentage is large, and in some localities it appears to approach the figures given for Denmark. When the numbers of infected animals in a herd range from 0 to 100 per cent. it is evident that no resulting average would be of any significance.

**Increase of the Disease.**—Is bovine tuberculosis on the increase? Statistics are so uncertain as to make any conclusion difficult. Certainly we hear much more of the disease than we did a few years ago, and the percentages reported to-day are much higher. The knowledge of the disease is, however, of very recent date, and 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 last 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 or slowly increasing or remaining stationary. But taking all facts together, the practical uniformity with which the percentage of reported cases has increased in the last years, has led to the general belief that the disease is actually and somewhat rapidly increasing among our herds.

But although no definite statistics can be given, either as to the prevalence of the disease or its increase, bovine tuberculosis is abundant enough. 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 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 any other disease. 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 usually had in mind the protection of the public rather than the assistance of the farmer.

## THE COMBAT AGAINST BOVINE TUBERCULOSIS.

**Resistance of Cattle.**—The foundation of a successful contest against the disease is a herd of animals 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, impossible absolutely to prevent the bacillus from being distributed by 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. In the attempt to keep animals warm, they have been too closely shut up in badly ventilated rooms, and they breathe the warm air over and over again. Such a condition, wholly independent of the tubercle bacilli which might be present, has a debilitating effect upon cattle, just as it does 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 the cows 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 show why so many animals yield to 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.

Anything which will induce a vigorous life will decrease the tendency to the disease. Proper food is an important factor in determining health. It may be difficult under the conditions of modern farming to allow the cattle to have proper exercise in the winter, but the lack of it is certainly one of the factors tending to increase the liability to tuberculosis. Too great attention paid to the increase in the yield of milk lessens the resisting power of cattle. Our agriculturists, by overfeeding with certain kinds of food, and by special high breeding for the purpose of increasing the yield of milk, are trying to turn an animal into a milking machine. The highly bred animals are, of course, useful for the purpose for which they are bred; but the agriculturist must remember that he cannot turn his cow into a simple milking 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.

## THE PROTECTION OF THE HERD.

While the treatment of the cow as an animal and not a milking machine must be the foundation of a healthy herd, the care of the farmer most not stop there. The animals must be guarded against infection and to this end much attention has been given in recent years.

**The Tuberculin Test.**—Any method of protecting a herd against tuberculosis must start with some method of detecting the disease in animals. Certain forms of the disease, especially when in an advanced stage, are easily discovered by clinical means, the veterinarian being able to detect them by the examination of the cattle. But there are other cases where no visible signs appear, and these cannot be found by clinical means. The tuberculin test has been devised to meet this difficulty and to dectect even the mildest cases. Tuberculin was first prepared by Koch. It is made by causing the tubercle bacilli to grow in a broth containing glycerin. While growing in such a broth, the bacilli produce certain toxic products which are soluble and which dissolve in the broth. The material is then treated in such a way as to remove the bacilli, and the clear, toxic-holding solution is *tuberculin*. Inasmuch as it does not contain any living bacilli, it cannot possibly cause the disease, and its use among animals cannot incite tuberculosis, as has sometimes ignorantly been 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 poison it. 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 marked rise in temperature, which soon disappears.

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. 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 much dispute. It has been found subject to some error. If animals are tested under abnormal conditions, as, for example, when in new barns, or if 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 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 may heal 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 postmortem 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. But this entirely too radical plan proved quite impracticable. Nevertheless, the use of tuberculin has become of great value to the farmer in his attempts to get rid of this disease among his cattle.

**The Preservation 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 and expensive to stamp it out. Tuberculosis does not develop spontaneously in a herd of animals, but *is always 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 great vigilance. To prevent the entrance of the disease into his herd from without, the farmer must exercise care in several 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. The matter is made more difficult by the fact that after an animal has been tested and responded, she is for some time protected from a second test. A dishonest dealer, therefore, may inoculate his cows privately and then put upon the market all those that respond to the test, knowing that for some time they will not again respond. 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.

*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 soon be distributed through the separating station over the entire 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. The only safe procedure is for the farmer either to bring up his calves on the milk from his own healthy herd or to insist that all milk fed to them shall first be subjected to the process of pasteurizing or boiling. So convinced are the agriculturists in Denmark that this mixed milk is the cause of much of the bovine tuberculosis, that a law has been passed 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.

**Treatment of a Tuberculous Herd.**—There appear to be four general methods of treating a herd after this disease breaks out in it:

1. All advanced cases which are recognized as dangerous to the public, including all cases of udder tuberculosis, may be removed and the animals destroyed, the others being left undisturbed.

This does away with most of the danger to the public consuming the milk, but does nothing toward eradicating the disease from the herd.

2. All animals that have the disease as shown by clinical or tuberculin test may be slaughtered. The attempt to enforce by law such a treatment of the disease has failed wherever tried. It involves too great a loss and dooms to slaughter many animals in the incipient stages of the disease that might recover and are still useful animals. It is sometimes done voluntarily by private owners in their determination to keep a herd free from the disease. It protects the herd and the public at the same time.

3. The great losses that are frequently involved in the slaughter of all reacting animals have led to the adoption of other plans for freeing the herd of the disease without such sacrifices. A plan was devised some fifteen years ago by Bang, consisting of separating the reacting animals from the others. The first step is to detect by tuberculin all tuberculous animals. The advanced cases are slaughtered. The other reacting animals are separated from the others and placed by themselves, removed from every possible contact with the rest of the animals in the herd. This is not because all reacting animals 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 about it. The healthy (non-reacting) animals are then placed by themselves, either in a new barn or the old one after it is thoroughly disinfected. By this means a practical isolation is accomplished.

If the farmer wishes to preserve the healthy herd from future attack, he must take precautions to have the isolation thorough. It may be effected by simply building a partition in his cattle shed; but in this case there should be no door in the partition, for that would surely result in a carrying of bacilli from one compartment to the other. The farmer should remember the facts already pointed out as to the methods of distributing 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 on the milk of the healthy herd alone. The healthy herd must be tested every six months or so, and if any show reaction they must at once be removed from the rest. The tuberculous herd may be kept and milked, but the milk should be sterilized. By keeping up this procedure for a few years, it is possible to eliminate the infected animals and have left a herd of healthy animals.

4. A still more recent plan has been widely adopted in Germany and seems at present to offer the simplest and most hopeful solution. It consists in separating all calves, as soon as born, from their mothers, and rearing them separately from the rest of the herd. Tuberculosis is not hereditary, and the calves of tuberculous mothers are, when born, free from the disease (except in rare instances). If, therefore, they are at once separated from their mothers, brought up on pasteurized milk, and not allowed any possible contact with the other animals, they may be reared free from the disease, becoming in a few years a herd of healthy cattle, and if they are guarded from outside sources of contamination they will continue to be free from the disease. Meantime the animals in the infected herd, whose milk may be used if throughly pasteurized, may be slowly disposed of, while the healthy, growing herd gradually replaces them with the smallest possible loss to their owner. Of course the healthy animals must not be allowed to enter the quarters formerly occupied by the diseased herd until there has been a thorough disinfection of the premises.

Which of these methods of procedure it is best to adopt depends upon circumstances. If a man has only a small number of animals and only one or two of them are tuberculous, his simplest plan will be to slaughter the reacting animals at once. If his herd is a large one, it is best to build up a healthy herd by one of the methods outlined. He must remember that a single tuberculous animal is a menace to his entire herd and he should begin his fight against the disease at the very first discovery of an affected animal. Neglect in using the tuberculin for fear that some reacting animals be found in the herd is the height of folly. Half-way measures in handling this subject are no better than none.

**Preventive Inoculation.**—In recent years claims have been made that a method of preventive inoculation against this disease has been found by using dried human tubercle bacilli for rendering cattle immune. Considerable data have been collected as to the success of this method, and it seems pretty certain that a considerable degree of immunity can thus be given to cattle. It is yet too early to say, however, whether this procedure will ever be a practical method of handling the tuberculosis problem.

## THE USE OF FLESH AND MILK FROM TUBERCULOUS ANIMALS.

The practical question of the disposal of milk and flesh from tuberculous animals is constantly arising. The answer is clearly dependent upon whether the disease in men and animals is the same. Since it is generally agreed that, if not the same, the two are so nearly alike that they may be transmitted from one to the other, it is the concensus of all that the possibility of transference through flesh or milk should be guarded against.

**Flesh.**—Tubercular matter when fed to 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. 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. 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 (somewhere about 1 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. 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. It is sometimes doubted that mankind can ever acquire tuberculosis from this source. Experiment has shown that large numbers of the bacilli must be swallowed at once to produce infection even in susceptible animals. 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. 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 cutomers 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.

# CHAPTER XIX.

## OTHER GERM DISEASES.

### ANTHRAX OR SPLENIC FEVER.

Anthrax is a disease of domestic animals which has been known for centuries. It is mentioned in the writings of Moses, and Homer refers to it in the Iliad. It occurs practically all over the globe, in all latitudes where cattle are kept, and seems to be entirely independent of climate. Every country of Europe suffers from it. 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 cattle 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 readily spread from herd to herd.

Cattle and sheep are the only animals in which it normally occurs as a spontaneous infection. Many other animals are, however, capable of infection with it. *Horses*, *goats*, *deer* and *mice* are very subject to the disease, while *dogs*, *cats* and *white rats* are not susceptible. The disease is also found in *man*, and is then known by various names, the most common 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.

**Cause.**—The discovery of the cause of this disease was one of the first triumphs of bacteriology. Its exciting cause is a bacterium, *Bact. anthracis*, which, though first seen in 1849, was not really demonstrated as the cause of the disease until 1875, by the work of Koch, and shortly afterward by Pasteur (Fig. 52). After some twenty-four years of dispute the final demonstration was due to such experiments as the following. It was easy to show by the microscope that this organism is present in the blood of the animals suffering from the disease, and that a drop of such blood, containing the organism, when injected into healthy animals would inevitably produce the disease in the inoculated animals. But this did not necessarily prove their causal agency, for it was possible to claim that there were some other poisons in such blood. For final proof it was necessary to separate the bacteria from the drop of blood, cultivate them, and inoculate animals with the pure cultures. At the time that this disease was first being studied no methods were known of obtaining isolated bacteria in pure cultures, and hence

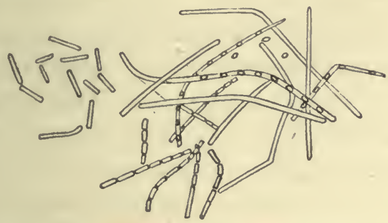

FIG. 52.—*B. anthracis*, the cause of splenic fever.

the long dispute. Pasteur finally procured his results as follows. Finding that the bacterium would grow in a solution made by steeping yeast in water, Pasteur inoculated a sterile flask of such yeast-water with a drop of anthrax blood. In a day or two his flask was filled with bacteria 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 were the bacteria present in the original drop of blood. Nevertheless, he found that though he continued these inoculations indefinitely, every flask was equally virulent, 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 in its blood of the bacilli in countless myriads. These results left no loophole for criticism, proving that this bacterium was the cause of anthrax, and thus for the first time demonstrating 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, *Bact. anthracis*, is a rod of moderate size (Fig. 52). 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. When in an active growing condition, this bacterium is readily killed by ordinary disinfecting agents and by a moderate heat, a temperature of about 160° F. easily destroying the rods. But it produces resisting spores which can easily be distinguished inside the rods as clear, glistening bodies. It is their resistance to ordinary 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 having 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 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. 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 evidently make the disinfecting of an infested locality a matter of very great difficulty.

**Method of Infection.**—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 out into a new pasture. In some of these cases which have been investigated the explanation is simple. In such pastures bodies of animals dead from anthrax have been buried, and the spores have remained alive for many years. Now, although these spores may have been buried some distance below the surface, 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, and the spores are later taken into the stomachs of cattle feeding on the grass. These spores resist the 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, producing the disease. These facts readily explain 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 may terminate fatally. The name *malignant pustule* is appropriately applied to this form of disease. In susceptible animals such recovery is very rare. 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. There are other modes of infection, but among animals the disease is most usually acquired through the intestine or through skin abrasions.

In the body of the infected animals the bacilli grow with great rapidity. An extremely small number of them inoculated into the body of a sheep may produce its death in about two days, and after death the whole body is found to be filled with the bacilli in incalculable numbers. 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 literally be crammed with bacteria.

This bacillus is extremely virulent in its action upon susceptible animals, so virulent, indeed, that a single bacillus, inoculated under the skin, 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 occurs among them only as the result of artificial experiments. 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 *wool-sorter's disease*.

**Preventive Inoculation.**—Although anthrax is an extremely fatal disease to animals and has, in the past, caused heavy losses to agriculturists, it is a source of less loss to-day than in former years, since it can be fairly well controlled by preventive inoculation. We have noticed in the last chapter that Pasteur demonstrated the important principle of preventive inoculation by his experiments upon anthrax; the discovery has been of great practical value. Cattle can be protected from anthrax by inoculation, and from the time that Pasteur pointed out the method, hundreds of thousands of animals have been thus inoculated and protected. But the protection is not found to be very lasting, and animals must be inoculated about once a year to be thoroughly safe from the disease. This, of course, reduces the value of the inoculation, and confines it to localities where, for special reasons, the disease is quite common. It also explains why the method is not so widely in use now as at first; but, nevertheless, large amounts of the inoculating material have been used in this country as well as elsewhere, and it is thought that immense losses have been prevented by this means since its discovery by Pasteur.

