AGRICULTURAL BACTERIOLOGY

RUSSELL AND HASTINGS

AGRICULTURAL BACTERIOLOGY

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AGRICULTURAL BACTERIOLOGY

FOR STUDENTS IN GENERAL AGRICULTURE

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FOREWORD

The art of agriculture has long been practised, but the science of agriculture is of comparatively recent origin. This science rests upon the fundamental sciences—chemistry, physics, and biology. One phase of biology, bacteriology, has within the last three decades assumed a most important relationship. The early researches of Pasteur, Koch, and their successors opened the field of inquiry as to the causation of animal disease. More recently, exact knowledge of the influence of microörganisms on soil processes, on dairying, and on foods in general has been greatly extended. It is of the utmost importance for the farmer and the student of agriculture to have a proper conception of these relations.

The purpose of the text here presented is to give to the reader and to the student the essential facts concerning the relation of microörganisms to daily life, and especially to that of the farm, without a confusing mass of detail, both chemical and biological, the presentation of which often hides the essential information the student should gain. The terminology is simple. Descriptions of specific organisms have been avoided; and, in general, the various phases of the subject are presented in their broad outlines in order to acquaint the student with the fundamental principles, which can be applied to subjects not considered.

A full conception of the relation of microörganisms to agriculture can not be gained without working with them in the laboratory. Without such experience the organisms remain intangible to the student. Much can also be done by the teacher in the class-room, through the use of more extended illustrative material than it is possible to give in the text, and through examples of the practical application of the organisms, to increase the interest of the student in this subject that has so many points of contact with the daily life of every individual.

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PART I

PROPERTIES OF MICROÖRGANISMS

AGRICULTURAL BACTERIOLOGY

CHAPTER I

THE RÔLE OF MICROÖRGANISMS

Animate nature is commonly divided into two great groups: the plants and the animals. It is possible, however, to make the division of life along other lines than form and function. Every living form must have building material; the various chemical elements that are essential for its structure must be available in fitting combinations. Every living form must also have energy; for work is being done by even the simplest forms of life, and without energy no work is possible. The sole source of energy for our world is the sun. The energy is transmitted in some inexplicable way through the space that separates the earth from the sun.

One group of living organisms is able to receive directly this radiant energy, and to use it in the work of growth and development. This power is limited to those forms that are provided with the compound known as *chlorophyl,* the substance that gives to the higher plants their green color. They obtain their building materials from the soil, the water, and the air, in compounds that contain but little or no energy. They combine these simple compounds into complex forms that contain a great store of energy. Thus the plant uses as food carbon-dioxide, water, oxygen, nitrates, phosphates, and sulphates, and forms from them all of the varied substances found in its tissues—the woody fiber, the sugars, the starches, the fats, the gums, the waxes, and the proteins. The green plants are builders of organic matter and storers of energy. They represent the *constructive* or the *synthetic* group.

Living forms that do not possess this wonderful energy-receiving and -utilizing compound, chlorophyl, can not use the radiant energy of the sun directly, but must rely upon that which the green plant has stored in its structure. These forms use vegetable matter as food, and obtain therefrom their building materials and the energy necessary for all their life processes. They utilize the energy and leave behind simpler types of compounds than those ingested. They break down vegetable matter, and are to be classed as *destructive* agents, or as *analytic* factors. The animal that lives upon the tissues of another animal is still relying upon the green plant for its food and energy. It is to be seen that the basis of classification is, whether the energy needed by the organism is obtained directly from the sun, or indirectly through the medium of another organism.

Destruction of man-made structures is as necessary as is the building thereof, and so it is in nature. The supply of plant food is limited, and it is essential that the elements in vegetable matter be returned to a form that permits of use by another plant. This is the work of the destructive group. The green plant furnishes to all other forms of life food and energy. They, in their turn, supply the green plant with food. Each group is absolutely dependent on the other for its continued existence.

Not all the members of the destructive group are animals. In it are placed many forms that every one recognizes as plants. They are devoid of chlorophyl, and demand the same kind of food as does the animal; their food must supply them with both building materials and energy. The term *"fungi"* or *"fungus plants"* is applied to them. One does not think of the animals as agents in the destruction of organic matter, for our interest in them is wholly along other lines. The waste products of animal life are very simple chemical compounds. Some of the fungus plants change their food relatively little as far as its chemical complexity and energy content is concerned. Their by-products are almost as complex as is the food itself, and in many instances possess economic value.

The destructive work of animals and of the fungus plants is included under the term *decomposition*. Other terms, such as *fermentation*, *decay*, *putrefaction*, and *rotting*, are synonymous. Usually, however, these expressions are applied to the decomposition of certain chemical substances, or to a particular type of decomposition; for example, one says that milk ferments and that meats putrefy.

The greater part of the decomposition of organic matter is occasioned by fungus plants of microscopic size that find their home in the soil and in the water. The body of an animal is buried; within a short time it completely disappears. An immense amount of waste organic matter may be placed in a stream—as, for example, in the great drainage canal that receives the sewage of Chicago. Before the stream that receives the effluent of the drainage canal reaches central Illinois, the organic matter has completely disappeared under the influence of the microscopic life of the water.

From this organic matter are formed carbon-dioxide, water, sulphates, phosphates, and nitrates. From organic matter minerals have been formed; therefore the term *mineralization* is often applied to the process. An element passes from the soil, the water, or the air into the green plant, and is built into some one of its compounds. These are used by some member of the destructive group, or more commonly by a series of members of this group, with the result that the element becomes again available to the green plant.

This passage of the elements from one form of life to another is called the *cycle of the elements.* An atom of carbon may be in the air to-day in the form of carbon-dioxide; to-morrow it may be in a sugar molecule of a plant; the next day in the tissues of an animal; and the succeeding day it may be again present in the air in a molecule of carbon-dioxide; ready for another of its ceaseless passages, carrying with it a supply of energy for the animal and the fungus plant.

The chief agents in the decomposition of organic matter are the protozoa, or simple animal forms, and the simple plant forms, which include the bacteria, the yeasts, and the molds. The term *microörganism* is often applied to these various types, and *microbiology* to their study. It is with these forms that this volume treats, and especially with the ways in which they influence the life of man. He meets them in the soil he tills; he makes use of them in the preparation of foods and products of industrial value; he is constantly striving to protect his food supplies from their action, and to protect himself and his animals from the diseases that they cause. They present themselves to him at every moment of his life, to his benefit or his injury. He must employ them, and fight them, either conscious or unconscious of the nature of his acts; and he who has intelligent acquaintance with them will certainly fare far better than one ignorant of the part they play. A knowledge of the rôle of microörganisms in nature is as essential as knowledge concerning the higher plants and animals.

CHAPTER II

THE DEVELOPMENT OF BACTERIOLOGY

Discovery of bacteria.—The study of bacteria is one of the most recent developments of biologic science. The facts that had been gathered concerning the bacteria were not grouped into an independent phase of biology until about 1880. It was not until 1882 that the new science received its name, *bacteriology*. The bacteria had first been seen in 1683 by Leeuwenhoek. Apparently he was the first to use an instrument of sufficient magnifying power in such a way as to make the bacteria visible.

The compound microscope was first made by Johannes Janssen and his son, in Holland, in 1590. The objects to be examined by such an instrument were illuminated by light coming from above, and reflected from the surface of the object into the lens of the microscope, and thence to the observer's eye. With such an arrangement the bacteria could not be seen. Leeuwenhoek used a simple microscope in his work, of lower power than others had employed. He, however, examined his objects by placing them between the source of light and his lens: he used transmitted light, or the kind one uses when he wishes to determine the freedom of a liquid from suspended matter, and places it between his eye and the window. The solid objects refract the rays of light, and thus their presence in the liquid is made evident to the eye. This simple modification of his microscope made Leeuwenhoek the discoverer of many microscopic objects, among them the yeasts and the bacteria. He is frequently called the father of microscopy.

Decomposition and its cause.—From 1683 to 1850 little was learned concerning the importance of the bacteria in nature. The biologists of those days were more interested in classifying and naming the various plants and animals than in studying what they were able to do. They were interested in morphology rather than in physiology.

It had been known to man ever since he attempted to preserve plant or animal matter that change in it was inevitable. The microscope revealed in decomposing material an immense number of microörganisms, among which the bacteria predominated. It was believed by many that these organisms were the cause of the decomposition. Justus von Liebig, the founder of organic and agricultural chemistry, believed that decomposition was purely a chemical process that in some way occurred in matter brought in contact with the decomposing material. He was the dominant figure in the chemical world from 1840 to 1860, and when he stated that "those who pretend to explain the putrefaction of animal substances by the presence of microörganisms reason very much like a child who would explain the rapidity of the Rhine by attributing it to the violent motion imparted to it in the direction of Bingen by the numerous wheels of the mills of Mayence," there were few bold enough to contradict him, and none whose reputation carried conviction.

Spontaneous generation.—Numerous experiments had shown that an infusion of meat could be boiled for some time and sealed immediately thereafter in the vessel in which it had been heated, and yet it would often undergo decomposition, and would be found teeming with microscopic life. Such experiments had given rise to what seems now a curious theory, that of *spontaneous generation* of life; that is, the creation of life from dead matter. The scientists of those days could not imagine that any living form could endure the temperature of boiling water for even the briefest period of time. The infusion of meat had been protected from the entrance of bacteria after it had been heated; therefore it was believed the forms found in the decomposing infusion must have arisen in some way from the lifeless material.

Many experimenters tried to disprove this theory. Schultze, in 1836, heated infusions to the boiling-point, and the air that entered his flasks when removed from the fire was passed through strong acid or alkali. In 1837, Schwann passed the air that entered the flasks through tubes heated by a direct flame. Schroeder and von Dusch, in 1853, plugged the tubes leading from the flasks with cotton wool, which filtered the air as it was drawn into the flasks when they cooled. Usually infusions thus treated did not decompose.

The adherents of the theory claimed that the exposure of air to the high temperature of the heated tube, to acid or alkali, or even to cotton wool, removed some life-maintaining principle therefrom. It was not possible by such experiments to disprove the theory. In 1860, the Paris Academy of Science offered a prize for an attempt to throw new light by suitable experiments on the question of spontaneous generation.

Pasteur, the father of bacteriology.—Louis Pasteur was born in the Jura district of France in 1822. He was a diligent student of chemistry, and became interested in the effect of certain crystalline substances and their solutions on polarized light. Among the substances he studied was tartaric acid and its salts, products of one of the great fermentation industries, the wine industry. In 1854 he was made a member of the Faculty of Sciences in the University of Lille, a great industrial city. He began the study of the manufacture of alcohol from beet-sugar. In 1857 he read a paper on the lactic-acid fermentation. He had discovered in sour milk a trace of grayish substance, and had proved it to be a ferment of milk. He had before him one of the most important of the bacteria. This work was the beginning of the new science of bacteriology.

Pasteur accepted the challenge of the Paris Academy, and on April 7, 1864, he gave his results to the world in a famous lecture at the Sorbonne. He showed that if any solution containing organic matter is heated long enough, and protected from the microörganisms in the air, it will remain unaltered. He avoided the objections that had been urged against the experiments of others by allowing air to pass in and out of his flasks through long curved tubes, on the walls of which all dust and bacteria would be deposited. He showed for all time that life comes from life, that every form is the progeny of preëxisting forms of like nature.

The importance of this work of Pasteur can not be overestimated, for it led him to continue the study of microörganisms until his death, in 1895. Pasteur's influence on the material side of human life has probably been greater than that of any other man.

Aniline dyes.—The discovery of the bacteria by Leeuwenhoek, and the recognition of their relation to decomposition by Pasteur, are two great landmarks in the history of bacteriology. Another was the accidental discovery of the aniline dyes by Perkin in 1856. The recognition of the bacteria, as they occur in many places, especially in the fluids and tissues of the animal body, is impossible unless they can be differentiated by stains from the other materials. The aniline dyes were first used for the staining of bacteria by Weigert in 1876.

Another great advance in the progress of the science was the development by Robert Koch, a German physician, of a method of separating one kind of bacteria from other kinds with which it might occur. His work enables the activities of a single kind of organism to be studied, and its power for the good or ill of man determined. The development, in 1882, of the gelatine-plate method for the separation of kinds of bacteria, enabled Koch and his followers to prove the bacterial nature of the cause of many of the most important diseases of man and the lower animals. Scarcely a year has passed, since 1860, that has not been marked by discoveries of the greatest importance to humanity in bacteriology and its related sciences.

Bacteriology has revolutionized the life of civilized man. Without it our great cities would be impossible, for they could not be provisioned. It has doubled the average span of human life. It has made surgery possible. It touches the life of every one of us in a multitude of ways, each day, from birth to death.

CHAPTER III

THE MORPHOLOGY OF MICROÖRGANISMS

The cell.—The unit of life is the cell, which consists of a limiting membrane inclosing semi-liquid contents. Within the cell are carried on all of the activities of the organism: herein the food is assimilated, by-products are formed, and energy is liberated for all of the vital processes.

The higher plants and animals are constructed out of great numbers of these unit cells, which, however, are collected into groups that are so related to each other as to form tissues. These tissues are often so differentiated in their physiological activity that they may perform a limited and highly specialized function. With the simpler forms of life, such as the bacteria, all of the complicated chemical processes essential to the life of the organism take place within the limits of a single cell. Between the two extremes there are to be found organisms in all degrees of complexity as to structure and function. The types that are of greatest importance in the decomposition of organic matter are either unicellular or the simpler multicellular forms.

With the multicellular organisms, variation in structure of the individual is limitless; consequently, *morphology,* or the science that describes the variation in form, is of major importance. The simplicity of the one-celled plants, the bacteria, and the yeasts, makes a description of their morphology much less complex.

The division of life into plants and animals, so advantageous in the study of the higher forms, is not especially helpful when the lower forms are under consideration. The organisms most important in decomposition processes are morphologically more closely related to the plants than to the animals; physiologically, they are more directly allied to the animals. It is customary, however, to consider the bacteria, yeasts, and molds as members of the plant kingdom.

Morphology of bacteria.—The bacteria may be defined as unicellular plants, devoid of chlorophyl, and reproducing by division of the cell into two daughter cells. This mode of reproduction has given to them the name of *schizomycetes,* or splitting fungi.

The lower or the true bacteria occur in three form types: spheres, rods, and spirals. A spherical organism is termed a *coccus* (plural, *cocci*); a rod is designated as a *bacillus* (plural, *bacilli*); a spiral organism is called a *spirillum*

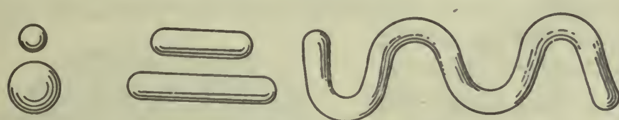

Fig. 1. Forms of Bacteria

A spherical organism is termed a coccus; a rod-shaped one a bacillus; a spiral cell is called a spirillum

(plural, *spirilla*). The spheres can vary only in size; the rods may vary in the ratio of the two axes, being either long and slender, or short and plump. If the two axes are of almost equal length, the rod will approach a sphere in appearance. Confusion, therefore, may develop in such cases; as, for instance, in the lactic-acid organism, which is so short a rod as to be called a coccus type by some writers. The ends of the rods may vary, being rounded or square cut, or even in a few instances concave. The spiral types may present all the variations of the rods, and may also vary in the extent to which the cell is bent. The curvature may be very slight, or it may be a true spiral. The rigidity of the bacterial cell is well illustrated in the spiral forms. It is evident that there may be a gradual gradation in form from the rounded coccus type through to the spirilla.

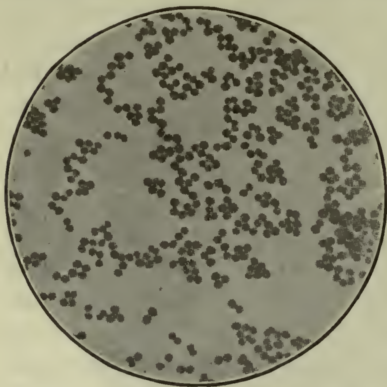

Fig. 2. Cocci
A spherical organism in which the cells occur in irregular shaped masses is called a staphylococcus After Günther.

Frequently under the conditions of growth in the laboratory and less frequently in nature, cells of abnormal shapes are noted, known as *involution* forms. It is commonly believed that these cells are degenerate forms and are not capable of reproduction. The deviation from normal cell type is probably occasioned by growth under unfavorable conditions.

Fig. 3. Large Bacilli
A rod-shaped organism in which the cells occur in chains is called a streptobacillus After Günther.

Reproduction. — The bacterial cell divides into two daughter cells by an infolding of the protoplasm in the middle of the cell until the protoplasm is completely divided. The cell wall is then formed, and finally splits, forming the opposing ends of the new cells. In the case of bacilli and spirilla, the division is always at right angles to the long axis. With the cocci, the plane of division may have any direction, since all axes are equal.

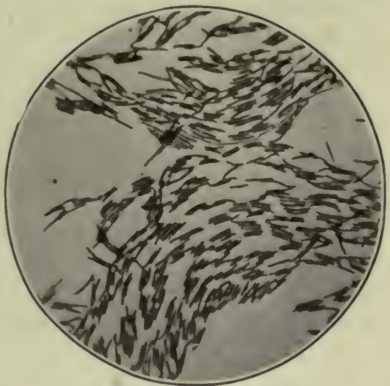

Fig. 4. Small Bacilli
After Günther.

Cell reproduction in the multicellular forms of life results in an increase in the size of the individual; in the unicellular forms it results in multiplication of the number of individuals. Immediately after cell division, the daughter cells are much smaller than the original mother cell at the time division began. They increase rapidly in size; to this process the term growth can be applied. Commonly one speaks of the growth of bacteria when reproduction is referred to.

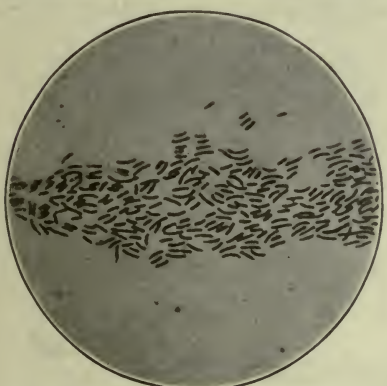

Fig. 5. Spirilla
The organism causing Asiatic cholera, frequently called the comma bacillus
After Günther.

The *generation period*, in the case of bacteria, is the time required for a mature cell to divide and for the resulting cells to reach maturity. With many forms of bacteria it requires only a short time for the process of division to be completed; in some instances it has been found to occur in twenty minutes. Such a rapid rate is attained only under the most favorable conditions of food and temperature.

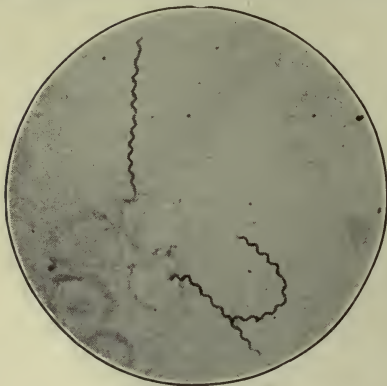

Fig. 6. Spirilla

The organism causing recurrent fever. The cells show many turns instead of only a portion of a turn as in the cholera organism After Günther.

This cell division is termed *vegetative reproduction,* in contrast to a second method that is found in a small number of the bacteria, *viz.,* reproduction by the formation of *spores.* In this process a portion of the protoplasm is condensed in one part of the cell to form a small spherical or oval, highly refractile body, to which the term

Fig. 7. Spores

A portion of the content of the cell is condensed into a body which appears very bright under the microscope. On germination a rod similar to the one that produced the spore results

endospore is applied. The production of the spore is followed by the death of the cell and its dissolution, so that the spore is ultimately set free. Spore formation is virtually limited to a few of the bacilli, and does not occur as long as nutritive conditions admit of vegetative reproduction. It is stimulated by lack of food or by the accumulation of by-products of cell activity. Unfavorable temperature conditions for growth tend to prevent the formation of spores. It is a specialized function of the organism, and, like most such functions, occurs within a narrower range of conditions than does reproduction.

The diameter of the spore in some species is greater than that of the cell itself, thus producing a distortion of the cell. In the case of *B. tetani,* the organism causing lockjaw, the enlarged spore is at the end of the cell forming a drumstick, in which case it is called *capitate.* If the spore is centrally located, giving to the cell a spindle-like appearance, it is called a *clostridium* type. The spores are readily differentiated from the cell proper by their higher refractile power, which gives them the appearance of bright dots under the microscope. In stained preparations they still appear as bright, unstained spots in the stained cells, since they do not take the stain in the ordinary methods of treatment.

Fig. 8. Spores

The unstained body to be noted in most of the cells is a spore formed by the condensation of a portion of the cell content
After Günther.

The bacterial spores possess greater powers of resistance to various physical and chemical agents than any other form of life. They are especially resistant to heat. Some spores will withstand the temperature of boiling water for sixteen hours. They have been found alive on dried herbarium specimens after ninety-two years, and in the author's laboratory the spores of *Bact. anthracis* remained alive in water for seventeen years. They are also resistant to chemicals. They assume an important function in the preservation of food and in the prevention of diseases, since their destruction is often a matter of great difficulty.

The spore, placed in a favorable environment, germinates and produces a cell similar to the one that formed the spore. Since a cell produces but a single spore, spore formation is not a matter of growth, but of reproduction. The germination may result in the rupture of the spore at the end, and the young cell emerges with its long axis parallel to that of the spore. In other types the cell may emerge at the side of the spore. In some instances the spore swells and the spore wall is absorbed in the cell substance. The type of spore germination may enable the differentiation of closely related morphological forms to be made.

Cell aggregates.—The arrangement of the cells frequently makes possible the recognition of species among

Fig. 9. Arrangement of Cocci
Streptococci, Sarcinae, Staphylococci

the higher plants and animals. Something of similar nature can be used in the study of bacteria. After cell division has occurred, the daughter cells may separate at once, or the cells may cohere for a time. If the cohering cells continue to reproduce, a chain of cells will result, which may consist of a few cells or of many. If the organism is a coccus, the term *streptococcus* (chain coccus) is applied; if a bacillus, *streptobacillus* is used.

Fig. 10. A Streptococcus
One of the organisms concerned in the souring of milk
After Orla-Jensen.

In the case of the bacilli this is the only cell aggregate with regularity of form that can occur. It is probable that the cells in a chain are inclosed in a common sheath. Various other forms of aggregates are found among the cocci. If the planes of division are always parallel and the cells cohere, a chain results, as noted above. If the tendency is for but two cells to cohere, the term *diplococcus* is used. If the planes of division have no definite direction, the progeny of a single cell will form an irregular cell mass. Such an organism is called a *staphylococcus*, from its similarity to a bunch of grapes. Cocci may occur

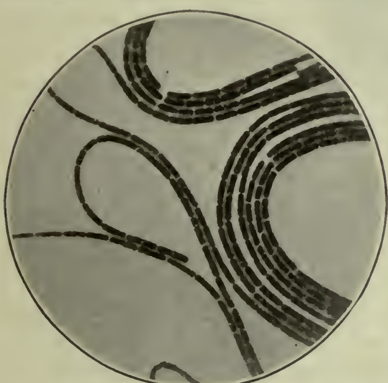

Fig. 11. Bact. Anthracis
Threads consisting of many cells characterize this organism
After Günther.

in bunches of fours. Such grouping is called a *tetracoccus*. Again, the successive planes of division may be in three dimensions of space, resulting in packets of cells, to which the name *sarcina* is applied.

The spirilla exhibit the same cell grouping as do the bacilli, although, as a rule, it is less pronounced.

Cell structure.—The bacterial cell wall is a relatively firm membrane, through which all food must pass by diffusion. Lining the cell wall is a layer of protoplasm, the ectoplast, which has a selective action on substances in solution in the cell sap, or in the liquid in which the cell occurs. Since the action of this cell structure determines what substances enter or leave the cell, it is one of the most important elements of the cell. If the cells are placed in a strong sugar or salt solution, the protoplasm shrinks and the cell is said to be *plasmolyzed*, a condition in which growth is impossible.

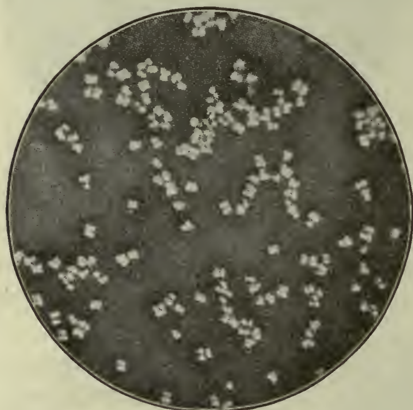

Fig. 12. A Tetracoccus
It will be noted that the cells tend to occur in aggregates of four
After Orla-Jensen.

The nucleus of the cell of the higher plant and animal is a structure of the utmost importance, since it governs for the most part the physiological activities of the cell. It plays the most important rôle in cell division. Its importance is such, in the cells of the higher forms, that it would seem impossible for any cell to function without a nucleus. A definite nucleus is not found in the typical bacterial cell.

From the relation of cells to stains, the conclusion has been drawn that the substance of the bacterial cell is essentially nuclear in character.

Inclusions of various kinds are sometimes to be observed in the bacteria. These bodies, termed *metachromatic granules,* may react to the stains in such a way as to differentiate them from the rest of the cell contents. Granules of glycogen and of sulphur are found in some species, as are also droplets of oil.

The outer layer of the cell wall is often of a gelatinous nature and more or less thickened, forming what is known as the *capsule.* It is probable that the presence of some of the capsulated bacteria causes the solutions in which they are growing to become ropy or slimy as is the case with ropy bread and slimy milk. Some bacteria are embedded in a mass of gelatinous material secreted by the cells, in which case the mass of organisms is termed a zoöglœa. The mass may be so firm as to have a leathery nature.

Fig. 13. Capsules

Each cell is surrounded by a gelatinous layer, which appears as a clear space in the photograph After Orla-Jensen.

Motility.—Many species of bacteria have the power of independent motion when they are in a suitable liquid. The organs of locomotion are delicate whiplike appendages variously distributed on the cell. The term *flagella* (singular *flagellum*) is applied to them. A *monotrichous* cell is provided with a single flagellum at one end. If two or more flagella are present at one end, the term *lophotrichous* is used. When clusters of flagella are at either end, *amphitrichous* is applied. And when the flagella are distributed over the entire cell, it is known as *peritrichous.*

The flagella are exceedingly delicate, and are so small that they are not visible in ordinary microscopic preparations. To make them apparent, the preparation is treated in such a way as to precipitate something upon them, thus increasing their apparent diameter. These locomotor appendages, which are usually much longer than the cell itself, are not found on the cocci. Many of the bacilli and nearly all of the spirilla are motile.

The space that can be traversed by even the most actively motile cell is small, probably not more than an inch or two in an hour. In comparison to their size the motion is quite rapid. Some have been shown to travel twenty times their own length in one second, a relatively much faster rate than that of a speeding race-horse. Some of the motile spirilla can reverse their direction without the turning about of the cell. In others, the cell must turn to reverse the direction of movement. It should be remembered that in the case of the peritrichous forms there must be coördination of movement among all of the flagella of a single cell. These arrangements give some idea of the complexity of the bacterial cell.

Size of bacteria.—The unit of measurement in microscopy is the *micron,* which is one thousandth of a millimeter, or approximately one twenty-five thousandth of an inch. It is usually expressed by the Greek letter μ. The great majority of the bacteria are from 0.5 to 5 microns in length or diameter.

It is difficult to obtain any appreciation of the minuteness of the bacteria. If they are represented by tiny cubes, a micron on each edge, one billion of them would be contained in a cubic millimeter, and one thousand billion in a cubic centimeter. If a sample of milk contains a billion bacteria per cubic centimeter, it means that less than one thousandth of its volume consists of bacteria.

Ultramicroscopic organisms.—It is known that there are forms of life so small that they are invisible under the highest powers of the microscope. An object having a dimension less than 0.2 micron can not be demonstrated by the microscope. The term *ultramicroscopic* organism is applied to such. The proof of their existence lies in the fact that an animal can be inoculated with a minute quantity of the blood of another animal suffering from some one of certain diseases. The presence of organisms in the blood injected can not be demonstrated microscopically. The inoculated animal acquires the disease. From it another animal can be inoculated. The process can be extended through as long a series as is desired, with constant results, which can be explained only by the presence of a living organism in the original material used. Only a living form could thus perpetuate itself. The term *filterable virus* is also applied to such minute organisms, for the reason that they pass through filters that remove the bacteria.

One gains some conception of the size of atoms and molecules when it is recognized that in each of these minute organisms there are many different chemical substances, and many molecules of each. Chemical processes and transformation of energy, that man can not and probably never will be able to duplicate, since they represent the life process, the power of self-perpetuation, are going on in each minute cell.

Higher bacteria.—Between the various typical groups of life, one finds transition forms that have the characteristics of both of the related groups to a greater or less degree. Such forms occur between the true bacteria and the true molds, or, more properly speaking, the filamentous fungi. The term higher bacteria, or *trichobacteria*, is applied to these in opposition to the *haplobacteria*, or strictly unicellular forms.

The higher bacteria occur in threads or filaments. A number of cells are contained in a common sheath. It is probable that the individual cells are capable of independent existence. Since they occur in filaments, they give evidence of a certain differentiation in function. Some of the cells are concerned with reproduction, others with the anchoring of the thread to its substratum. In such organisms is found the beginning of the division of labor, the distinguishing characteristic of the higher forms of life. The filaments are usually unbranched. Some, however, show true branching, a property that allies them to the molds. Still others have false branching, due to the misplacement of a cell in the thread. A cell forced out of its position in the chain by its division gives rise to a new thread.

Another property that allies the higher bacteria to the molds is the production of spores, or conidia, by the division in the three dimensions of space of the upper cells of he sessile thread. The conidia are motile in some instances; in others, non-motile. They leave the sheath in which they have been formed, and float away to establish new threads vhen they lodge on a favorable substratum. The spores may germinate while still in the sheath, giving rise to a whorl of threads.

Among the higher bacteria are to be found some of great practical importance, such as the iron bacteria, which frequently cause the clogging of water-mains and drains; and certain disease-producing organisms, as the one causing lumpy jaw in cattle.

Classification of bacteria.—Many attempts have been made to erect a classification of bacteria comparable to the classifications used in botany and zoölogy. The latter are based on morphology. Due to the simple morphology of the bacteria, a classification based thereon is unsatisfactory and of limited value. For purposes of identification, not only must morphology be considered, but also the action of the organism on the food material, the *physiology* of the organism, and the appearance of the masses of cells as they occur in the artificial cultures of the bacteriologist, *i. e.* the *cultural characteristics.*

The classification that is in most common use is that of Migula. It is as complete as possible where classification is based on morphology alone. As modified by Buchanan and presented in simple form, it is as follows:

MIGULA'S CLASSIFICATION OF THE EUBACTERIA

(Modified)

(*Forms of economic importance only*)

Suborder I. *Haplobacteria.* Bacterial cells not permanently united into filaments, without sheaths.

Family I. *Coccaceæ.* Cells spherical, at least when free.

1. Cells non-motile:
   - *a.* Cell division in one plane, cells frequently remaining attached in chains........................*Streptococcus*
   - *b.* Cell division in two planes (sometimes irregular), resulting in formation of flat plates or of masses of cells ....*Micrococcus*
   - *c.* Cell division in three planes, all at right angles, the cells remaining united after division, forming cubes or packets ....*Sarcina*
2. Cells motile:
   - *a.* Same as Micrococcus, but with flagella......*Planococcus*
   - *b.* Same as Sarcina, but with flagella.........*Planosarcina*

Family II. *Bacteriaceæ.* Cells cylindrical in shape, not bent.

1. Cells non-motile ....*Bacterium*

2. Cells motile:
   - *a.* With polar flagella .... *Pseudomonas*
   - *b.* With peritrichous flagella .... *Bacillus*

Family III. *Spirillaceæ.* Cells elongated and bent, usually spirals or segments of spirals.

1. Cells non-motile .... *Spirosoma*
2. Cells motile:
   - *a.* Cells short, comma-shaped, one to three polar flagella .... *Microspira*
   - *b.* Cells longer, with tuft of polar flagella........ *Spirillum*
   - *c.* Cells very long and slender, flexible. Flagella, if present, demonstrated only with difficulty...... *Spirochæta*

Suborder II. Trichobacteria. (Family...... *Chlamydobacteriaceæ*) Cells cylindrical, united in threads or filaments, surrounded by a sheath.

- *a.* Filaments unbranched .... *Leptothrix*
- *b.* Filaments showing false branching .... *Cladothrix*
- *c.* Filaments showing true branching:
  1. Spores produced .... *Nocardia*
  2. No spores observed .... *Actinomyces*

The terms applied to specific organisms have various meanings. Usually they refer to the type of decomposition caused by the organism, the disease produced by it, its habitat, or its respective group morphologically Examples are as follows: *Bact. lactis acidi,* a non-motile rod that produces lactic acid as its chief by-product; *B. typhosus,* a motile rod causing typhoid fever; *B. coli communis,* a motile rod the habitat of which is that part of the digestive tract known as the colon; *B. Welchii,* a motile rod named from its discoverer, Dr. William Welch, one of the most prominent of American bacteriologists.

The subjects of classification and nomenclature in bacteriology are much confused. The same organism may be described and referred to in the literature under many names.

Morphology of yeasts.—The yeasts, or *saccharomycetes,* as they are freqently called because of their growth in sugar solutions, are unicellular organisms like the true bacteria, but they are more complex in structure, possessing a definite nucleus which is small, and recognizable with difficulty, if at all, in unstained preparations. It can, however, be demonstrated by proper methods of staining. The protoplasm contains granules, oil globules, and vacuoles that are

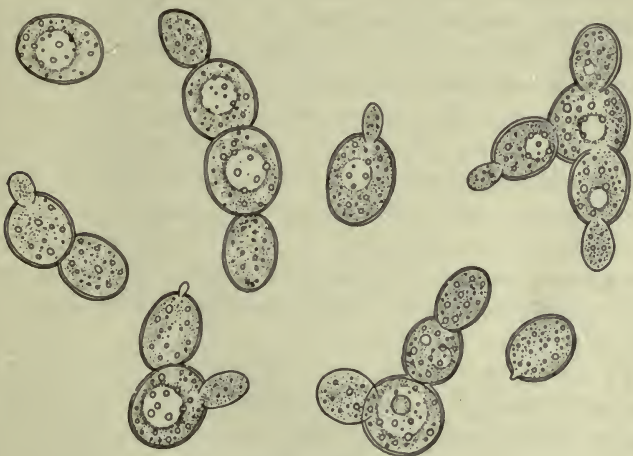

Fig. 14. Yeasts

The cells reproduce by budding. The large clear areas in some of the cells are vacuoles, which are filled with cell sap

filled with cell sap. The wall of the yeast cell is a relatively firm structure of yeast cellulose.

The yeast cell may be oval, ellipsoidal, or cylindrical, rarely spherical. The shape is not so constant in form for a given species as is the case with the bacteria. The yeasts are, as a rule, larger than the bacteria, the cells commonly ranging from 2.5 to 12 microns in width. The grouping shows little of the regularity that is noted among some of the bacteria. Reproduction is accomplished by the vegetative method, or by the production of spores. The former takes place by the formation of buds on any portion of the cell. A portion of the nucleus passes into the minute protuberance, which then enlarges, and is separated from the mother cell by a constriction. The daughter cell may remain attached, or may separate at once from the mother cell.

Spore production in the case of the true yeasts is a method of increase in numbers, since, as a rule, more than one spore is produced, from two to eight being usual. The spores are often termed *ascospores,* and the spore-producing cell an *ascus.* The spores are not normally produced in growing cultures. They have various shapes and markings. In one species, *Saccharomycetes anomalus*, they are shaped like a derby hat. The ascopores germinate by swelling, bursting the wall, and budding. The true yeasts are those that reproduce by budding and spores. The false yeasts do not produce spores. The term *torulæ* is often applied to the latter.

The yeast spores are not so resistant to heat as are the bacterial spores; indeed they are but little, if any, more resistant than the vegetative cell. The non-resistance of the yeast spore to heat is of great importance in food preservation.

Molds.—The molds represent a more highly developed and more complex group of organisms than do the bacteria and yeasts. Many hundred genera are known. It is possible to give only a few of the more important points of their morphology, especially of those that are of practical importance. The molds are multicellular, and exhibit a division of work among the cells. Some are concerned with nutritive processes, others with reproduction.

The mold plant consists of a network of branching threads, called *hyphæ.* The threads are formed by cylindrical cells placed end to end. The threads concerned with nutrition are termed *vegetative hyphæ;* those concerned with reproduction, *fertile hyphæ.* The term *mycelium* is applied to the entire mass of growth resulting from a single spore.

The filaments may be divided by cross-walls, or *septa,* or they may not be so divided. In the septate mycelium each cell contains a nucleus. In the non-septate mycelium, nuclei occur at intervals along the thread in the protoplasm. Therefore even the non-septate molds are to be classed as multicellular. The threads branch at frequent intervals. The microscopic appearance of the mycelium is that of a mass of tangled filaments. The filaments are not usually all in contact with the food, but rise into the air to some extent, giving to the typical mold a fluffy appearance. The hyphæ are usually colorless.

Reproduction is by the formation of spores, which are produced in enormous numbers. In some species both sexual and asexual spores are produced. The former are of small practical importance and will not be treated. The asexual spores are most often borne on hyphæ that rise into the air and are thus out of contact with the moist food material, a fact of importance when their distribution is considered. The manner of production, their size, shape and color, are the most important characteristics used in the classification of molds. The spores are often colored, brown, black, and green being the most common.

In a number of the more important groups of molds, the spores are produced on the ends of the filaments. Such filaments are called *conidiophores,* and the spores *conidia.* In others they are formed in closed sacs, called *sporangia,* the filament bearing the sac being termed a *sporangiaphore.* The latter type of spore formation is limited to the non-septate molds. The mature sporangium usually bursts, and the numerous sporangia spores are set free. The sporangium is quite comparable to the ordinary puff-ball. Some

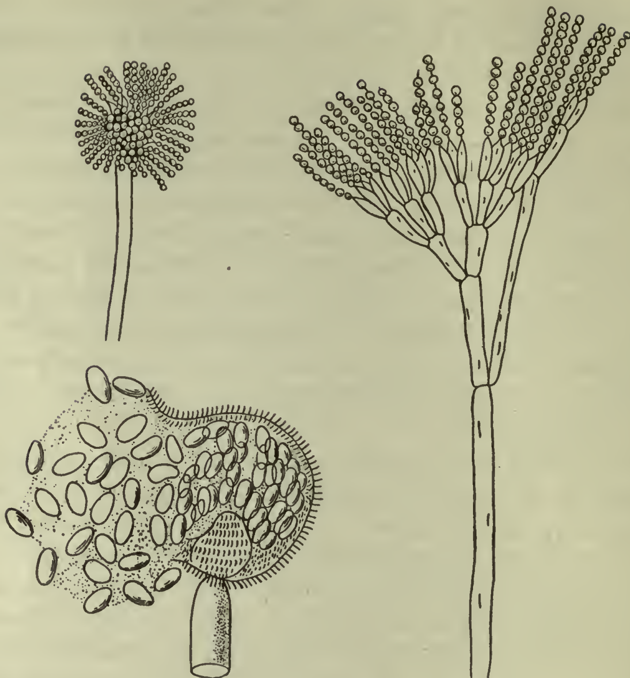

Fig. 15. Spore-formation by Molds

The manner of spore-formation by the aspergillus molds, upper left, by the penicillium molds, right, and by the mucor molds, lower left

of the molds form spores by the breaking up of entire filaments into short segments. Such spores are called *oïdia. Oidium lactis,* the mold that develops on milk, is the most important from a practical standpoint.

The most common molds appearing in foods are the *mucors,* the *penicillia,* and the *aspergilli.* The former produce the spores in sacs. The most common is *Rhizopus nigricans* producing the soft rot on vegetables like sweet potatoes. The spores are black, and at the point at which the fertile hyphæ branch from the vegetative hyphæ clusters of rootlike hold-fasts or rhizoids are produced.

In the genus *Aspergillus* the spores are borne on the ends of club-shaped stalks, called *sterigmata* (singular, *sterigma*), which are grouped on the enlarged end of the conidiophore. The spores are green, yellow, orange, brown, or black. *Aspergillus niger* is the most common of the black-spored types; *Aspergillus glaucus,* which occurs on grain, silage, canned fruits and vegetables, is the most common green-spored one.

In the case of the penicillia, the conidiophore branches one or more times, producing a cluster of parallel hyphæ, from the end of which a chain of conidia is abstracted. This results in a broom- or brush-like appearance, which gives the genus its name of *Penicillium,* from the Latin word for brush. The most common members of the group have green spores.

The mold spores, like those of the yeasts, are easily killed by moist heat.

In the case of the bacteria and yeasts, true unicellular organisms, it is necessary for each cell to be in direct contact with the food. In the case of a mass of cells growing on a solid, the nutrients may pass by diffusion through the mass of cells not in direct contact with the medium. In the case of the molds, cells in actual contact with the food may pass the same to other cells not in contact with it. This enables the molds to invade vessels that are absolutely protected from the bacterial invasion, such as the cultures of the bacteriologist.

The production of spores in the air, and thus out of contact with a moist substratum, enables the spores to be easily carried away from their point of production by air currents. This method of transportation is favored by their lightness. Their ubiquitous distribution on every object is the result of the profuseness with which they are produced and the ease with which they are distributed.

Protozoa.—This term is applied to unicellular animals, some of which are of interest to us because of their relation to bacteria, and because some of them produce diseases in man and in domestic animals in much the same way as do the bacteria.

The animal cell wall lacks the rigidity and firmness of the plant cell wall. For this reason, the protozoa do not have the definiteness of form that marks the unicellular bacteria and yeasts.

Many of the protozoa ingest solid food, again a differentiation from the unicellular plants. Bacteria serve as food for some of the protozoa to be found in soil and water.

CHAPTER IV

CULTIVATION AND STUDY OF BACTERIA, YEASTS, AND MOLDS

The studies of the botanist and the zoölogist are more largely confined to the structure of the individual than to its physiology, to form rather than to function. The bacteriologist finds that little of practical importance can be gained from such a study of the organisms in which he is interested. He must extend his observations to the part they play in the decomposition of organic matter. The bacteria, the yeasts, and the molds occur in nature in such confusion and in such mixture of kinds that a study of them in their native habitat is of no avail. Before one can learn anything of the functional activities of a single kind, it must be separated from all others, and it must be grown under controlled conditions. This work involves the isolation and the cultivation of *pure cultures,* that is, those that contain but a single kind in which each cell is like every other cell.

Culture media.—Almost any kind of organic matter will serve as food for some type of organism. Under laboratory conditions it is desirable to use as few kinds of nutritive materials as possible, and to have these supply conditions for the growth of as many kinds of organisms as possible. These nutritive materials, when used in the laboratory for the growth and cultivation of microörganisms, are called *culture media* (singular, *medium*). It is essential that all or a portion of the material used be soluble in water, otherwise it can not pass through the cell membrane and the layer of cytoplasm lining the cell wall. It is essential that the chemical reaction be favorable for the organism to be grown.

The bacteriologist has succeeded in devising what may be termed basal culture media, to which may be added amendments to adapt them better to the growth of specific organisms. The great basal culture medium is broth or bouillon, prepared by infusing one part of chopped lean meat (beef or veal most commonly) in two parts of water. The soluble constituents are extracted from the meat. The insoluble portion is filtered off, and to the filtrate is added 1 or 2 per cent. of peptone. The latter is prepared from meat or blood by digesting it with one of the agents that are active in the intestinal tract of animals until the meat or blood is changed into a mixture of simpler products that are soluble in water. The infusion of meat to which peptone has been added is boiled to coagulate the albumens present. Its reaction is then adjusted to the desired point by methods that can not be discussed in sufficient detail to warrant their treatment here.

Acidity and alkalinity are due to the presence of hydrogen (H) and hydroxyl ions (OH) respectively. Any solution in which the former are in excess will be acid in reaction. If the hydroxyl ions are in excess of the hydrogen ions, the solution will be alkaline. If both are present in equal numbers, the material will be neutral in reaction. Pure water is the great example of a neutral substance. For most microörganisms the nutrient medium should be approximately neutral. It is doubtful whether there is any microörganism that will not grow in a neutral reaction when other conditions are favorable.

The broth is filtered, after its reaction has been corrected, to remove all coagulated and suspended matter, since it is desirable to have the medium clear in order that the growth of bacteria therein can be more easily seen.

Commercial extract of beef may be used in place of fresh meat, and is widely employed on account of its convenience. Sugars of various kinds, glycerin, and other substances may be added to the broth to adapt it to the needs of specific organisms. This simple culture medium will permit the growth of the great majority of organisms in which the bacteriologist is interested.

Milk, which contains a mixture of sugars, proteins, and mineral substances, is admirably adapted to the cultivation of bacteria.

Materials that are naturally solid or that are solidified by heat are used as media. Prominent among these are slices of various vegetables. Potato is most commonly used, as are the mixture of the white and yolk of the egg, and blood serum.

Liquefiable solid media.—It is necessary to have a medium that is liquid at high temperatures and that becomes solid on cooling for purposes of isolation, as will be explained later. Such a medium can be obtained by the addition of 10 per cent. of gelatin to the beef broth. This medium will remain solid up to 77° F. Since some bacteria do not grow at such low temperatures, gelatin can not be used for their cultivation. Certain bacteria have the power of digesting or liquefying it, an advantage or a disadvantage, depending on the service the medium is to yield.

The broth may also be made into a liquefiable-solid medium by the addition of from 1 to 1.5 per cent. of agar, a substance obtained from certain seaweeds found in Japan and China. The medium to which the agar is added exhibits the peculiar property of melting at 98° C. (208° F.), but of not solidifying until it is cooled to 38° C. (100° F.). This enables the bacteria to be mixed with it while it is at a temperature that will not injure them, and for the cultures to be kept at any desired temperature without becoming liquid. The agar is not liquefied by any of the ordinary bacteria; it serves simply as a solidifying material, while gelatin may serve as food for many bacteria. Gelatin is a protein; agar, a carbohydrate.

The introduction of gelatin in 1882, by Robert Koch, made possible the great developments in bacteriology that took place in the last two decades of the nineteenth century.

Sterilization of culture media.—The media as prepared will contain many microörganisms that were in the ingredients or the vessels used. These organisms must be destroyed if the media are to be of any value. This is most commonly accomplished by heating the media until they are free from living forms. They are then said to be *sterile.* The process is termed *sterilization.* The media ordinarily will contain bacteria, yeasts, and molds, and the spores of each. A short exposure to the boiling-point will destroy all except the bacterial spores. These can be destroyed with certainty only by prolonging the period of exposure to 212° F. for a number of hours. This is not practical, and therefore other methods must be used.

The culture medium in appropriate containers is placed in a steamer, so that the containers will be surrounded by streaming steam. An exposure for fifteen minutes after the temperature of the medium has reached that of the steam will suffice to kill everything except the bacterial spores. If the medium is now placed at ordinary room temperature, the resistant spores will germinate within a few hours, and the resulting vegetative cells may then be readily destroyed by a second heating. In practice, three exposures are made on successive days. The process is called *intermittent sterilization.*

If the temperature of the steam can be raised, it will, of course, have a more destructive effect on the spores. This is done by placing the media in their containers in a steam-tight apparatus. Steam is generated therein, and the air is allowed to escape so that the entire space will be filled with steam. The chamber is then closed. The temperature obtained will depend on the steam-pressure. A steam-pressure of 15 pounds to a square inch yields a temperature of 120° C. (248° F.). Exposure for fifteen minutes to this temperature is usually sufficient to sterilize the media. Where any amount of organic matter is to be sterilized, the latter method is used, as in bacteriological laboratories and in canning factories. The apparatus employed is ordinarily termed an autoclave.

The same method can be used for the sterilization of glass and metal objects, and of clothing.

The culture media rendered sterile can be kept for an indefinite time if protected from contamination by microorganisms and from desiccation.

Sterilization by dry heat.—It is sometimes more convenient to use dry heat, such as that of an oven, for the sterilization of objects that will not be injured thereby, as glass and metals. Dry heat is far less destructive to life than is moist heat. It is thus necessary to prolong the period of exposure and to increase the temperature over that used in the autoclave in order to insure sterilization. A temperature of 150° to 170° C. (302–338° F.) must be maintained for an hour. All organic matter will be injured by such treatment.

Small objects that will not be injured by heat may be sterilized in the direct flame of a gas-burner. The needles used for transferring bacteria from one culture-tube to another in the laboratory are thus rendered germ-free.

Sterilization by filtration.—It is essential that sterilized culture media and other organic materials be protected from contamination after treatment. In the canning industry this is accomplished by hermetically sealing the container. Such a method is impossible in the laboratory, where glass containers must be used which must be easily opened and closed, and yet be protected from contamination. This is accomplished by plugging the tubes and flasks with cotton wool. The air will pass freely into the container, but all definite bodies such as microörganisms will be removed. The air is thus effectively sterilized. If the cotton becomes damp, molds may grow on it and will soon penetrate it, thus contaminating the contents of the vessel.

Water and many other liquids can be sterilized by passage through filters of unglazed porcelain. Such a process is sometimes used for the sterilization of culture media that would be so altered by heating as to be rendered worthless.

Sterilization by chemicals is possible, but can not be used in the laboratory; for if chemicals are added to the culture media, they can not be removed or rendered non-effective so as to permit the growth of bacteria in the media.

Isolation of pure cultures of bacteria.—As previously stated, little can be learned concerning the relation of specific kinds of organisms to a particular type of decomposition or to disease production until the organism in question has been separated from all other kinds, and thus a pure culture obtained.

The most common way of securing such pure cultures is by the use of gelatin or agar. The medium is melted and cooled to 45° C. (113° F.), at which temperature agar remains liquid, and which is not injurious to any of the bacteria. A small amount of the material from which one desires to isolate the pure culture is intimately mixed with the liquid medium. This is then poured into a shallow flat glass dish, which is allowed to cool. The agar quickly solidifies, holding the contained organisms in place. In the favorable nutrient medium, growth occurs with the formation of a mass of cells sufficiently large to be visible to the unaided eye. Such masses are termed *colonies.*

Each colony represents the progeny of a single cell or a group of cells. The colonies of many kinds of bacteria ex-

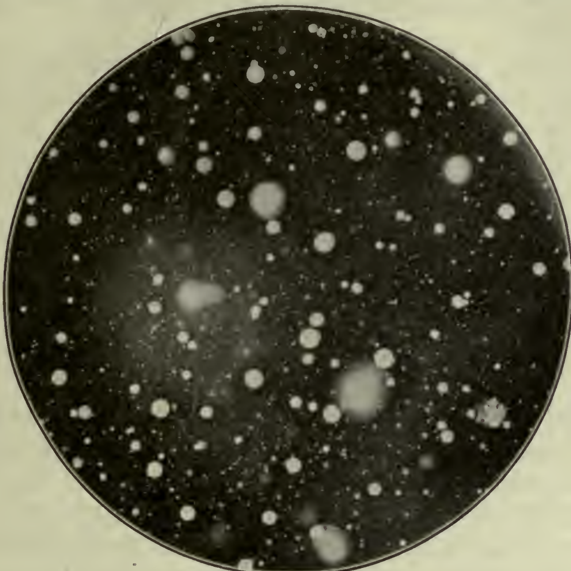

Fig. 16. A Plate Culture

Each of the spots or colonies on the plate has resulted from the growth of a single cell or group of cells of the same kind in the substance with which the plate was seeded. Plate cultures are used for determining the number of bacteria in various substances and for the isolation of pure cultures of bacteria

hibit more or less characteristic appearances, with reference to the size of the colony, its shape, the nature of its edge, and its interior structure. These are termed *cultural characteristics.* The colonies resulting from cells that were on the immediate surface of the medium or very close to it give rise to *surface* colonies. These exhibit the more characteristic appearances, since the growth can develop freely.

The *deep* colonies that result from the growth of cells embedded in the agar must push the culture medium aside in order to make room for the mass of cells. Since the colony is under considerable pressure, it can not develop freely. The form of such colonies is most commonly lenticular, or spherical.

It is, of course, essential that all materials and objects used in making the plate culture, as it is commonly called, be sterile, with the exception of the material to be examined, otherwise one could not be assured of the exact source of the resulting growth. It is also essential that the colonies be not too numerous, otherwise their size will be limited by the competition for food. It is usual to prepare, not a single plate culture, but several plates containing varying amounts of the material, so that on some one of the plates the resulting colonies will not be too numerous for successful isolation of the pure cultures.

A minute portion of a colony may be transferred to a tube of sterile agar or other appropriate media, producing what is called a *tube culture.* The exact form of the organism under observation can now be observed under the microscope, and its physiological properties determined by seeding it on various kinds of culture media and noting the resultant growth. The growth of the organism in the tube culture soon ceases, owing to the accumulation of its waste products or to the exhaustion of food. The cells will remain alive for varying periods of time, depending on the organism. The culture can be kept alive by furnishing it with fresh food through the transfer of a few of the cells to a new tube of nutrient medium. Such cultures form the so-called *stock cultures,* and are the pure seed supply of the bacteriologist.

In many instances it is impossible to isolate an organism from its native habitat by the plate-culture method. This method will be successful only when the desired organism is present in approximately as great numbers as the other bacterial forms that can develop on the plate culture. If only a few of the organisms are present, it will usually be necessary to seed some special selective medium that is more favorable for the desired organism than for the other types with which it may be associated. This will enable the organism in question to gain the ascendancy in the culture, which can then be plated with assured success.

An animal that is susceptible to a disease may be inoculated with a mixture of kinds of bacteria among which the producer of the disease is present. The body of the animal will act as a selective medium, and from its tissues a pure culture can often be obtained. Many modifications are employed to obtain pure cultures of some of the bacteria. More extended texts on laboratory methods must be consulted for the methods employed.

Quantitative methods.—The plate culture is also used for the determination of the number of microörganisms in any material. If a known amount of the material is added to the melted nutrient agar or gelatin, the number of colonies developing in the plate culture can be counted, and from these results the bacterial content of the original material may be determined. Each colony must have developed from at least one cell. It is thus possible to obtain a number that will, as a rule, approximate the number of cells actually present in the material used. The method is used constantly in the control of water and milk supplies.

It is, of course, impossible to obtain a medium that will enable *all* kinds of bacteria that may be present in such materials as milk or water to develop. The results thus obtained represent only the number of organisms that are able to grow under the conditions present in the culture plate. It is usually possible to adjust the conditions so that the organisms that one desires to study will develop, and thus the plate-culture method of determining bacteria is of great value in spite of its limitations.

Microscopic examination.—An object having dimensions less than 0.2 micron can not be demonstrated even under a high-powered microscope. Some of the bacteria approach this limit. Indeed, the great majority of the spheres are less than one micron in diameter. It is therefore essential to employ instruments that will give the maximum degree of magnification; but, since magnification is secured only with a reduction in illumination, a practical limitation is placed on the possible increase in the magnifying power of the microscope.

The two developments of the microscope that greatly accelerated the development of bacteriology were concerned with an increased illumination of the object. The first was the immersion objective, which prevents the refraction or bending of the rays of light as they pass from the glass on which the object to be examined is placed, into the air. This is accomplished by placing a drop of liquid having the same index of refraction as glass between the lens and the glass slide. Cedar oil dissolved in xylol is used for this purpose.

The light rays are reflected from the mirror through the object into the lens of the microscope. The Abbé condenser is a series of lenses placed beneath the stage of the microscope which concentrates the light rays coming from a certain area of the mirror and focuses them on a much smaller area of the object, thus illuminating it brightly.

Examination of unstained bacteria.—The motility of bacteria can be determined only by examining a liquid that contains the living cells. For this purpose, a drop of the liquid containing the bacteria is placed on a very thin piece of glass, known as a cover-glass, which is inverted over a cavity ground in a thicker piece of glass, the slide. The evaporation of the drop in the unsealed space causes currents, the effect of which makes it difficult to determine whether the movement of the contained bacteria is a really independent one, or is due to the currents. By sealing the edges of the cover-glass to the slide with vaseline, evaporation is prevented.

All finely divided matter suspended in a liquid shows a purely physical movement known as the *Brownian* movement. Each particle has a trembling or vibratory motion, but no progressive movement of the particles from place to place is noted. The bacteria are sufficiently small to show this molecular motion. Those forms of bacteria that are provided with flagella have the power of independent progressive motion, which can be readily demonstrated under the microscope.

Staining.—The live bacteria are difficult to demonstrate in any material, except in a liquid in which they are the only bodies in suspension. In such materials as milk, blood, and other body fluids, the recognition of bacteria by the microscope is impossible, unless the material can be so treated as to differentiate the organisms from the other materials present. This is done by staining the organisms with solutions of certain dyes. The material to be examined is spread in a thin film on a slide or cover-glass, and allowed to dry. The film is then treated in a way that will cause it to adhere firmly to the glass when it is subsequently moistened. This process is termed ''fixing'' the preparation, and is accomplished by gently heating the dried film in a gas flame to such a temperature as to cause the protein to coagulate, or by treatment with some chemical that will produce the same effect. The film can now be flooded with a dye and the material will not be washed from the slide.

The stains most commonly used are the aniline dyes, which were first discovered accidentally by Perkin in England in 1856. They were, however, not used in the study of the bacteria until 1876, when they were employed by Weigert. They have been of the greatest service in the development of bacteriology.

The bacteria can be differentiated from the other materials in which they occur, either by a contrast in depth of color, or by staining all or a portion of the bacteria one color and the other materials another color. The first method can be used in demonstrating the bacteria in milk; a film of milk stained with methylene blue will reveal the bacteria as dark blue objects in a light blue field. The casein and albumen form the background of the preparation. The organisms that cause tuberculosis may be detected in sputum, milk, and in other materials by staining them a bright red, while the other bacteria and the material in which they are embedded are stained blue. The tubercle bacilli are thus differentiated from the other bacteria and the background of the preparation in a striking way. This is made possible by a property of the tubercle bacillus which will be discussed later.

Stained preparations are usually employed for the study of the morphology of bacteria, for the demonstration of spores and flagella. The spores do not stain as easily as do the vegetative cells. It is thus possible to have the spores appear as a bright unstained area in a stained cell. If the spores have been set free by the dissolution of the cell, they will appear as bright oval bodies in the microscopic field.

The flagella are so minute that, unless their apparent diameter is increased, they can not be seen with the microscope. They can be made visible by precipitating some dye on them and thus increasing their apparent diameter.

The staining properties of bacteria aid in their classification. The most valuable of the methods used for this purpose is that devised by Gram. If bacterial preparations are stained with gentian violet dissolved in a saturated solution of aniline oil in water, and then treated with iodine dissolved in a solution of potassium iodide, the combination of the protoplasm of the cell and the dye is, in the case of certain organisms, of such a nature as to be soluble in alcohol; in the case of other bacteria it is insoluble. The latter retain in the presence of alcohol the color imparted to them by the dye, and are termed *Gram-positive* organisms. The others are called *Gram-negative*, since the color is removed by washing the preparation in alcohol. This method is of value in demonstrating Gram-positive bacteria in tissues that are themselves Gram-negative.