## OTHER GERM DISEASES AMONG ANIMALS.

No other diseases among animals have acquired so much interest as tuberculosis and anthrax, although several others are known to be produced by microörganisms and are of considerable importance to agriculture. Only a brief mention of these is possible, but the following list includes all of the important diseases of domesticated animals, that have been proved to be caused by microscopic parasites.

**Swine Plague, Fowl Cholera, Rabbit Septicemia, Rinderseuche, Wildseuche** (*B. pleurosepticus*).—These names are applied to a variety of affections of animals, but they all appear to be essentially the same thing. The cause is a bacterium which was first identified by Pasteur as the cause of fowl cholera and later identified as the inciting agent in all these diseases. They are all contagious and often produce considerable havoc among domestic animals. The names given indicate the variety of animals attacked. *Rinderseuche* is the name given when it attacks cattle, and *Wildseuche* when it attacks deer; *septic pleuropneumonia* and *pneumoenteritis* are also names applied to it.

The bacterium causing all these diseases is a short rod, so short as sometimes to be called a Micrococcus. The cultures obtained from different animals have been given different names, *B. bovisepticus*, *B. suisepticus*, etc., but the most careful study fails to show differences sufficient to warrant their separation, and the name *B. pleurisepticus* has been suggested as indicating its relation to its many hosts. While it attacks many animals it is, so far as known, harmless to man. It produces a type of disease quite similar to the forms of blood-poisoning which have been, in medical practice, called septicemia. It is extremely fatal to some animals, fowls and rabbits succumbing to its action with extreme rapidity and with almost absolute certainty. Among the larger animals its course is not necessarily so fatal, but in all those referred to above the disease is a serious one and almost always fatal. When attacking the hog it produces one form of *swine plague*, this being the type of the disease most commonly found among domestic animals, and the one which will usually be most interesting to the agriculturist.

**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 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 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 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 of the whole body 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 a 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 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 common.

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 and successfully adopted in the United States.

**Tetanus** or **Lockjaw** (*B. tetanus*).—This 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 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 milk-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 must 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.

**Takosis of Goats.**—This is a disease of goats only recently studied and found to be caused by a bacterium. It brings on a general weakness and wasting away, which finally results in death. It has caused great loss among the Angora goats in the northern states. It is always fatal.

**Lumpy Jaw, Malignant Tumor, Wooden Tongue.**—These three names are applied to the same disease, located, however, in different places. The cause of the trouble is one of the higher types of fungi rather than a bacterium. The name of the organism is **Actinomycosis** and it differs from bacteria in forming longer threads and in branching (see page 12). When it finds entrance into cattle, generally through the mouth, it may invade the tissues and produce the diseases named above. Sometimes it is found in the throat, lungs, and skin. It is most common in cattle, but it also occurs in swine, and may be given to other animals by inoculation. It occasionally occurs in man. The disease is not common, though it causes considerable loss at times, since it is serious and apt to be fatal. It produces hard tumors that invade the bones or other tissues, causing great distortion. The disease is not contagious and its source is as yet unknown.

**General Inflammatory Troubles.**—Inflammatory, suppurative, and tumor-forming troubles are liable to occur in almost any part of the body of man or animal. These are commonly caused by bacteria, particularly by the class called *Streptococci*. The affections do not form any specific disease, but receive a variety of names according to the location of the trouble. For example, when the streptococcus produces inflammation of the udder it is called *garget*, *mammitis*, or *mastitis*, while *hoof rot* and *navel ill* represent other types of inflammation located elsewhere. The streptococci that cause garget have been found abundantly in the milk of cows and are believed to be the reason for some of the illnesses in mankind that follow the drinking of raw milk. Various forms of *sores*, *boils*, *abscesses*, and the *inflammations* following wounds are also caused, largely or wholly by streptococci, and most types of inflamed tissue in an animal may be rightly attributed to the action of this class of bacteria.

Another bacillus associated with a variety of troubles among animals is named *B. necrophorus*. This organism produces more than an inflammation; it gives rise to a general decay of the tissues attacked (called *necrosis*) and, since it attacks many parts, it has a variety of effects. In the skin it causes numerous inflammatory diseases. It produces the *foot rot* of sheep and also of cattle. It attacks the bones in the nose, causing their destruction; it may bring about troubles in the alimentary canal, and it is the source of some of the cases of hog cholera, as well as several other affections.

**Foul Brood of Bees** (*B. alvei* and *B. larvæ*).—Foul brood is a disease attacking the larvæ of bees while still within their cells causing them to become sickly and eventually killing them and producing a decomposition of the body. The hive becomes vile-smelling from the decomposition and the whole economy of the hive is interrupted. The bees fail to collect honey and the hive may be ruined. There are really two different diseases going by this name, the American and the European foul brood, resembling each other and yet being easily distinguished. Both are produced by bacteria, the European by *B. alvei* and the American by *B. larvæ*. In this country the latter is the more common, though both are found. Both diseases are readily carried from hive to hive. Sometimes this is done by robber bees that steal honey from hives, and sometimes it is carried by the bee-keeper who handles a diseased colony and then a clean colony, or who places in a clean hive honey or combs from an infested hive. Its very infectious nature should be thoroughly appreciated by the bee-keeper and great care should be taken in handling bees. It is also doubtless carried from locality to locality by the custom of selling bees. The two diseases are widespread over America, Europe, Africa, and Australia. It spreads rapidly, sometimes infesting a whole district in the course of a single season so as nearly to ruin the industry of the bee-keeper.

## DISEASE CAUSED BY UNKNOWN PARASITES.

The causes of several well-known diseases have not yet been discovered; nevertheless it must be recognized that they are caused by microörganisms too small to be seen by our microscopes. That they are caused by living agents of extremely minute size is shown by two series of facts: 1. Material may be obtained from animals suffering from the disease which will produce the disease in others, but its power is destroyed by the same disinfectants as those used to kill bacteria. 2. The infectious agent will pass through porcelain filters, whose pores are too small to permit even the smallest bacteria to pass, while it will not pass through some of the very fine porcelain filters with pores still smaller, but large enough to allow liquid to pass through them. There are other reasons for the conclusion, but they cannot be given here. Although these organisms have never been seen, quite a little is known of their general nature. The animal diseases produced by invisible organisms are the following.

**Foot-and-mouth Disease.**—This disease, manifesting itself chiefly in the mouth and feet of cattle, varies much in its severity. Although not often causing death, it does result in great financial losses to dairymen. It is readily transferred to other animals, most kinds being susceptible to it. It occurs rarely in man, being transported through the milk of diseased animals, but it is not a very serious matter, being a mild infection only. For many years it has caused heavy losses in the cattle-raising communities of Europe. It has not been common in the United States, though a few cases have occurred at intervals, and there have been two rather severe epidemics within the last ten years. These epidemics have been vigorously handled by the agricultural department and have been speedily stamped out. It is hoped that by the vigorous measures taken in killing all cattle attacked, the disease may be prevented from gaining a foothold in the country, and that our dairymen may thus be protected from the troubles and losses experienced elsewhere. Hitherto the efforts have been successful. No other remedy is known save that of isolation or slaughter.

**Rinderpest. Cattle Plague.**—This is a very serious disease, originally found in Asia, but for centuries periodically invading Europe, and recently very rife in Africa. It attacks cattle chiefly, and its death rate is very high. Man is immune against it.

**Rabies,** or **hydrophobia,** is also produced by some agent not yet surely known. It attacks dogs chiefly, although occasionally it is found in horses, cattle, and man. So far as known the only source of the disease is the bite of infected animals, and the great majority of cases come from the bite of dogs. The name hydrophobia applies to the disease in man only, where a dread of water is one of the symptoms. Since this dread of water does not appear in dogs suffering from the disease, the name rabies is best applied.

Other diseases of the same category are: **pleuropneumonia**, a serious disease of cattle; **horse sickness**, a destructive disease of horses in South Africa; **bird pest** (Vogelpest) a highly infectious and fatal disease of chickens; **sheep-pox**, a disease of sheep in the Mediterranean countries. In all of these the exciting agent is unknown and is probably too minute to be seen with the microscope.

## ANIMAL PARASITES.

There are some diseases of animals caused by microscopic *animal* parasites. Of these the only well-known example in this country is *Texas fever* or, as it is frequently called, the *tick fever*. This latter name is given to it from the fact that the disease is distributed by means of the cattle tick. Other diseases caused by animal parasites are *surra* and the *tsetse fly* disease or *nagana*, neither of which is found in this country.

**Diseases of Other Animals.**—Parasitic diseases are found in all animals bred upon the farm, each animal having its own peculiar types. Ordinary fowls have *fowl cholera*, *roup*, *diarrheal diseases*, etc., and turkeys, geese, and ducks have diseases of their own. Even fishes are subject to diseases produced by bacteria of other fungi. It is impossible in this work to consider this subject further, but it is well to bear in mind that the list of parasitic diseases is a long one and is being increased constantly as investigation is being extended.

## CHAPTER XX.

### THE PARASITIC DISEASES OF PLANTS.

It is by no means easy to draw a sharp line between plant disease and the phenomenon of decay. If the tissues of a living plant show signs of decay it is called a disease; if the decay occurs in fruit or vegetables after they are harvested we speak of it as decay. But there are some parasitic organisms that may grow in the living plant and thus find access to the fruit so that the fruit will decay after harvesting. Should this be called a disease? In some cases the parasites seem to do no injury to the living plant, but live in its tissues to injure the stored fruit or vegetable later. In such cases it is manifestly difficult to say whether the phenomenon should be called a disease. In the types given in the following pages the parasites in most cases do injury to the living tissues, although one or two are exceptions.

While most parasitic diseases of animals are due to bacteria, with a considerable number caused by animal parasites and almost none by the higher fungi, a different condition of things is found among plants. The larger majority of plant diseases are caused by the higher fungi, a considerable number by bacteria, while, so far as known, none are caused by microscopic animal life. In our brief survey of this important field we may best divide the subject into two divisions: 1. *The Fungoid Diseases.* 2. *The Bacterial Diseases.*

### THE FUNGOID DISEASES OF PLANTS.

This is by far the largest class of plant diseases, but they can only be touched upon in this work. The Fungi that cause this class of diseases are mostly of some size and can hardly be called micro-organisms. They do not therefore strictly belong to a discussion of bacteriology, but their very close relation to germ life makes it necessary to consider them briefly.

As stated on an earlier page, the higher fungi are characterized by developing a *mycelium*. This delicate branching, usually colorless thread, grows in profusion in or upon the substance that furnishes the fungus with its nourishment. It is this mycelium that makes these plants especially adapted to live as parasites upon plants. A spore of some fungus falls upon the surface of a leaf and germinates, sending out its tiny thread. This finds some opening into which it can thrust its way. Sometimes the opening is a wound in the cuticle, but in other cases it is the breathing pore of the plant, the stomata. Once it has entered through this cuticle it finds the tissues soft and moist and there is nothing to prevent its growing through the plant. The mycelium can readily grow among the plant cells, winding its way in all directions and may in time penetrate to all parts of the plant. Living thus as a parasite and drawing its sustenence from its host it naturally produces more or less effect upon the plant life, resulting in what are called plant diseases.

The mycelium is the growing part of the plant, but not its reproducing part. These plants are reproduced by spores. Although differing in their method of origin, the spores are always minute, microscopic bodies, produced in immense numbers by the fungus. Generally, though the fungus grows below the surface of its host, the spores are produced on its surface. The mycelium is usually out of sight, while at certain spots the parasite breaks through the cuticle of its host in order to produce spores and discharge them into the air. The mycelium is white or colorless, but the spores show a variety of colors. It is evidently by these superficial spores chiefly that the fungus is spread from plant to plant.

When one of these spores gets carried to the surface of another plant it must first germinate before it can do any injury. In order to germinate it must absorb moisture, a fact that explains the great influence of the weather upon the fungoid diseases. In moist weather the spores find plenty of moisture upon the surface of the leaves, while in dry weather the necessary moisture is lacking. Once it has germinated and its mycelium has entered the plant, it finds plenty of moisture within so that it is no longer dependent upon the weather.

The effects produced by these fungi growing in the plant tissues are extremely varied. Any part of the plant may be affected, some diseases showing in one place and others elsewhere. The leaf may become covered with *spots* of various colors, or it may *wilt*, or *roll up* or *drop off*. *Scabs* may grow on the plant or its fruit, or the whole may show signs of *rotting*. Plant diseases have received various popular names that are loosely applied and not very clear in their meaning. The more common descriptive names are the following:

**Wilts** are characterized by the wilting and withering of the plant.

**Rots** are characterized by a tendency of the plant tissue to soften and decay.

**Smuts** show a mass of black or blackish spores.

**Mildews** show a whitish, powdery growth over the surface of the host.

**Rusts** show spots of a reddish color, due to reddish-yellow spores.

**Anthracnose** is a name frequently applied to diseases causing spots on the leaves or elsewhere.

**Blight** is a term with no definite meaning, but is generally applied to almost anything that causes a general wilting and destruction of the plant.

These terms are all in a measure descriptive terms of the effects produced by the parasites on the host. None of them are specific diseases, but all are produced by many different parasites on many different hosts, and in some cases the same parasite may produce different types of disease at different stages of its life.