Systematic study of bacteria.—The classification of higher plants and animals and their identification is based wholly upon their morphology, their form, and their structure. In the case of such simple organisms as the bacteria, the variation in form is so slight as to make this character of limited value in the study and identification of a particular pure culture. Many different kinds of bacteria are so alike in the form of the cell itself that it becomes necessary to include other characteristics in differentiating one type from another.

Cultural characteristics.—The appearance of a mass of cells that has developed in or on a culture medium is often so characteristic that it can be used in separating one species from another. For example, if a pure culture produces a uniform turbidity in a liquid, it is evident that the cells will be found as single cells, or in very small aggregates. If the growth is massed at the top or the bottom of the culture medium, it implies that the cell-aggregates will be large, and that the cells may be found in long chains, or in zoöglœa-like masses. The medium may remain perfectly clear except for the growth at the surface or bottom.

The culture growth may vary widely in its profuseness, from that which is invisible to the eye to one that may occupy a considerable part of the volume of the medium. The profuseness of growth is a reflection of the morphology and physiology of the organism concerned. If pronounced capsules are formed, the mass of growth will be large. Again, if the by-products are extremely toxic to the organism, growth can continue but a short time; while, if they are not toxic, the growth may be limited only by lack of food.

The consistency of the growth, whether moist or dry, friable or slimy, is also noted, as is the color. The great majority of the bacteria produce a grayish white growth, which is more or less opaque. Some produce pigments, and are called *chromogenic* organisms. The most common colors noted are yellows, varying from a pale lemon to a deep orange. Blue, violet, green, red, brown, and black are less common. In some instances the pigment is a by-product, produced under certain conditions of growth and not under others. Its production is thus not an essential part of the physiology of the cell. In other cases it is a constant property, and undoubtedly is a part of the vital processes of the cell. If the pigment is soluble in water, the medium in which the organism is growing will be colored. If it is insoluble, the growth alone is colored. Among the manifestations of the chromogenic bacteria that have attracted especial attention are green pus, blue milk, and bright red spots on bread, the latter due to *B. prodigiosus*.

The relation of the organism to air will determine the location of its growth in culture media, and thus affect the appearance of the culture. The by-products of the organism will also aid in determining the cultural characteristics. The formation of gas is an example.

Physiological characteristics.—In the detailed study of an organism, attention is directed chiefly to the chemical changes that it produces in the substances in which it is growing, *i. e.*, to the biochemical properties of the organism. Culture media are prepared containing definite chemical substances, and the products formed therefrom are determined. The points to which attention is chiefly directed are the fermentation of various carbohydrates with the formation of acids, alcohols, and gases; as well as the action of the organism on different proteins, *e. g.*, casein and gelatin. The ability of the organism to produce disease in plants and animals may also be studied.

The final criterion in the identification of any organism is the determination of its ability to cause a specific effect. Thus the identification of the anthrax bacillus rests on the production, in animals known to be susceptible to anthrax, of a disease that has the characteristics of anthrax. An organism may have the same morphology, the same cultural characteristics, and the same physiological characteristics, as determined by the usual laboratory tests, as the true anthrax bacillus; but, unless it produces death in guinea-pigs, with certain definite changes in the tissues, it can not be identified as the anthrax organism. The identification of a pure culture of bacteria is a difficult task, in the solution of which all the tests that science has devised may be used.

CHAPTER V

PHYSIOLOGY OF MICROÖRGANISMS

In a general way, the simple presence of microörganisms has no significance, as far as the decomposition of organic matter is concerned. Decomposition always implies the growth, the proliferation, of the organism. Two problems present themselves daily to almost every individual. One is connected with the prevention of the growth of organisms in order that organic materials may be preserved; the other is the facilitation of the growth of organisms in order that the chemical change that they cause may be hastened. The conditions favoring or retarding the growth of microorganisms thus become of great practical importance.

Moisture.—Water is an essential condition for the development of all life. It makes up a large part of the weight of every organism; it is needed for the transportation of the food and waste products. It is impossible to make any general statement in regard to the amount of water that must be present before growth can take place. Some types of materials hold water in such a way that the organisms can not make use of it. The material is then said to be *physiologically dry*. Bacteria can grow in butter containing 15 per cent. of water, while they can not grow in most types of organic matter unless a much greater percentage of water is present. The reduction of the water content of organic matter is the most common way of preserving it from the attacks of microörganisms.

The growth of microörganisms may be prevented in the presence of large amounts of water by its content of materials in solution. The cell must have an internal pressure—*i. e.*, it must be *turgid*—before growth can take place. This pressure is known as *osmotic pressure*, and is due to the fact that the concentration of the liquid in the cell is greater than that of the liquid in which the cell is found. The protoplasmic layer that is in direct contact with the cell wall functions as a *semi-permeable* membrane, which will allow the passage of certain substances in and out of the cell freely, and will not permit others to pass. Certain of the cell constituents are thus prevented from leaving the cell. In an effort to maintain an equilibrium of concentration inside and outside of the cell with reference to these compounds, water is drawn into the cell. The passage of water into the cell creates the internal pressure. If a cell is placed in a solution of some material that can not pass into the cell on account of this semi-permeable membrane, water will be withdrawn from the cell to assist in establishing an equilibrium of concentration. The internal pressure of the cell will be destroyed by this process. The cell is then said to be *flaccid* or *plasmolyzed.* In this condition no growth can take place. The plasmolyzed condition may be a permanent one, or it may be overcome by the slow passage of the substance into the cell and the reëstablishment of a turgid condition.

The yeasts are more resistant to the effect of materials in solution than are the bacteria. Many of them can grow in an almost saturated solution of cane sugar, while a 15 per cent. solution will inhibit the growth of most bacteria. The molds are still more resistant to the action of concentrated solutions. In fact, the inhibition of mold growth by increasing the concentration of the liquid by the addition of sugar or salt is so slight as to be of little importance. The molds are likewise able to grow on materials from which bacteria and yeasts can obtain no water. In other words, a material may be physiologically dry for one form of life and not for another. The growth of molds on bread, cheese, dried meats, paper, and clothing is evidence of their ability to grow in the presence of a small percentage of water.

If a cell is transferred from a concentrated solution to one that is less concentrated, the water will be drawn into the cell and may cause the rupture of the cell wall. The term *plasmotypsis* is applied to this process.

Many of the bacteria can not withstand desiccation, the cells being almost immediately destroyed. Still others can remain alive in a dried condition for long periods of time. Their resistance to desiccation becomes of importance in disease transmission. The bacterial spores are extremely resistant to drying. Yeasts are, in general, less easily injured by desiccation than are the bacteria. The mold spores are very resistant to drying.

Temperature.—As is well known, decomposition goes on more rapidly as the temperature is increased, up to a certain limit. This, of course, implies that the growth of the microörganisms is accelerated as the temperature rises. The temperature at which any organism thrives most rapidly is known as the *optimum temperature* for that organism. As the temperature changes from the optimum, the rate of growth decreases. As a rule, a few degrees above the optimum temperature, growth is no longer possible. The *maximum temperature* has been reached. As the temperature falls below the optimum, the rate of growth is reduced until a point is reached at which it ceases. This is known as the *minimum temperature,* and is, as a rule, far below the optimum temperature.

The temperature zone in which growth of bacteria is possible varies widely with different organisms. In the case of some, it is but a few degrees in extent; with others, it may be very wide, ranging from the freezing-point to that of the animal body. The great majority of microörganisms have their optimum-growth temperature between 20° and 40° C. (68°–104° F.). The term *mesophilic* is applied to these. For others the optimum is lower; these are called *psychrophilic,* while those that grow at high temperatures are called *thermophilic.* The minimum temperature for the psychrophilic organisms is usually below 0° C. (32° F.). The maximum temperature for the thermophilic may reach 70° C. (158° F.), a temperature at which most vegetative bacteria are quickly destroyed. It is thus seen that the temperature zone in which the growth of bacteria is possible is an extremely wide one, extending from below the freezing-point of water to what are ordinarily termed scalding temperatures. No one organism is able to grow through this entire range. Organisms that are able to grow at high and others that are able to grow at low temperatures are common in soil and in the feces of animals.

Organisms can grow below the freezing-point of water only when the freezing-point is depressed by the presence of soluble material in the water of the food.

The temperature zone of existence is far wider than that of growth. Freezing does not normally destroy either vegetative bacteria or the spores. If the organisms are kept in a frozen condition for long periods, they gradually die. The death rate is so slow that food removed from cold storage may spoil as quickly as it would have done at the same temperature before being frozen. Indeed, the spoiling is often more rapid in certain foods that are so changed by freezing as to adapt them better for the invasion and growth of bacteria therein. Repeated freezing and thawing is more harmful than being kept continuously in a frozen condition. Still lower temperatures do not seem to have much additional effect.

As the temperature is increased above the maximum for growth, a point is soon reached at which injury to the cells takes place. The weaker cells are first destroyed; the more resistant only as the heat is applied for a longer period or a higher temperature is used. The point at which all of the cells of a given culture are destroyed is known as the *thermal death point.* This temperature will depend on the length of exposure to heat, and on the amount of moisture present. The smaller the percentage of water, the less injurious will be the effect of the heat. Heat injures the cell through coagulation of the protoplasm. The temperature required to cause coagulation rises rapidly as the water content of the cells decreases. This fact is the reason for the greater effectiveness of moist heat as compared with dry heat in sterilizing processes.

The chemical reaction of the liquid is also a factor in determining the destructive action of heat on microörganisms. In an acid-reacting liquid they are more easily destroyed than in a neutral solution. Bacterial spores, as has been stated, are extremely resistant to heat.

Relation to oxygen.—Oxygen is an essential element for the growth of all life. The higher forms of life, both plant and animal, are adjusted to rather definite percentages of oxygen in the air. This is not true of the microörganisms as a class. Some can grow in the total absence of free oxygen; still others can grow in percentages far above that of the atmospheric air.

The term *anaërobic* is applied to those that can grow in the absence of free oxygen. They can also grow in the presence of reduced amounts of free oxygen. When they grow in the absence of free oxygen, they must draw their oxygen supply from combined sources, as from the sugars, nitrates, or sulphates. In this mode of securing oxygen, they are not unlike the unit cells of the animal body that derive their oxygen from a combined source, the oxyhemaglobin of the blood.

The bacteria that can grow only in the presence of relatively large amounts of oxygen are termed *aërobic*. In liquids the growth of such organisms will be confined to the surface. The organisms that can grow through a wide range with respect to the amount of oxygen, from a total absence to that of atmospheric air, are called *facultative*. If the organism grows better in the absence of free oxygen than in its presence, it is termed a *facultative aërobe*. If the reverse is true, the term *facultative anaërobe* is used. The facultative bacteria make up the greater part of the known species.

The cultivation of anaërobic bacteria is accomplished by replacing the air of the culture container with hydrogen, nitrogen, or carbon-dioxide, by absorbing the oxygen of the air with some substance such as an alkaline solution of pyrogallic acid. Most of them can be grown in appropriate culture media without the artificial exclusion of air. It seems probable that in the soil, and in other places in nature, they can grow in the presence of larger amounts of oxygen than will permit their growth in the laboratory.

The yeasts are facultative. It seems, however, that, while growth may go on for a period in the absence of oxygen, it can not continue for an indefinite time, as is apparently true with the anaërobic bacteria.

The molds are all aërobic, a fact that becomes of great importance in food preservation.

Reaction.—The true chemical reaction, the relative number of hydrogen ions as compared to hydroxyl ions, is a factor of great importance in influencing the growth of microörganisms. The bacteria, as a rule, grow best in neutral materials. The yeasts will grow in neutral substances,

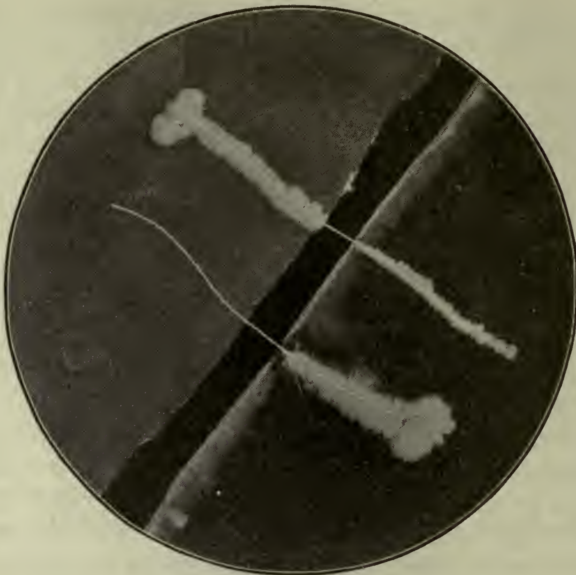

Fig. 17. Effect of Acid on Bacteria and Yeasts

The lighter portion of the plate contains an acid agar, the darker portion a neutral agar. Threads carrying bacteria and yeast respectively were placed on the plate. The bacteria on the lower thread grew only on the neutral agar while the yeast grew on both but more profusely on the acid medium

and also in those that are quite acid, as in fruit juices. The molds will tolerate a still greater acidity.

Light.—One ordinarily thinks of light as favorable to the development of animals. It is, of course, essential for the growth of the green plants, since from it they secure their energy. Any portion of the animal body not protected by a covering or by a pigment in the cells will be quickly injured by the direct rays of the sun. Sunburn is evidence of this action. By the development of pigment or the darkening of the skin, the injurious action of light is prevented. The cells of microörganisms are likewise easily destroyed by the direct rays of the sun. Artificial light is used for the destruction of bacteria in water. The source of light is the mercury-vapor lamp, which is especially high in violet and ultra-violet rays, which are most efficient in the destruction of bacteria. Glass prevents the

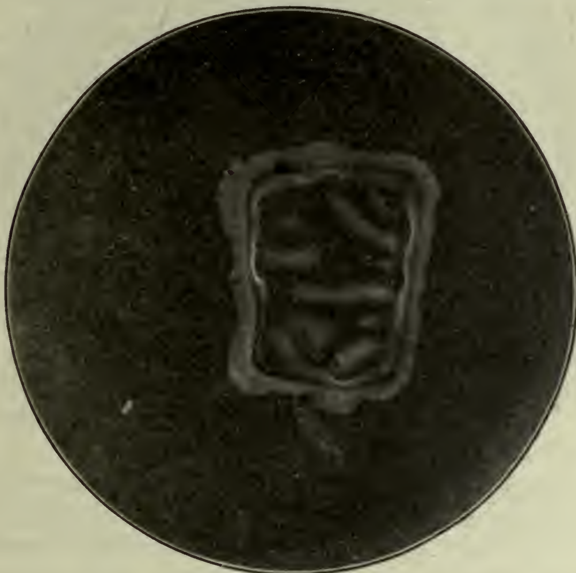

Fig. 18. Effect of Light

The agar plate was seeded over the entire surface with bacteria. The center of the plate was protected and the plate was exposed to the direct rays of the sun for one hour. All organisms except those protected from the sun have been killed.

passage of these rays to such an extent as to make a lamp with a glass tube of little value, while one with a quartz tube will be very effective.

The germicidal action of sunlight in the destruction of disease-producing organisms in houses and stables has been greatly over-emphasized. It requires but a thin layer of dust to destroy the effect of the light. Again, but a small portion of the walls inclosing any space is exposed to the sun.

Chemicals.—Many chemicals, both inorganic and organic, are toxic to animals. The same is true of microörganisms, and in a general way the same chemicals are injurious to both forms of life. The term *disinfectant* is applied to those substances that have a marked action on microörganisms; to those that have a less pronounced effect the term *antiseptic* is applied. The action of the latter may be limited to the prevention of growth. When such substances are used in foods, they are called *preservatives.* A discussion of the various chemicals used, in one connection or another, will be included later.

Food.—The food of bacteria, yeasts, and molds must be soluble in water, otherwise it can not pass into the cell. All naturally occurring organic matter serves as food for some kind of an organism; and yet, much of it is insoluble in water. The question then arises as to how the insoluble material can be used.

The animal ingests insoluble food and prepares it for passage through the intestinal wall by pouring into the alimentary tract at various levels agents that change the insoluble food into soluble compounds. These digestive agents are known as *enzymes.* One acts upon starch, another on protein, still another on fats. In fact, each animal is provided with such an array of enzymes as will enable it to use all the compounds of its normal food.

The cell of the microörganism may be compared to the animal in this respect. It forms enzymes that diffuse into the material in which the cell finds itself, and that change the insoluble compounds into soluble ones. This is a process of decomposition. It is a form of decomposition, however, in which energy is not dissipated. If it were, it would be a most wasteful process as far as the organism is

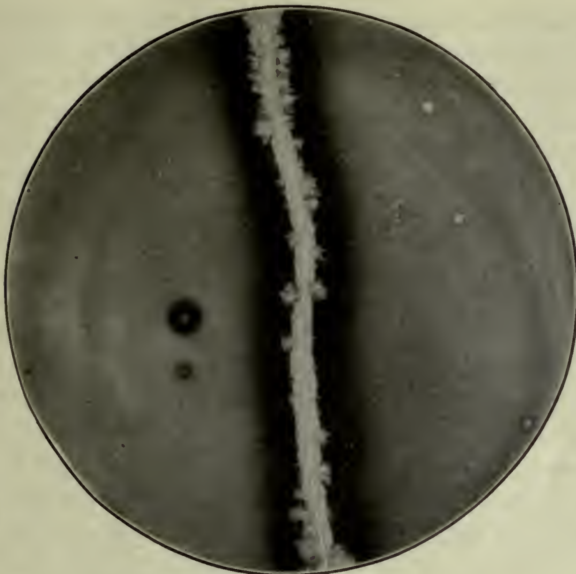

Fig. 19. Enzyme Action

The light portion of the plate is due to the presence of casein in the agar. The digesting enzyme has acted on the casein not only in contact with the bacteria which form the white streak, but on that a distance away. The enzyme has changed the casein to soluble products which can pass into the cells

concerned. The processes that go on inside the cell, and that are connected with the assimilation of the food, always involve a loss of energy.

The more varied the enzymes produced by an organism, the more varied may its food be. For example, no organism that does not produce an enzyme that can change gelatin into soluble compounds can utilize this substance as food. The classification and naming of enzymes is very confusing, and need not be introduced here. The enzymes that act on protein are commonly called proteolytic enzymes. Some can act on a number of different proteins, others on only a few.

The enzymes are always the product of life. They seem to stand between the living and the dead, in that they have some of the properties usually ascribed to life alone. They are destroyed by heat, and are injured or destroyed by those chemicals that have an injurious action on the cell itself. They are usually compared to the inorganic catalysts of the chemists, substances that favor in some way a chemical reaction, and yet do not enter directly into the reaction. This comparison comes from the fact that a minute quantity of an enzyme may act on a large amount of material—as, for example, one part of rennet may curdle one million parts of milk in a short time.

It is usually considered that the unicellular organism has no dependence on other cells of its own kind. This is probably not true. It seems probable that the enzymes formed by a cell do not serve the originating cell, but a later one. Many of the enzymes seem to be set free only on the death of the cell. If this is true, the popular concept of the independent existence of a unicellular organism is not the correct one in all cases.

In a pure culture of bacteria, the dead cells undergo dissolution. Since there are no outside sources for the decomposing agents, it is clear that they must come from the cells themselves. Such decomposition by the inherent enzymes of the cell, or mass of cells, is termed *autolysis*. It is noted especially in the dissolution of bacterial cells after spores have been formed. It is also met in the tissues of higher plants and animals.

The food requirements of microörganisms vary widely. Those that live on dead organic matter are called *saprophytic;* those that grow in the bodies of plants and animals are termed *parasitic*. Many of the bacteria that grow in the animal body are perfectly harmless to the host; such are called *commensal* organisms. The disease-producing organisms are parasitic. This classification of the organisms as they grow in nature does not hold under laboratory conditions, since many of the parasitic bacteria can be grown on dead matter. The parasitic organisms find favorable conditions for growth in the body of plant or animal. If these conditions can be duplicated or approximated in the laboratory, the organism will grow independent of the living host.

The bacteria are of far greater importance in causing decomposition than are the yeasts and molds. This rests largely on their cosmopolitan nature as far as growth is concerned. They grow over a wider range of temperature than any other group. They grow with or without air, and can make use of the most varied materials as food. These facts, coupled with the extreme rapidity of growth, insures the predominance of bacteria over other organisms whenever the reaction or concentration of the food material permits them to develop.

Metabiosis.—Organic matter is decomposed by the agency of all forms of life that do not use the energy directly from the sun. The decomposition is complete; each element is returned to that form in which it was when it was utilized by the green plant. No one form is able to cause the complete decomposition of a mixture of organic substances. The process is usually carried on by a sequence of forms, the second using the by-products of the first as food material. The by-products of the second supply food and energy for a third, and the process goes on, with a gradual dissipation of energy and the formation of more and more simple compounds. This action of a sequence of organisms is termed *metabiosis*. It is of great importance, not only in nature, but also in the fermentation industries.

The gradual degradation of organic matter by a series of organisms is usually known only in part, either with reference to the causal forms or to the chemical processes involved. In the decomposition of certain carbohydrates the process is more simple and may be followed in its essential details. Starch may be acted on by certain organisms through their enzymes, and a complex sugar formed, as maltose ($C_{12}H_{22}O_{11}$). The latter, by the same organism or by another, may be changed to a simpler sugar, glucose ($C_6H_{12}O_6$), which may be fermented by yeasts with the formation of carbon-dioxide and alcohol (C_2H_5OH). The former compound is available for the green plant. The latter may be used by certain bacteria which oxidize it to acetic acid ($C_2H_4O_2$), which, in its turn, is changed by molds to carbon-dioxide and water. The elements contained in the sugar are now all available to the green plant. It is not to be understood that this is the particular path always followed in the decomposition of starch. Innumerable deviations, both as to organisms and products, may occur. It is presented simply as an example of metabiosis.

The initial steps in the process described can be carried out under anaërobic conditions. The decomposition of acids is a process for which the molds are especially fitted. These are all aërobic. Therefore, if the process is to go on to completion, aërobic conditions must be established. It may be stated as a general fact that the initial processes in all decomposition may take place under anaërobic conditions, but that the final steps can go on only under aërobic conditions. This fact, which is of great importance in many practical relations, finds its explanation in that the initial steps are accomplished largely by the addition of water to the molecules to be broken up. This process is termed *hydrolysis*. A simple splitting up of the molecule may also occur. The final stages involve the addition of oxygen to the molecule. All of these processes are illustrated in the equations presented:

$2\ C_6H_{10}O_5 + H_2O = C_{12}H_{22}O_{11}$
Starch + Water = Maltose
$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$
Maltose + Water = Dextrose + Dextrose
$C_6H_{12}O_6 = 2\ C_2H_5OH + 2\ CO_2$
Dextrose = Ethyl Alcohol + Carbon-dioxide
$C_2H_5OH + O_2 = C_2H_4O_2 + H_2O$
Ethyl alcohol + Oxygen = Acetic acid + Water
$C_2H_4O_2 + 2\ O_2 = 2\ CO_2 + 2\ H_2O$
Acetic acid + Oxygen = Carbon-dioxide + Water

It must not be thought that these equations present the entire process. They simply present the chief reactions. For example, the following equation, $C_6H_{12}O_6 = 2\ C_2H_5OH + 2\ CO_2$, implies that all of the sugar appears as alcohol and carbon-dioxide. The true reaction is more like the following: Dextrose + Yeast = Alcohol + Carbon-dioxide + products of unknown nature + yeast ^{n}. In other words, a part of the sugar is used in the formation of numerous products other than the chief by-products, and another part of the sugar is used in building the new yeast cells, for decomposition goes on only as the organisms proliferate.

Relation of the organism to its by-products.—It is evident that, if an organism changes sugar to carbon-dioxide and water, it will derive more energy from a given amount of sugar than will another organism that forms an organic acid from the sugar. The organic acid contains much energy, while the carbon-dioxide and water do not. If the two organisms are to secure an equal amount of energy from sugar, the second must decompose a much greater amount of sugar than the first. With the organism that causes complete decomposition of the sugar the amount of by-products may not greatly exceed that of the mass of cells resulting from the decomposition. In other words, a large part of the food is used for the building of cells. This is made possible by the fact that the organism uses all the energy contained in its food. In the case of the organism that forms by-products that contain much energy, the weight of the cells will be insignificant in comparison to that of the by-products. The first type of organism will be of no value in the manufacture of products of industrial value, nor will it be of importance in disease production, except as it may cause mechanical injury. It is this property of partial decomposition of their food that gives to the bacteria and the yeasts their great importance in industrial fermentations, and that makes the bacteria such potent agents in the causation of disease. The animals and the strictly aërobic forms of microörganisms carry on the decomposition of their food to a very complete degree. In other words, their waste products are simple in composition, and contain little energy. It should be kept in mind that the microörganisms derive both building material and energy from their food. Certain constituents of the food may contain no energy, and can, of course, be used only for the formation of the cell substance. Other foods may serve both purposes. Indeed, the greater part of the food will be thus used. It is customary to speak of food for energy and of food for growth.

Classes of organic matter.—The greater part of vegetable and animal matter is to be included under the following great groups: carbohydrates, which include the celluloses, the starches, and the sugars, compounds containing carbon, hydrogen, and oxygen; proteins, which in addition to the above elements also contain nitrogen, sulphur, and sometimes phosphorus; and the fats, which contain the same elements as the carbohydrates. The latter are, however, less easily decomposed by microörganisms than are the carbohydrates. The chemical transformations that these organic compounds undergo as they are acted upon by microörganisms can be most clearly represented in connection with the applied phases of our subject. The cycle of each element is as important in the economy of nature as is that of any other element. The interest in decomposition processes is chiefly limited, however, to the cycles of carbon, nitrogen, and sulphur.

Distribution of microörganisms.—It is evident, from what has been said, that microörganisms will abound wherever organic matter is present and where other conditions will permit them to grow. The annual crop of organic matter finds its way to the soil, there to be destroyed by the microörganisms and by the lower forms of animal life. Virtually every soil yields sufficient vegetation to support an abundant crop of microörganisms. The soil is the most important of the habitats of the bacteria. In it are found the most varied kinds, both from the standpoint of morphology and physiology. The number, as determined by the plate-culture method, ranges from a few thousand per gram to several million; but it must be remembered that the number determined by the plate culture represents only a portion of the total bacterial flora of the soil. It is certain that each gram of cultivated soil contains hundreds of millions of organisms.

The number of bacteria in the soil decreases rapidly with the depth, so that at varying depths the soil ultimately becomes sterile. The decrease in bacteria with increasing depth is probably due to a number of factors, such as absence of free oxygen and lack of food. In the denser soils the filtering action is undoubtedly important. Liquids can be sterilized by passing them through a filter of unglazed porcelain. The soil acts in a similar manner, removing mechanically the bacteria from the water percolating through it. The soil also contains yeasts, molds, and protozoa in varying numbers.

The bacterial content of the ground water will depend on the depth of the stratum from which it comes. That derived from superficial layers will contain many bacteria, while that from the deeper levels is free from them. The surface waters vary widely in bacterial content, the chief determining factors being the amount of organic matter that they receive and the amount of soil carried by the water. The bacteria are found in the greatest depths of the ocean that have been examined.

Factors that keep the number of bacteria reduced are operative in both soil and water. The cycle of life in water is something as follows: Many of the protozoa feed upon bacteria; the protozoa in turn are used as food by the crustacea, which make up a large part of the food of many fish. If it were not for these restraining factors, many of our waterways into which sewage is discharged would become so offensive as to cause a nuisance.

Lack of food and moisture do not permit the growth of bacteria in the air. They are found there, however, in widely varying numbers. The soil is undoubtedly the chief point of origin. The greater the dust content of the air, the greater will be its content in microörganisms. The air has a characteristic bacterial flora, which consists of forms that are able to resist desiccation.

The number of dust particles in the air is greater in the city than in the open country. It has been shown that only a few of the dust particles carry bacteria. The studies of Whipple on the air of school-rooms showed an average dust content of 871,000 per cubic foot, and but 113 bacteria in the same volume.

The tissues of healthy animals are free, or relatively free, from bacteria; but within the alimentary tract they grow profusely at all levels, except within the stomach, where they are restrained by the acid of the stomach juices. In the intestine conditions are especially favorable with reference to temperature, moisture, food, and removal of by-products. The feces of all animals are especially high in bacteria. The bacteria are present in the superficial layers of the skin.

The tissues of plants are undoubtedly free from bacteria. They are present on the surface of the leaves in such numbers and vary so much from the normal soil flora that it is certain growth occurs thereon. The occurrence of bacteria on plants has been studied but little.

PART II

SOIL BACTERIOLOGY

CHAPTER VI

THE RELATION OF MICROÖRGANISMS TO SOIL FERTILITY

The basic work of the farmer is the growing of plants of economic value. All other work is incidental and insignificant in comparison with the growing of the green plant that is to supply food for man and for the animals he has brought to his service. The farmer's primary interest is therefore in the soil, as the place in which the plants he desires to cultivate will grow.

There are many factors that determine whether the soil is a place in which the plant can grow well and yield to the farmer a large return for his labor. The texture of the soil must be such that the roots of the plant can penetrate it freely, in order that they may draw food and water from a large volume of the soil. The water content of the soil must vary within certain limits. If it is too low, the growth of the plant will be checked; if it is too high, the factors preparing the food for the plant will be interfered with, and again growth will be retarded. The temperature must be favorable for the plant, and also for the agents that are active in making the food ready for it. Lastly, there must be a supply of food in fitting form for the crop. If the farmer is to be successful in the highest sense, he must control these various factors.