**Methods of Combating Fungoid Diseases.**—There are several general methods by which these diseases may be kept in check: 1. By the *selection* of *resistant varieties* of the cultivated plants. Experience has shown that some varieties yield readily to the parasites while others are highly resistant. A careful selection of the varieties, guided by experience, is sometimes of value in checking disease. 2. By *regulating the conditions* of the cultivated plant in such a way as to render it best able to resist the disease. This involves the matter of cultivation, fertilizing, controlling weeds that serve as hosts of the parasite, etc., and demands a knowledge of the methods and seasons for the spore distribution of the fungi, and each disease has to be studied as a separate problem. 3. By the use of *fungicides*. These are properly selected chemicals that act as powerful germicides upon the fungi, but do not injure the host-plant. They are mostly applied by spraying, and the spray reaches the surface of the plants only, being, therefore, of little or no value after the mycelium has actually entered the plant tissue. Hence to be useful they must be applied at just the right time, and each disease must be carefully studied as to its time of sporing in order that spraying may be a success. A majority of the successful fungicides contain *copper* that seems to be especially efficient upon this class of fungi. 4. With some of these diseases a *rotation of crops* is efficient, since a fungus that attacks one host may be without influence upon another species of plant. 5. *Clean seed selection; i.e.*, selection of seed free from disease.

To describe the various fungoid diseases is impossible in this work. A list of the more important ones is given below, classified according to their host plant, with the popular name and the name of the fungoid parasite.

**Alfalfa.** Leaf spot, *Pseudopeziza Medicaginis* (Lib.) Sacc.

**Apple.** Bitter rot, *Glomerella rufomaculans* (Berk.) Sp. and von Schr. Black rot, *Sphæropsis malorum* Pk. Canker, *Nectria ditissima* Tul. Leaf spots, *Phyllosticta*. Powdery mildew, *Podosphæra leucotricha* (Ell. and Ev.) Salm. Rust, *Gymnosporangium macropus* Lk. Scab, *Venturia inæqualis* (Cke.) Aderh. Sooty blotch, *Phyllachora pomigena* (Schw.) Sacc.

**Asparagus.** Anthracnose. *Fusarium*, Rust, *Puccinia asparagi* DC. Rust, *Cladosporium herbarium* Link.

**Beans.** Anthracnose, *Colletotrichum lindemuthianum* (Sacc, and Magn.) Bri. and Cav. Downy mildew, *Phytophthora Phaseoli* Thax. Leaf blotch, *Isariopsis griseola* Sacc. Rust, *Uromyces appendiculatus* (Pers.) Lk.

**Beet.** Leaf blight, *Cercospora beticola* Sacc.

**Cabbage.** Club root, *Plasmodiophora Brassicæ* Wor. Leaf molds, *Alternaria Brassicæ* and *A. macrospora* Sacc.

**Celery.** Leaf blight, *Cercospora Apii* Fr.

**Corn.** Leaf blight, *Helminthosporium turcicum* Pass. Rust, *Puccinia Sorghi* Schw. Smut, *Ustilago Zeæ* (Beckm.) Ung.

**Cucumber.** Downy mildew, *Plasmopara cubensis* (B. and C.). Anthracnose, *Colletotrichum lagenarium* (Pass). Scab, *Cladosporium cucumerinum* (Ell. and Arth.).

**Grape.** Anthracnose, *Sphaceloma ampelium* DeBy. Black rot, *Guignardia bidwellii* (Via. and Rav.). Downy mildew, *Plasmopora viticola* (Ber. and De Ton.) Gray mold, *Botrytis*. Powdery mildew, *Uncinula necator* Bur.

**Muskmelon.** Anthracnose, *Collototrichium lagenarium* Ell. and Hals. Downy mildew, *Plasmopora cubensis* Humph.

**Oat.** Black-stem rust, *Puccinia graminis*. Smut, *Ustilago Avenæ* Jens.

**Onion.** Black mold, *Macrosporium Porri* Ell. Black spot, *Vermicularia circinans* Berk. Downy mildew, *Peronospora schleideni* Ung. Smut, *Urocystis Cepulæ*.

**Peach.** Brown rot, *Sclerotina frutigena* Schrt. Leaf blight, *Cercosporella Persica* Sacc. Leaf curl, *Exoascus deformans* Fckl. Scab, *Cladosporium carpophilum* Thm.

**Pear.** Black mold, *Fumago vagans* Pers. Leaf spot, *Spetoria piricola* Desm. Rust, *Gymnosporangium globosum* Farl.

**Plumb.** Black knot, *Plowrightia morbosa* Sacc. Brown rot, *Sclerotina frutigena* Schrt. Powdery mildew, *Podosphæra oxyacanthæ* DeBy.

**Potato.** Blight, *Phytpphora infestans* (one of the most destructive of our plant diseases). Dry rot, *Fusarium oxysporum* Schl. Scab, *Oospora scabies* Thaxt.

**Squash.** Anthracnose, *Colletotrichum lagenarium* (Pass.) Ell. and Hals. Black mold, *Rhizorpus nigricans* Ehr. Metallic mold, *Choanephora cucurbitarim* Tha.

**Strawberry.** Fruit rot, *Botrytis vulgaris* Fr. Leaf blotch, *Ascochyta Fragariæ* Sacc. Leaf spot, *Sphearella Fragariæ* Sacc.

**Tobacco.** Frost fungus, *Botryosporium pulchrum* Cda.

The above list contains only a few of the very large number of known fungoid diseases of plants, but will serve the purpose of showing their variety. The fungi that produce these diseases are by no means closely related to each other. The higher fungi are divided into many classes and the disease-producing parasites are distributed among them all. For these distinctions, however, the student must be referred to books upon botany.

## THE BACTERIAL DISEASES OF PLANTS.

Only within recent years has it been appreciated that bacteria are important agents in producing plant diseases. Even after their agency in causing diseases in animals had been fully recognized it was denied that they could produce troubles of this sort in plants. Up to very recent date, it was claimed that it was an impossibility for bacteria to penetrate plant tissue so as to produce trouble. Plant cells are provided with hard cell walls of cellulose and wood, which protect the living protoplasm within; and, since these cells form the bulk of the plant and are adherent to each other, it was difficult to see how bacteria could penetrate into the plant at all. The mycelium of the higher fungi can do this readily since it can thrust itself between the cells, and thus grow easily within the solid tissues; but it seemed impossible to believe that bacteria could penetrate the hard tissues. Within recent years, however, it has been demonstrated that this is possible and the last ten years have disclosed many bacterial diseases of plants, until to-day we know of more bacterial diseases of plants than of animals.

**The Black Rot of Cabbage** (*Pseudomonas campestris*).—An illustration will best show the general course of such a disease and at the same time indicate how conclusive is the proof of the agency of bacteria. For this purpose will be chosen the black rot of the *cabbage, cauliflower, turnip,* and several other members of the family *Cruciferæ.* The disease appears first, as a rule, upon the edges of the leaves, as brown spots, that spread down the leaves following the veins to the midrib and petiole and finally into the main stem of the plant. It then travels rapidly through the whole plant causing the leaves to wilt, turn yellow, dry up and become thin and parchment like. The veins in the leaves and stem are particularly affected and turn black, this being the characteristic feature of the disease and the source of the name black rot (Fig. 53). Sometimes the veins alone are affected. Sometimes the trouble does not appear in the growing plant, but only in the cabbage after storing, extending through them rapidly and ruining them.

When these black veins are studied with the microscope they are found to be filled with bacteria and it is easy by proper methods to remove them and cultivate them in the laboratory. Pure cultures of an organism are thus obtained, *Pseud. campestris* (Fig. 53). It is easy to keep this growing in the laboratory for months under strict observation. Having thus obtained a pure culture it can be demonstrated at any time that it will produce the disease. It is only necessary to dip the tip of a needle into the pure culture and then prick the leaf of a healthy plant with it. This inoculation is followed in a few days by the appearance of the characteristic symptoms of the disease, starting at the point of the needle and travelling down the plant in the usual way. By proper study it is possible to show that the bacteria multiply in the plant following, the vascular bundles which they first turn black and then destroy. Since these bundles convey the water to the plant their destruction shuts off the usual water-supply, and the plant wilts. It is possible at any time to isolate the bacterium from these diseased plants and obtain it again in pure culture. Cabbage plants pricked with sterilized needles show no evil result, proving that it is the inoculated bacteria that produce the disease. Such experiments as these, repeated many


FIG. 53.—The black rot of cabbage. *a*, a bit of the stem showing the blackened fibrovascular bundles; *b*, cells, highly magnified, showing some filled with bacteria; *c*, the bacteria.

times by different experimenters, leave no room to doubt that the bacteria are the cause of the disease in question.

The method by which the bacteria make their way into the plant is interesting. We have learned in an earlier chapter that bacteria frequently secrete enzymes. This *Pseud. campestris* secretes such an enzyme and one that has the power of softening and dissolving cellulose. As the bacteria multiply at the inoculated point they secrete this enzyme, called *cytase*, which at once softens and disintegrates the walls of the adjacent plant cells. The contents of the cells thus exposed are quickly killed by the action of the bacteria, a *toxin* being probably secreted by them for the purpose, and the bacterium, feeding upon the food thus furnished, multiplies further. More cytase is produced, dissolving more cell walls, and the disease progresses as the bacteria thus enter the plants. In this way they travel through the plant, chiefly in the vascular bundles, and finally may affect the plant throughout. The cellulose-dissolving enzyme has been found to be secreted not only when the bacterium is growing in the host, but also in the laboratory in the bacteriologist's test-tubes.

The bacteria have apparently three methods of entering the plant. Through the uninjured cuticle they are unable to enter, nor can they enter through the stomata of the plant. But if the cuticle be broken by a wound or scratch, no matter how tiny, the broken cuticle will offer an entrance to the germs. Further, there are on the edges of the leaves minute openings called water pores. Through these pores the bacteria also can enter. Seemingly they can also enter through the roots, especially through the tips of the rootlets which are likely to be exposed and broken during transplanting.

No effectual remedy against this disease has yet been found. Its method of distribution from field to field is not well known, and hitherto no means of checking it after it has made its appearance in a field has been discovered. Wet weather, which is best for the growth of the cabbages is, unfortunately, also best for the growth of this parasite. That it lives in the soil from year to year seems proved, and hence after it makes its appearance in a field it is likely to recur year after year. Since it is confined to members of the

Cruciferæ, it is a natural suggestion that a change of crop to some kind of plant not in this family should be made when any particular plot of ground becomes infested with the disease. The destruction of all weeds of the mustard family in the vacinity of cabbage plots is also to be recommended..

## OTHER BACTERIAL DISEASES OF PLANTS.

The illustration given will serve to show the kind of evidence that is sought for in the study of plant diseases. In the list that follows, demonstrative evidence has been obtained in practically all cases, so that all of the diseases in this list may be accepted as caused by bacteria. The list given is a long one, and if it be compared wi'h the list of animal diseases given in the last chapter it will be seen to surpass that list. These plant diseases have not the importance nor have they developed the interest that attains to some of the animal diseases, but nevertheless they are of great significance in farming operations, sometimes causing very large losses. As in the case of animal diseases the bacteria causing them are not confined to one plant host. The black rot, for example, attacks the *cabbage*, the *cauliflower*, the *turnip*, *kale*, *Brussels sprouts*, *collards*, *rutabagas*, *radish*, as well as some other plants, all of the family *Cruciferæ*. So, as with the other diseases, the same parasite may attack several hosts.

**Classification of Bacterial Diseases.**—Plant diseases are less clearly defined and classified than animal diseases. Popular names have been applied to them without careful discrimination till the popular names have ceased to have any sharp meaning. The bacterial diseases may, however, be fairly well divided into three types, distinguished by the kind of effect they have upon the host. These are: 1. *The Wilts*. In these the bacteria attack chiefly the vascular bundles, either destroying their cells or clogging them. This shuts off the ordinary water-supply to the plants and causes them to wilt and wither. 2. The *Bacterioses and Rots*. In these diseases the bacteria invade the tissues generally, not being confined to the bundles, and destroy the plant cells at once. They may cause the tissues to become much softened, thus producing the *soft rots*, or they may fail to cause this softening but injure them in other ways, so that the plant does not rot but becomes filled with bacteria and the tissues are much injured. These are sometimes called *Bacterioses*. 3. *The Tumor Diseases*. In these cases the bacteria cause the formation of unusual growths, tubercles, or tumors, on the various parts of the plants.

**The Wilts.**—The black rot of cabbage belongs to this class and is really not a "rot," but a wilt. In addition, three others will be briefly described.

*Brown Rot of Potato, Egg Plant* and *Tomato* (*B. solanacearum*).—Although frequently called a rot, this disease is really a wilt. It is a widely distributed disease of the potato, especially in the northern part of United States and Canada. The leaves of the attacked plant first wilt and shrivel and then the stem turns brown or black. The affection extends down the vascular bundles and may reach the tuber. In this it spreads through the vascular bundles, causing in time a destruction of the potato that has given to it the name of *brown rot*. The bundles are found to be filled with bacteria in great numbers, that destroy the cell walls, finally causing the complete disintegration of these tissues. The isolation of the bacterium is easy and inoculation experiments show that it is capable of producing the same disease in various members of the potato family. The bacterium appears to be carried from plant to plant by insects, and the potato beetle is an important agent in its distribution.

The term potato rot is applied to any form of disease that is followed by the rotting of the potato. There are several different parasites that produce this phenomenon, some of them belonging to the higher fungoid types. It is thought that this bacterial disease is the cause of the larger part of the rots in our Northern States. A second bacterial rot of the potato is caused by a bacterium named *B. solanisaprus* (Har.). It is also a wilt rather than a rot, as we have used the terms, although after it affects the tuber itself it produces a general decay of the tissues. It is common in Canada, where it was first described. A third bacterial potato rot is caused by *B. atrosepticus* (VanHall).

It is important to note that the bacterium that causes the brown rot of the potato can also live as a parasite in the *tobacco* plant where it produces what is known as the *Granville wilt.*

*The Wilt Disease of the Gourd Family* (*B. tracheiphilus, Sm.*).—This bacterium attacks various members of the gourd family, being best known in the *cucumbers, muskmelons, pumpkins,* and *squashes.* The bacterium that produces it will grow readily in laboratory media and invariably produces the disease when it is inoculated into healthy plants. It causes the wilting of the plant by clogging up its vascular ducts. The bacteria appear to be distributed by insects which inoculate the plant, chiefly on the leaves, by puncturing or by eating holes in them. The cucumber beetle and the squash bug are especial offenders in this respect, and anything that will keep these insects in check will help to reduce the troubles from the disease.

*The Corn Wilt* (*Pseudomonas stewarti*).—This disease affects sweet corn in the early summer. The leaves wilt without apparent cause and the plant gradually withers and dies, at times in four days and at others as much as a month is required. Sometimes the attacked plants will recover. Usually the leaves are affected one after another, but sometimes the whole field seems to be attacked at once. If the stem is cut lengthwise the vascular bundles will appear as yellow streaks, which become black in the dead stems. If cut across, these bundles exude a yellow viscid substance that is composed mostly of bacteria that are the agents that produce the disease. The germs are thought to be distributed by the seeds of diseased plants, and no remedy has been suggested except to select resistant varieties of corn, and to use care not to plant seed from infected plants.

While this bacterium attacks only sweet corn, there is another species that injures field corn. This has been variously named (*B. Zeæ, B. cloacæ*). It causes quite a different type of trouble, producing dark purplish discolorations on the leaf sheath, giving a yellow coloration to the plant and causing the ears to undergo a moist rot. It also attacks the broom corn.

A wilt of the sugar-cane is produced by *Pseud. vasculans.*

**The Bacterioses and Rots.**—A single illustration of this type must suffice.

*The Fire Blight of the pear, quince, apple, etc.*, (*B. amylovorus.*)—This bacterium attacks various members of the apple family and a number of other plants as well. The disease has been known for over a century and almost every conceivable explanation has been given for it. That it is caused by a bacterium has been finally demonstrated by the isolation of the organism and the reproduction of the disease by inoculation experiments. In the form known as the twig gall, the first indication of the disease is commonly seen in a browning or blackening of the leaves of the young shoots, which soon die. It then extends into the stem by the way of the inner bark, causing it to become blackened. The whole of this tissue is destroyed by the bacterium, causing a girdling of the tree. Then it extends down the stem, sometimes going at the rate of an inch a day, and eventually causing great injury or complete destruction. It particularly attacks the stored starch, converting it into a gummy substance. The diseased area may extend for a distance down the stem causing a patch of "canker", and if checked in its growth by the onset of winter, it remains alive in the stem till warm weather, when it once more begins its work of destruction. In moist weather a viscid mass extends from the canker spots, containing bacteria. Little is known of its means of distribution, although it is thought that it may find entrance through the flowers and be carried to them by bees. It may, however, enter through wounds in the bark elsewhere. The only feasible method of fighting it of any value is to cut away the diseased parts as soon as the trouble appears, great care being taken to be thorough in the pruning and to cut away every bit of diseased wood.

Other examples of this type of bacterial diseases are the following.

*Bean blight*, produced by *Bact. Phaseoli.*

*Cotton bacteriosis*, produced by *Bact. malveacarum.*

*Walnut bacteriosis*, produced by *Pseud. juglandis.*

*Mulberry blight*, produced by *B. cubonianus.*

*Black spot of plum*, produced by *Pseud. Pruni.*

*Wakker's disease of hyacinth*, produced by *Bact. Hyacinthi.*

*Soft rot of turnips*, etc., produced by *B. oleraceæ*.

*Soft rot of carrots*, produced by *B. caratovorus*.

*Soft rot of sugar beet*, produced by *B. tenthium*.

*Soft rot of stored celery*, caused by *Pseud. fluorescens*.

*Rot of iris*, produced by *Pseud. iridis*.

*White rot of turnip*, produced by *Pseud. destructans*.

*Gummosis of beet*, produced by *B. Betæ*.

*Soft rot of onions* is also caused by bacteria, and there are some other diseases less well known.

**The Tubercular or Tumor Diseases.** *The Olive Knot* (*B. savastanoi*). This disease, first studied in 1886, and attributed upon insufficient proof to a bacterial origin, has recently been demonstrated to be a bacterial disease. The bacillus is a motile one with several flagella at one end and grows in ordinary culture media in the laboratory. Several different bacteria have been found associated with this disease, but the one to which the above name has been given is its cause, as shown by the fact that the inoculation of olive trees with cultures of the organism is invariably followed by the appearance of the characteristic symptoms of the disease at the point inoculated. The effect of the bacillus is to stimulate the plants to unusual growth. The various tissues of the stem multiply more profusely than common, producing a swollen growth on the stem which is called the olive knot (Fig. 54). This injures the trees and sometimes kills them. The organism, so far as known, enters the plant exclusively through wounds. It occurs in the various olive-raising countries of Europe and Africa, and also in California.

FIG. 54.—The black knot of the olive.

*The Crown Gall of the Peach and Other Plants* (*B. tumifaciens*.)—This disease, until recently attributed to a different class of fungi, has now been proved to be caused by a bacterium. In the peach it commonly produces an enlarged growth at the crown of the plant, between the stem and the root. The parasite that causes it has the power of growing upon a large series of plants, producing tumors in various parts of the plant which injure it more or less, according to the extent of the infection. Among the plants that may be infected with it are the *raspberry* (Fig. 55), the *daisy*, the *hop*, the *radish*, the *cabbage*, the *tobacco*, the *sugar beet*, the *grape*, the *tomato*, the *oleander*, the *apple*, and some others. It is unusual for a parasite to have such a long list of possible hosts, but in all these plants it has been demonstrated by Smith that tubercles will be produced by the inoculation of pure cultures of the organism. It is the cause of considerable losses to horticulturalists.

FIG. 55.—The crown gall on the root of the raspberry.

*Root Tubercles of Legumes*.—These have been considered in a different connection (Chapter VII), but they are properly classed here. They are certainly caused by parasitic bacteria, although in this case apparently both the parasite and the host are benefited by the association, a condition sometimes called *symbiosis* rather than parasitism.

*Remedies*.—Remedies for the bacterial diseases are not as yet very satisfactory. Spraying, so frequently efficient against fungoid diseases, is of no value here, because the bacteria are always within the tissues of the plant where the spray cannot touch them. Hence in dealing with plant diseases in general it is always desirable to know whether they are fungoid or bacterial, since in the latter case spraying is always useless. Each disease has to be met by devices adapted to the peculiar nature of the disease, and no general principles can be given beyond that already pointed out on page 295.

# PART VI.

## APPENDIX.

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## CHAPTER XXI.

### LABORATORY WORK.

Laboratory work should, so far as possible, accompany the study of the text. It is impossible to make a series of experiments that will closely follow the order of topics in the text, since a large amount of preliminary work has to be done in making media before actual study of bacteria begins. The order of experiments below given will be found a convenient one to follow, but may be modified to suit convenience.

#### APPARATUS NEEDED.

Steam sterilizer.

Autoclave.

Hot air sterilizer—A common gas oven used for cooking will do.

Stew-pan for cooking media.

Flasks.—Liter and half-liter.

Test-tubes, heavy—Board of Health pattern.

Petri dishes—four inches in diameter.

Pipettes—1 c.c. and 2 c.c. Some larger ones are also convenient. Each of the smaller ones should have a glass tube holder (Fig. 56) or there should be a metal box with cover to hold fifty or more pipettes at once.

Wire baskets to hold test-tubes.

Test-tube racks, or common tumblers with a little cotton in the bottom to hold test-tubes.

Beakers.

Evaporating dishes.

Fermentation tubes.

Burette holder with four burettes.

Evaporating dishes.

Measuring cylinders—1 liter and 100 c.c.

Counting plate or counting card.

Platinum wire to be fused into glass rods.

Culture oven with constant temperature of 98°.

Bunsen burners.

FIG 56.—1 c.c. pipettes inclosed in glass tubes for sterilizing.

Forceps—Common and Cornet forceps.

Microscope with 1/12 immersion lens and plenty of slides and cover-glasses.

## MATERIALS.

| | |
|---|---|
| Peptone (Witte) | Normal NaOH and Normal HCl. |
| Salt | Phenolphthalein |
| Beef extract | Alcohol |
| Gelatin—Gold label | Corrosive sublimate |
| Agar-agar | NaOH |
| Litmus, dry in cubes | HCI |
| Absorbent cotton | Azolitmin |
| Fuchsin | Methylene blue |
| Dextrose, lactose, and saccharose | Common cotton, good quality |

No. 1. **Washing and Sterilizing Glassware.**—All glassware must be thoroughly washed in hot water and soap. New glassware should first be treated with 1 per cent. HCl. Used glassware, containing remains of gelatin or agar, should first be boiled in water containing powdered soap or sal soda. Follow this with thorough washing in hot water, rinsing in cold water and draining.

To begin with, the student may wash 50 test-tubes, 2 one-liter flasks, one dozen Petri dishes and several 1 c.c. pipettes. After drying, place the pipettes in glass holders or a metal box, plug the test-tubes and flasks tightly with cotton and place these with the Petri dishes in the sterilizing oven. Heat for one hour at 310° F. (155° C.) for one hour. All glassware should be subsequently sterilized in the same way before using.

No. 2. **Preparation of Agar Culture Medium and Bouillon.**—Measure out the following:

| | |
|---|---|
| Water . . . . . . . . . . . . . . . . . | 1 liter |
| Liebig's extract of beef . . . . . . . . | 3 grams |
| Common salt . . . . . . . . . . . . . | 5 grams |
| Peptone . . . . . . . . . . . . . . . . | 10 grams |

*a.* Divide in two lots, set one-half aside (see below); place the other half in a stew-pan and carefully weigh the dish and its contents, noting the combined weight for future use. Dissolve the mixture at about 150° F. (60°C.). Add 6 grams of agar-agar (1.2 per cent.), cut into small pieces, and dissolve by heat. It takes considerable heat to dissolve the agar and may require boiling. Boil till the agar is *completely* dissolved and then replace the water that has evaporated by adding cold water till the original weight has been restored.

*b.* Adjust the acidity to 1.5 per cent. as follows:

Measure out 10 c.c. of the mixture, placing it in an evaporating dish with ten times its bulk of water. Bring to a boil and add a few drops of phenolphthalein solution.* This solution is colorless so long as it is acid, but turns red when alkaline. The agar medium remains red after the addition of phenolphthalein, showing it to be acid.

Place the evaporating dish under a burette containing 1/10 normal NaOH (one part normal NaOH diluted with nine parts of distilled water) (Fig. 57). Take the reading on the burette. Allow the solution to fall drop by drop into the evaporating dish, stirring between each drop. As long as the material remains acid no color will appear. Continue adding the NaOH until a faint red color appears and does not disappear upon stirring. When this point is reached the contents of the evaporating dish are neutral.

Take the reading upon the burette and determine the difference between the first and second reading. The difference gives the number of cubic centimeters of 1/10 normal NaOH needed to neutralize 10 c.c. of the culture medium. Divide by 10 to determine the amount necessary to neutralize one c.c., and multiply by 990 to determine the amount necessary to neutralize the rest of the agar medium.

Add to the agar medium sufficient NaOH to neutralize it. Instead of adding 1/10 normal, add *normal* NaOH, using of course only 1/10 as many cubic centimeters as the above calculation shows would be needed of 1/10 normal NaOH. This will bring the whole to the neutral point.

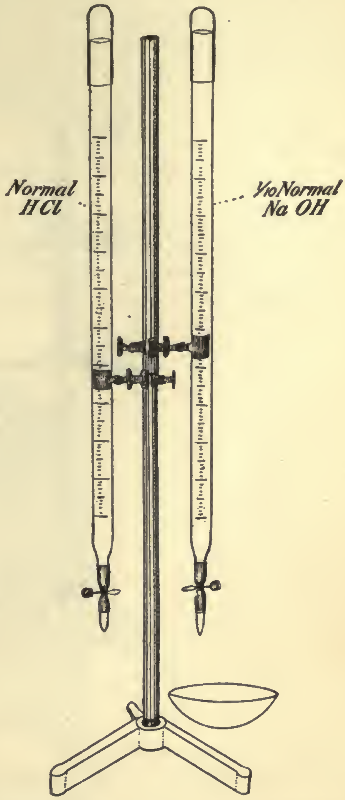

FIG. 57—Two burettes arranged for neutralizing culture media.

Add normal HCl to the amount of 15 c.c. per liter. In this case, there being only 990 c.c. left, the amount added should be 14.9 c.c. This makes an acidity of 1.5 per cent., the best reaction for most bacteria.