The control can not be absolute, but it may be exercised to a high degree. The farmer must have before his eyes not only the crop of the year, but of the next year, of the next decade, and of the coming century. He must so handle his soil that it shall be now and in the future a place in which the green plant will find favorable conditions for development. In the handling of the soil, the farmer must consider the multitudes of microörganisms that are growing therein.

Unavailable and available plant food.—The green plant obtains its carbon from the carbon-dioxide of the air, its oxygen from the same source, its hydrogen from water. Nitrogen, phosphorus, calcium, magnesium, potassium, sulphur, iron, and silicon must be supplied from the soil. The plant must be furnished with each of these elements in certain definite chemical compounds. One property that all these compounds must possess is solubility in water. The food must pass into the roots through the firm cell wall by the process of osmosis.

The great agricultural districts of the world are in the humid regions, where the rainfall is sufficient to allow the larger part of the water to pass through the soil until it reaches the so-called ground-water layer. The water, as it passes downward, tends to dissolve and transport with it all soluble soil constituents. The storage of plant food in a form available to the plant is incompatible with this condition. The food must be stored in an insoluble form, and thus be protected from the leaching action of water. This available food must be prepared for use by the plant through the action of some slowly acting factors, such as the solvent effect of water on materials that are ordinarily classed as insoluble. Weathering, the mechanical reduction in size of soil particles under the action of frost, and the effect of biochemical agents such as bacterial activity, are factors that release plant food for use by the green plant.

The fertility of any soil depends upon the amount of unavailable plant food stored therein, upon its potential fertility, and upon the rate at which the chemical, physical, and biological factors are preparing it for the green plant. Of these the biological factors are the most important. The various kinds of microörganisms feed upon the organic matter, changing it into simpler compounds, until the various elements are in a form usable by the green plant. At the beginning of each growing season there is a minimum of available plant food; hence the unavailable reserve must be changed into an available supply. This means that the farmer must grow a crop of microörganisms to prepare the way for the visible crop. The supply of food for his field crop, and therefore the yield of the same, depends in no small degree on the success with which he grows his microscopic crop.

In the newer portions of the world, the farmer pays little attention to any other source of plant food than the soil. In the older agricultural regions, the store of plant food has been partially exhausted. The plant must grow on the material the farmer adds to the soil in the form of organic matter. Under these conditions, the chief interest is in the efficiency of the soil organisms, and in their ability to transform the added potential plant food to the available food supply.

From this point of view the soil becomes a factory where multitudes of workers are working over the raw material, converting it into the finished food for the green plant. The soil must therefore be favorable for the growth of the microörganisms that are concerned in the cycles of carbon, nitrogen, sulphur, and phosphorus, the four main elements the plant derives from the soil. The lack of any one of these elements in compounds available to the plant will limit the growth of the crop. Nitrogen is most often the element that acts as a limiting factor to the development of the plant; for, in the final form in which it appears in the decomposition of organic matter, nitrates, it is easily leached from the soil.

The soil as a culture medium.—The amount and kind of bacterial growth in the soil, and therefore the quantity of food available to the green plant, will depend on many factors. The chief factor is the food supply. If large amounts of organic matter are added to the soil, as will be the case under natural conditions where the entire crop is returned to it, the amount of food will be large. If the crop is removed and sold in its entirety, as in the case in grass farming, the amount of organic matter returned to the soil will be reduced to a minimum. Again, a farmer may purchase feed, hay, and grain for the use of his animals, and by adding the manure produced to his fields, he increases the food supply of the bacteria in the soil. In market-gardening the addition, in the form of manure from the cities, of many tons of organic matter grown on the soil of distant farms permits a large development of bacteria, and therefore an increased crop of vegetables. Without a beginning there can be no end; without bacterial food in the soil there can be no food crop for man or beast.

Moisture and air.—The amount of water in the soil has an influence not only on the total amount of bacterial growth, but also on the types of organisms that may grow—a factor of great importance in determining the kinds of green plants that may develop in any soil.

The soil is a porous structure made up of minerals in varying states of subdivision. The interspaces in the soil may be completely filled with water, as in a water-logged soil. Under such conditions only facultative and anaërobic organisms can grow. If the soil spaces are filled with air, aërobic as well as facultative bacteria can develop. The so-called hydrostatic water is removed from the soil by gravity. It tends to carry with it the soluble constituents of the soil. Evaporation of moisture is constantly taking place at the surface. To replace this loss by evaporation, through capillary action of the soil particles, the water moves upward toward the surface. Ultimately a point is reached at which the water films surrounding the soil particles become so thin that this upward movement does not continue to replace the loss due to evaporation. Such a soil is said to be air-dry, and contains only hygroscopic moisture. It is doubtful whether bacteria can grow in such a soil. It is probable that the soil of humid regions never becomes so dry as to destroy any large portion of the non-spore-forming bacteria by desiccation.

In coarse-grained soils the extent of surface of the particles in a unit volume of the soil is much less than in a finer soil. It is thus evident that in a fine soil a given amount of water will form a thinner film, and be more tightly held by the soil particles, than will be the case with a coarse soil. A state of physiological dryness will therefore be reached at a higher water content in fine soil than in a coarse soil.

The air supply of the soil is in an inverse ratio to its water content. As the water is removed by drainage or evaporation, the pore spaces become filled with air. In coarse sandy soils the air supply is usually sufficient to favor rapid bacterial action. Indeed, often the farmer is desirous of limiting the air supply by packing and rolling the soil in order to retard decomposition. The ventilation of close-textured soils, owing to variations in atmospheric pressure and to the effect of wind, is not sufficient to cause any rapid replacement of the soil air laden with carbon-dioxide by atmospheric air. Under the most favorable natural conditions, the aëration of such soils is insufficient to permit the rapid growth of such pronounced aërobic organisms as the nitrifying bacteria, except at the immediate surface. In the close-textured soils the decomposition of organic matter is slow and often incomplete. Here the efforts of the farmer must be directed toward more perfect aëration. All processes of cultivation aid, as does drainage.

Temperature.—The temperature zone in which bacterial growth is possible is a wide one, extending from below 0° to 70° C. (32°–158° F.). Representatives of both psychrophilic and thermophilic bacteria are found in the soil. The great mass of bacteria in the soil are mesophilic, and are favored by relatively high temperatures, from 16° to 40° C. (61°–104° F.). It is customary to speak of cold or late soils, and of warm or early soils. The former are those of close texture, which in the spring become dry enough to cultivate only after a considerable period. Water has a high specific heat. A soil filled with water will therefore increase in temperature slowly as compared with one containing a smaller amount of moisture. The time required for the temperature of the soil to reach a point at which bacterial growth will be rapid will be prolonged in a close soil. The supply of available plant food is largely removed from the soil by the percolating water of fall, winter, and spring. Before the crop can make any marked growth, opportunity must be afforded for the soil organisms to form available plant food. This process will be retarded in a wet soil, hence the expression "late soil." The loss of water and decomposition of organic matter take place quickly in open-textured soils. The expression "early soils" is therefore a fitting one to apply to them. The gardener needs a loose, sandy soil for his early crops.

Reaction of the soil.—The bacteria are favored by a neutral or slightly alkaline rather than by an acid reaction. An acid reaction in the food medium is not injurious to yeasts and molds. The greater part of the elaboration of plant food from organic matter in the soil is occasioned by bacteria. It is, therefore, essential that the soils in which the acid reaction is pronounced be neutralized by the addition of lime, in order that the decomposition of the organic matter be favored, and hence conditions established for a luxuriant growth of the seeded crop.

The decomposition of organic matter under anaërobic conditions is incomplete; acids persist—thus marsh lands are often acid in reaction. By drainage of such lands, air is admitted and the growth of molds is made possible. These organisms will gradually destroy the acids, and the reaction of the soil will be changed in time so as to be more favorable for bacterial action and for plant growth. Drainage and cultivation may soon make a marsh soil a fitting place for plants that are very susceptible to acid.

Number of organisms in soil.—The number of bacteria in a soil, as has been shown, is dependent on many factors, chief among which is the amount of organic matter. The bacterial content of a sandy soil may be but a few hundred thousand per gram, that of a rich garden soil several million per gram. The bacterial content of soil is determined by the plate-culture method, using some particular kind of culture medium. It should be understood that only a portion, probably a very small portion, of the total bacterial flora of the soil is thus revealed; for only the bacteria that can grow on the medium used, and at the temperature and in the oxygen tension at which the cultures are kept, can develop. It seems probable that, in any soil that will permit the growth of higher plants, the bacteria are to be measured by the hundreds of millions per gram. The higher the fertility of the soil, the higher will be its bacterial content; for the bacteria are the direct cause of the former, through their elaboration of plant food.

The soil also contains many molds and yeasts. Little is known of the effect they produce in the soil. They are, however, to be considered as agents in the decomposition of organic matter and in the elaboration of plant food.

Protozoa, unicellular animals, are present in the soil in numbers reaching several thousand per gram. It is to be remembered that every animal is a factor in the decomposition of organic matter. The relatively large size of the protozoa makes them an appreciable factor in the elaboration of plant food. Many protozoa live on bacteria, and it has been claimed by some students of the soil that they are the cause of the reduced fertility of many soils, since they cause the destruction of certain classes of bacteria that are essential in the cycle of nitrogen. It is a well known fact that the bacterial content of surface waters is kept at a low level by the protozoa.

The macroscopic lower animal forms in and on the soil are likewise abundant and also function in the decomposition of organic matter. It has been shown that from ten to fifteen million insects of macroscopic size may be found on a single acre of meadow-land. Within the soil the common earthworm occurs in varying numbers. Determinations indicate that as high as 350 pounds per acre of these organisms may be found in the more fertile soils. All of these forms are living on complex, organic compounds, and are giving off simple waste products. They also function in the pulverization of organic matter, and thus make it more easily attacked by microörganisms. The earthworm is also of importance in the aëration of the soil through its burrows. The soil is passed through the alimentary tract of the earthworm, and is brought by them to the surface in their ''castings.'' This reworking of the soil particles materially improves the texture of the soil. When land is plowed that has been under grass for a number of years, it will be found to possess a granular structure, due to the action of earthworms and the roots of the higher plants.

The ordinary concept of the soil, that it is an inert mass of mineral matter, is therefore far from the fact. It is filled with the most varied kinds of living things of microscopic size, the work of which is as vital to animal existence as it is to the green plant. The farmer must care for these tiny inhabitants of the soil as well as for his crops and animals.

CHAPTER VII

THE DECOMPOSITION OF ORGANIC MATTER IN THE SOIL

Almost every conceivable organic compound is added to the soil in the plant and animal matter that finds its way therein. All is grist for the mill of the microörganisms of the soil. So far as is known, no substance is able to resist their action; even such resistant substances as chitin, horn, and gums ultimately disappear, as do the bones of animals that are placed in the upper layers of the soil. If, in some way, they have been deposited in the lower layers of the earth or in a place where they are protected from the action of microörganisms, they will persist for ages.

From the standpoint of the action of the decomposing matter on the fertility of the soil and from the viewpoint of the farmer, all organic matter may be divided into two great groups: first, those compounds that contain only carbon, hydrogen, and oxygen, of which the sugars and starches are examples; second, the nitrogen-containing substances that are best illustrated by the proteins. These latter compounds also contain sulphur and phosphorus, the cycles of which are of interest to the farmer.

The decomposition of all of these substances, with the formation of those compounds in which the various elements are available to the green plant, is an intricate process from the standpoint of the chemical reactions concerned and the organisms involved. Knowledge of the complete process is yet fragmentary and incomplete. Only a few general facts are known, and little of the details of the gradual degradation of organic matter by microörganisms.

No single form can act on an organic compound and change it into the final products of decomposition. Many forms are concerned in the complete decomposition of the simplest forms of organic matter. The various organisms concerned may bear different relations to one another. The most common and the most important of these relations is that already discussed, to which the term metabiosis is applied. It is to be noted, from the example of metabiosis given on page 59, that the processes of hydration are first involved, later dextrose is split into alcohol and carbon-dioxide, and this is followed by oxidation. The former processes can take place in the absence of free oxygen under the influence of facultative organisms. The completion of the decomposition process must be occasioned by aërobic forms. This fact is noted also in the decomposition of all organic matter; the final stages are always due to aërobic organisms. This fact implies the accumulation of partially decomposed organic matter under conditions where oxygen is not abundant, a phenomenon of the utmost importance in the soil.

The degradation of starch does not always occur on the lines laid down; from sugar, acids rather than alcohol may be formed. Lactic, acetic, formic, butyric, and propionic acids are the most common. The formation of acids from sugar and alcohol is chiefly due to bacteria, while yeasts are the prime factors in the production of alcohol.

It is to be noted that, in the decomposition of carbohydrates, acids of varying strength are formed, down to the weak carbonic acid. This accounts for the acid reaction in decomposing organic matter in which carbohydrates predominate over proteins, which in their decomposition yield ammonia. The reaction of decomposing protein will be alkaline, as exemplified in eggs and meats. It may be stated as a general truth that, in material consisting of a mixture of sugars and proteins, the former will be the first to be attacked in processes of decomposition, with the result that an acid reaction will be established which will prevent further decomposition, except as it may be occasioned by such organisms as the molds which use acid as food. If any condition, such as the absence of free oxygen, does not permit these aërobic forms to grow, the partially decomposed material will not suffer further action. This fact is of great practical importance in the soil.

In the decomposition of organic matter under the action of a sequence of forms, each of which obtains its building material and its energy from the food consumed, the material is gradually changed to a more stable form and its energy content reduced until it reaches a stage in which the various elements are available for the use of the green plant.

Humus.—The soil was originally formed from the disintegration of the rocks, and at first contained no organic matter. In the evolution of life, plants began to grow in the soil. Following their death, decay occurred. If the decomposition had been complete, the soil would have remained purely inorganic matter, since the final products of decomposition are readily soluble in water. If decomposition is not complete, a residue of partially decomposed organic matter remains in or on the ground. To this residue the term *humus* is applied. The content of the soil in humus varies from an almost complete absence to soils in which it is the most prominent constituent. The accumulation of humus is exceedingly slow. It has required many thousand years to accumulate the amount found in rich prairie soils. Under cultivation it is removed at an infinitely more rapid rate than it was formed.

The rate and extent at which humus accumulates bears an intimate relation to the aëration of the soil, and the opportunity for the growth of the aërobic organisms that are the most prominent agents in the final decomposition of organic matter. In marsh soils the accumulation of humus is great, due to the water-logged condition. The opposite extreme is noted in the coarse, sandy soils in which the air supply is at a maximum. Neither of these soils represents the highest type of fertility. Marsh soils are low in fertility, because the kind of compounds demanded by the most important cultivated crops have not been formed therein. Sands are relatively infertile, because no store of new plant food is accumulated. The maximum of fertility is attained in the loam soils, which contain enough oxygen to enable a portion of the organic matter to be completely decomposed, and yet permit of the gradual accumulation of humus.

All types of soils are much better aërated under cultivation than under natural conditions. The result is that the store of humus is gradually reduced and the fertility of the soil is diminished. The humus affects the fertility not only by forming a store of plant food, but by its effect on the water-holding capacity of the soil and on its texture. If the farmer does not return to the soil most of the organic matter it has produced, soil exhaustion is inevitable.

The maintenance of the humus content of the soil is one of the important problems of the farmer in the older regions of the world. The type of farming has much influence on this. If grass-growing is the chief industry, no organic matter is returned to the soil and the humus content will be constantly depleted. The same is true of grain-farming when no effort is made to return the straw to the soil, as is the case in the grain-growing sections of this country where the straw is burned. If the farm crops are fed to animals, and what they produce is sold in the form of dairy products, live stock, wool, and the like, while the manure is returned to the land, the tendency will be to maintain the humus content of the soil.

The crop grown on most cultivated lands is larger than the land would produce under natural conditions. In other words man by his tillage operations establishes more favorable conditions for the action of microörganisms in the soil than obtains in nature. This results in the more rapid change of the unavailable to available plant food, a process that hastens the reduction of the humus content.

The difference in the rate and completeness of the decomposition of organic matter in the various types of soils is shown when manure is added to them. An application of barnyard manure to a sandy field may show its effect in an increased crop only during the season in which it was applied, while in the case of a clay soil its effect may be noted for two or three years. Again, when it is desired to use land for the disposal of organic matter, as in the case of sewage, the best conditions are found in an open, sandy soil, in which the process will go on rapidly and completely with little or no accumulation of humus. In denser soils, a much larger area is required, as the applications can be made with less frequency, or the soil becomes clogged with the accumulation of organic matter which can not be decomposed by reason of the lack of growth of aërobic organisms. The disposal of sewage by conducting it on to the land is used only when areas of sandy land are available.

CHAPTER VIII

THE CYCLE OF CARBON

The cycle of carbon may be best presented in the decomposition of the carbohydrates. The carbon in fats and in proteins is ultimately changed into the same form as in carbohydrates, *e. g.* carbon-dioxide. Many of the decompositions of carbohydrates are of industrial importance and will be discussed later. Others can most conveniently be considered in connection with the soil processes.

During the growing season, the green plants draw heavily on the carbon-dioxide in the air. It is, of course, clear that, if agencies are not operating to return the carbon to the air as carbon-dioxide, the growth of green plants will ultimately be limited. The air contains from 0.03 to 0.04 per cent. of carbon-dioxide.

Every living form produces carbon-dioxide through its respiratory processes. The green plant during daylight hours is both producing and consuming this gas; the former is accomplished in respiration, the latter by photosynthesis. The two processes tend to balance each other. In the dark photosynthesis is stopped, while carbon-dioxide production goes on. All animals and fungus plants produce carbon-dioxide. The green plant robs the air of its carbon-dioxide; the rest of living things replace it, and thus life is able to continue its round. The decomposition of organic matter taking place in the soil abstracts oxygen from the soil air, and increases its content in carbon-dioxide, until as much as from 2 to 9 per cent. of this gas may be found in the soil. Poor ventilation of the soil tends to maintain the carbon-dioxide therein. This is dissolved in the soil water, thereby increasing materially its solvent effect on certain of the minerals of the soil.

Organic acids are always produced in the decomposition of carbohydrates. In poorly aërated soils these tend to accumulate, forming the raw or acid humus noted in marshy soils. Hydrogen and also methane may be formed. The latter is commonly called marsh-gas, from its formation in water-logged areas by anaërobic and facultative bacteria.

Cellulose decomposition.—The decomposition of certain carbohydrates is of special importance to the farmer. The great mass of plant tissue consists largely of cellulose, a compound that is insoluble in water and resistant to decomposition. The plant fibers, such as cotton and hemp which are used for industrial purposes, consist of cellulose. The larger portion of organic matter in barnyard manure also consists of cellulose. It is believed that cellulose is acted on by microörganisms with the formation of sugars, as in the case of starch. In the soil these are at once changed to still simpler compounds.

Quantities of cellulose are contained in the rough feed, hay and grass, consumed by animals. It has been found that ruminants can digest about 75 per cent. of the cellulose contained in the feed; horses about 50 per cent., man about 25 per cent. of that contained in young, tender plants, while the dog can not digest cellulose at all. As has been previously stated, all insoluble foods ingested by the animal are supposed to be changed to soluble compounds by the action of enzymes elaborated by the animal body. No enzyme capable of acting in cellulose has, as yet, been demonstrated in the animal body. The only explanation that can be offered is that bacteria, which change the cellulose to sugars, are present in the alimentary tracts of animals and that these sugars are utilized by the animal.

The animals that can digest cellulose most completely are those in which the food is retained in the body for a long period of time. In the case of the cow and the sheep this extends to six or seven days. In the laboratory the decomposition of cellulose is slow, but the conditions are not so favorable as in the animal, where the temperature and moisture conditions are at the optimum; the constant removal of the by-products is again of the utmost importance in determining the rate at which any biological process will be maintained.

Whether this is the only service to the animal that is rendered by the immense numbers of microörganisms growing in the intestinal tract of animals may be doubted. Many experiments have been made in the hope of determining whether the life of the higher animals would be possible without the presence of bacteria in the alimentary tract. It is very difficult to maintain an animal in a perfectly sterile condition and still keep it otherwise normal. The general conclusion to be drawn from such experiments as have been most successful is that, while the bacteria may not be necessary for the life of the higher animals, they are of great importance in aiding the animal to utilize its food, and that probably such action is not confined to the celluloses. The relation existing between the animal and the bacteria of the intestinal tract is one in which the animal is deriving some benefit. There is a more or less characteristic flora for each kind of animal. If this flora is replaced by an abnormal one, the helpful relation may be changed to one in which the animal is injured. It is believed that the condition known as *autointoxication* in man is due to the replacement of the normal acid-producing flora by one that acts primarily on proteins, with the production of poisonous substances that are absorbed from the alimentary tract and exert a cumulative effect on the animal. Metchnikoff, the Russian bacteriologist, also asserts that what are usually regarded as symptoms of old age in man are due to the gradual replacement of the normal flora by a harmful one.

Retting of fiber plants.—In the preparation of the fibers from such plants as hemp and flax, it is necessary to decompose the binding substances that hold the fibers together. These bodies are carbohydrates and are known as pectins. In the rotting or "retting" process, it is essential that the cellulose fibers shall not be acted on so as to weaken their strength. In the case of flax, the straw may be immersed in water for a short time, or it may be allowed to lie on the surface of the ground. The bacteria and molds quickly decompose the pectins, and by breaking the straw into short pieces the fiber may then be freed from the binding material. In some parts of the world certain streams have been found to be very favorable for the retting of flax. The river Lys in Belgium is a famous place for flax retting. The content of the water in pectin-decomposing organisms is supposed to be the explanation of the favorable action of this river. Efforts have been made to isolate pure cultures of the pectin-fermenting organisms, and to use them in the retting of flax in tanks, instead of exposing it in natural waters. Pectin-decomposing organisms play an important rôle in the spoiling of fruits and vegetables.

Oxidation of hydrogen and methane.—Hydrogen and methane are commonly formed in the decomposition of carbohydrates. Neither the hydrogen nor the carbon is here available for the green plant. Bacteria have been found that are able to oxidize such energy-containing gases, forming the ultimate decomposition products, carbon-dioxide and water.

CHAPTER IX

THE ACTION OF BACTERIA ON THE MINERALS OF THE SOIL

The mineral portion of the soil consists almost wholly of carbonates, sulphates, phosphates, chlorides, and silicates, which are, of course, relatively insoluble, otherwise they would be removed by the percolating water. The water that falls on the soil in the form of rain contains no mineral matter, or at most only traces; but drainage or well water contains considerable quantities of mineral matter in solution. It is thus apparent that processes are at work in the soil by which some of the minerals of the soil are being converted into soluble compounds. In all ground waters will be found carbonates, sulphates, nitrates, and chlorides of calcium, magnesium, potassium and sodium.

Probably none of the minerals of the soil is absolutely insoluble in pure water, but most of them are so slightly affected by the water as to be classed as insoluble. As has been shown, various organic acids are formed in the decomposition of organic matter. A strong acid, nitric acid, is also the final product in the decomposition of nitrogenous compounds. Ultimately all the carbon of organic matter appears as carbonic acid. These various acids are dissolved in the soil water, and influence its action on the minerals of the soil, which by their fine state of division present an enormous surface to it.

Calcium.—The limestone that is found in the soil was removed from the water of the sea by the action of the shell-forming animals. At the death of the animal the shell was deposited on the floor of the sea, and in time limestone deposits were produced. By some movement of the earth's crust the floor of the sea was lifted above the water-level and

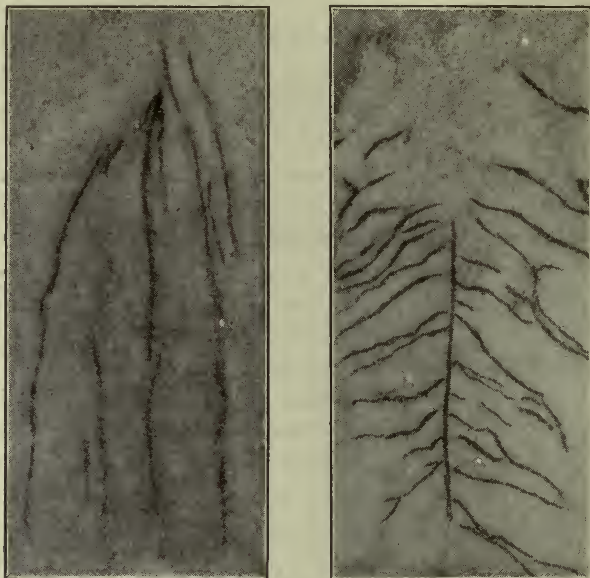

Fig. 20. Etching of Marble by Plant Roots

The picture on the left shows the solvent action of plant roots on a marble slab in the absence of bacteria; the one on the right the action in the presence of bacteria. The latter have formed acids from the material given off by the plant and have increased the solvent action on the calcium carbonate
After Fred.

subjected to weathering. These deposits were also ground by the action of the ice in the great glaciers that swept over a large part of the country, or they were disintegrated by the weather, so that a great mass of limestone found its way into the soil.

The carbon-dioxide dissolved in the soil water increases the solvent action of the water on the calcium carbonate, bringing it into solution in the form of calcium bicarbonate, which is removed in the drainage water. Thus ultimately all of the limestone that was formed in the sea is returned to it, largely by virtue of the indirect action of soil organisms. It is estimated that lime is being removed from our soils at the rate of from 400 to 1,000 pounds an acre each year. The increased amount of decomposition of organic matter due to cultivation of the soil hastens the removal of the limestone therefrom. Ultimately the lime is removed to such an extent that the soils tend to become acid, and limestone must be added. This again favors decomposition of the organic matter, and the removal of the lime is hastened.

It is improbable that the organic acids hasten to a great extent the removal of calcium carbonate from the soil. It is true that they will act on this mineral: salts of the respective acids which are soluble in water will be formed. The acid radicle of these salts will be decomposed and carbon-dioxide will be produced, which will combine with the calcium to form calcium carbonate again. The calcium nitrate formed in the soil may be leached therefrom. In the ocean this salt is decomposed by certain bacteria, forming therefrom gaseous nitrogen and calcium carbonate.

It is probably true that the calcium contained in organic matter appears as calcium carbonate at the end of the decomposition process. The amount thus formed is but a small fraction of that removed from the soil.

Lime is added to the soil in the form of ground limestone (carbonate of calcium, marl) or burned lime (calcium oxide). It is essential that lime be in as fine a state of division as possible, so that it can be mixed thoroughly with the soil and be brought in intimate contact with each soil grain.

Phosphorus.—Phosphorus is another element that is needed by the growing plant, and is often found in such small amounts in an available form as to limit the yield of the crop. It occurs in the soil in the form of calcium phosphate, iron, and aluminum phosphate, and in organic matter. All these forms are insoluble in water.

In the Southern States there are great deposits of calcium phosphate, or rock phosphate as it is often called. This rock is ground to a very fine powder which is sold under the name *floats*. This source of phosphatic fertilizer is the most important one. Superphosphate, which represents a more soluble form of phosphate, is often used. It is produced by treating the ground-rock phosphate with sulphuric acid, forming the acid phosphate. Phosphoric acid contains three atoms of hydrogen that can be replaced by such a base as calcium. The acid phosphates that are obtained when but one or two of the hydrogen atoms are replaced by calcium are soluble in water, while the normal phosphate is not. Superphosphate is added when a quick acting phosphatic fertilizer is desired.

If the insoluble phosphates in the soil are to be made available to the green plant, they must be rendered soluble by processes similar to those used by the manufacturer of superphosphate. The acids to accomplish the change must be those formed in the decomposition of the organic matter added to the soil. It is generally recommended that ground-rock phosphate be mixed with barnyard manure, or that it be applied directly to the land when the green manuring process is to be tried. It would be useless to add rock phosphate to a light sandy soil that is quite devoid of organic matter. The phosphorus in organic matter becomes available to the green plant on the decomposition of the material. The various chemical changes through which it passes are not known.

Potassium.—Potassium is found in the soil largely in the form of an insoluble silicate, which also contains aluminum. A soil may contain thousands of pounds of this potassium compound to an acre and yet may respond to the application of soluble potassium salts. It is believed that the by-products of bacterial action have an effect upon the insoluble compounds, tending to bring some of the potassium into soluble form. For example, the bicarbonate of lime is supposed to react with the aluminum-potassium salt, forming potassium bicarbonate. It is probable that stronger organic acids formed in the decomposition of organic matter exert a solvent action on the potassium compounds of the soil.

It is essential to have soluble potassium compounds not only for the green plants but for some of the important classes of bacteria in the soil.

Sulphur.—Sulphur is an essential constituent of every plant or animal cell. The green plants derive their supply from the sulphates of the soil. The amount of sulphur needed is so small that in most soils there is an abundant supply for the needs of the crop. Some soils, however, respond to the application of sulphur. It is probable that the favorable action of superphosphate is sometimes due to the sulphur it contains.

The sulphur of organic matter appears as a sulphide when the material undergoes decomposition. When the decomposing material is high in sulphur, as in the case of the egg, the odor of this gas is very apparent. The odor may also be noticeable in sewage, in which the formation of hydrogen sulphide can be easily shown by adding a little ferrous sulphate. The iron is precipitated as iron sulphide, which is black in color.

Hydrogen sulphide contains much energy and forms a source from which certain bacteria derive the energy for growth. They oxidize the sulphide to free sulphur, which may be stored in their cells to be further oxidized to sulphuric acid, which will combine with bases to form sulphates, producing compounds in which the sulphur is available to the green plant. Such bacteria are found in the soil, and in great abundance in sulphur springs. Their action results in the formation of a strong mineral acid, which exerts a solvent action on many of the soil minerals. Sulphur is lost in the drainage water in the form of sulphates.