*c.* Boil vigorously for fifteen minutes and then restore the original weight by adding water. Test the reaction again as before to see if it is

* Eight per cent. phenolphthalein in 50 per cent. alcohol.

correct. It should require 1.5 c.c. of 1/10 normal NaOH to neutralize the acidity in 10 c.c. If the reaction is right, cool to about 60° F. and add the white of an egg which has been mixed with a little water, adding slowly with stirring. Heat slowly and allow to simmer until the egg has coagulated and the liquid is clear. This may take half an hour or more. Add water to bring to the original weight again, plus the weight of the added egg, and then filter as follows:

*d.* Place a considerable quantity of absorbent cotton in a large funnel (Fig. 58). Over the top of a second funnel above the first, place some cheese-cloth. Pour the hot agar medium into the cheese-cloth. It will run through

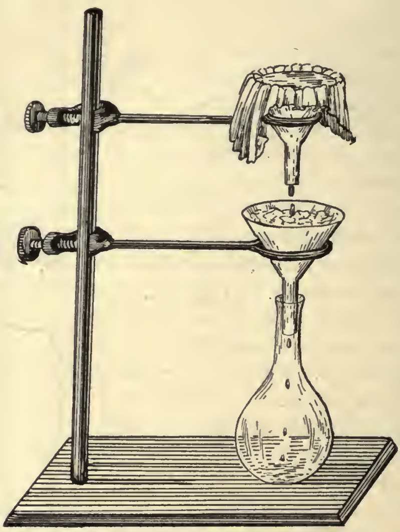

FIG. 58.—Showing method of filtering culture media.

rapidly; then filter through the cotton to be caught below in a beaker or flask. It should be transparent, clear, and of a yellowish-brown color. If it is not clear a second boiling with a second egg will usually clear it.

Place 10 c.c. of this agar medium into each of 25 test-tubes, replacing the cotton plug. In filling these tubes use either a 10 c.c. pipette or a funnel, taking care not to allow the agar to touch the sides of the tubes where the cotton is to be inserted.

*e.* Place the test-tubes, together with the flask containing the remainder of the medium (tightly plugged with cotton) in the autoclave and sterilize at 15 lbs. for 30 minutes. Cool, and after the pressure is down to zero, remove from the autoclave. While still hot lay the test-tubes down on a table with their mouths slightly raised, so that the agar will form a slanting surface (Fig. 59). Allow them to harden in this position and set aside for future use. Keep this and all other culture media in a cool dark place until used.

**Bouillon.**—To make bouillon the procedure is as above, except that agar is not added. The other half of the dissolved mixture of water, peptone,

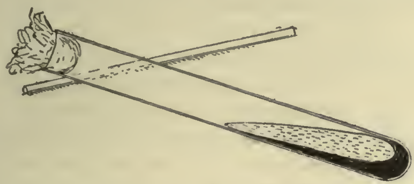

FIG. 59.—Showing method of hardening agar slants.

salt, and beef extract, in *a*, is brought to the acidity of 1.5 per cent. in exactly the same way as above described, and, after a boiling (the egg white is not necessary here) the bouillon is filtered through filter-paper, placed in tubes, and sterilized in exactly the same manner as agar medium.


FIG. 60.—A steam sterilizer (*Fowler*).

If it is not convenient to use the autoclave, either of these may be sterilized by *discontinouus heating* as follows: Place the tubes and flask in a steam sterilizer and steam for one-half hour (Fig. 60). Set aside for twenty-four hours and then steam for another half-hour. Set aside once more for twenty-four hours and then steam a third time. If properly sterilized these media will keep indefinitely.

No. 2. **Determination of the Number of Bacteria in Water.**—Melt three tubes of agar as above made in hot water and cool to about 125° (50°C.) With a sterilized pipette place a cubic centimeter of water from any source in each of three Petri dishes (Fig. 61.), and pour the agar from one of the melted tubes into the Petri dish. Replace the cover again at once and by gentle agitation, thoroughly mix the agar in the dish with the water first added. Do not do this violently enough to spill or throw the agar up on the sides of the plate. After mixing set on a level table to harden and then place in the culture oven. These preparations are called **agar plates.** After twenty-four hours the plates will be seen to be dotted over with **colonies**, each supposed to come from a single bacterium. Count the number of colonies; this will give the number of bacteria in the original c.c. of water that have been able to grow in the medium.

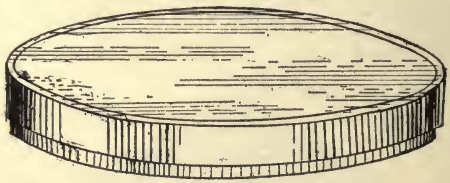

FIG. 61.—A petri dish for making "plates."

No. 3. **Water Blanks.**—Place in a considerable number of test-tubes 9 c.c. of water. In a series of small flasks or bottles place 99 c.c. of water (*i.e.*, add 100 c.c. and then remove 1 c.c.) After plugging tightly with cotton, place in the autoclave and sterilize at 15 pounds for one hour. It is very desirable to have test-tubes and bottles used for these water blanks with a mark etched upon them at the 9 c.c. and 99 c.c. level. When they are to be subsequently used for dilution care should be taken to see that they are filled, *exactly* to the mark, since evaporation frequently withdraws some of the water, and unless they contain the exact quantity, errors will be introduced.

No. 4. **Determination of the Bacteria in Milk.**—Milk commonly contains so many bacteria that it must be diluted before the bacteria are determined. The amount of dilution needed will vary widely with the age of the milk. For most market milk a dilution of 1,000 will serve. Proceed as follows: Into a 99 c.c. water blank place 1 c.c. of the milk to be tested. Shake vigorously so as to distribute the bacteria uniformly. With a second pipette transfer 1 c.c. of this mixture to a 9 c.c. water blank and again thoroughly mix. With a third pipette place 1 c.c. of this in a Petri dish and then pour upon it the contents of an agar tube. Agitate as in the last experiment, allow to harden and incubate in the oven for twenty-four hours. Use a counting plate in counting the colonies. If they are too numerous to count directly, count the number in one of the areas in the counting plate and multiply by the number of such areas covered by the whole plate. This will give the number of colonies on the plate, and this multiplied by 1000, will give the number in the original milk. There may be any where from a few thousand to several millions. It frequently happens that the dilution of 1000 is insufficient, giving too many colonies on a plate. Higher dilutions are needed in such instances, but whether to make a higher dilution can only be determined by a knowledge of the age and temperature of the milk and by experience.

From these and from the plates of the following two experiments isolate and purify cultures as directed in No. 7.

No. 5. **Bacteria in the Air.**—Pour the contents of several tubes of agar into Petri dishes, replace the cover and allow to harden. Remove the cover from one of them and allow it to remain open in the laboratory for two minutes. Then replace the cover and place in the incubating oven. Expose a second in the same way in a barn; a third out of doors; a fourth in a barn after hay has been thrown down in front of cattle. Other Petri dishes may be exposed in other localities. After twenty-four hours' incubation count the number of colonies and compare the relative number of bacteria in the air at the different places.

No. 6. **Bacteria on the Fingers.**—Pour a Petri dish as above directed and allow to harden. Remove the cover of one and touch it gently with the fingers in several places. The hands may then be thoroughly washed, and a second Petri dish treated in the same way. Incubate for twenty-four hours and see if bacteria colonies have grown where the fingers touched the agar.

No. 7. **Isolation and Purification of Bacteria.**—Any of the plates above prepared will show after proper incubation a number of colonies. Comparing the different colonies noticeable differences will be seen between them. These differences in the colonies commonly indicate different kinds of bacteria, for the same kind of bacteria produce, commonly, the same kinds of colonies.

FIG. 62.—Platinum needle and platinum loop.

*Isolation.*—Sterilize a platinum needle (Fig. 62), in a flame until red-hot and after allowing it a few seconds to cool, dip the tip of it into one of the colonies. Transfer the bacteria adherent to the needle to one of the agar slant tubes by removing the plug and drawing the tip of the wire over the surface of the agar. Sterilize the needle again before laying it down. Label the tube with a gum label telling its source. In a note-book make a brief record of the kind of colony from which it was obtained. Allow the inoculated tube to grow, either in the incubating oven or in the room, until a growth appears on the surface of the agar. This is an **agar slant.**

*Purification.*—If the colony thus isolated has grown from a single bacterium this growth on the agar will be a pure culture. But this is not always the case and therefore the culture must be purified. With the tip of a platinum needle remove a minute quantity of the growth on the agar and place it in a 9 c.c. water blank. Thoroughly shake and transfer two platinum loopfuls of the water to a melted agar tube. Shake gently so as to mix, but not to produce bubbles, and then transfer two loopfuls of this agar to a second agar tube, mixing as before. Pour the contents of each agar tube into a Petri dish, harden and incubate as usual. If the culture was pure the colonies should be all alike. Pick out one of them with the platinum needle, inoculate it upon another agar slant and label it a *pure culture* from milk or whatever may have been its source. In this condition it may be set aside and preserved for a long time. As long as the agar remains moist the bacteria will usually be alive.

In the above manner isolate and purify a considerable number of cultures of bacteria from the plates made in Nos. 4–6, and keep these in a cool. dark place for use in various experiments given below.

No. 8. **Microscopic Study of Bacteria.**—Prepare one or both of the following staining solutions:

*Methylene Blue.*

| | |
|---|---|
| Saturated alcoholic solution of methylene blue, | 15 cm. |
| Potassium hydrate (1 : 10,000),* | 50 c.c. |

*Fuchsin Solution.*

| | |
|---|---|
| Saturated alcoholic solution of fuchsin, | 5 c.c. |
| Five per cent. solution of carbolic acid, | 45 c.c. |

In the middle of a clean microscopic slide place a drop of water (sterilized). With a platinum needle remove a very small quantity of the bacteria growth from the surface of one of the slant cultures prepared in No. 7 and place in the drop of water. Stir this drop with the needle, to distribute the bacteria and then (*a*) **spread** it over the slide. Allow it to dry in the air, and then pass the slide three times slowly through a gas flame. The purpose of this is to (*b*) **fix** the bacteria firmly to the slide so that they will not be washed away. It is necessary not to use too much heat. This may also be done by leaving the slide for a few minutes on a water-bath. After fixing, cover the bacteria completely with several drops of one of the staining solutions and allow to (*c*) **stain** for several minutes. The length of time necessary for this varies with conditions, one to five minutes being usually sufficient. Wash off the stain in running water and then dry the slide by gentle heat. Place a drop of immersion oil upon the stained bacteria and place the slide under the microscope. Use a 1/12 inch immersion, lowering the objective into the immersion oil and focusing very carefully. If the microscope has an Abbe condenser or a diaphragm it is best to have this widely open. The bacteria are so minute that it is hardly possible to study them with a lower magnifying power than a 1/12, although they can be

* To make this solution add 1 c.c. of a 10 per cent. KOH solution to 99 c.c. of water, and then add 5 c.c. of this to 45 c.c. of water.

seen with a 1/6 inch. Examine the bacteria and sketch. In this way make a microscopic examination of all of the cultures isolated and purified, and compare with Figs. 7 and 9. If it is desired to preserve the specimen place a drop of Canada balsam on the bacteria after drying, and then cover with a cover-glass.

*Motility.*—To determine the motility of bacteria transfer a small quantity from an agar slant to a bouillon tube, and allow to grow for 24 hours. Place a drop of the 24-hour-old bouillon culture on a slide and put upon it a cover-glass. Examine this with a 1/6 inch objective and with the diaphragm nearly closed. The best light for the purpose is artificial light (electric) placed near the microscope and reflected through by the plain surface of the mirror. It will be very difficult at first to see the bacteria, but with careful focusing they will appear as transparent dots or rods. Examine carefully to determine whether they are stationary or motile, calling only those motile that move back and forth across the stage and not those that simply dance back and forth without locomotion (the Brownian motion). It is sometimes desirable to keep the specimen under observation for some time in which case a **hanging-drop** method may be used. A concave slide is to be used and a ring of vaseline painted around the depression. The drop containing the living bacteria is placed in the middle of a large cover-glass and inverted over the concavity of the slide. By pressing it firmly into the vaseline ring it will be sealed so as to prevent evaporation and may be kept under observation for hours.

No. 9. **Bacteria in the Mouth.**—With a clean knife scrape a little of the material attached to the teeth and spread it in a very thin layer over a slide. Dry, fix and stain, and with a microscope note the large numbers of bacteria present. Sketch the varieties seen.

No 10. **Gram Stain.**—Prepare the following:

*Anilin Oil Gentian Violet.*

| | |
|---|---|
| Saturated alcoholic solution of gentian violet, | 6 c.c. |
| Absolute alcohol, | 5 c.c. |
| Anilin water,* | 50 c.c. |

*Grams Iodin Solution.*

| | |
|---|---|
| Iodin, | 1 gm. |
| Potassium iodid, | 2 gm. |
| Distilled water, | 300 c.c. |

Spread and fix on a slide, a little of one of the cultures of bacteria, and stain for one and one-half minutes in the gentian violet solution. Pour off stain, without washing, and place in the iodine solution for one and one-half minutes. Apply 95 per cent. alcohol until the drippings do not stain white filter-paper. This will take about three minutes and the specimen will be largely *decolorized.* Wash in water and study with microscope to

* Made by adding 2 to 3 c.c. anilin oil to 50 c.c. of water and shaking thoroughly, with subsequent filtering.

see if the bacteria are still stained blue or have been decolorized. Different species differ in this respect. If they are still stained they are *Gram-positive;* if decolorized, they are *Gram-negative.*

No. 11. **Microscopic Study of Yeast.**—Rub up a bit of an ordinary yeast cake in a little water. Place a dilute drop on a slide and proceed to stain exactly as above described for bacteria. Study with 1/12 immersion lens and compare with yeast as to size and shape. Look over the specimen and find some cells that show buds.