The reduction of sulphates by bacteria is a common process when sea water is mixed with water carrying organic matter. Such conditions occur when sewage is discharged into tidal rivers. The dissolved oxygen of the water is soon exhausted by the bacteria feeding on the organic matter. The facultative bacteria must utilize some other source of oxygen, and find a supply in the sulphates, which are thus reduced to sulphides. This process may be so pronounced as to create a nuisance in cities located on the sea.

Iron.—Iron is often present in ground water as ferrous carbonate. Certain bacteria known as the iron bacteria facilitate the precipitation of the iron from the water. The insoluble iron accumulates in the sheaths of the bacteria, among which many of the higher bacteria are to be noted. Some of the iron bacteria have peculiar forms, such as flat, twisted ribbons.

It is believed that they have been the causal agents in the deposition of many of the great iron-ore deposits such as are found in northern Minnesota. They often cause the accumulation of a deposit on the inside of water-mains through which an iron-bearing water passes. They also may cause the plugging of drain-tile in marshy land. The acids produced from the decomposition of organic matter dissolve the iron, which is carried by the percolating water into the drains, there to be precipitated. These bacteria are often to be noted in masses in iron springs.

It is quite probable that the soil bacteria influence the tilth of the soil; that they influence the loss of moisture from it; and that they are the cause of the earthy odor that is so characteristic. This is due to volatile substances formed in the decomposition of the organic matter in the soil.

CHAPTER X

THE CYCLE OF NITROGEN

While it is perhaps impossible to characterize any one process as more essential than some other, where all are necessary steps in a complex relationship, it is undoubtedly true that the cycle which nitrogen undergoes in nature is invested with the greatest interest of any of the elements, because of the intricacy of the changes involved, their dependence on one another, the completeness with which the various steps have been traced, and the possibility of controlling by scientific knowledge the progress of these changes.

Nitrogen is constantly being removed from the soil by growing plants, and it is essential that in some manner the nitrogen be returned to the soil and again made available to the plant. In the discussion of the cycle of carbon it was shown that not only were microörganisms instrumental in the return of the carbon of organic matter to a form in which the plant can again make use of it, but that both animals and plants are giving off carbon-dioxide as a product of their respiration. In the case of nitrogen it will be seen that microörganisms are the only agents by which the nitrogen in plant and animal matter can be made available to the green plant.

The amount of free nitrogen in the world is enormous. The air contains 80 per cent. of this element. Over every acre there are 35,000 tons of this gas. Nitrogen is an inert element and does not readily enter into combination with other elements, such as the oxygen of the air; but there are both chemical and biological methods of bringing nitrogen into combination. The difficulty of bringing about these reactions is indicated by the fact that, in spite of the immense amount of free nitrogen, combined nitrogen, under existing commercial conditions, costs considerably more than any other food element.

The nitrogen of the soil.—The store of nitrogen in the soil is in the humus, the residue of the organic matter that has undergone decomposition in the soil. The content of average arable soils in nitrogen is from 0.1 to 0.2 per cent., but the nitrogen in humus is not available to the plant, because the humus is insoluble. It is a very fortunate provision of nature that a portion of the nitrogen that has been added to the soil in the organic matter has been thus stored. If it were all immediately available, that which was not used by the growing plant would be quickly leached from the soil in the drainage water.

Under natural conditions, some nitrogen is lost in the drainage water. As will be seen later, factors are operating in every soil by which combined nitrogen is added to it. Where the entire crop is returned to the land, as is true under uncultivated conditions, the nitrogen content of most soils slowly increases. Thus has accumulated through many thousands of years the present nitrogen supply of our soils. Under cultivation, the removal of the crop and the loss by leaching more than balance the gain, and the soil becomes depleted of nitrogen. There comes a time when nitrogenous fertilizers must be added to maintain the crop-producing power. This addition may be made in a variety of ways. Besides the plant residues, animal material may be purchased, such as blood and bone meal, fish scrap, wool waste, etc. Guano, the excrement of birds, originally formed an important source of nitrogenous fertilizers. Besides natural plant and animal material, ammonium sulphate and sodium nitrate are used as fertilizers.

It is probable that plants can make use of nitrogen in a number of different compounds, varying with the kind of plant. Some plants, as potatoes and rice, can use ammoniacal nitrogen; oats make use of either ammoniacal or nitrate nitrogen, showing no preference for either; while corn and beets must have their nitrogen needs supplied in the form of nitrates. Most of our cultivated plants demand all or a portion of their nitrogen in the form of nitrates, and can make a normal growth only in their presence. It is thus essential that the nitrogen added to the soil be changed from protein nitrogen to nitrate nitrogen before it can be generally available. This process can be effected only by the action of the microorganisms of the soil.

Ammonification.—This term is applied to that portion of the cycle of nitrogen in which protein nitrogen is changed to ammonia. The proteins are very complex substances of high molecular weight. Legumin, the characteristic protein of leguminous plants, has been held to have the following formula: $C_{718}H_{1158}O_{238}N_{214}S_2$. The path from this complex molecule to carbon-dioxide, water, hydrogen sulphide, and ammonia is a long and largely unknown one, both chemically and biologically.

The organisms that use the native proteins as food must form proteolytic enzymes which will change the protein into more soluble and diffusible compounds that can pass into the cells. The intermediate products are divided into three great classes, the proteoses, the peptones, and the amino acids, the simplest of which is glycocoll or amino-acetic acid, having the formula $CH_2(NH_2)COOH$. From such compounds ammonia is easily formed.

A great number of molds and bacteria, aërobic, facultative, and anaërobic, can form ammonia from protein. The process can thus go on in the most open soils, and also in those that are constantly saturated with water. The conditions that favor the process are those that favor bacterial growth in general, a temperature from 20 ° to 40 ° C., (68 °–104 ° F.), an abundant air supply, and a neutral reaction.

The rapidity with which the process proceeds is largely dependent on the material undergoing decomposition. Some nitrogenous fertilizers are quickly ammonified, as dried blood, ground fish, and tankage; while cotton-seed meal and leather wastes are very resistant to decomposition. A material resistant to decomposition can not be used with success as a source of nitrogen for a quickly maturing crop.

The importance of ammonification is seen when it is remembered that every atom of nitrogen built into the tissues of the green plant must be converted into ammonia before it can again be used by the plant, either as ammonia or as nitrate nitrogen. Since the process of ammonification is an essential step in the cycle of nitrogen, it is necessary that it go on at such a rate that the plant crop will not be limited by the lack of nitrogen in an available form. The establishment of a neutral reaction by the addition of lime, the aëration of the soil by the removal of water by drainage and by cultivation, are conditions that favor the process of ammonification. One of the theories of diminished soil fertility seeks to account for reduced yields by the destruction of the ammonifying bacteria by the protozoa in the soil. The partial sterilization of soil by heat or by volatile antiseptics, such as carbon-disulphide, often results in an increased plant growth. It has been believed by some that in such treatment of the soil the protozoa were destroyed, while the development of the ammonifying bacteria went on unrestrained; hence a greater amount of nitrogen became available for the crop.

The nitrogen that has been built into the tissues of the animal body is broken down into simpler compounds as a result of the metabolic activities of animals. The nitrogen waste from animals is eliminated in the urine in the form of urea, hippuric acid, and uric acid. The amount and relative proportions of these three compounds vary in the different animals. It is estimated that 60 per cent. of the nitrogen of the food is eliminated by the horse in urine, 42 per cent. in the case of sheep, and 31 per cent. in cattle. In all cases the nitrogen in the three compounds mentioned is changed into ammonia by means of a group of bacteria that are most often termed the *urea-fermenting* organisms. They differ widely in their form and structure, but all have the common property of forming an enzyme, called *urease,* which changes the urea to ammonium carbonate. This group is found in the soil and in the solid excrement of animals. The mixing of the solid and liquid excrement results in the rapid change of the urea and allied compounds to ammonia. The strong odor of ammonia often noticed in horse stalls is due to the fermentation of the urea.

The urea-fermenting bacteria require oxygen for their growth. They are also favored by a strong alkaline reaction, and are able to continue growth in the presence of quantities of ammonia that would quickly stop the growth of the common putrefactive bacteria. The urine of herbivorous animals is alkaline in reaction, while that of man and the carnivorous animals is acid. The former is a better medium for growth of the urea-fermenting organisms. Uric acid is changed directly to ammonium carbonate or to urea and then to ammonia. Hippuric acid is likewise fermented with the formation of ammonium carbonate, or first may be changed to benzoic acid and glycocoll. The latter is then ammonified. The ease with which the ammonification of the nitrogenous bodies in urine takes place accounts for their high availability as nitrogenous fertilizers.

Nitrification.—While some of the plants having economic value can make use of ammonia, others can not, and it is necessary to have the ammonia oxidized to nitric acid. This process can be accomplished by purely chemical means. Many porous substances have the property of occluding gases or of condensing them. The modification of platinum known as spongy platinum has this property in a high degree. If ammonia is brought in contact with spongy platinum, it will be oxidized to nitric acid.

Before anything was known of the activity of bacteria in such processes, it was thought that the soil represented such a porous medium. This view was strengthened by the manner in which nitrates were made in earlier times. Large amounts were needed for the gunpowder used in the almost unceasing war operations. The natural deposits then known were insufficient for these purposes, hence the necessity of manufacturing nitrates arose. This was accomplished by mixing organic matter with earth. The mixture was piled about brushwood, which served to make the pile more porous. The pile was kept moist, and a neutral reaction maintained by the addition of limestone to the mixture of soil and organic matter. After a period the pile was leached with water, and the nitrates that had in some manner been formed were recovered. These piles were known as saltpeter plantations. The calcium or sodium nitrate thus obtained could be changed to potassium nitrate by treatment with the lye leached from wood ashes. Until the discovery of the great deposits of sodium nitrate in Chile, the larger part of the nitrates used in the industries were thus prepared. The Chinese are still using the process. Nitrates can also be obtained by the leaching of the soil to which large quantities of organic matter have been added and which has been protected from leaching. During the Civil War the Confederate States were forced to leach the soil beneath old tobacco barns. The Chinese remove the dirt floors from their houses and dissolve the nitrate that has there accumulated.

Many of the characteristics of the change of ammonia to nitric acid in the soil related the process to a biological cause. The causal bacteria were isolated by Winogradsky in 1889. He confirmed earlier observations that the change of ammonia to nitric acid is not a single chemical change, but involves two steps: the ammonia is first oxidized to nitrous acid, and this substance further oxidized to nitric acid. Winogradsky isolated two groups of bacteria concerned in these changes. The first could grow only when ammonia was available to it. This compound represented the sole source of energy and of nitrogen for the organism. The second group was limited to the nitrous acid formed by the first for its energy and nitrogen. The change of ammonia to nitric acid is an example of metabiosis. The acids formed, of course, unite with bases to form neutral salts, nitrites, and nitrates.

The nitrous acid-forming bacteria differ in their morphology in various parts of the world, but apparently all are invested with the same peculiar physiological characteristics. Earlier it was thought that the presence of chlorophyl was essential for the use of carbon-dioxide as a source of carbon, and that no other type of life than the green plant was able to utilize so stable a substance as carbon-dioxide. The nitrifying bacteria are able to grow in the total absence of carbon, except in the form of carbon-dioxide. Winogradsky showed that the energy needed for the process is

Fig. 21. Effect of Nitrifying Bacteria on the Growth of Barley

In the absence of nitrogen in the soil the growth of the plant has been small. The addition of sulphate of ammonia has resulted in a far better growth. The addition of the same substance and of nitrifying bacteria that change the ammoniacal nitrogen to nitric nitrogen has enabled the plant to make a normal growth

After Fred.

obtained through the oxidation of ammonia or nitrous acid, depending on the group of nitrifying bacteria concerned. The nutrient solution must contain the potassium, sulphur, and phosphorus needed for the structure of the bacteria, but these may be present in inorganic form. The ammonia in the soil or in the nutrient solution may be in the form of a neutral salt, such as ammonium sulphate. Through the activity of the bacteria, an alkaline-reacting radicle, ammonium, is changed to an acid—nitrous acid. This, together with the sulphate radicle, causes the rapid increase of acidity in the soil or in the solution in which the first step in nitrification is going on. It is essential to have a neutralizing agent present, such as calcium carbonate, else the process will soon stop. An abundant supply of oxygen is also essential, as it is for all oxidizing processes. The organisms grow best at about 30 ° to 35 ° C., (86 °– 95 ° F.).

Nitrification in the soil.—If nitrification is to go on rapidly in the soil, conditions that will permit the rapid growth of the causal bacteria must be established. One of the most important conditions is a well aërated soil. If the pores are filled with water, anaërobic conditions prevail and no nitrification can go on. In those soils that naturally are well drained, as sandy soils, or in which the excess of water is removed by drainage or otherwise, the air supply will be greater, and other conditions being equal, the oxidation of the ammonia formed by the ammonifying bacteria will go on quickly. It is difficult to establish the most favorable condition with reference to oxygen in our soils. Only by frequent cultivation can the nitrifying process be kept at its maximum. The formation of nitrates is most rapid in the soil on which a cultivated crop is growing, such as corn or roots. This accounts, in part at least, for the large amount of dry matter these crops will produce per acre. In the making of composts the frequent stirring of the pile favors the process of ammonification, and of nitrification especially.

Nitrification goes on very slowly in acid soils, such as marsh or peat soils. If these are treated with lime in such quantities as to establish an alkaline reaction, the formation of nitrates will be greatly increased. In water-logged soils the decomposition of the organic matter is incomplete, and the acid produced accumulates. The removal of the water by drainage permits the air to enter, and thus gives opportunity for the growth of aërobic microörganisms, such as molds, that will decompose the acids, making the soil a better home for the nitrifying bacteria.

The nitrification process goes on slowly at low temperatures. It is probable that it continues as long as the soil is not frozen.

Conservation of nitrogen.—When moisture and temperature conditions are most favorable for crop production, the yield is often limited by the lack of sufficient quantities of some one element. Generally speaking, this limiting element is most often nitrogen. It is again probable that the ammonia is oxidized as rapidly as it is formed, but not sufficient ammonia is formed for the needs of the crop. As has been indicated, the process of ammonification is favored by the same conditions as are known to favor the process of nitrification. The plant leads a sort of hand-to-mouth existence, as far as the supply of nitrates is concerned, since in the growing season the nitrates are used as fast as they are formed. After the crop is removed the process of decomposition continues, and the nitrates accumulate in the soil, to be removed in the wet periods of the fall, winter and spring.

Since nitrogen is most frequently the element limiting the growth of the crop, and since the store of nitrogen in the soil is none too large, it is essential that the farmer use every means to conserve the nitrogen supply of the soil. Much can be done in this regard by keeping a crop on the land constantly. For example, after the removal of corn the land may be planted to rye, which will use up the nitrates in the soil. If this crop is plowed under in the spring, the organic matter will decompose, and the nitrogen be made available for the coming crop. It has been determined that four times as much nitrogen is lost in the drainage water as is removed in the crop. This loss is particularly heavy in the South, where the long exposure of the soil to the winter rains gives a most favorable opportunity for leaching.

The fallow method of handling the soil results in the establishment of favorable conditions for decomposition, because of the well aërated condition of the soil and the retention of moisture in the summer months. Plant food thus accumulates in the form of nitrates, so that when a crop of winter wheat or rye is sown in the fall, rapid growth occurs.

Nitrate deposits.—Almost all of the nitrate used in the industries and as a fertilizer is obtained from natural deposits in Chile. It is believed that the deposits are due to the accumulation of large amounts of organic matter in some arm of the sea. This was raised above sea-level, and underwent decomposition in a region in which the rainfall was not sufficient to leach the nitrate into the deeper levels of the soil, so it accumulated in some such manner as it is now accumulating in some parts of the West. In sections of Colorado the nitrate content of orchards and of fields has become so high as to destroy all vegetation.

Denitrification.—Nitrogen is removed from the reach of green plants by both chemical and biological processes. For example, in the discharge of explosives of all kinds, which contain as a rule great amounts of nitrogen, this gas is set free. The nitrates in the soil may be destroyed by bacteria. These processes are termed *denitrification*. There are still other processes by which the nitrogen is not lost from combination, but is changed into forms in which it is not available to the plant. In the absence of air and in the presence of organic matter, many bacteria can use the oxygen contained in nitrates for their respiratory processes, as the ordinary anaërobic bacteria use the oxygen of sugar. This ability to reduce nitrates to nitrites and to ammonia is a very common property of bacteria, and is made use of in the detailed study of organisms. When aërobic conditions are restored in the soil, the ammonia and nitrites will be reoxidized by the nitrifying bacteria. There is no loss of nitrogen in the process, except such as may occur in a secondary reaction that may take place between the nitrites and ammonia in which the nitrogen of both compounds is set free. It is not certain that this secondary reaction is of any importance in the soil, although it may be elsewhere, as in certain methods of sewage disposal.

A much smaller number of bacteria are able to reduce nitrates to free nitrogen. The conditions necessary for the process are first the presence of nitrate, second a supply of organic matter, and third an absence of free oxygen. The organic matter is essential to furnish the energy needed to decompose the nitrate. It was seen that the nitrifying bacteria obtain energy from the oxidation of ammonia to nitrites and nitrates. If energy is set free in a chemical reaction, energy will need to be absorbed to carry on the reverse operation. In the presence of air these organisms use the free oxygen and leave the nitrate untouched.

The denitrifying bacteria are found in the soil and in manures, especially in horse manure. It is not believed that the process is of great economic importance, since conditions in the soil essential for the process do not obtain for any length of time. If nitrates were added to a rich soil or were applied simultaneously with barnyard manure, a portion of the nitrogen might be lost; but in ordinary soils no great loss of nitrogen can occur because of this process.

Many soil bacteria can obtain the nitrogen needed to build their cells from nitrates. If any considerable amount of growth of the organisms takes place at the same time the demand of the crop for nitrate is greatest, the crop may be limited in its growth, since nitrogen is most frequently the limiting element. There would be the same objection to these bacteria as to weeds among a cultivated crop, namely, the removal of food that might otherwise be used by the crop. These bacteria ultimately die and the nitrogen is ammonified, so they do not permanently remove the nitrogen from the soil, but merely take it temporarily from the reach of the plant.

CHAPTER XI

BARNYARD MANURES AND SEWAGE DISPOSAL

Manures.—One of the most important by-products of the farm, as far as fertility of the soil is concerned, is the excrement of farm animals and the litter that is used as an absorbent in the stalls. Manure contains the four elements necessary for the maintenance of fertility of the soil—nitrogen, phosphorus, potassium, and sulphur. None of these is available for the plant in the form in which it is excreted by the animal, but must undergo decomposition by microorganisms. Fresh manure is harmful to plants rather than helpful. The elements must pass through the cycle of changes that have been previously detailed.

The farmer desires to conserve the value of the manure as far as possible, and should handle it in such a manner that he may return to the soil the maximum amount of the fertilizing elements. Loss of the elements may occur by leaching of the piles. This is true for all the elements. Nitrogen may be lost by being converted into volatile substances. The farmer should also remember that organic matter is of value to the soil as a source of humus and to furnish energy for classes of bacteria yet to be described.

From the standpoint of the kinds of microörganisms that grow in manures, this animal refuse may be divided into two classes, the basis of division being the amount of water it contains. Horse and sheep manure contain a smaller amount of water than cow and hog manures. They are also more porous and lose water more rapidly. The solid excrement of the cow dries slowly. The lack of moisture and the porosity of manures from the horse and sheep permit the introduction of air, and hence favor the growth of aërobic organisms, especially molds. The respiration of the aërobic forms results in the production of heat, which is not readily radiated on account of the non-conductivity of the organic matter. As the temperature increases the more rapid growth of the organisms is made possible. This growth continues until the decomposition of the manure is complete and the loss of nitrogen and organic matter is marked. These so-called hot manures are subject to fire-fanging. The loss of organic matter can be prevented by the close packing of the piles to exclude air, or by the addition of water. In the absence of the air the decomposition will be due to anaërobic forms, and while the rotting will be complete in that the vegetable matter loses its identity, there is not so great a loss as under aërobic conditions.

Cow and hog manure are cold manures on account of their high moisture content and their close texture, giving no opportunity for air to penetrate. These manures do not overheat or fire-fang. In two piles of manure of the same composition, one of which was piled loosely while the other was closely packed, the following losses were noted. The loss of nitrogen from the loose pile amounted to 34 per cent. and the loss of organic matter to 53 per cent., while from the closely packed pile the loss of nitrogen was 28 per cent. and of organic matter the same.

The conservation of the fertilizing value of manures involves the stopping of the processes of decomposition short of completion. The last steps in decomposition are, as has been mentioned, always due to aërobic organisms. The anaërobic organisms will disintegrate the fibrous materials of the manure and enable it to be easily distributed over the soil. In the anaërobic processes a considerable portion of nitrogen, phosphorus, potassium, and sulphur in the manure is converted into soluble products. The decomposing manure should, therefore, be protected from leaching. Thoroughly packed piles that expose the minimum of surface tend to conserve the value of the manure. The accumulation of manure in deep stalls in which sufficient litter is used to absorb the liquid manure, and in which the constant tramping of the animals excludes the air, is undoubtedly the best way of handling manure. It can best be used with sheep and feeding cattle rather than with dairy cows. The direct application of the fresh manure to the land is also an excellent method of conserving its fertilizing value.

Sewage disposal.—The disposal of the waste material of man represents an important problem to the family living in the country. When great numbers of people are crowded together, as in cities, and when to the household waste is added the waste of great industrial establishments like the packing-houses of Chicago, the problem becomes still more important. The organic matter in this material, ordinarily called sewage, must be decomposed by the action of microörganisms into the simple mineral substances, the salts of nitric, phosphoric, and sulphuric acids. The decomposition of the organic matter must be so controlled that it shall not become a nuisance or injurious to health.

It is, of course, desirable, from the standpoint of conservation of the elements having great fertilizing value, that the organic matter be returned to the soil. In many of the larger Oriental cities, the night soil is collected and carried to the cultivated lands near the city. This process, commendable as it may be from the standpoint of the conservation of plant food, can be used only where human labor is cheap.

The American and European cities use water as a vehicle to transport the sewage from the point of production through the sewers to the place of disposal. A considerable amount of decomposition occurs in the sewers as the sewage flows through them. The larger amount of decomposition must, however, occur in the soil, in water-courses or in artificial disposal plants. In all, the same organisms function. The sewage may be flooded over the land in a manner comparable to the application of water in irrigation. The water leaches through the soil, leaving the organic matter behind, there to be decomposed by the soil organisms. Sandy land is desirable for the successful use of this method of sewage disposal. From the open soil the water passes quickly, while the air drawn into the pore spaces facilitates the decomposition process. Within a short time this change is effected. If a fresh application of sewage is then made, the soluble products resulting from the first will be removed, and another quantity of organic matter will be left in the soil.

The process can be continued indefinitely; for, owing to the highly aërobic condition of the sand, decomposition is complete. If an attempt is made to employ a close-grained soil for such purposes, failure will result. The organic matter left by the percolating sewage will not be completely decomposed, because of the small supply of oxygen, and the residue will increase in amount. Soon the land will become of no value for the disposal of sewage. The cities of Berlin and Paris dispose of a portion of their sewage by applying it to sandy land. The farm home can use this same method with success if it is provided with a modern water supply, so that water can be employed to carry the sewage on to the land.

In most cities the sewage is turned into a body of water, and the decomposition processes occur in the liquid rather than in the soil. If the body of water is large in proportion to the volume of sewage, the decomposition will take place without the production of objectionable odors or without injury to the water life. If the amount of sewage is large in proportion to the water, the organisms will soon exhaust the oxygen, and the decomposition will not be complete. Products having offensive odors and an injurious action on the higher animal forms of the water will be produced.

The application of sewage to the land increases its fertility. On the sewage farms great crops of grasses and roots may be raised. The addition of sewage to a stream will, of course, produce an increase in the number of bacteria. This will cause an increase in the forms that live on bacteria, such as the protozoa. The greater number of these low animal forms will cause an increase in the crustacea that serve as food for fish. Hence the addition of not too large quantities of sewage to a body of water usually results in an increase in the number of fish. If these are consumed as food, some portion of the organic matter is again made use of by man, and is not wholly lost. The farm home can make use of this method for the disposal of its sewage if a body of water of some size is available. In case any city draws its water supply from this source, it should not be used for the disposal of household sewage, because of the danger of spreading typhoid fever, as will be explained later.

If the sewage is allowed to undergo partial or complete decomposition before it is discharged into a body of water, a much greater amount of sewage can be added to the water without injuring it in any way. Since it is not easy to establish conditions so that the decomposition can be carried out by aërobic organisms, as in the soil, the larger part of the decomposition is allowed to take place under anaërobic conditions in large tanks, called *septic* tanks because the processes are carried on by bacteria. The tanks are so arranged that the sewage flows slowly through them; the solid matter settles on the bottom and forms a sludge. There soon accumulates on the surface a scum in which a portion of the gases formed in the decomposition is held. This excludes the air in a very perfect manner. If the

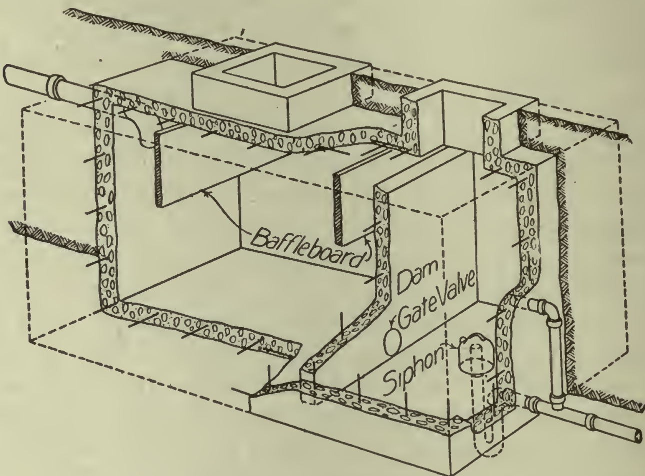

Fig. 22. A Septic Tank

The larger compartment is the septic tank proper in which the organic matter of the sewage is exposed to bacterial action. The second compartment or dosing chamber is gradually filled from the septic tank and then emptied by the automatic syphon into tile drains

sewage is allowed to remain in the tank from twenty-four to forty-eight hours, the solid matter will be liquefied, the protein changed to ammonia, the carbohydrates to acids and gases. The tanks are frequently called *digestion* tanks because of the nature of the changes that go on in them. The effluent from the tank is turbid and has a disagreeable odor. This partially decomposed material is often turned into a body of water, or it may be so treated that the decomposition will be more complete. The more complete changes, which include such processes as the oxidation of ammonia to nitrates, can go on only in the presence of air. The sewage may be conducted on to filter beds of porous material such as cinders, through which it is allowed to trickle slowly. The final stages of decomposition here take place, and the effluent from the filter beds is as clear as water and is harmless when added to water in any amount, as far as the water life is concerned.

The decomposition may be carried on entirely under aërobic conditions. This is accomplished by conducting the sewage into tanks having false bottoms of a porous material through which air can be forced. Under such conditions there soon becomes established a bacterial flora that is capable of immediately decomposing the organic matter. After a period of aëration, the air is turned off. The bacteria settle rapidly, so that the upper two thirds of the contents of the tank can be drained off, carrying the soluble products, and leaving the bacteria, to act quickly and completely on the next quantity of organic matter presented to them. This process is called the "activated sludge" process.

The farm home will find it most convenient to use the septic tank for the disposal of the sewage, and to apply the effluent of the tank either to the surface of the soil or beneath the surface by means of tile. It is necessary to establish conditions that will be favorable to the growth of the essential classes of bacteria. The installation of such a plant is a separate problem for each home. All that can here be done is to point out the necessary conditions that must be established. The tank must be of such a size that it will hold at least twice the amount of sewage produced daily. Before the sewage passes into the septic tank, it is desirable to have it flow through a grease trap to remove the fat that is found in the kitchen waste; for the grease is quite resistant to decomposition and may clog the drain tile.

The tank must be so arranged that the sewage will enter without disturbing the sediment or the layer of scum. The inlet must be below the surface, and cross-partitions should be placed in the tank to prevent currents and to keep all portions of the sewage in the tank for the same time. A second chamber is provided, into which sewage enters from the first tank. The septic tank proper is thus kept continually full.

If the digested sewage is applied to the surface of the soil or discharged into underdrains, it is necessary to discharge it at intervals rather than constantly, so as to give the water time to drain away and the air to enter in order that the decomposition may be completed. The second tank should hold from one third to one quarter of the daily volume of sewage. It is called the *dosing* chamber, and some means must be provided by which it can be emptied when full. This can be accomplished by installing a gate-valve at the bottom which can be opened and closed from the surface. A more convenient arrangement is the automatic syphon, by which the tank is emptied whenever the sewage reaches a certain depth.

The drains into which the sewage is discharged are placed from eight to ten inches below the surface, and are laid to grade with open joints, as in the case of ordinary tile drains. When the tank is emptied, the sewage flows into the tile and fills the entire length of the drain. It passes out of the open joints and percolates into the soil, where the last steps in the decomposition of the organic matter take place, just as in the filter beds used in the disposal of municipal sewage. If the sewage were allowed to flow constantly from the tank in a small stream, it would find its way into the soil through the first few joints of the tile; the soil in the immediate neighborhood would be kept water-logged, and the oxidation processes could not go on. The soil would soon become clogged with the undecomposed organic matter. The tiles should be laid near the surface of the soil, so that oxygen shall be available for the aërobic bacteria.