No. 12. **Gelatin Culture Medium.**—*a.* Weigh out the same ingredients as directed in No. 2, omitting the agar. After the mixture has dissolved add 12 per cent. of first-grade gelatin. Allow to soak till soft and almost melted. Weigh the dish with its contents. Bring to a boil slowly and boil for five minutes and add water to restore original weight.

*b.* Determine the acidity and bring the reaction to 1.5 as described in No. 2.

*c.* Boil 15 minutes. Cool and add the white of an egg dissolved in a little water. Heat slowly to boiling and allow to boil gently till the egg is coagulated and the liquid clear; usually about 15 minutes. Replace the evaporated water.

Heat once more to boiling and filter through absorbent cotton. Place 10 c.c. in each of about fifty tubes and the rest in a flask, plugging both with cotton. Sterilize in the steam sterilizer for twenty minutes. Set aside for twenty-four hours and steam a second time; this time allow half an hour steaming. Set aside for another twenty-four hours and steam again. Gelatin cannot be sterilized in the autoclave satisfactorily, since too high a heat will prevent its subsequently hardening when cooled. Too long boiling will in the same way ruin the gelatin.

No. 13. **Litmus Gelatin and Litmus Agar.**—These are used chiefly to detect acid-producing bacteria. Make agar or gelatin in the manner already described, except that 1 per cent. lactose is added, and 1.5 per cent. agar instead of 1.2 per cent., or 15 per cent. gelatin instead of 12 per cent. These are to be filtered in the usual way, and are known as *lactose agar* and *lactose gelatin.*

Prepare a litmus solution by soaking 50 grams of dry litmus cubes with 250 c.c. water. Soak for twenty-four hours and filter through filter-paper. Add enough of this to the agar or the gelatin to give it a blue color. Tube the medium as usual and sterilize.

Instead of using the litmus solution *azolitmin* may be used. This is a powder that may be dissolved in a little alcohol and sufficient added to the lactose agar or lactose gelatin to give a blue color. Its use is simpler and in some respects better than litmus solution.

No. 14. **Gelatin Plates from Milk.**—Procure some milk that is not more than six hours old. Dilute 1000 times, as directed in No. 4. Place 1/2 c.c. of the dilution in two Petri dishes and 1 c.c. in two others. Pour into each the melted gelatin from one of the gelatin tubes prepared in No. 12. Thoroughly mix by gentle agitation and place in a cool place to harden. Set aside at a temperature of about 70° for the bacteria to grow. If the temperature rises above 80° the gelatin is likely to melt and spoil the plate. It takes about two days for the colonies to appear. After two or three days carefully study the plate, noting the liquefying colonies and the non-liquefying colonies. Note also other differences. Isolate and inoculate upon agar slants several of the different colonies, including both liquefiers and non-liquefiers.

*Litmus gelatin* or *litmus agar plates* should be made in the same way, and the appearance of a red color around some of the colonies will make it possible to detect the acid-forming bacteria.

No. 15. **Potato Tubes.**—Select a large fair potato, carefully wash and peal. With a special cutter or with a broken test-tube with sharp edges, bore out some cylindrical plugs of potato. Cut them obliquely so as to make two wedge-shaped pieces of each plug, and soak in running water overnight. In the bottom of some large test-tubes place a little cotton and enough water to cover it (Fig. 63). Place a single potato slant in each tube and sterilize by one-half hour steaming upon three successive days.

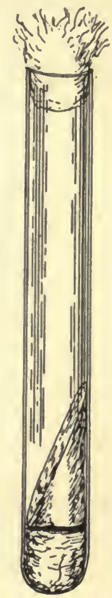

FIG. 63.—Potato tube.

No. 16. **Milk Tubes.**—Place about 10 c.c. of *skim* milk in test-tubes and sterilize for one-half hour on three successive days. The milk should be first tested with litmus-paper, and if acid, should be made neutral by adding NaOH.

*Litmus Milk.*—This is made as above, except that enough litmus solution is added before sterilizing to give a moderately blue color.

No. 17. **Fermentation Tubes.**—Prepare 200 c.c. of bouillon as described in No. 2, adding to it 2 grams lactose and adjust the reaction to the neutral point. To a second lot of bouillon add the same amount of dextrose and to a third lot the same amount of saccharose. After dissolving and filtering pour into fermentation tubes, enough to fill the closed arm and half the bulb (Fig. 64). A dozen or more tubes of each of these bouillons should be prepared and labeled. Sterilize by steaming on three days. If gas collects in the closed arm remove by tilting the tube.

No. 18. **Testing Characters of Bacteria.**—Several isolated and purified cultures of bacteria have been prepared in No. 7. After having prepared the several culture media above described, use them as follows: Make a fresh agar slant from each purified bacteria culture and allow to grow about 24 hours. If possible use a culture of *B. coli* for one of the series of tests. Then inoculate with a small quantity of the growth the following.

*a.* Two agar slants. *b.* One gelatin stab. This is made by dipping a straight platinum needle into the bacteria and then thrusting it straight into the gelatin of a gelatin tube, in the middle of the tube, and forcing the needle to the bottom, carefully withdrawing without disturbing the gelatin. *c.* A fermentation tube of each of the three sugars. *d.* Two milk tubes. *f.* Two litmus milk tubes. *g.* Two potato tubes, inoculating the potato on its surface only. Place one of the agar tubes, one of the milk tubes, litmus milk tubes, and potato tubes in the incubating oven at 98°. Place all others at room temperature. Allow to grow several days, examining each day, For each species of bacterium note the following points:

*Agar Slants;* Color. Is growth thick or thin, moist or dry; does it spread?

*Morphology.*—With the microscope study stained specimens; note shape—formation of chains—spores. Determine motility.

*Gelatin Stab.*—Note liquefaction, needle growth, surface growth. Compare growth with Fig. 35.

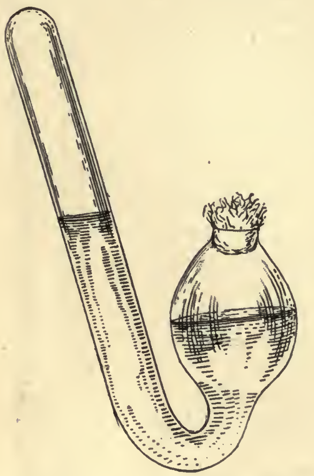

FIG. 64.—Fermentation tube with closed arm containing gas.

*Bouillon.*—Note turbidity, scum, sediment.

*Milk Tubes.*—Note at 98° and 70° the development of acid as shown by its action on litmus, curdling, separation of whey, appearance of gas bubbles, subsequent softening and solution of the curd, called digestion.

*Potatoes.*—At 98° and 70°, note color, abundance of growth, texture of growth, discoloration of potato.

*Fermentation Tubes.*—After several days note whether gas has collected in the closed arm. If not, record the bacterium as forming *no gas*. Test the liquid in the bulb with litmus-paper to see if acid. If gas is formed in any tube place a mark on the tube at the top of the liquid to mark the amount of gas. Then fill the bulb completely with a 2 per cent. NaOH solution. Place the thumb over the opening of the bulb so that there will be no gas bubble between the thumb and the surface of the liquid. Invert the tube, allowing the gas to flow into the bulb, and by turning back and forth mix the gas with the NaOH solution, keeping the thumb in position all the time. After mixing turn the tube once more so that all the gas will be in the closed arm. Remove the thumb and it will usually be found that the level of the liquid in the closed arm rises, because some of the gas has been dissolved in the NaOH. This dissolved gas is $CO_2$. By determining the level of the gas before and after the treatment the proportion of $CO_2$ to the undissolved gas may be determined. This is called the *gas ratio.*

Tabulate the characters of the different species of bacteria tested, determining whether any two of them are alike in all respects. It is by such characters that bacteria are described. To determine species is too difficult for a beginner.

No. 20. **Test for Indol.**—Make the following:

*Dunham's Solution.*

| | |
|---|---|
| Peptone, | 1 gm. |
| Sodium chlorid, | 0.5 gm. |
| Distilled water, | 100 c.c. |

Dissolve, place in test-tubes and sterilize as usual. After sterilization inoculate tubes with several different pure cultures of bacteria and allow to grow for 10 days. Add 1 c.c. of a 0.01 per cent. solution (fresh) of potassium nitrite and a few drops of concentrated sulphuric acid. Heat gently. If a pink color appears it indicates the formation of indol, a character used to distinguish certain species of bacteria (*e.g.*, *B. coli*).

No. 21. **Putrefaction.**—Place in a series of test-tubes with a little cold water the following: *a.* A bit of raw meat; *b.* some white of egg; *c.* some flour; *d.* some crushed beans; *e.* sugar; *f.* starch; *g.* a bit of melted butter. Set in a warm place for two or three days and determine which will putrefy and which will not.

From the tube containing the meat and the egg, remove a bit of the liquid as soon as putrefaction begins and examine under a miscrocope (both stained and unstained). Examine the liquid in the other tubes in the same way. Remove a little of the putrefying mass from one tube and dilute by placing it in a water blank. Transfer a platinum loopful of this dilution to a melted gelatin tube and another to a melted agar tube. Mix by gentle agitation and pour into Petri dishes. Allow to grow for two days and examine the colonies. Are both liquefiers and non-liquefiers present?

No. 22. **Ammoniacal Fermentation of Urea.**—Fill a test-tube or a flask half-full of urine and allow to stand for a day or two in a warm place. Note the odor of ammonia. Suspend a bit of red litmus-paper* in the mouth of the tube and note that it turns blue from the ammonia fumes. Remove a bit of the liquid with a platinum loop and examine (stained) under microscope. Note the immense number of bacteria.

No. 23. **Manure and Sewage.**—Place a small drop of sewage and a small bit of manure in separate water blanks. After thorough mixing remove a loopful in each case and transfer to a second water blank for further dilution. After again mixing transfer a loopful of this second dilution to melted agar or melted gelatin, gently agitate and then pour into Petri dishes. Allow to grow for two or three days and note the number of colonies, indicating the great numbers of bacteria in the original materials. A quantitative determination can be made if desired by using 1 c.c. of the sewage or 1 gram of manure, and diluting 100,000 times.

No. 24. **Soil Bacteria.**—For general study use standard media as already described, adjusting the reaction to 0.5 per cent. acid instead of 1.5 per cent. and using 1.2 per cent. agar and 12 per cent. gelatin. Plates made with these media inoculated with soil will not fail to show numerous soil organisms, bacteria and molds being very abundant. To obtain proper samples of

*Filter-paper moistened with Nessler's solution (used by chemists) is better, which should turn yellow to reddish-brown if ammonia fumes arise.

soil for the study of various soil problems requires special methods and special care too difficult for elementary work. The relative number of bacteria in different soils may be determined as follows: Select two soils for study, preferably one rather sandy and the other filled with humus. Obtain a sample by mixing the soil well with a spade and take to the laboratory about 100 grams. Mix thoroughly the sample and weigh out one gram. It is best to do this after passing the soil through a sieve. Place in a 99 c.c. water blank and shake vigorously for two minutes. Transfer 1 c.c. of this to a second 99 c.c. water blank and mix well. Transfer 1 c.c. to a 9 c.c. water blank and mix again. From this transfer 1 c.c. to a Petri dish and then pour upon it the contents of a melted agar or gelatin tube. Mix in the usual way, harden, and after two to four days count the number of colonies in the two soils. This will give the relative numbers approximately only. To obtain them exactly allowance must be made for the water in the two soils.

No. 25. **Denitrifying Bacteria.**—Make a broth containing 1000 c.c. water, 1 gm. peptone and 2 gm. potassium nitrate. Fill a few fermentation tubes and sterilize by steam. Inoculate several with a little soil from different localities. Incubate at ordinary room temperature for several days. Gas will appear in the closed arm if denitrifiers are present. This gas is mostly nitrogen and represents so much loss to the soil. The bacteria can be isolated by the plate method if desired.

No. 26. **Nitrogen Fixers.**—Their action may be shown as follows: Make a solution containing: $MgSO_4$ 2 gm., $K_2HPO_4$ 2 gm., CaCl 0.2 gm., dextrose 2 gm., citric acid 5 gm., $FeCl_3$ traces, water, (distilled), 1000 c.c. Make the reaction neutral, taking great care not to pass beyond the neutral point. Place some of this in a flask, sterilize by steam, and inoculate with a little soil. Incubate at ordinary room temperature. After two or three weeks, growth will be evident and usually a membrane appears on the surface. This membrane contains nitrogenous matter and since there is no nitrogen in the culture medium as above made, the nitrogen must have been assimilated from the air. The isolation of these bacteria is very difficult.

The study of the *nitrifiers* is too difficult to be undertaken by elementary students.

No. 27. **The Presumptive Test for Bacillus coli.**—This test is frequently made to determine whether water is suspicious. Fill fermentation tubes with lactose bouillon and inoculate five tubes with 1/2 c.c. and five more with 1/10 c.c. of the water to be tested. Place in the incubating oven for twenty-four hours. If gas appears in the closed arm sufficient to fill it from 1/10 to 1/4 full, the test is positive and the water *probably* contains *B. coli.* To determine this absolutely requires further tests that cannot be given here. The purpose of using the different amounts of water is to give a rough idea of numbers. If gas appears in all of the 1/10 c.c. tubes it will indicate that there are probably more than 10 of the gas-producing organisms per c.c. of the water. If it appears in the 1/2 c.c. tubes but not in the 1/10 it indicates that there are less than 10 per c.c. This presumptive test is only of value in suggesting suspicion, but insufficient to state the presence of sewage contamination.