The tile may be laid in cinders or gravel if the soil is heavy in character. A sandy soil is best adapted for such

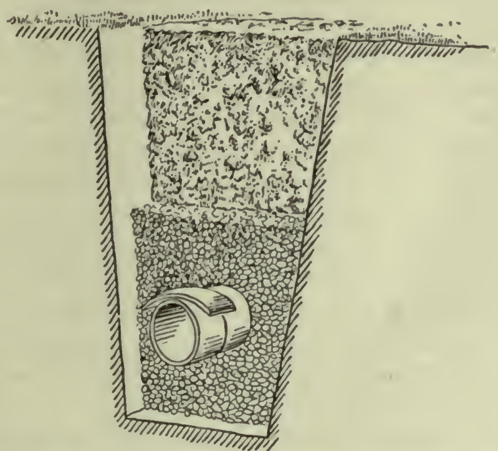

Fig. 23. The Tile Drain of a Sewage Purification System
The tile are laid near the surface of the ground and surrounded with coarse material like gravel or cinders in order that the sewage may pass freely out of the drains into the ground in which the final steps in its decomposition take place. These processes require an abundance of oxygen

a disposal system, but the method can be used in most types of soil. Since but little solid matter other than organic enters the septic tank, the accumulation of sludge is slow, and the tank will not have to be cleaned for several years. The drains may have to be dug up and the tiles cleaned once in three or four years, as earthworms bring in a good deal of soil. The system will work with little attention, and furnishes a means by which the farm home can dispose of the household sewage in a convenient and harmless manner. This, together with an abundant and well arranged water supply, adds much to the comfort and healthfulness of the farm home. It is thus possible for the country dweller to have all the conveniences that the city home possesses in this respect, and at practically no greater cost.

CHAPTER XII

THE FIXATION OF NITROGEN

As has been seen, the supply of nitrogen in the soil is the result of processes that have been going on from time immemorial under natural conditions. When human activities enter into consideration, the equilibrium of natural forces is upset. The decomposition of the organic matter goes on more rapidly and more completely in cultivated soil than in the virgin forest or prairie. The more aërobic conditions favor the more rapid decomposition of the humus that has accumulated, and, unless care is taken to add increased quantities of organic matter to the soil, it soon becomes so depleted that profitable crops can no longer be grown.

The depletion of organic matter is of especial importance from the standpoint of the nitrogen supply, because the humus is the chief source of nitrogen for the soil organisms. Some nitrogen is lost in the drainage water, and also in the crop removed from the land. It is probable that some is also lost in the decomposition of nitrogenous matter, and certainly in the process of denitrification. The depletion of the nitrogen content of the soil has been considered by some observers to be a most serious problem. Some have maintained that the population of the world will be limited because of the constant loss of nitrogen from the soil. It is probable that such fears are unfounded, for many factors are at work tending to maintain the nitrogen content of the soil.

Chemical processes have been devised by which the nitrogen and the oxygen of the air can be brought into combination. The most successful of these is the fixation of nitrogen by electric discharges. Where cheap electric power can be had, nitrates can be made at prices that enable them to compete with the natural product from Chile. Large quantities of such nitrates are made in the Scandinavian countries, where water power is abundant and conditions are not such as to enable the power to be used for other purposes. Constant progress is being made in the development of methods by which the nitrogen of the air is made to combine with other elements, and it is highly probable that the world has little to fear from an insufficient supply of combined nitrogen in the years to come.

Nitrogen added to the soil by rains.—Every electric discharge occurring in the atmosphere results in the production of oxides of nitrogen. These and the ammonia in the air are returned to the soil in the rain water. It has been determined that from three to six pounds of nitrogen are thus added to each acre in a year. About 70 per cent. of this is in the form of ammonia, the remainder in the form of oxides of nitrogen.

Nitrogen fixation in soil.—The same crop can be grown on the land for hundreds of years; the yield will soon reach a level below which it will not fall. This level is usually established by the rate at which some one element is made available. The limiting factor most frequently is nitrogen. The yield of the crop is usually larger than could be accounted for by the amount of nitrogen added to the soil in the rain water. It would thus seem that there must be factors at work in the soil that tend to maintain the nitrogen content. In the latter part of the last century, Berthelot, a French chemist, studied the increase of nitrogen in soils on which no crop was growing, and which were protected from the rain. He found that a constant increase in nitrogen took place. He estimated that the nitrogen thus added to the soil in the fields amounted to from 50 to 75 pounds an acre each year. When the soil was heated no increase in nitrogen occurred. This indicated a biological process.

It is now known that there are at least two groups of bacteria in the soil that are able to fix nitrogen. One of these is an anaërobic group. The first organism studied was given the name of *Clostridium Pasteurianus.* The organism will grow in a nutrient solution containing inorganic salts, which supply the essential mineral elements, and sugar, which supplies the energy. No nitrogen need be present in the solution. In the process of growth, nitrogenous organic matter is formed, and since the only source of nitrogen is the free nitrogen of the air, the organism must in some way bring it into combination. It was found that for every gram of sugar fermented about two milligrams of nitrogen were combined.

Another group of nitrogen-fixing bacteria in the soil belonging to the aërobic type is known as the *Azotobacter* group. Most of its members have the ability to form a black pigment; the name *Azotobacter chroöcoccum* has been given to this type. This type is more efficient in fixing nitrogen than the previous group, in that for every gram of sugar fermented from 10 to 20 milligrams of nitrogen are fixed. Members of this group are found in nearly all soils; they are more abundant in the more fertile soils. They are also found in water, frequently in combination with green algæ. It is supposed that they live in a symbiotic relationship, the algæ furnishing the carbohydrate to the bacteria, and the latter nitrogen in an available form to the cells of the algæ. Not only can sugar be used as a source of energy, but also organic acids. It is thought that cellulose is also made available to the nitrogen-fixing bacteria by the cellulose-fermenting bacteria through the formation of sugars.

It has been maintained that the nitrogen-fixing power of a soil could be increased by inoculation with cultures of these organisms. Such a hope has not yet been realized. Their action in the soil must be favored by the establishment of a suitable environment. A sufficient supply of both phosphorus and potassium is essential, as is organic matter from the fermentation of which they may obtain the energy necessary for the combination of the nitrogen. As noted in the discussion on manures, it is desirable to add to the soil as much organic matter as possible, irrespective of whether it contains any of the four elements that are known to be most important from the standpoint of the soil. One important rôle of the organic matter is to favor the growth of these nitrogen-fixing organisms. There is good reason to believe that these two groups of bacteria are important factors in the maintenance of the nitrogen content of the soil, rather than simply scientific curiosities, as some have considered them. The nitrogen fixed by these groups of bacteria is built into organic matter, which must be ammonified and nitrified before the nitrogen drawn from the air can be used by the green plant.

Leguminous plants.—It has long been recognized that the leguminous plants have different properties from the grains and grasses in that they are able to produce luxuriant crops on lands on which the non-legumes will make but a meager growth. They also seem to enrich the soil, since the yield of the crop following them is often greater than that obtained from the same land on which a non-legume had been grown. This property has led to the inclusion of some type of leguminous plant in most systems of crop rotation. It was found by Liebig, the father of agricultural

Fig. 24. Nodules on Soy Beans
The nodules are large and rough on the surface

chemistry, that in some unknown manner the leguminous plants were able to increase the content of the soil in nitrogen, and that they seemed to have sources of nitrogen that were not open to other classes of plants.

It had also long been known that there were commonly on the roots of the leguminous plants, nodules or tubercles, which were usually looked upon as galls similar to those produced on many plants by the stings of insects, and by other causes that stimulate the growth of the plant cells in the immediate vicinity to which the stimulus is applied. The tubercles were thought to be injurious, or at least not of service to the plant. It had been found that the tubercles contained bacteria. Hellriegel and Wilfarth, German investigators, found, in their study of the ability of different plants to grow in the absence of some one element, that the non-leguminous plants were able to make but a slight growth in the absence of combined nitrogen. Some growth would always take place because of the content of nitrogen in the seed, but when this was exhausted, the plant would die of nitrogen starvation. When legumes were studied, the results in some instances were identical with those obtained with the non-legumes, while at other times the legumes showed an ability to grow in the absence of combined nitrogen in the soil. No prediction could be made as to the outcome of any experiment when legumes were used, as could be done with the other kinds of plants.

These investigators found that the ability of the leguminous plants to grow in the absence of combined nitrogen was correlated with the presence of tubercles on the roots of the plant. If the soil was sterilized, no tubercles appeared, and the plant was unable to grow except when nitrogenous fertilizers had been added to the nitrogen-free soil. A few drops of the leachings from the soil on which the legume in question had been grown was sufficient to induce tubercle

Fig. 25. Effect of Inoculation on Alfalfa
The soil contained no nitrogen. By the aid of the nodule-forming bacteria the inoculated plants have been able to secure nitrogen from the air

formation and consequently growth. If the leachings were heated, no effect was noted. It was thus evident that the causal factor was a biological one. Soon after the discovery of the relation of the nodule to the nitrogen needs of the plant, the nodule-forming bacteria were isolated by the Dutch bacteriologist, Beyjerinck.

When leguminous plants are spoken of, the cultivated legumes come to mind, such as the clovers, alfalfa, the peas and beans, the vetches, lupines, and serradella. The native flora of all soils and of all parts of the world is made up in large part of leguminous plants. It has been determined that 20 per cent. of the flora of the Western prairies consists of wild or native legumes, while still higher figures have been obtained in the Eastern States. In size they vary from the smallest clovers to full sized trees, such as the honey locust. All, as far as is known, have the same relation to the nodule-forming bacteria, and possess the ability to use the free nitrogen of the air. Leguminous plants are found growing on every type of soil. Some are adapted to acid soils, while others grow best on alkaline soils. Many are adapted to high land, and a few are water plants.

It is certain that the leguminous plants and the associated bacteria have been the chief factors in the gradual storing of nitrogen in the soil. Under their influence, the elemental nitrogen of the air is brought into combination in the form of proteins, which, as they have undergone decomposition in the soil, have left a residue of humus.

The legumes are differentiated from the grasses and grains by their peculiar relation to a group of bacteria and by their composition. Both the seed and vegetative parts contain much more nitrogen than is the case with the nonleguminous plants, as is shown in the following table:

Average Fertilizing Constituents and Digestible Nutrients to the Ton

Kind of Forage	Fertilizing Constituents in One Ton			Digestible Nutrients in One Ton			Total
	Nitrogen	Phosphoric acid P_2C_5	Potash K_2O	Crude protein	Carbohydrates	Fat	
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Alfalfa hay	47.6	10.8	44.6	212.0	780.0	18.0	1,032.0
Red clover hay.	41.0	7.8	32.6	152.0	786.0	36.0	1,018.0
Soybean hay ..	51.2	13.6	46.6	234.0	784.0	24.0	1,072.0
Field-peas hay..	48.4	13.4	24.8	244.0	802.0	38.0	1,132.0
Corn fodder ..	21.4	6.6	17.8	60.0	946.0	30.0	1,074.0
Timothy hay ..	19.8	6.2	27.2	60.0	856.0	24.0	990.0
Kentucky blue-grass hay ..	26.6	10.8	42.0	94.0	870.0	30.0	1,032.0
Oat hay	26.8	16.0	65.4	90.0	762.0	34.0	928.0

Inoculation of the soil.—It is, of course, evident that if the soil does not contain the bacteria that form the nodules and bring the free nitrogen of the air to the service of the plant, the legume must draw all of its supply from the soil. If the crop is removed, the soil will be more rapidly depleted of its nitrogen content than with a grain crop, and if the crop is turned under, no increase in the nitrogen content of the soil will have taken place. If the legumes are to be used to enrich the soil, the fields must contain the bacteria. The recognition of this fact led to the inoculation of the soil. At first soil from a field on which the legume in question had been grown was employed. Later the use of pure cultures of the bacteria was attempted. For many years it met with little success, due, apparently, to the fact that the bacteria on artificial cultivation lost their ability to form the nodules. More recently improved methods of growing the bacteria in the laboratory have been devised, and at present the use of artificial cultures for the inoculation of legumes is successful.

The success attained in the use of the cultures for the inoculation of seed depends on many factors, chief among

Fig. 26. Effect of Inoculation on Peas
The soil of the field was a light sand, very low in nitrogen. The inoculation of the seed resulted in a luxuriant crop. Without the bacteria the crop was a failure

which is the vitality of the culture. If it is old and made up of weakened bacteria, good results will not be obtained. The culture must be grown under favorable conditions and be fresh to give good results. It is possible to bring large numbers of the bacteria into intimate contact with the seed by inoculating it directly with the culture.

The bacteria that will produce the nodules on the roots of one legume will not necessarily do so on a different legume. The legumes may be divided into separate groups within which the bacteria from one legume will produce nodules on the roots of the other legumes in the group, or "cross-inoculate." The following list gathers the common leguminous plants into groups that cross-inoculate:

1. To inoculate *red clover,* use the bacteria from red clover, mammoth red, alsike, crimson, Egyptian, or white clover.

2. To inoculate *alfalfa,* use the bacteria from alfalfa, white sweet clover, yellow sweet clover, bur clover, yellow trefoil, or fenugreek.

3. To inoculate *garden pea,* use the bacteria from garden pea, field pea, hairy vetch, spring vetch, wild vetch, broad bean, lentil, sweet pea, or perennial pea.

4. To inoculate *cowpea,* use the bacteria from cowpea, partridge-pea, peanut, Japanese clover, velvet bean, lima bean, wild indigo, or tick trefoil.

5. To inoculate *garden bean,* use the bacteria from garden, field, navy, kidney, or scarlet runner bean.

6. To inoculate *lupine,* use the bacteria from lupine, serradella, or wild lupine.

7. To inoculate *soybean,* use only the bacteria from the soybean.

The bacteria enter the plant through the root hairs. They stimulate the growth of the cells at the point of entrance, and the nodule is produced. If this nodule is ex-

Fig. 27. Effect of Inoculation on Sweet Clover
The crop was grown on a very poor sandy soil. Inoculation enabled the plant to draw the necessary nitrogen from the air

amined under the microscope, the plant cells will be found filled with myriads of motile bacteria, which in the young nodules are rod-shaped, but in the older ones assume abnormal shapes known as *bacteroids*. In some not well understood manner, the bacteria are able to obtain their nitrogen from the air and to make it available to the plant. The bacteria derive from the plant the fermentable material necessary to secure the energy demanded for the fixation of the nitrogen. The relation is thus a mutually helpful or a *symbiotic* one.

The plant can thus obtain a sufficient supply of nitrogen to make a good growth, but when growing under natural conditions the plant derives a greater or less amount of its nitrogen from the soil in the same way as do other plants. No one can state the proportion of nitrogen taken from the air or from the soil under any given set of conditions. All that can be said is that the plant is unable to use the free nitrogen of the air unless the nodules are present on the roots. If the nodules are few, a small part of the nitrogen may come from the air, while if the roots are well covered with nodules, the plant will undoubtedly take the major part of its nitrogen from the air. Every farmer should make an effort to have all the legumes he may grow well inoculated.

Other conditions must be made as favorable for the legume as possible. There should be an adequate supply of potash and phosphorus in the soil, and the reaction should be favorable for the particular legume. When these conditions are met and the appropriate bacteria are present or have been added, nodule development should be abundant. It must be remembered that the only way in which the legume can increase the fertility of the soil is with reference to the single element nitrogen. A leguminous crop may be grown on a field and be removed, and the soil remain as high in nitrogen as before the crop was grown; but this can never be true of potassium, phosphorus, and sulphur.

The legume bacteria are motile. There is, however, no reason to believe that they can pass through the soil in a horizontal direction for any distance. The plant root must come in contact with the bacteria before infection can take place. Since the plant roots do not fill all the spaces of the soil, it is essential that the soil contain great numbers of these organisms in order that an abundance of nodules may be formed. If artificial inoculation is to be resorted to, it is important to bring the organisms in intimate contact with the roots of the young plant. This is best accomplished by the inoculation of the seed rather than by the inoculation of the soil. In the case of inoculation with soil, the seed may be moistened slightly and the fine soil thoroughly mixed with it. The seed should be treated shortly before it is to be sown. The pure cultures are usually added to water, which is sprinkled over the seed. It must be remembered that unfavorable conditions, such as drying and sunlight, may destroy the organisms on the seed. If soil containing the organisms is available in unlimited amounts, it may be broadcasted over the field, or it may be applied with a drill and well harrowed in, so as to mix it as intimately as possible with the soil.

No very definite direction can be given as to the amount of soil that should be used in the inoculation, since this will be determined by the number of bacteria in it. In case it is broadcasted over the land, several hundred pounds an acre should be added. It has been found in experimental work in the greenhouse that the bacterial content of the soil to which sugar has been added may reach a point where one half pound an acre will produce a good inoculation.

Another practical method of securing optimum conditions favorable to the growth of a different kind of legume is to sow a small quantity of the seed in question with the regular crops. Thus, if it is desired to secure a catch of alfalfa on soil that has not grown this crop, instead of inoculation with a pure culture or infected soil, some farmers follow

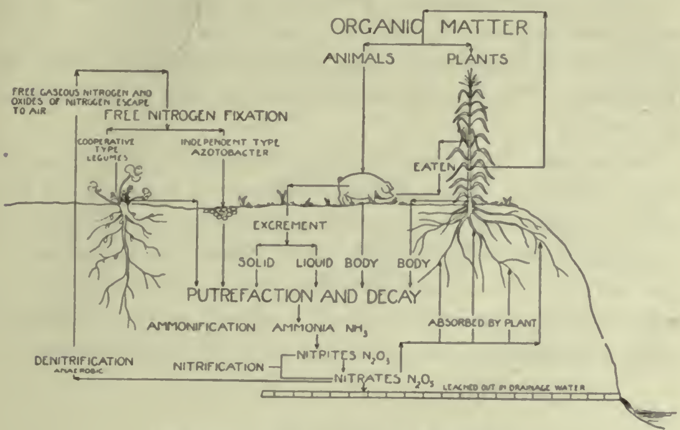

Fig. 28. The Cycle of Nitrogen

After Wright.

the practice of sowing a small quantity of alfalfa seed with all of their grain crops, even if the land is seeded down to red clover. In a few years this preliminary inoculation suffices to infect the soil sufficiently so that inoculation of the crop can be readily secured later.

The matter of inoculation is especially important when a new legume is to be grown or when a legume is to be sown on a field on which it has not been grown for a number of years. The bacteria are able to grow in the soil itself. Experience has shown that they gradually decrease in number, and after five or six years will be so diminished that inoculation is advisable if the legume is to be grown again.

It has been shown that the composition of the plant is changed by the presence of the nodules in that the nitrogen content of the aërial parts of the plants bearing nodules is higher than plants on which nodules are not present. The composition of non-legumes growing with legumes is also changed in the same manner.

A few non-leguminous plants may bear nodules on the roots, and apparently have the same relation to free nitrogen as do the legumes. The most important of these are the alders. The development of the nodules is very sparse, as a rule.

The legume furnishes the cheapest way of preventing the rapid reduction of the nitrogen content of the soil. Where sandy lands are to be reclaimed and worn-out soils restored, the legume is to be considered a most important factor. The nitrogen thus added to the soil is estimated to cost only from one half to five cents a pound, as opposed to the usual commercial price which is from 25 to 30 cents.

PART III

THE RELATION OF MICROÖRGANISMS TO FOODS

CHAPTER XIII

THE CONTAMINATION OF FOODS

The decomposition of organic matter is due to the action of microörganisms that utilize the various compounds as food, and leave, as a result of their life processes, more simple substances, or *by-products.* Since most of these changes affect the quality of foods that are used by man, or even the domestic animals, it is desirable to protect food supplies in general, so far as practicable, from the action of such microörganisms. Especially in the temperate zone is this question of food preservation of great importance, for the season during which plant growth takes place is short, and vegetable matter must be stored for use during the colder period of the year. Under the complex conditions in which we now live, the question of protection of food during the process of distribution is likewise of great importance.

While the action of most microörganisms in food supplies does not enhance the nutritive properties of foods, certain types are used to advantage in the preparation of some foods, as in the fermentation industries, in which the raw materials are transformed by the action of living organisms. Some by-products are used as food, or they may be of service in the preparation of food, as is the case with carbon-dioxide, formed by the action of yeast on sugar, which serves as a leaven to "raise" or lighten the dough in bread-making.

Milk.—In the following pages the discussion is limited chiefly to milk and dairy products, as virtually all phases of the relation of microörganisms to foods are well illustrated with milk and its products. There are special reasons why a detailed discussion of the action of microörganisms on this food product is desirable. Milk is one of the most important foods in the dietary of the American and European people. It forms about one sixth of the food of the population of this country, and for children a much greater proportion of their nutriment. One milch-cow is kept for each 4.5 persons. A large portion of the milk consumed is used as raw milk, and hence its contamination with disease-producing bacteria is of great importance. Again, its preservation is a problem that is presented to the producer and to the consumer daily; for, in the production and handling of milk, it becomes seeded with great numbers of bacteria, which find in it a most favorable place for growth. The manufacture of butter and cheese was originally carried out on the farm. Their preparation has now been largely removed therefrom; but the farmer is still the producer of the raw material, the quality of which determines the quality of the product.

Factors governing decomposition.—In the decomposition of any substance the rapidity of the changes involved are determined by the number of organisms from foreign sources that are brought in contact with the material, and by the rapidity with which growth occurs, since decomposition processes can not occur without growth, no matter how great the initial contamination of the food. The question of food preservation, therefore, may be divided into two divisions: first, the contamination of the food; second, the destruction of the microörganisms contained in the food or the inhibition of their growth.

The first is especially important with liquid foods, such as milk, because the organisms can be uniformly incorporated with the liquid, and their growth will not be limited to any one point, as in the case of solid foods. Again, when once introduced, they can not be removed therefrom as from the surface of a solid.

The source of contamination of foods in general is readily traced to contact with matter from the soil, water, or the contents of the alimentary tract of animal life. These materials harbor the bacterial life that is the cause of the changes involved, and if foods can be kept from direct contact with such organic wastes, it is comparatively easy to prevent in large measure the decomposition changes that will otherwise occur. In the protection and care of food products, it is desirable to do only those things that are of real necessity and value, rather than to waste time and effort in carrying out a mode of procedure that is unnecessarily refined. So much exaggeration is frequently found in the public prints, relative to germ life and its dangers, that not infrequently unnecessary alarm is engendered in the minds of many people. This makes it important that consideration be given to the various sources of contamination from which milk becomes seeded with bacteria. It is especially essential that the relative importance of the various sources of contamination be understood, for if improvement of the product is to be attempted, the first efforts should be directed to those sources from which the greatest return for money and labor expended will be obtained.

All milk will contain bacteria, no matter how carefully it may be produced. It is impossible to maintain the same standards of cleanliness in the stable as in the kitchen, the bakery, and the meat-shop. More than any other food, milk is subject to contamination with materials rich in bacteria.

Contamination of milk from the interior of the udder.— When milk leaves the milk-producing cells of the udder of a healthy animal, it is probably free from these organisms; but this condition does not long obtain, for before it is drawn from the animal it comes in contact with the bacteria that have invaded the udder through the opening of the teat and have established themselves throughout the spaces or channels which ramify through this organ. The greater number of organisms are found in the lower portion of the udder, in the milk cistern and in the large milk ducts. The opening of the teat comes in contact with material that contains the most varied kinds of bacteria, and it is probable that the milk ducts are invaded by many kinds. Only certain types, however, are able to grow in the udder, and these only to a limited extent. As will be seen later, all body fluids have a germicidal action. The germicidal action of milk probably explains why the growth of the bacteria that invade the udder is not nearly so rapid as one would expect under the favorable conditions with reference to food and temperature. It has been shown that no bacterial increase occurs in milk for a period after it is drawn. This has usually been ascribed to the germicidal action of the milk. Whatever action milk may exhibit in this direction, it is of small importance in the practical handling of the product.

Fig. 29. A Section of an Udder

The milk is conducted from the secreting cells by the milk ducts which empty into the milk cistern from which it is drawn through the teats. Bacteria enter the teats and penetrate into the smallest of the milk ducts

The kinds of bacteria that are able to grow in the udder are not those that are actively concerned in the spoiling of milk; hence, this source of contamination of milk, although one that can not be avoided, is of small commercial importance. At times the udder may be invaded by bacteria upon which the milk has no germicidal action. Growth will then be unchecked and serious trouble may result.

The milk at the time of withdrawal generally contains a few hundred bacteria per cubic centimeter. Great differences in individual animals are to be noted. In the same herd two animals were found that showed an average bacterial content of more than 30,000 per cubic centimeter during a period of over one year. Another animal, kept under the same conditions, gave milk in which the average germ content was but 800 per cubic centimeter. The mixed milk of a number of cows will contain from a few hundred to a thousand or more bacteria per cubic centimeter, even when the contamination from outside sources has been prevented as far as is practically possible.

Since the greater number of bacteria are found in the lower part of the udder, and hence in the first milk drawn from each teat, it is the custom to discard the fore-milk, or the first few streams from each teat. This reduces the number of bacteria found in the milk, but will have little, if any, influence on the keeping properties of the milk, since the organisms found in the udder grow very slowly at ordinary temperatures.

Contamination from the air.—The air in a stable contains varying numbers of bacteria adherent to the dust particles. Some of the manure becomes dry, and is ground into fine particles by the movements of the cattle. This is supplemented by the soil that is brought into the stable on the hoofs of the cattle. The dry feed is covered with dust and minute particles of soil; the bedding and coat of the cow are also covered with dust. Any operation that serves to throw the dust from these sources into the air facilitates the passage of some of it into the milk. The particles settle rapidly. Therefore, if dust-raising operations, such as bedding, brushing the cattle, and feeding dry feed are carried out some time before the milking-time, the contamina-

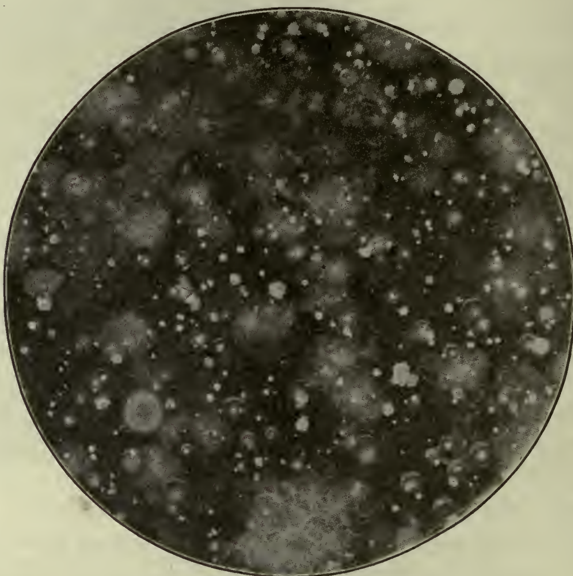

Fig. 30. Contamination from the Air
A culture plate exposed for thirty seconds in a dusty stable shows numerous colonies of bacteria and molds

tion of the milk from the air will be slight. The amount of foreign matter introduced into the milk from the air is very small compared to that introduced from other sources.

The types of microörganisms found in the air are not those primarily concerned in the spoiling of milk. The contamination of milk from the air is therefore relatively unimportant, both quantitatively and qualitatively. Nevertheless, the contamination from this source should be avoided as far as practicable, since only by directing attention to all sources of contamination can the bacterial content of milk be kept at a low level.

Contamination from the animal.—The larger part of foreign matter introduced into milk comes from the udder and flanks of the animal. In improperly constructed stables, and where the bedding is not sufficiently abundant, the flanks often become coated with manure. The udder may also become soiled. If the yards are muddy, if the cows have access to mud-holes or muddy streams, the udder and teats will be soiled. Even on pasture the udder becomes coated with dust.

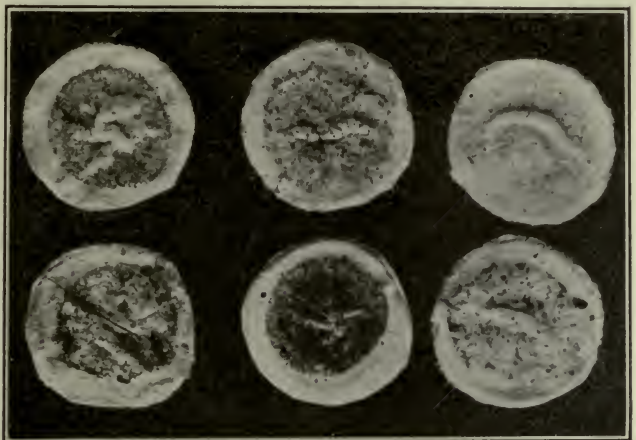

Fig. 31. Dirt Tests

A pint of milk was passed through each of the cotton filters. The amount of dirt in the respective samples of milk is shown by the color imparted to the cotton

The extent of the contamination from the animal depends on her condition as to cleanliness. It is impossible to draw milk from a dirty animal without grossly contaminating it. The farmer can not afford to clean soiled animals before milking; he must exert his efforts to prevent the soiling of the animal. The yards should be well drained, and covered, if possible, with some material that will not become muddy, such as cinders or gravel.

It should be kept in mind that the stable in which dairy cattle are kept represents a factor in the determination of

Fig. 32. Bacteria on Hairs
Cow hairs were placed on the surface of an agar plate. The adherent bacteria developed and formed colonies

the quality of the food that is to be produced therein. The stalls should be so constructed that the cows will automatically be kept clean. They should be of a proper length for each animal, and the gutters should be deep and wide in order that the manure that accumulates between the periods of cleaning will not reach the level of the floor of the stall.

The stable should be cleaned each day; and an ample supply of bedding should be provided in order that the manure carried on to the stall floor by the animal will be absorbed. The bedding should be of such a nature that it will not contaminate the coat of the animal. Fresh, clean straw,

Fig 33. A Dirty Stable
It is impossible to produce good milk in such an environment

shredded corn stover, sawdust, and shavings are good absorbents, and are relatively free from dust and bacteria. Moldy or rotten straw and litter from the horse stalls are objectionable because of their influence on the quality of the milk. Every effort should be made to keep the animal in a clean condition, since this is one of the ways by which a great deal of contamination can be prevented at a minimum of expense.