No. 28. **Bacteria from the Root Tubercles of Legumes.**—The bacteria that have been commonly supposed to cause root tubercles may be obtained as follows:

Add 5 gm. of wood ashes to 1,000 c.c. water, heat in steam, boil for one minute and filter. To the filtrate add 10 agar and 4 grm. maltose (or some other sugar); heat in steam till dissolved, boil one minute and filter. Place in test-tubes and sterilize by steaming on three successive days, or in an autoclave for one hour at 10 pounds pressure.

Select some vigorously growing legume, not too large, and with a spade dig up its mass of roots still embedded in soil. Wash away the soil from the roots and probably plenty of tubercles will be found attached. Remove a small tubercle with forceps, wash in tap water and then immerse in a solution containing 500 c.c. water, 1 gram corrosive sublimate, and 2.5 c.c. HCl. It should be immersed in this for two to three minutes to sterilize the surface. Then the tubercle is held between folds of filter-paper that has been moistened in the sublimate solution and a gash is cut in it with a hot knife. A platinum needle is then sterilized and some of the central mass of the tubercle removed and placed in a drop of sterile water on a slide. Place a drop of sterile water in each of several Petri dishes and transfer a small loopful of the drop on the slide containing the tubercle contents, into the water drop in each of the Petri dishes. Pour into each a tube of the maltose agar, allow to harden and incubate at 70°. The colonies will appear in three to four days and may be isolated and studied in the usual manner.

No. 29. **Bacteriological Analysis of Market Milk.**—Collect in sterile bottles a number of samples of market milk from different milkmen. Keep cool with ice until they are brought to the laboratory and then plate *at once*. Dilute 1,000, place 1 c.c. in each of two or three Petri dishes and add a tube of ordinary agar. Incubate at 98° for twenty-four hours, count the bacteria in each plate and determine the average. This is the procedure commonly used in making bacteriological analyses of miscellaneous samples of market milk. If there is any reason for thinking the milk is very old a higher dilution is necessary. If, on the other hand, the examination is to be made of milk known to be good and in cool weather, a dilution of 100 is better.

No. 30. **Bacteria in Sour Milk.**—Allow some milk to stand till sour, but not curdled. Dilute it 100,000 times (two 99 c.c. and one 9 c.c. water blanks) and make plates in litmus gelatin or litmus agar. Incubate at 70° for two or three days and count the number of bacteria. Count the number of acid colonies.

No. 31. **Bacteria in Cream.**—Make plates as in No. 30 from *fresh* cream, diluting 1,000 times and using litmus gelatin and litmus agar. Make other plates from some *ripened* cream in gelatin and in agar and diluting 100,000 times. Incubate at 70° for three days and compare the plates from the two lots of cream as to number of bacteria and relative proportion of acid bacteria.

No. 32. **Efficiency of Pasteurization.**—Obtain some milk ten to fifteen hours old. Make three plates as described in No. 30. Place the milk in a jar, and heat in water to 140°, keeping it at that temperature for one-half hour. Make from it a second series of plates. Incubate plates at 98° for twenty-four hours. Count the number of colonies in the two sets of plates.

No. 33. **Bacteria on Hay.**—Place a handful of hay in water and warm slightly for ten minutes. The water should be hardly more than luke warm. This is called a *hay infusion*. Put a platinum loop of the infusion into a water blank and inoculate a loopful of this dilution into an agar plate. Incubate at 98° for one day and count the bacteria.

No. 34. **Study of Common Molds.**—Place under a bell glass, or in some other closed box which will prevent evaporation, some pieces of bread, slightly moistened, two or three pieces of cheese, and some slices of a lemon. Keep these in a warm place for a few days until molds make their appearance. Examine day by day until they become covered with spore masses. Note the mycelium, its fineness of texture and the color of the spore masses. Determine, if possible, whether the mold masses on the different objects are the same or different species. Remove a bit of the mycelium, place in a drop of water on a slide under a cover-glass and examine under the microscope. Sketch the threads and their method of branching. Place some of the spores under the microscope. Sketch. How do they compare in size with bacteria and yeasts?

No. 35. **Germination of Mold Spores.**—Melt two or three agar tubes and two gelatin tubes and add a few drops of HCl, just sufficient to make the medium slightly acid. Pour out in Petri dishes and allow to harden. With a platinum needle transfer the smallest possible quantity of spores from one of the molds and touch the surface of the agar and gelatin in several places with the tip of the needle. Examine with low-power microscope and note that spores have been planted on the surface. Set in a warm place and examine in twenty-four hours to see if the spores are germinating. Sketch a germinating spore. Allow to grow till spores are formed, studying daily with microscope.

No. 36. **Yeast and Fermentation.**—Grind up a few apples in a meat-cutter squeeze the juice through cheese-cloth and then filter through filter-paper. Fill six fermentation tubes with the juice, filling the closed arm full and the bulb half-full; plug with cotton. Set two of them in a warm place. Sterilize the other four by steaming for half an hour. After sterilizing inoculate, two with a little yeast (from a yeast cake) and set in a warm place. Examine in eight to twelve hours. Note the gas bubbles rising in the closed arm. Remove a little of the sediment and examine under the microscope (both stained and unstained). Note the clusters of budding yeast cells. How do they compare with the cells in the yeast cake? After the arm is about half-full of gas, test with NaOH as described in No. 18. By the amount of gas dissolved by the NaOH determine how much of the gas is $CO_2$ Does any fermentation occur in the sterilized tubes? In the original unsterilized and uninoculated tubes? If fermentation occurs in the latter examine with a microscope to see if yeast is present.

No. 37. **Decay of Fruit.**—Select some sound apples and inoculate them with mold spores by dipping the tip of a knife-blade into a mass of mold spores of the growths in No. 34 and thrusting the tip through the skin of the apple. It will be best to inoculate different apples with each of the kinds of mold that have grown on the objects in No. 34, some of which will probably be the species to produce decay. Place the apples in a fruit jar, close the mouth, not too tightly, and set aside in a warm place. Examine day by day and note that decay soon begins, starting at the inoculated points. Allow the decay to continue till the mold breaks through the surface and produces spores. If decay does not occur, it means that the species of mold used were not those that produce decay, and the experiment should be repeated with molds from other sources.

## DISINFECTION.

No. 38. **Disinfectants.**—Of the many disinfectants in use three are of particular practical value. These, in the strength commonly used, are the following:

*Carbolic Acid Solution*, 1–20.

| | |
|---|---|
| Carbolic acid crystals, | 25 grams |
| Water, | 500 c.c. |

In weighing the crystals of carbolic care should be taken not to touch them with the fingers since they will burn the skin.

*Corrosive Sublimate Solution*, 1–1000.

| | |
|---|---|
| Corrosive sublimate, | 1 gram |
| Water, | 1000 c.c. |

In making and handling these solutions it should be borne in mind that they are *very poisonous*.

*Chlorid of Lime.*

| | |
|---|---|
| Fresh chlorid of lime, | 25 grams |
| Water, | 500 c.c. |

This disinfectant is cheap and effective if fresh, but it will not keep, and should be made up at the time of using. One pound of the chlorid of lime in six gallons of water will make up an efficient, cheap disinfectant for disinfecting walls and floors of rooms and has the advantage of being non-poisonous.

No. 39. **Testing Disinfectants.**—Mix the white of an egg with ten times its bulk of water, and place the material in a series of test-tubes filling each about one-third full. To the different tubes add the following: *a.* no addition; *b.* 1/4 gram salt; *c.* 1 gram salt; *d.* 2 grams sugar; *e.* 5 grams sugar; *f.* one drop of corrosive sublimate solution (1–1000); *g.* six drops of corrosive sublimate solution; *h.* one drop of formalin; *i.* three drops of formalin; *j.* one drop of carbolic acid solution (1–20); *k.* four drops of carbolic solution; *l.* ten drops of carbolic solution; *m.* 1/8 gram of borax; *n.* 1/4 gram of borax. Place all test-tubes in the incubating oven and examine at intervals to see which of them undergo putrefaction and which are thoroughly disinfected. Note how very much more efficient some disinfectants are than others. Which proves to be the most efficient? It is well in this experiment to close the tubes loosely with a cork to prevent evaporation of the volatile disinfectants.

**The Use of Disinfectants.**—The ordinary use of disinfectants is in connection with disease, their purpose being to destroy disease germs and thus to prevent the spreading of disease. They are sometimes used for other purposes, such as reducing offensive odors, etc., but primarily they are for the checking of infection. There are various methods of killing bacteria which may be applied under different conditions. *Heat*, *sunlight*, *drying*, *chemicals*, and *disinfecting gases* are all of use in certain connections. The determination of which is to be used will depend upon conditions. The first problem to be settled in all cases of disinfection is when and where the disinfecting agent should be applied to produce the desired results. A few practical suggestions as to methods may be of value.

**The Person.**—Of all sources of danger the one of greatest importance is the person; first the *patient*, especially after recovery, when he is to mingle with other people, and secondly the *attendants* on the patient. Disinfection of the patient during the disease is rarely possible, though his skin should be kept clean by bathing in water to which a little glycerin is added. The nurse, however, should keep scrupulously clean. Her hands should be carefully washed in soap and water followed by strong alcohol, or the corrosive sublimate solution above described. Such cleaning should follow every time that the nurse handles the patient or any article of clothing or eating utensils touched by the patient. Other parts of the body also need attention, but not so frequently. The hair should be kept in a cap to prevent its getting contaminated, for it is difficult to clean and almost impossible to sterilize it. When the patient has recovered so as to leave quarantine he should receive the same treatment.

Carbolic acid solution is especially useful as a skin wash, and is extremely valuable in cases of cuts or skin abrasions. If all cuts and bruises be washed at once in the carbolic solution (1/20), many a serious sore and many a case of blood poisoning will be prevented. Every household should have a carbolic acid solution on hand for such purposes.

**Clothing, Bedding, Etc.**—These articles offer a difficult problem. The following general directions may be given.

*Burn* everything which is of no great value.

*Boil in water* all articles that can be so treated. The boiling should continue for half an hour and will be sufficient for complete disinfection. Steaming is sometimes employed for articles too heavy for boiling, such as mattresses and carpets. This requires special apparatus and can rarely be performed at home. Any article that can be soaked in water may be disinfected by soaking it three or four hours in water containing one part formalin to 5,000 parts of water. Exposure to air and sunlight are good disinfectants for light clothing, but not for heavy articles like mattresses. There is no good method for their disinfection. Where the infection of such articles is considerable the only safe thing to do is to destroy them.

*Excreta.*—The *feces*, the *urine*, and all *discharges* from patients are most likely to contain infectious organisms and must be handled and treated with great care. One of the best methods of treating is to place the excreta in a vessel and cover completely with a chlorid of lime solution prepared as above described. This should be allowed to act at least an hour before the mixture is thrown into the sewer or otherwise disposed of. Ordinary milk of lime or dry slacked lime is useful in earth closets or privies.

*The Sick Room.*—While a room is occupied by a patient little can be done to disinfect it. In case of a contagious disease it is desirable that curtains, hangings and carpets should be dispensed with, since these catch and hold dust. Little else can be done beyond care in keeping the room clean. After the room is vacated it is frequently desirable to disinfect it before it is again occupied. Carpets, curtains, and bedding should be removed and disinfected as above suggested. All surfaces in the room, including walls, ceilings, floors, tables, chairs, and especially cracks around mopboards and the floor should be washed freely with a disinfectant. Corrosive sublimate solution is frequently used, or the chlorid of lime solution. If care is taken to wash all surfaces thoroughly, putting plenty of the disinfectant into the cracks, the disinfection is complete and satisfactory. Since this plan of washing is rather long and troublesome, a simpler plan has been widely adopted in recent years of using a *gaseous* disinfectant. The gas most commonly used is formaldehyd gas. This is applied in various ways, but the simplest is as follows: All cracks in the room are first sealed by pasting gummed paper over them, this including cracks around mop boards, chimneys, as well as fire-places and key-holes. Then a pailful of steaming water is placed in the room to give moisture. Lastly, one or more "formalin candles" are lighted. These are mixtures of solidified formalin which give off the desired gas when heated and in the candles there is added a certain amount of paraffin that will burn. These candles can be obtained from any drug store and upon the wrapper there is always stated the number of cubic feet that the candle is supposed to disinfect. The number of these candles to be burned must therefore be determined by estimating the space in the room and using candles accordingly. It is best to use about half as much again of the formalin candles as recommended on the wrapper, since they will rarely be as efficient as is claimed for them. After lighting the candles leave the room quickly and seal the door on the outside with gummed paper. Leave closed for about twelve hours and then open windows and doors so as to allow the gas to pass from the room. If a sufficient amount of formalin is used this disinfection is thorough.

**The Stable.**—The disinfection of the stable is difficult because of the roughness of the lumber with which the stable is made. A satisfactory method of disinfection is as follows: 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 (1–1000). 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 solution of chlorid of lime may be used in the same way in washing the walls and floors. A disinfection of a stable with formaldehyd or any other gaseous disinfectant is impossible, since the stables are never tight enough to prevent the gas from escaping rapidly.

**The Dairy.**—The disinfection of the dairy must follow along essentially the same lines as the stable. Everything must first be cleaned as thoroughly as possible, and then all woodwork may be washed with corrosive sublimate, or better, with a 3 to 5 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 and steam are legitimate. After the disinfection of all parts, the whole must be washed with water.

**Other localities** inhabited by animals. To disinfect the *barn-yard* 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.

# INDEX


Abortion, 288
Abscesses, 289
Acetic fermentation, 30
Acidity, determination of, 309
Acidity of soil, 120
Acid liquefiers, 152, 198
Acquired resistance, 257
Actinomyces, 12, 13
Aerobic bacteria, 19
Agar culture medium, preparation of, 308
Air, bacteria in, 313
Air, relation to, 19
Alcoholic fermentation, 2, 25, 42, 212, 322
of milk, 157
Alinit, 119
Ammoniacal fermentation, 53, 319
Amyolytic fermentation, 25, 27
Anabolism, 23
Anaerobic bacteria, 19
Analytical processes, 24
Animalculæ, 2
Anthracnose, 295
Anthrax, 258, 280
method of infection, 282
preventive inoculation, 284
Antiseptics, 243
Ascococcus, 12
Aspergillus, 7
Autoclav, 18
Azolitmin, 316
Azotobacter agilis, 94

Bacillus, 12, 13
alvei, 289
anthracis symptomatici, 287
atrosepticus, 302
betæ, 305
Bulgaricus, 148, 159
carotivorus, 305
choleræ suis, 286
cloacæ, 303
coli, 130, 148, 320
cubonianus, 304
cyanogenes, 156
danicus, 94
denitrificans, 67
erythrogenes, 156
Bacilus, fluorescens, 52
lacto rubifaciens, 156
lactis viscosus, 154
larvæ, 289
mallei, 286
mycoides, 55
necrophorus, 289
olericeæ, 305
pleurosepticus, 285
prodigiosus, 156
radicicola, 98, 99, 100
solanacearum, 302
savastanoi, 305
solanisaprus, 302
stutzeri, 55
subtilis, 52
suipestifer, 285
tetanus, 287
tobacci, 226
tracheiphilus, 303
tuberculosis, 261
tumifaciens, 305
Zeæ, 303
Bacillus carriers, 162
Bacteria, meaning of term, 9
Bacterial treatment of sewage, 81
Bacterioses, 301, 304
Bacterium, 12, 13
Bact. aceti, 218
acidi lactici, 145, 183
aerogenes, 147, 202
anthracis, 280
hyacinthi, 304
malveacarum, 304
phaseoli, 304
Bedding, disinfection of, 324
Beggiatoa, 116
Bird pest, 291
Bitter butter, 184
cheese, 202
milk, 155
Black leg, 287
rot, 298
spot in cheese, 203
Blight, 295
Brie cheese, 204
Blue milk, 156
Blue spot in cheese, 203
Boiling of milk, 172

Bouillon, preparation of, 311
Bread raising, 214
Brown hay, 241
Budding, 8
Burnt hay, 241
Butter, bacteria in, 192
By-products, 25

Camembert cheese, 204
Canning, 245
Carbolic acid, solution, 323
Carbon cycle, 45
  transformation of, 41
Cattle plague, 291
Cellulose, 43
Cheddar cheese, 197
Cheese ripening, 195
Chlorid of lime, 323
Cholera, Asiatic, 129
Cider, 214
Cisterns, 133
Cladothrix, 12
Clostridium Pasteurianum, 93
Clothing, disinfection of, 324
Cocci, 10
Coccus, 13
Cold as a preservative, 242
  relation to, 18
Colonies, 9
Combustion, 42
Commercial fertilizers, 122
Compost heap, 35, 77
Concentrated milk, 176
Condensed milk, 176
Contact bed, 84
Cooling of milk, 169
Corn wilt, 303
Corrosive sublimate solution, 323
Cow, care of, 165
Cream, bacteria in, 321
Cream ripening, 181
  control of, 185
Crown gall, 305

Dairy, disinfection of, 326
  inspection, 179
Decay, 26, 33, 50, 51
Decomposition, 24, 50
Denitrification, 30–36
Denitrifying bacteria, 67, 320
Diarrheal diseases, 163
Digestion by bacteria, 151
Digestion of curd, 151, 196
Dill pickles, 224
Diastase, 28, 215
Diphtheria in milk, 163
Diplococcus, 12
Discontinuous heat, 18
Disease germs in milk, 161
Diseases of plants, 293
Disease, relation of bacteria to, 2
Disinfectants, 323
  use of, 324
Drinking water, sources of, 130
Drying, 237

Edam cheese, 197, 203
Eggplant rot, 302
Eggs, preservation of, 248
Excreta, disinfection of, 325
Excretions of bacteria, 24
Enzymes, 29, 30, 226, 231
  list of, 30
  secreted by bacteria, 33

Fallowing, 122
Farcy, 286
Farm Sewage, 87
Faults in cheese, 201, 208
Fermentation, 2, 25, 29, 35, 36
  tubes, 317
Filter bed, 84
Filtered water, 133
Filtering milk, 170
Fire blight, 304
Fission fungi, 9
Flagella, 11
Flavors in cheese, 196, 199
Flesh, preservation of, 238
Food of bacteria, 20
  supply, 37
Foods of plants, 38
Foot and mouth disease, 290
Food rot, 289
Fore milk, 139, 169
Form of bacteria, 10
Foul brood, 289
Fowl cholera, 285
Fruit, decay of, 323
  preservation of, 239
Fruity cheese, 202
Fuchsin solution, 314
Fungi, 5, 6
Fungoid diseases of plants, 293
  list of, 296
  methods of combating, 295

Galactase, 138, 196
Garget, 153, 289
Gelatin culture medium, preparation, 316
  plates, 316
Germicidal power, 159
Glanders, 286
Goiddu, 158
Gorgonzola cheese, 206

Gram stain, 315
Granville wilt, 303
Green manuring, 108, 123

Hard cheese, 197
Hay, bacteria in, 322
curing of, 239
Hemp, 234
Higher fungi, 6
Hoof rot, 289
Hog cholera, 285
Horse sickness, 291
Humus, 40

Ice, 134
Indol, test, 318
Inflammation, 289
Inoculation, protective, 258
diseases controlled by, 260
Inorganic foods, 39
Iron, 117
salts, 38
Immunity, 253, 257
Isolation of bacteria, 313

Katabolism, 24
Kefir, 158
Kummys, 157

Lactic acid bacteria, 143
protective action of, 160
Lactic fermentation, 30, 34
Leben, 158
Leeuwenhoek, 1
Legumes, use of, 104
value of, 95
Leptothrix, 12
Limburger cheese, 207
Lime, 39, 111
Liming of soil, 56, 94, 121
Linen, 234
Liquefaction of gelatin, 151
Lockjaw, 287
Lophotrichic bacilli, 11–12
Lumpy jaw, 288

Magnesia, 39
Malignant tumor, 288
Manure, 120, 122
bacteria in, 319
fresh and ripened, 75
heap, bacteria in, 70
composition of, 69
fermentation of, 71, 74
losses from, 70
protection of, 73
Mastitis, 289
Mazoon, 158
Metabolism, 24
Metacoccus, 12
Methylene blue, solution, 314
Micrococcus, 12
Microörganisms, meaning of term, 5
Microscopic study of bacteria, 314
of yeast, 316
Microsporon, 12
Mildews, 295
Milk, bacteria in, 137
bacteriological analysis of, 321
Milk bacteria, growth of, 159
sources of, 138
Milker, 42, 166
Milk faults, miscellaneous, 157
Milking machines, 168
room, 167
Milk pail, 168
standard, 178
vessels, 141, 167
Mineral ingredients in soil, 40
Moisture, relation to, 19
Molds, 6
study of, 322
Monotrichic bacilli, 11, 12
Motility, 11, 315
Mucor, 7
Multiplication of bacteria, 14
Mushrooms, 6, 44
Mycoderma, 217
Navel ill, 289
Neutral types, 153
Nitragin, 107, 119
Nitrates, 38, 47, 57, 59, 77
Nitrification, 30, 57, 63, 64, 65
Nitrifying organisms, 58, 60, 61
Nitrites, 57, 59
Nitrobacter, 60
Nitrogen cycle, 89
fixation, 92, 101, 320
fixing bacteria, 93, 95
Nitrogenous food, 47
Nitrogen, loss of, 90
Nitrosococcus, 60
Nitrosomonas, 60
Number of bacteria, determination of, 312

Oidium lactis, 224, 240
Oleomargarine, bacteria in, 193
Olive knot, 305
Ophidimonas, 116
Organic foods, 39
Organized ferments, 26, 31
Oxidizing fermentation, 30

Parasites, 21, 251

Pasteurization, 173
efficiency of, 322
Penicillium, 7
Camembertii, 205
Roquefortii, 206
Pepsin, 28
Peptonizing bacteria, 55, 150
Percolating filter, 84
Peritrichic bacilli, 11
Phosphates, 38
Phosphorus, 113
Pleuropneumonia, 291
Potash, 38, 115
Potato rot, 302
tubes, 317
Powdered milk, 177
Preservatives, chemical, 243
in milk, 171
Preservation of food, 236
Presumptive test for B. coli, 320
Proteids, 48
Proteolytic fermentation, 30
Proteus vulgaris, 52
Pseudomonas, 12
campestris, 298
destructans, 305
fluorescens, 305
iridis, 305
prunii, 304
Stewarti, 303
vascularis, 303
Ptomaines, 25
Pure cultures, 9
in butter-making, 187
tobacco curing, 228
vinegar-making, 221
Purification of cultures, 313
Pus in milk, 138
Putrefaction, 26, 33, 50, 319
Putrid butter, 185
cheese, 202

Quarter-evil, 287

Rabies, 291
Rancidity of butter, 192
Rauschbrand, 287
Red milk, 156
Rennet-forming bacteria, 150
Rennin, 33
Reservoir, 133
Resistance against microörganisms, 252–257
method of increasing, 254
Rinderpest, 291
Rinderseuche, 285
Root tubercles, 96, 100, 306
Root tubercle bacteria, 98–101, 119, 321
Roquefort cheese, 206
Rots, 295, 301, 304
Rotz bacillus, 286
Rusts, 295
Rusty spot, 203

Saccharomyges, 8
Salt as a preservative, 244
Saltpeter plantations, 76
Saprophytes, 21
Sarcina, 12
Sauer kraut, 223
Scarlet fever in milk, 163
Self-purification of soil, 55
Septic tank, 83
Sewage, bacteria in, 319
composition of, 78
farming, 79
treatment of, 78, 81
Sheep-pox, 291
Sick-room, disinfection of, 325
Silage, 229
Silo, 35
Slimy bread, 216
milk, 153
whey in Edam cheese, 203
Smuts, 295
Soft cheeses, 203
Soil, acidity of, 120
aeration of, 121
bacteria, 319
infusion, 97
inoculation, 106, 119
microörganisms in, 39
nitrogen in, 65
origin of, 39
Sour bread, 216
fodder, 233
milk, bacteria in, 321
Soured beans, 224
Spices as a preservative, 245
Spirillum, 12
Spoiling of food, 235
Spores, 7, 15
Springs, 133
Sprinkling filter, 84
Stables, care of, 166
disinfection of, 325
Staining of bacteria, 314
Starches, 43
Starters in butter-making, 186
in cheese-making, 200
preparation of, 187
use of, 189
value of, 191
Sterilization, 17

Sterilization, of milk, 171
Sterilizing, 311
soil, 118
Stilton cheese, 206
Streams, contamination of, 135
Streptococci, 138, 289
Streptococcus, 12
bacteria in, 145
Streptothrix, 12, 13
Sugar, 42
as a preservative, 244
bacteria in, 250
Sulphates, 38
Sulphur, 115
Sweet cheese, 202
curdling, 150
Swelled cheese, 148, 201
Swine plague, 285
Swiss cheese, 197
Symptomatic anthrax, 287
Synthetic processes, 23

Tainted butter, 184
Takosis, 288
Tallowy butter, 184
Temperature, relation to, 16
Tetanus, 287
Texas fever, 291
Thermophiles, 16
Thunder storms and sour milk, 144
Toad stools, 6
Tobacco-curing, 224
Tobacco, diseases of, 228
Tomato rot, 302
Torula amari, 155, 202
Toxins, 252
Transportation of milk, 177
Trickling filter, 84
Tuberculin test, 271
Tuberculosis, 261
animals subject to, 263
bovine and human, 264
combat against, 269
Tuberculosis, distribution of, 266
preventive inoculation, 277
resistance against, 263
treatment of in a herd, 273
use of flesh, 277
milk, 278
Tumor diseases, 302, 305
Turnip-tasting butter, 185
Turnip taste in milk, 157
Typhoid in milk, 131, 162
fever in water, 129

Udder, 140
Unfermented grape juice, 213
Urase, 54
Urea, 49

Vaccination, 258
Vinegar as a preservative, 244
eels, 222
making, methods, 216, 219
organisms, 218
preservation of, 222
Viscogen, 176

Washing glassware, 308
Water, bacteria in, 127
Water blanks, 312
Water-bone diseases, 129
Water, sewage contamination of, 128
Wells, 130
Wildseuche, 285
Wilts, 295, 301, 302
Wines, 212
Wood, 44
Wooden tongue, 288

Yeasts, 8, 27, 212, 316
Yoghourt, 158

Zymase, 33