Prevention of contamination from the animal.—Even

Fig. 34. A Clean Stable
Appetizing food can be produced under these conditions

under the best of conditions in both summer and winter, the coat of the animal will become dusty, and it is advisable to remove this dust, as well as the loose hair, before milking. This can be done by brushing the flanks and udder shortly before milking time, in order that the dust thus created shall have time to settle. A still better way is to wipe the udder

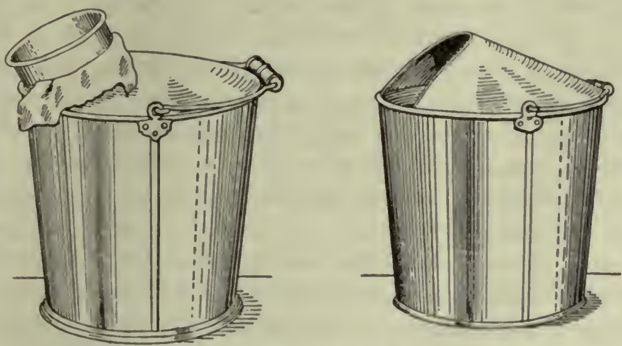

Fig. 35. Sanitary Milk Pails
The Stadtmueller and the Truman pails are two of the most practical of the many that have been devised

with a clean, damp cloth. To avoid most completely this source of contamination, the udder should be washed, and the excess water removed with a clean cloth. The udder is thus left damp and no dust can leave it. The latter method is the one used on farms on which the highest grade of milk is produced. Clipping the udder and flanks serves to prevent the entrance of dust from the animal, and to make the cleaning of a soiled animal much easier than if the hair is long.

The exclusion of dirt from the animal may also be attained by the use of a small-topped milk-pail, in which the opening is restricted in some way. Such pails (Fig. 35) are very effective in the exclusion of dirt, and are nearly as convenient to use as is the common open pail. The larger part of the dirt comes from the flank of the animal rather than from the udder. The milking-machine is also an effective way of preventing the introduction of mud and dirt into the milk, since the milk passes from the teat directly into tubes that lead to covered pails. The effect that such factors have in producing clean milk is dependent on the condition of the coat of the animal. If the latter is very clean, the use of the covered pail will have little, if any, influence in improving the quality of the milk; but if the animal is dirty, the influence will be great. The extent to which any producer can apply methods for the prevention of the contamination of milk is to be determined by the results produced, and whether he can obtain compensation from the consumer for the additional expense incurred. Common decency, however, demands that the introduction of visible quantities of mud and manure be avoided. Prevention of contamination should begin with those operations that will have the maximum effect.

Influence of the milker.—The milker should appreciate the relative importance of the various sources of contamination, and should know the most effective means of preventing the introduction of microörganisms. The dress and hands of the milker should be clean, and the methods of milking such as to avoid contamination. Milking should not be done with wet hands. If the conditions demand something to soften the teats, vaseline may be used. The whole hand should be used in milking, rather than stripping with the thumb and forefinger.

Contamination from the utensils.—It is difficult to clean dirty utensils with sufficient thoroughness to remove all traces of organic matter to the extent that bacterial growth will not take place in case the temperature and moisture conditions permit. The thoroughness with which the utensil can be cleaned is dependent on the material of which it is made. Woodenware can be cleaned less easily and less thoroughly than can metal vessels. Again, if the utensil is so constructed that joints and angles exist which can not be readily reached in the cleaning by the cloth or brush, it will be difficult to remove accumulations of organic matter. The sharp angles encountered in milk-cans, and the open joints that are found in the cheaper grades of tinware, make such utensils difficult to clean.

The condition of the utensil is another factor that determines the ease with which the cleaning process is carried out. The smooth surface of a new tin vessel is much easier to clean than that of a rusted and dented utensil. Such complicated utensils as milking-machines and cream-separators are difficult to keep in a sanitary condition. The rubber tubes that conduct the milk from the teat-cups to the receiving can of the machine can not be entirely freed from milk, and it is impossible to dry them. In order to avoid a large amount of contamination from the tubes, it is necessary to place them in an antiseptic solution in such a manner that they shall be entirely filled with the solution and not partially filled with entrapped air. No solution has been found entirely satisfactory. If a few pieces of fresh quick or stone lime are kept in the tank in which the rubber parts of the machine are to be placed, the alkalinity of the solution will be such as to prevent bacterial growth. A saturated solution of common salt may also be used, but this will not completely prevent the growth of bacteria. Certain kinds will grow very slowly therein.

The addition of a small amount of a solution of calcium hypochlorite or bleaching powder to the brine will destroy the bacteria. The addition of one part of a solution obtained by stirring one pound of bleaching powder in one gallon of water, and allowing the insoluble portion to settle, to two hundred parts of the brine at semi-weekly intervals, will suffice to keep the tubes in good condition. The action of the solution must be supplemented by a thorough cleaning of the parts each week. As the tubes are used, minute cracks form on the inner surface and become filled with milk. The antiseptic solution enters them slowly, and the bacterial growth that may occur therein will be forced out of the cracks under the influence of the constantly changing pressure in the tubes when the machine is in use.

The tubes may also be kept in a satisfactory sanitary condition by placing them in water and heating to 176° F. for a few minutes. The tubes may be allowed to remain in the water until they are to be used again.

Cleaning of milk utensils.—Milk utensils should be washed as soon as possible after using, for if the milk is allowed to dry on the surface of the utensils it is difficult to remove. They should be rinsed with cold water, then washed with a hot solution of a washing powder. Soaps and soap powders are difficult to remove by rinsing, and are not as effective in the removal of milk and grease as are washing powders. A stiff brush should be used for scrubbing, for much of the dirt can be removed only by mechanical force. Finally, the vessel should be rinsed with boiling water, using it in such quantities that the vessel will be heated sufficiently, so that rapid and complete drying will take place. The growth of microörganisms can not occur in the absence of water. Imperfect washing will not be so serious, if the utensil is perfectly dried, as will a more thorough washing with imperfect drying. If steam is available, the utensils can be so thoroughly heated as to destroy all organisms that are likely to injure the keeping quality of the milk. Furthermore, steaming facilitates the drying process. In the washing-rooms of city milk depots, can driers form an important part of the equipment. The amount of bacterial growth that can take place in a few cubic centimeters of water in a milk utensil is often sufficient to add as many as 50,000 bacteria to each cubic centimeter of the milk when the utensil is filled. It is doubtful whether such a number of bacteria are ever added to the milk through the introduction of dirt.

All utensils should be washed after each period of use. This is especially true of cream-separators. It is impossible to remove the accumulation of organic matter that collects on the wall of the bowl by running water through the machine. It is essential that the machine be taken apart, well washed, and thoroughly dried. Strainer cloths should be washed as free from milk as possible, and placed where they will dry quickly, so that no growth can occur in them.

Contamination from factory by-products.—The cans in which milk is transported to the creamery and cheese factory are also used to carry the whey and skim milk back to the farm. This custom would have no disadvantage if the cans were thoroughly washed before being used again. This, however, is the exception rather than the rule, and hence bacteria find their way from the whey-tank to the cheese-vat. The great opportunity for the whey-tank to become seeded with harmful types of bacteria or yeasts makes this source of contamination of much importance in the manufacture of cheese. Such trouble can be avoided by heating the whey to a temperature of 140° to 155° F. as it passes from the cheese-vat to the whey-tank, where it is stored until the following day. If the volume of whey is large, it will require considerable time for the temperature to fall to a point where any bacterial growth can take place. Heating to the above temperature is sufficient to destroy most non-spore-forming bacteria. Whey so treated will be sweet when returned to the farm, and will have a higher feeding value than sour whey. It will also be free from disease-producing organisms that may thus be carried from one farm to another. It has been found that the enhanced value of the butter and cheese is more than sufficient to pay for all cost of treatment.

Factors determining the number of bacteria.—The bacterial content of any sample of milk is dependent on the number that have been introduced into it from the various sources that have been considered, and on the extent to which the bacteria have grown in the milk. It has been shown that, under ordinary conditions, the utensil is the most important source of contamination, and the animal the next in importance. Even the most simple utensil that is apparently in a satisfactory condition may add an unbelievable number of bacteria to the milk.

In seeking to improve the quality of milk from the standpoint of contamination with bacteria, the utensils should be considered first. Prevention of growth therein by the complete removal of water should take precedence over attempts to sterilize the utensils by steam, unless the steaming can be so prolonged that the utensil will dry quickly from the heat imparted to it. Steaming for a few seconds, and leaving the utensil in a moist condition, defeats the aim in view. Utensils in which no bacterial growth has taken place, supplemented by wiping off the visible accumulations of mud and manure from the udders of the cows, and the use of small-topped pails, will prevent the entrance of the major portion of organisms into the milk.

The problem connected with the second factor, that of the growth of bacteria in milk, must be solved by means to be treated in a subsequent chapter. A high bacterial content does not necessarily mean a milk produced under undesirable conditions with reference to cleanliness, but it does imply a milk of undesirable quality from the standpoint of the consumer.

Straining and clarifying milk.—It might be thought that the foreign matter introduced into milk could be removed, thus reducing the bacterial content as well. For the removal of dirt, straining the milk is generally resorted to. This practice may be carried out so as to remove the insoluble material that has found its way into milk, but it will have little if any influence on the reduction of bacteria, since they are readily washed off from the surface of solid matter, and are able to pass the pores of the finest strainer that may be used. All processes of straining can serve only to improve the appearance of the milk, but can have little if any influence on its keeping quality or healthfulness.

Much of the milk now sold in cities is subjected to a process known as clarification by passing it through a machine that is comparable to a cream-separator. The insoluble material that has been introduced into the milk will be completely removed by the strong centrifugal force applied to the milk in the rapidly revolving bowl of the clarifier. This material will be supplemented by cellular elements from the udder, and by casein, the whole forming a slimy mass known as separator slime. The color is white if the milk treated is relatively free from dirt. Usually it is grayish in color, because of the dirt therein. Since the bacterial content of the slime is much higher than that of the untreated milk, the process serves to remove a portion of the bacteria from the milk. The actual reduction is, however, so small as to be of no practical importance in influencing the keeping quality or the healthfulness of the milk. Like straining, it improves only the appearance of the product.

Influence of feed on contamination of milk.—The bacterial content of the feed or water consumed by the cow can not have a direct influence on the kinds or number of bacteria that are introduced into the milk, as these organisms do not pass from the intestine through the blood and appear in the milk directly. They may, however, have an indirect influence by changing the type of the bacterial flora in the manure, some of which nearly always finds its way into the milk from the dirty flanks of the animal. If the feed is such as to make the manure more liquid than usual, it is more difficult to keep the animals clean.

The use of improper feed may, however, alter the taste of the milk or its value as food. Cabbage, turnips, rape, wild onions, and some weeds that may be eaten in the pasture contain certain volatile principles which are absorbed directly into the circulation and may then appear in the milk, just as the odor of onions appears in the exhaled breath. Where such substances are eliminated in the milk, the normal taste is changed. This, of course, does not injure the healthfulness of the milk, but it decreases its commercial value. Certain drugs, as mercury, arsenic, or strychnine, if given to cows, may be eliminated with the milk. The milk of an animal receiving medicine should not be used for human food, and especially for the feeding of children. Fats readily absorb any odors with which they come in contact, and milk, by reason of its cream content, thus absorbs some odors, such as that of bananas, with especial avidity. In order not to injure the flavor of the milk, it should be kept and handled in an atmosphere that is free from odors of all kinds. These absorbed odors are often difficult to differentiate from those due to the growth of bacteria in the milk.

Contamination of other foods than milk.—There are few foods that are handled in such a condition as to make them comparable to milk. Most of them are protected from the invasion of microörganisms. Others, while subject to contamination, do not permit the growth of the organisms that come in contact with them. There are two important examples that are quite comparable to milk in every respect. Oysters can not be subjected to any preservative agent other than cold. They are constantly exposed to contamination, and form an ideal medium for many bacteria. The water in which the oyster is grown supplies the initial contamination. They are commonly immersed in fresh water for a short period for the purpose of "plumping." The streams in which they are placed are often contaminated with sewage. A contamination with both saprophytic and pathogenic bacteria is thus possible. In opening the shells, the oyster is exposed to contamination from the hands of the worker, and the utensils also serve to add their quota of bacteria. The juice of the oyster is rich in protein and neutral in reaction, supplying an ideal environment for the growth of putrefactive bacteria.

Chopped meats present similar problems. In their preparation the bacteria are uniformly mixed with them. Food and moisture are abundant. Spoilage quickly occurs unless the bacteria are restrained in their development.

Contamination of foods is not confined to the handling they receive in the channels of commerce, but occurs in the home. Unused portions of food left over from the day's meal are peculiarly liable to bacterial activity. The contamination in the home is largely from utensils, the adequate sterilization and drying of which will do much to enhance the keeping of food from one day to another.

It is essential that all foods that must be used without previous cleaning, and especially those that are eaten without cooking, be protected from contamination, both for esthetic and sanitary reasons. Bakery goods, candies, etc., should be handled with due regard to cleanliness. Dust contamination and pollution incident to handling food products are especially to be considered.

CHAPTER XIV

THE CONTAMINATION OF FOODS WITH PATHOGENIC BACTERIA

The diseases of man and the lower animals that are due to the growth of microörganisms in the body of the living animal are propagated by the passage of the organism from the body of the diseased individual into the body of a still healthy individual. Many diseases are transmitted only by very direct contact of the healthy with the diseased. With others the organism may be transported over long distances in time and space. Many objects may serve as transporting agents. Prominent among them are certain foods.

The causal organisms are usually contained in some of the discharges of the body, which in one way or another come in contact with food materials. Frequently the organisms are not resistant to desiccation, and in this case can not be distributed on dry solid objects, but may be carried by moist foods such as milk and water. There are a number of reasons why these foods are especially important in the causation of disease. In the case of milk, the product of many farms is brought together and mixed in the plant of the milk distributor. A contamination of the product on any one farm may thus result in the introduction of the harmful organism into hundreds of city homes. The original contamination may be slight, but by the time the milk is consumed, it may contain an innumerable number of the organisms; for certain of the pathogenic bacteria find in milk a favorable nutrient medium, and can grow at the temperatures at which it is often stored. There is opportunity for milk to serve as a transporting agent of disease-producing organisms from the farm to the city, not at infrequent intervals, but daily throughout the year. The milk is subject to contamination with the organisms causing disease in the milk-producing animal, and also with those of man. Man is susceptible to a number of diseases that primarily affect cattle. These facts place milk first among the inanimate objects in the distribution of disease. The diseases most often spread by milk are tuberculosis, typhoid fever, diphtheria, and scarlet fever. Some idea of the importance of milk as an agent in the distribution of disease is shown in the following summary of epidemics that were traced to milk in Boston in the interval 1907 to 1911.

1907	Diphtheria	72 cases
1907	Scarlet Fever	717 cases
1908	Typhoid Fever	400 cases
1910	Scarlet Fever	842 cases
1911	Tonsilitis	2,064 cases

Water is probably to be classed as second in importance to milk in the distribution of disease-producing organisms. While some disease organisms can live in water for a varying period of time, this medium does not offer the opportunity for actual growth that milk does. Typhoid fever is the principal disease that is water-borne.

Bovine tuberculosis.—A detailed discussion of this disease will be presented in a subsequent chapter. Only the facts relevant to the relation of the disease in cattle to tuberculosis in man will be presented here. Tuberculosis is a disease that affects many portions of the body. It is characterized by the formation of nodules or tubercles, which gradually increase in size, giving rise to abscesses, the contents of which contain the tubercle bacilli. When these abscesses are located in parts of the body from which the bacilli may escape to the exterior on the breaking of the abscesses, the disease is said to be of the open type. This stage in the progress of the disease is not reached for a considerable number of months, or even years, after the inception of the disease. Approximately 25 per cent. of tubercular cattle have the open form of the disease. The milk of such animals is likely to be contaminated with the organism, the frequency and extent of the contamination depending upon the location of the abscesses. They may be present in the udder, in which case the milk is almost sure to be contaminated. If the abscesses are located in the lungs, as is most commonly the case, the infectious material will be coughed up from the lungs; a portion is ejected from the mouth, while the remainder is swallowed. The tubercle bacilli therein are not destroyed in their passage through the alimentary tract, and are eliminated in the feces. Since some fecal matter inevitably finds its way into milk, the opportunity is offered for contamination of the milk with tubercle bacilli coming from the lungs. Abscesses in the intestine or liver may serve to contaminate the milk in the same manner.

Tubercular abscesses in the udder may discharge their contents directly into the milk-ducts. The extent of contamination from the udder is much greater than from other sources, and is probably of much greater importance; but it is probably less frequent than contamination from the lungs, either by means of the manure or the dust of the stable.

The percentage of milch-cows suffering from tuberculosis varies widely in different parts of the world, but it is definitely appreciable in all sections where dairy development has been considerable. It is probable that mixed milk supplies, such as those represented by the supplies of the larger cities, constantly contain tubercle bacilli to some extent. The dilution of the milk of diseased animals with that of healthy animals tends to diminish the danger, both to animals and to human beings consuming such milk. The alarming extent of tuberculosis in hogs is direct evidence of the constant and marked contamination of the mixed milk.

It is impossible to examine market milk in any effective manner for the presence of tubercle bacilli in order to determine whether an animal is eliminating the organisms; hence, under practical conditions, it is necessary to consider any animal that has the disease as a potential source of danger, although she may not be giving off the organisms. The usual method of preventing the contamination of the milk with bovine tubercle bacilli is to apply the tuberculin test to the animals, and to remove all animals that react to the test.

The importance of bovine tuberculosis as a factor in the occurrence of the disease in man has been established only within the last few years. Through detailed studies made on organisms isolated from fatal cases of the disease in people of all ages, it has been possible to ascertain whether the organism in question belonged to the human or the bovine type. The organism from cattle is more virulent for most experimental animals than is the organism from man. This, together with the differences in growth on culture media, enables the bacteriologist to tell whether the organism originally came from cattle or from man.

From the data that have been collected in various parts of the world, it is certain that bovine tuberculosis is responsible for a portion of this disease in man. The susceptibility to infection from contaminated milk is greatest in the case of the young. The bovine tubercle bacillus has been found but infrequently, as compared to the human tubercle bacillus, in people over sixteen years of age.

The organisms are able to penetrate the tissues of the throat, especially the tonsils, from which they pass to the lymph-glands of the neck. The bacilli may also pass through the intestinal wall, producing tuberculosis of the abdominal cavity. The methods for safeguarding the wholesomeness of milk will be discussed in connection with methods used to preserve it.

Septic sore throat and garget.—Inflammation of the udder is of frequent occurrence in cattle. The more serious cases are due to the invasion of the udder by bacteria, the development of which is not restrained by the germicidal action of the fluids of the udder. A considerable number of kinds of bacteria have been found associated with such troubles. It is probable that the majority of them are incapable of causing harm in persons consuming the milk. A more serious form of garget is believed to be due to organisms that come from cases of septic sore throat in man. It is supposed that the contaminated hands of the milker serve to carry the bacteria on to the teats. They invade the udder through the milk ducts. The inflammation caused by their presence may be so slight as not to attract attention, and yet the milk may produce widespread epidemics of throat trouble. All cases of udder inflammation should be considered as potentially dangerous, and the milk rejected.

In the case of certain diseases of milk-producing animals the organism is present in the milk at the time of its withdrawal from the udder, not only from animals that have the disease but from animals that are apparently in normal health. Malta fever, a disease of goats, and contagious abortion of cattle, are examples. In the case of the latter it is known that an animal may continue to excrete the bacilli for years. It is not known that the organism is of any sanitary importance, as far as man is concerned, but undoubtedly such animals are the cause of the spread of the disease to other individuals. The disease is often spread by purchase of affected stock.

In a general way it may be said that the milk of an animal that is suffering from any disease whatever should not be used for human food. It may not be true that the milk would be distinctly harmful, but it is always well to err on the safe side. Such troubles as abscesses on any part of the body, inflammation of the intestines, or any abnormal condition after calving should cause the rejection of the milk.

Typhoid fever.—Of those diseases that do not affect cattle, but are spread by means of milk, typhoid fever is the most important. The organisms producing the disease enter the body with the food or drink. They establish themselves in the intestines, and from there penetrate to other parts of the body. They are eliminated in the feces and the urine. From these infectious materials they are brought in contact with food and drink by a number of agents.

Not only milk, but many other foods and water are concerned in the spread of typhoid fever. The methods by which food products may become contaminated are so similar that all may be discussed together. Milk has one peculiarity that is not common to most other foods, in that the typhoid bacilli find in it an excellent culture medium; and since growth can take place at temperatures far below that of the human body, indeed at temperatures at which milk is often stored, the slight contamination that might be of small importance in other foods may be the starting point of a great epidemic when milk is concerned. The typhoid

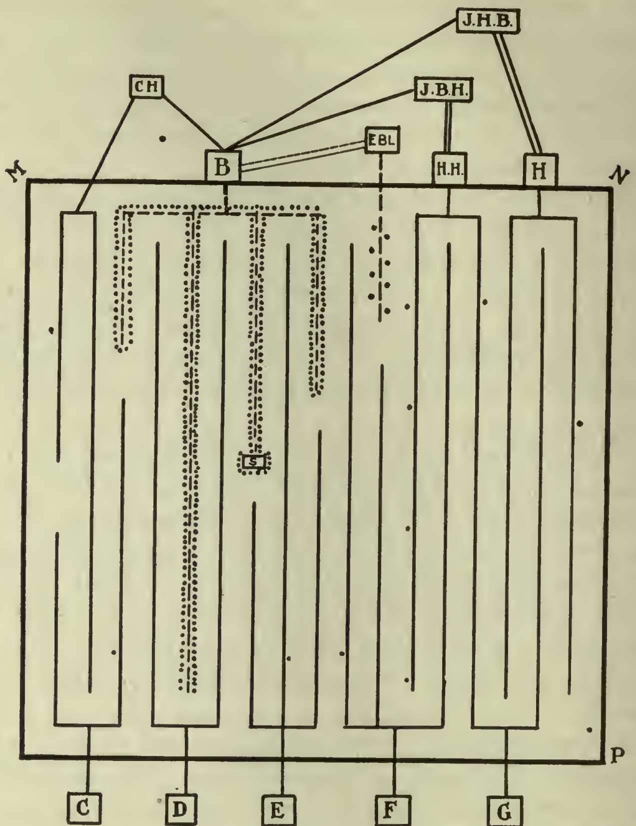

Fig. 36. Typhoid Fever Spread by Milk (Stamford, Conn.)
The small squares represent milk producers and dealers and the lines milk routes in a village. Three hundred and eighty-six cases of typhoid fever occurred on one route and but eighteen on the routes of the other nine distributors

organism is unable to grow in water, the bacilli gradually die, and within a week or ten days all have perished.

Contamination of water supplies.—The most frequent manner in which water is contaminated is by its pollution with sewage containing the organism. The methods of disposal of both municipal and farm sewage often are such as to permit of its introduction into the water. Municipalities often draw their water supplies from a body of water that is contaminated by sewage. In individual supplies, the farm well is often located in close proximity to the outhouse, and if the well is not protected from the

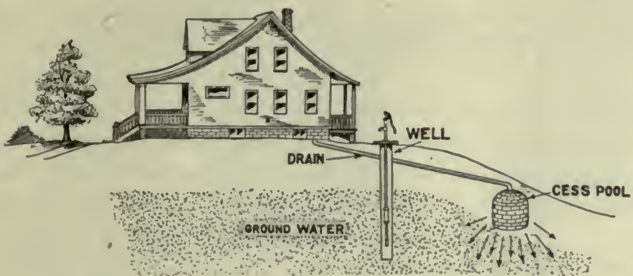

Fig. 37. Typhoid Fever Spread by Water
The entrance of infectious material into a cesspool is likely to contaminate the well unless it is some distance from the cesspool

entrance of surface water, or seepage from the upper soil layers, infectious material may be carried into the well by the drainage water. If the well is a drilled one, the iron casing should extend to an impervious layer of soil or rock, and the curb should be constructed so that no waste water can find its way into the well. If the well is dug instead of drilled, the upper portion of the protecting wall should be laid in concrete, and the surface properly protected by a concrete curb.

No definite statements can be made as to the distance infectious material may be carried by the drainage water, as this depends much on the porosity of the soil. If the soil is clay, gravel, or sand, the movement of infectious matter will be for only a short distance; but if the soil is underlaid with limestone, underground channels may develop by the solution of the limestone, which may carry the organisms for considerable distances. The well should not be less than one hundred feet from any possible source of pollution.

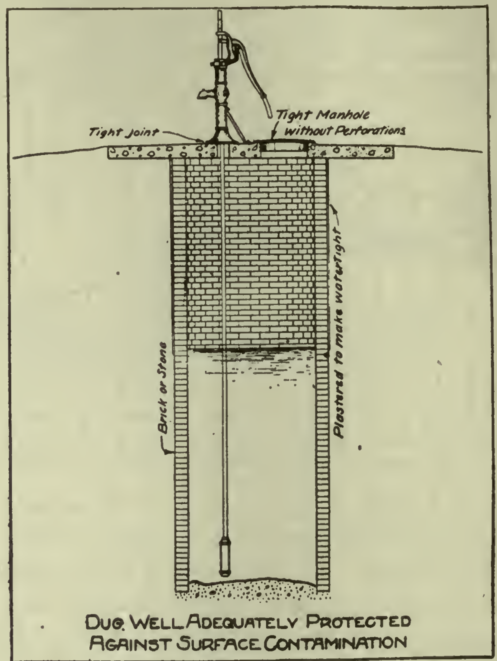

Fig. 38. Protection of a Well

All wells should be protected from surface water since this may carry disease-producing organisms

The chief protection is to be found in the filtering effect of the soil. If no water can enter the well until it has first passed through at least fifteen to twenty feet of soil, there will be little danger of infection.

There are many ways in which contaminated water may come in contact with milk, as in the case of rinsing the milk utensils with cold water, and the accidental or intentional addition of water to milk.

The protection of the farm water supply against contamination with typhoid bacilli is important, not only from the standpoint of the health of the farm home itself, but also from the point of view of the homes to which the products of the farm find their way. Milk is the most important product in this regard, because the typhoid organism can grow so luxuriantly in it, and because so large a proportion is used without previous heating.

Springs are the outlets of underground streams. Spring water is usually free from bacteria when it issues from the ground, but it immediately becomes seeded with organisms from contact with the organic matter in the soil, unless special precautions are taken to guard it.

Contamination from typhoid patients.—Direct infection of milk sometimes occurs where a person in contact with a typhoid case, as a nurse, also is concerned with the preparation of food or the handling of milk. Such infection can occur only when carelessness obtains with reference to the cleansing of the hands after handling the patient. In these days physicians give strict directions that all the discharges of a typhoid patient shall be treated with a disinfectant that will destroy the typhoid bacilli; but if care is not taken by those coming in contact with the patient, they may not only acquire the disease themselves, but may serve to spread it to others. The recognized case of typhoid is not so dangerous as those that are not clinically apparent, such as mild cases for the treatment of which no physician is consulted. The attack may be so slight that the individual may not be aware of any appreciable illness. Yet the virulent organism is eliminated in these cases to the same extent as in pronounced cases.

Typhoid carriers.—When recovery from typhoid takes place, the bacilli usually disappear from the body, but in about 4 per cent. of cases the organisms persist for a period of time. Exceptional cases have been recorded in which the organisms were eliminated for many years after recovery. Such people are known as *typhoid carriers.* It is estimated that about one in each thousand of the population is to be classed as a typhoid carrier. Whenever such a carrier is engaged in the preparation or handling of food, an epidemic of typhoid may result. An outbreak of four hundred cases in New York city was traced to a person who had had the disease forty-seven years before. As has been stated, the contamination of milk is important, due to the opportunity for the growth of the bacilli; but any food may become contaminated and thus become the cause of trouble. Since no one recognizes the typhoid carrier as such, and since generally he does not even know his own condition, the problem of protecting the public against this source of typhoid fever seems to the modern health officer impossible of solution.

Oysters and typhoid fever.—Shell-fish represent another food that is not infrequently concerned in the spread of typhoid fever. When oysters are removed from the salt water in which they have grown, they are placed in fresh water for a short period in order that they may be "fattened" or "plumped" by the absorption of water. If the water in which they are placed is polluted with sewage, the oyster will be contaminated, and since they are often consumed raw, opportunity is presented for the transmission of the organism.

The house-fly.—Another agency in the distribution of typhoid fever is the house-fly. If infectious material is deposited where flies have access to it, they may carry the organisms to the food with which they come in contact. All privy vaults should be so constructed that flies can not or will not enter them; all homes and all places in which food is prepared or sold should be effectively screened.

Diphtheria and scarlet fever.—Diphtheria and scarlet fever are also spread by means of milk. The opportunities for the contamination of foods by the causal organisms of these diseases are not so varied as in the case of typhoid fever. It is probable that the infection is largely from mild cases that are not recognized, or through the agency of a person acting in the dual capacity of nurse and milker.

The influence of bacterial content on healthfulness.—The question concerning the effect of the growth of saprophytic bacteria in food on its healthfulness is an important one, since a large proportion of the food materials that reach the market are in an incipient state of decomposition. Frequently it has progressed to such an extent as to be evident to the senses. The value of such food is lessened thereby. The question as to its absolute rejection as human food will depend on whether it is intrinsically dangerous, rather than on the impression it may make on those whose economic condition is such that they can afford to reject it. There is little reason to believe that decomposition processes in ordinary foods are capable of producing disease. This is especially true of fruits, and possibly less true of meats, fish, and certain vegetables.

Many common foods are used only when they have undergone certain types of decomposition. The same type of decomposition in another food would cause its rejection for esthetic rather than for hygienic reasons. In a general way, it may be said that the control of foods with reference to their bacterial content is an economic rather than a hygienic matter, and there would seem to be little reason for not allowing the sale of foods that show evidences of decomposition to people who wish to purchase them at a fair price.

It is believed that milk in which a considerable amount of bacterial growth has occurred is less healthful that milk of lower bacterial content. There is no reason to believe that the organisms of the *Bact. lactis acidi* group injure the healthfulness in any way, no matter how much they may have grown in it. There are many other groups of bacteria constantly present in milk for which such an assertion can not be made with assurance. Among these may be classed the liquefying bacteria and those of the *B. coli-aërogenes* group.

Infant mortality.—It is thought that the milk supply has much to do with the high death rate of young children. In some American cities more than 40 per cent. of the children die before they reach one year of age. The greater number of these are fed on some substitute for mothers' milk, cows' milk being most frequently used. It is claimed that if the milk were of better quality, if it contained less bacteria, and had undergone less decomposition, a great decrease in the death rate of artificially fed children would be noted. It is certain that the most powerful factor concerned in the great improvement of market milk that has taken place in recent years has been the effort to reduce the high rate of infant mortality.

Poisonous foods.—Substances that are very poisonous when swallowed are formed by certain bacteria, the most prominent of which is *B. botulinus,* a spore-forming anaërobic organism. The organism received its name from the fact that it was first found in sausage (Latin, *botulus,* sausage). For some time it was thought that only foods of animal origin permitted the growth of this organism. More recently, cases of botulinus poisoning have been traced to various vegetables. In almost all instances the trouble has been caused by the use of canned rather than fresh materials. The spores of the organism enable it to resist the heat treatment that the foods received, and its anaërobic nature enables it to grow in the cans, which are practically devoid of air.

The toxic substance produced by this organism is of great potency. As small a quantity as 0.01 cubic centimeter of a glucose broth culture can produce death in a monkey or rabbit when given through the mouth. The extreme toxicity of this by-product of the organism may make a food dangerous before any signs of decomposition are apparent to the senses. The poisonous substance is easily destroyed by heat. If a canned vegetable or meat shows the slightest sign of spoilage it should not be used for food until it has been heated to the boiling-point. The organism itself is unable to develop in the animal body. The injury is due to a preformed product which is ingested. Thorough heating of foods just before use is the factor of safety against organisms harmful either in themselves or because of their by-products.

CHAPTER XV

THE PRESERVATION OF FOODS

It is impossible to handle foods in such a manner as to prevent microörganisms from coming in contact with them. With greater or less rapidity, depending on whether the organisms find in or on the food favorable conditions for growth, decomposition changes will ensue and the food be rendered worthless. In a previous chapter the ways in which foods become seeded with microörganisms were studied. The more cleanly the conditions under which foods are handled, the smaller will be the number of organisms brought in contact with them, and the less rapidly will the changes take place. The greater the number of organisms initially present, the more rapidly the decomposition changes occur, other conditions remaining constant. Hence, one of the chief ways of preserving foods is to prevent their contamination with germ life. But mere prevention of contamination is not sufficient and must be supplemented by other means, which may be divided roughly into three classes: the removal of microörganisms from foods; their destruction; and the establishment of conditions that will retard or prevent their growth. These methods may be used singly or in combination.

The microörganisms that adhere to the surface of solid foods may often be removed in large part by washing. Microörganisms are practically always contained in dirt and other matter foreign to the food. Consequently, the removal of this dirt will tend to reduce such contamination. Washing or wiping of fruits, such as apples, tends to remove the molds that are concerned in rotting processes;

and the washing of meats and of many other foods will have some influence in retarding decomposition changes.

Purification of water.—Water may be classed as a food; and, while it is not subject to decomposition, and hence one cannot speak of preserving it, it does serve to distribute disease-producing organisms. The measures that are taken to preserve foods often serve to destroy any pathogenic organisms they may contain, just as they destroy the saprophytic organisms.

Bacteria, harmful and otherwise, may be removed from water by passing it through filters of unglazed porcelain. The pores of these filters are very fine and tortuous, so that the bacteria are held back even though the pore spaces are of greater average diameter than the organisms. If, however, the filters are used for a considerable period of time without cleaning, some forms of bacteria will multiply in the pores and the filtrate will no longer be sterile. The filters must be frequently cleaned and sterilized, if they are to function in an efficient manner. The purification of water in percolating through the soil is also due to the same principle.

Many cities purify their water supplies by filtering surface waters through filters composed of layers of gravel and sand, arranged so that the finer material is on the surface. The upper surface of the filter soon becomes coated with a gelatinous layer of bacteria and sediment which is so coherent that it prevents the organisms in the water from passing through the sand. As this sediment layer increases in thickness, it becomes less permeable, thus reducing the amount of water that will pass the filter. In time this filtering surface must be removed. For the first few days after this disturbance, the efficiency of the filtration process is reduced, until the gelatinous layer is reëstablished. By means of this process, it is possible to reduce the bacterial content of surface waters from 95 to 99 per cent. and to eliminate practically all danger from typhoid and other water-borne diseases.

Another method of purifying water supplies consists of producing gelatinous precipitates by the addition of chemical agents such as salts of iron or aluminum. When these are added to water containing lime in solution, insoluble compounds of a jellylike nature are formed. All fine particles, including bacteria, are enmeshed in the gelatinous material, and thus can be removed readily by sedimentation or filtration. When water so treated is passed through coarse filters, these gelatinous precipitates are readily removed, and the water thereby not only clarified, but its germ content materially reduced.

The fine turbidity present in wines is removed in a similar way. When small quantities of gelatin are added, the tannin naturally present in wines causes to be produced an insoluble gelatinous precipitate that is readily removed by filtration.

The harmful organisms in water may be destroyed by heating it to the boiling-point. They may also be killed by adding to the water a minute quantity of calcium hypochlorite or bleaching powder, which has a powerful germicidal action in the absence of all but traces of organic matter. The odor and taste of chlorine is at first evident in the treated water. This soon disappears and the hypochlorite is changed into harmless substances. One part of the reagent to one million parts of water is often sufficient to destroy 99 per cent. of the bacteria therein. The treatment is applicable to any quantity of water. Some large cities chlorinate their entire supply. The water supplies of the armies in the recent war were safeguarded by adding hypochlorite to the water-tanks whenever they were filled.

Inhibition of microörganisms.—It is impossible to produce and prepare foods without contaminating them with microörganisms. No appreciable decomposition can take place without the actual growth of the organisms. Any treatment that will inhibit or prevent the growth of bacteria and allied organisms will favor the preservation of food supplies. Many chemical and physical agents influence the proliferation of microörganisms.

Desiccation.—Nature's method of protecting organic matter from spoilage is by the removal of water. It is by far the most important way of preserving foods and fodders. The great staple foods, grains, flours, meals, hays, and other roughages, are thus preserved. Fruits as raisins, currants, and prunes are protected by drying in the sun. The artificial dehydration of vegetables has made great progress and is destined to become more important as time passes. The process obviates the transportation of large quantities of water and the use of expensive containers. When appropriate means of drying are used, the natural flavor and texture of the original materials are restored by allowing them to take up water.

The dry foods absorb water if kept in a damp atmosphere. The molds, due to their ability to secure water where other organisms can not, are the first to appear on the moist food. The molding of hay and grain are important examples of the ability of molds to grow in the presence of small quantities of water. Meats, fish, eggs, and milk are preserved by the removal of water. Edible oils, such as olive and cottonseed, owe their keeping qualities to their freedom from water.

Preservation by concentration.—In an earlier part of this book the relation of the cell to the density of the liquid surrounding it was presented. It was shown that the ease with which the cell is plazmolyzed varies widely with the different groups of microörganisms. As a group, the bacteria are the least resistant to the action of solutions of high osmotic pressure; the molds are the most resistant. This differentiation in the ability of organisms to grow in concentrated solutions is of great importance in food preservation. Concentration alone can not usually be relied upon to protect food materials, but must be supplemented by some other agent or process. Frequently heat is applied in concentrating the material, as in preparing syrups from the sap of the cane, the beet, or the maple; or heat is applied in the preparation of the food after the concentration has been raised by the addition of sugar or salt. The yeasts and molds that are most likely to grow in such materials are destroyed by the heat; and, unless recontamination occurs, the food should keep. The exclusion of air, thus preventing mold growth, is another supplementary factor.

Syrups owe their keeping qualities to their concentration. If these sugary liquids are insufficiently concentrated, they undergo most commonly an acid fermentation, caused by bacteria. Condensed milk is prepared by the concentration of fresh milk and by the addition of cane sugar. Jellies and jams are protected from bacteria by their concentration. The heating they receive in preparation frees them from yeasts, and mold growth is prevented by covering the surface with a layer of paraffin. The addition of salt to meats and fish, coupled with partial drying, is a common practice. The same materials may also be preserved by placing them in a saturated solution of salt.

Preservatives.—Many chemical substances exert an injurious effect on microörganisms, inhibiting their growth when present in such minute quantities that their effect can not be ascribed to physical action. They act chemically, and are usually called *preservatives*. Among those most commonly employed have been boric acid and borates, benzoic acid and its salts, salicylic acid, and formalin. Boric acid is used in butter, especially in that made in New Zealand and Australia to be shipped to the English markets. Benzoic acid is used in catsups and ciders, while salicylic acid is contained in the canning powders that have been widely sold in the past. Formalin has often been used in milk. One part of formalin to ten thousand parts of milk will have a marked inhibiting effect on bacterial growth. The use of such chemicals in foods is prohibited by law in most States, and by the national government in its control of the interstate commerce in foods. The use of benzoates is allowed in certain foods, but the amount used must be stated on the label.

As to the effect of such preservatives on health, different views are held by various authorities. The prohibition of their use is a wise one, since the foods in which they would be used can be preserved by other means concerning which there is no question as to their effect on the health of consumers.

Certain chemicals sometimes added to foods unite with definite decomposition products, thus masking the effect of the changes produced. If sodium bicarbonate is added to milk, it combines with the lactic acid, and thus reduces the acidity of the product. Sulphites are used with meats, particularly chopped meats, to impart a bright red color to the same and to neutralize the odors produced in putrefactive changes. Potassium nitrate is used in corned beef. Some of the nitrate is reduced to nitrite, which is an active agent. It also imparts a bright red color to the meat. The use of this latter substance is not regarded as dangerous. Generally speaking, such chemicals can be used in sufficient amounts to accomplish the desired result without being apparent to the taste. If their use were sanctioned by law, materials unfit for food would be sold.

Some of the condiments, such as cloves, cinnamon, and mustard, contain essential oils that have a preservative action that is more marked on molds than on bacteria. They are used especially in pickles, catsups, mincemeat, and fruit-cake. No regulations concerning their use are needed, for the reason that the amount that can be added to a food is limited because of their influence on flavors.

In the smoking of meats, chemical compounds of the creosote type are produced by the slow or imperfect combustion of wood, and are deposited on the surface of the meats. Certain woods, such as beechwood, yield a special flavor that is much prized. Of later years the so-called liquid smoke, which is a by-product of wood distillation, is often used as a surface application. Its value as a preservative depends on the disinfecting action of the creosote.

Organic acids.—Organic acids are widely used in the preservation of foods. The acids may be formed in the foods by decomposition processes, or they may be added, as in the case of the addition of vinegar to pickles. Sauerkraut is prepared by cutting cabbage and packing it tightly in vessels with 2 per cent. of common salt. The pressure and the action of the salt extract the juices from the plant tissue. This liquid, which contains sugar, protein material, and various salts, makes an excellent medium for the growth of lactic-acid-forming bacteria. The amount of acidity thus produced is sufficient to inhibit entirely all development of putrefactive bacteria. As long as the acid reaction is maintained, the kraut remains edible. The acid may, however, be destroyed by the growth of molds and yeasts on the surface of the liquid, but if the sauerkraut is placed in kegs and thus kept from the air, no mold growth can take place. The action of acid-forming bacteria is also important in the preservation of certain pickles, especially cucumber pickles made in brine.

Silage.—The preservation of green fodder, especially corn, has become of great economic importance. It is customary to cut the material to be ensiled into short pieces, so that it may be closely packed. This process permits the sap to exude, and gives the bacteria that were on the surface of the tissue access to it. Bacterial growth is rapid; lactic and acetic acids soon accumulate to such an extent as to exclude the growth of putrefactive forms. The fresh vegetable tissue carries large numbers of acid-forming bacteria on its surface, so that no inoculation is necessary.

The oxygen of the air between the pieces of ensiled material is soon exhausted by the respiratory processes of the cells of the plant tissue. The result is that the air consists of carbon-dioxide and nitrogen. No molds can grow under these conditions. If the wall of the silo is so constructed that no air can pass through it, and if the silage is closely packed, so that air can not penetrate deeply into it from the surface, the acid mass will keep for an indefinite time.

In those areas in which molds grow, as near the surface, the acid will be destroyed and conditions thus established that will permit the growth of putrefactive bacteria. The silage in these places will be dark in color and will have an offensive odor, and the tissues will have disintegrated, while that in which the growth of molds and putrefactive bacteria has been prevented will show none of these changes.

Preservation by low temperature.— The use of low temperatures to restrain or prevent the growth of microörganisms is of the greatest importance. The methods involved in artificial refrigeration and their application to the cold-storage industry are largely the outcome of the relation of cold to the preservation of food supplies.

The temperature zone within which bacterial growth can take place extends from a few degrees below the freezing point of water to about 158 ° F. It is true that no one organism possesses the ability of growing throughout this entire range of temperature. Most kinds are able to develop from temperatures in the neighborhood of 50 ° F. to somewhat above blood heat. Also there are groups capable of multiplying at or near the freezing-point, and others at temperatures of 120–140 ° F. Those types habituated to low temperatures are of much practical significance in the storage of foods.

The greatest importance of low temperatures in the preservation of foods is not found in the extreme temperatures that are employed in cold-storage warehouses, but in the range that occurs in daily life under the conditions obtaining in the ordinary household. The great majority of bacteria grow most rapidly from 60 ° to 100 ° F. If the temperature is reduced to 50°, the rate of bacterial multiplication is much retarded, as is to be noted from the following table in which is given the time required for the division of a single bacterial cell into two completely grown daughter cells at different temperatures.

The generation time of B. coli

45° C.	(113° F.)	20 minutes
40° C.	(104° F.)	17.2 minutes
35° C.	(95° F.)	22 minutes
30° C.	(86° F.)	29 minutes
25° C.	(77° F.)	40 minutes
20° C.	(68° F.)	95 minutes
16° C.	(60° F.)	120 minutes
10° C.	(50° F.)	14 hours, 25 min.

As ordinary refrigerators maintain a temperature varying from 45° to 50° F., it is evident that bacterial growth is greatly retarded by maintenance of food material under readily available low-temperature conditions.

The zone of bacterial existence is far wider than the zone of growth. This fact is of importance in the storage of foods, for when they are removed from storage, the microörganisms that were present when the foods went into storage begin to grow, and the decomposition changes rapidly occur. Indeed, with some foods, especially meats, these changes develop after freezing with even increased rapidity, owing to the fact that the muscle fibers are forced apart by the freezing process, allowing the cell juices to exude from the cells themselves. This permits the bacteria to act more readily on the meat than when confined exclusively to the surface. Even after storage at extremely low temperatures, below 0° F., foods spoil rapidly; for while alternate freezing and thawing are very injurious to bacterial life, microörganisms are able to withstand exposure to low temperatures for prolonged periods of time.

In the storage of foods at low temperatures, the effect of freezing on the physical properties of food must be considered. For example, eggs, fruits, and vegetables can not be stored without injury at temperatures at which freezing will occur. If milk is frozen and allowed to remain in this condition for any considerable period, the fat is altered physically, so that on thawing it does not mix thoroughly with the serum. Longer exposure causes the casein to separate.

In the case of foods that can be stored with impunity at temperatures below freezing, the period of storage may be greatly extended; but with such foods as milk and eggs, where storage must be above freezing, the holding period is limited because of the fact that bacterial development can occur at temperatures slightly above freezing. In milk the development of the acid-forming bacteria is stopped at these temperatures; consequently the milk may not undergo the customary souring change, but other bacterial types that act on the casein can grow slowly at these low temperatures, rendering the milk unfit for use within a few weeks. It is believed that many of the cases of poisoning due to ice cream have been caused as a result of the storage of cream, a practice that has now been largely abandoned.

Even in the case of foods that are not contaminated, by microörganisms, changes may go on that limit the time that the food can be held in storage. These changes are due to the enzymes that are normally present in many uncooked foods, and are usually termed autolytic changes. The *ripening* of meats is an example. In the case of eggs the white becomes less viscous, and loses to some extent its beating properties. Water also passes from the white into the yolk; the membrane surrounding the yolk weakens, so that when the egg is broken, it is difficult to avoid mixing the yolk with the white.

Preservation of eggs.—In preserving eggs it is necessary to prevent the invasion by bacteria and to limit the loss of water from the eggs. Eggs are practically sterile when laid, but, owing to the porous nature of the shell, bacteria are able to penetrate it readily if the surface of the egg is moist. These organisms penetrate the shell in the same manner as they do the wall of a porcelain filter, *i. e.*, by growing through it. If the eggs are left in a dirty nest, or placed in a damp cellar, the adhering moisture may be sufficient to enable the bacteria on the shell to multiply and so penetrate the shell. In cold-storage rooms it is essential that the temperature be kept constant, so that moisture will not condense on the surface of the eggs.

For home use, eggs may be preserved by placing them in some liquid in which bacterial growth can not take place, but the liquid must be of such a nature that it will not be absorbed by the eggs. For this purpose sodium silicate, or *water-glass,* is most frequently employed. The colloidal nature of this silicate prevents its passage into the egg, and the alkalinity of the solution stops all bacterial growth. No desiccation can, of course, take place. If the eggs have been invaded by bacteria before placing them in the water-glass, the growth of the bacteria will not be inhibited. In such cases, gaseous by-products may be formed in the egg to such an extent that rupture of the shell occurs, in which case the ill-smelling decomposition products will be absorbed by the remaining eggs to such a degree as to injure their commercial value. The best practice is to place the eggs in water-glass the same day they are laid.

Preservation by heat.—Virtually the only way by which the microörganisms present in any food can be completely destroyed is by the application of heat. The vegetative cells of bacteria, yeasts, and molds are easily destroyed when subjected to temperatures approximating 140°–150° F. The spore stages of all types are more resistant, but particularly so with the bacteria that require a temperature exceeding that of the boiling-point before they can be completely destroyed. It is therefore easy to free any food substance from all organisms except the spores of bacteria.

In practice two processes are used: first, the application of a temperature slightly exceeding the scalding-point of water, from 140° to 165° F. This process, known as *pasteurization,* from the fact that it was first employed by Louis Pasteur in the treatment of wines to prevent abnormal changes, does not destroy all microörganisms, but only those in the vegetative or growing stage. The more effective process, known as *sterilization,* utilizes temperatures equaling or exceeding the boiling-point. The former method is employed when it is not desired to keep the material for long periods, as in the commercial handling of milk. It is also used when the presence of bacterial spores is of no importance, because of the fact that their germination is prevented by the reaction of the material. The higher temperature is employed in the preparation of canned foods where complete destruction of bacterial life is necessary to preserve such material for relatively long periods of time.

Pasteurization of milk.—The organisms concerned in the spoiling of milk are non-spore-forming bacteria, and are easily destroyed. Milk may also contain pathogenic organisms which it is necessary to destroy. Fortunately, those disease germs that are likely to be spread through the agency of milk are non-spore-forming, so that protection of milk supplies may be secured through pasteurization.

In the treatment of any food with heat, the physical effect on the material must be considered. In many cases the chemical and physical changes are such as to injure the commercial value of the food. If milk is heated above 145° F. for any length of time, the process of creaming is much retarded. The fat globules in milk are not uniformly distributed throughout the entire milk, but are grouped in masses that present a relatively smaller surface in proportion to their volume than do the separate globules. The viscosity of the serum is such that it offers a certain amount of resistance to the rising of the fat. The larger the mass of fat the more readily does it rise to the surface.

In the commercial handling of milk in bottles, it is desirable that the creaming take place rapidly, since an indistinct or thin cream line is considered by the consumer as indicating a milk low in fat content. The cooked taste that is imparted to milk by too high a degree of heat is objectionable to many consumers. It has often been stated that heated milk is less digestible and likely to cause nutritional troubles in children.

Milk contains growth-stimulating substances of an unknown nature. These bodies are known as vitamines. The heating of milk partially destroys these important substances, and for that reason becomes an objectionable process. The advantages of pasteurization greatly outweigh the disadvantages. If it seems desirable, the loss of vitamines in milk, due to the heating thereof, can be compensated for by the feeding of fruit juice, especially that of the orange. Cases of malnutrition and abnormal development of the bones, rickets, have likewise been ascribed to heated or pasteurized milk. But there is no reason to believe that milk as now treated is more likely to be the cause of such troubles than is raw milk. In fact, milk heated to the boiling-point is successfully used with children.

In the treatment of any food the degree of heat necessary to destroy the organisms is dependent on the length of time the material is exposed to its action. Low temperatures for long periods of time may be as effective as higher temperatures for shorter exposures. In the pasteurization of milk, the exposure may be at 145° F. for from twenty to thirty minutes or 160° F. for one minute. Either of these methods will insure the freedom of the milk from pathogenic bacteria, and will destroy such a proportion of the acid-forming bacteria as to improve the keeping qualities of milk. In actual practice no method has been devised by which milk can be heated momentarily with perfect success. In machines designed for this so-called *flash* method of heating, the milk is allowed to flow through the heating-chamber in a continuous stream. In such a device the rate of flow is not uniform in all parts of the machine.

The milk in contact with the walls flows less rapidly than that in the middle of the machine; consequently, while some of it may be heated sufficiently long to permit of the thorough destruction of all disease-producing bacteria, if they are present, other portions may be insufficiently heated. When the possibility exists that such organisms as the typhoid and tubercle bacteria may be present, it is apparent that no method can be regarded as entirely satisfactory that will not insure the destruction of these types.

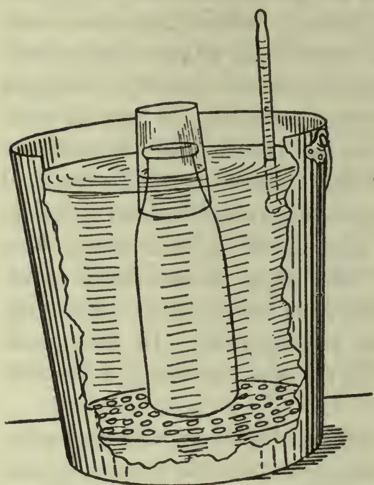

Fig. 39. A Home-Made Pasteurizer
Unless the milk is known to be safe, it should be pasteurized in the home. A tumbler is a convenient cover for the milk bottle during pasteurization and storage. A floating dairy thermometer is desirable

While this *flash* method of pasteurization was earlier adopted by many milk dealers for the treatment of milk supplies, it has not been with the full approval of health authorities, and gradually it has been displaced by the more efficient *holding* method. The fundamental principle of the holding process is that a given quantity of milk can be held at any desired temperature for any given length of time. This makes it possible to treat the milk in such a way as to insure perfect safety, through the complete destruction of disease-producing organisms.

In the methods described the destruction of all bacteria is not attained. To prevent the rapid development, it is essential that the pasteurized milk be cooled quickly and stored at a low temperature. As milk is commercially handled, not all of the acid-forming bacteria are killed; hence, pasteurized milk sours as does raw milk, but the process is materially delayed. If heated at the higher pasteurizing limits, all but the spore-forming bacteria will be destroyed. Such milk will not sour, but decomposition changes will occur in which the casein and albumen will undergo a change.

Pasteurization of other liquid products is frequently employed. Beer is heated to low temperatures after it is placed in bottles, to prevent the appearance of fermentations that might impart an undesirable taste and appearance. The presence of alcohol and some of the extractives from the hops tends to prevent the growth of the bacteria that have not been killed by the heating. Wines are also heated in a similar way to overcome the turbidity that sometimes results from bacterial changes.

Such acid products as tomatoes, rhubarb, and grape juice can be preserved by a short exposure at the boiling-point. This insures the destruction of all but the spores of bacteria, the development of which is prevented by the acid reaction. To obviate subsequent infection, it is advisable to treat such material after it has been placed in containers. Such a method has recently been introduced with milk, but the attendant expense is such that it has not been generally adopted by milk dealers, even though it would insure complete immunity from milk-borne disease.

Preservation by sterilization.—The indefinite preservation of foods in closed containers has been rendered possible through the application of the process of sterilization. On this principle rests the great development of the canning industry, which has assumed such tremendous proportions of late years. While it is impossible to raise the temperature of boiling water above 212° F., the temperature of steam when confined increases rapidly above that point. Under a steam pressure of fifteen pounds to the square inch, a temperature of 248° F. is attained, which is sufficient to destroy all forms of bacterial life, even the most resistant spores.

In the treatment of many foods, the heating process can not be continued for too long a period without injury to the physical properties of the product, as, for instance, with milk heating at excessive temperatures causes the casein to curdle. In the treatment of peas, if heated at too high a temperature or for a prolonged period, some of the peas crack open, allowing the contents to escape. This results in a turbid liquid which is unattractive and gives the impression of an abnormal fermentation. To prevent this the cans should be exposed for a longer time at a lower temperature. In the earlier days of the canning industry much loss was occasioned from the development of abnormal fermentations, due to the growth of gas-producing bacteria, the spores of which were not destroyed by insufficient sterilization. Even under the anaërobic conditions that prevailed in the closed container, luxuriant germ growth could occur.

With milk, peas, and vegetables, the reaction of the liquid is sufficiently neutral to permit of the ready germination of any spores that may have escaped destruction in the heating process. Since gas is generally produced as a result of such fermentation, spoiled food products preserved in tin containers can usually be detected by the bulging of the ends of the can. This pressure may develop to the point where the cans actually explode. In the treatment of meat products, there is no practical danger of overheating, so the losses that occur are relatively small.

In household preservation of vegetables, sterility can be secured by heating the product to the boiling-point on three successive days, as described on page 36. Small steam-pressure cookers have also been recently introduced for household use. In the canning of fruits in the home, the sugar that is added for flavor aids in the preserving process, through the fact that it increases the concentration of the liquid and also raises the boiling-point of the solution so that the spores are subjected to a temperature above 212° F.

It has been shown by recent investigation that much of the canned material is not sterile. It is not essential that any material be free from all living microörganisms or their spores in order to be protected from decomposition. If the organisms present are unable to grow under the conditions that obtain in the material, their presence is of no importance. The spores of many aërobic bacteria are among the most resistant, and are most likely to persist in foods after processing. In the sealed can that has been largely freed from air before being sealed, the aërobic spores can not germinate. The spores of anaërobic bacteria in the food are far more likely to cause trouble in canned goods than those of aërobic forms. The development of the spores of anaërobic forms may be prevented by concentration, acids, and preservatives.

CHAPTER XVI

THE FERMENTATIONS OCCURRING IN FOOD PRODUCTS

In the preparation and handling of foods, they inevitably become seeded with a great variety of organisms. This contamination is a more or less constant factor, in that representatives of the three great groups of microörganisms, the bacteria, yeasts, and molds, are always introduced. The relation between the groups will vary, depending on the nature of the food material. Whether one group or another is to be most active in the decomposition changes will depend on the composition and concentration of the material. Such foods as meats, which are high in protein and low in carbohydrates, undergo putrefactive changes, due to bacteria that act primarily on the protein. If the material contains a large amount of sugar in proportion to the protein, and the reaction is not acid, the type of fermentation will be similar to that noted in milk in which the sugar is fermented with the production of acid, and the protein is not attacked to any degree. If the material contains sugar and has an acid reaction, a condition found in many fruit juices, yeasts are likely to be the dominant type of organism concerned in its decomposition. Alcohol and carbon-dioxide are the chief products of yeast action.

The acid fermentation of milk.—The souring of milk is so common that it is looked upon as a normal change; in fact, its absence is more likely to be regarded as an abnormal condition than is its occurrence. If milk could be secured without microörganisms, it would remain unchanged for many days; but, in the normal course of events, it invariably becomes seeded with a variety of organisms, which in a short course of time are able to develop and produce the characteristic fermentative by-products that bring about the usual changes noted. These changes are not produced by a specific organism, but by a group of widely dissimilar species as far as form is concerned, which, however, are able to produce varying amounts of acids, particularly lactic acid. While this fermentation product is produced in such quantities as to characterize the change involved, yet other by-products are likewise formed, as other acids, gases, and various substances. Some of these flavor-forming products may be of value, as in the case of those producing agreeable flavors in butter.

The curdling of milk.—The casein of milk is not in actual solution, but exists in a colloidal condition in combination with calcium. As the bacteria multiply, a portion of the sugar that they use is changed to acid, which combines with the calcium, leaving the casein free, in which condition it is precipitated in the form of curd.

The bacteria that are primarily concerned in the acid fermentation of milk may be divided into two groups. The name *Bact. lactis acidi* is applied to the typical representative of the first group. The natural habitat of this organism is unknown. It is a facultative non-spore-forming organism. Its optimum temperature is from 86° to 95° F. It grows rapidly at from 60° to 68° F., and some strains at 50° F. Owing to its facultative nature, it grows throughout the entire mass of milk, causing a uniform curdling. Normally it is not introduced into milk in as large numbers as many other kinds of bacteria; but, as it finds in this medium an exceedingly favorable environment, it is responsible for most of the decomposition that is noted in milk. No gas is formed by this organism. It produces chiefly lactic acid and small quantities of volatile acids, such as formic and acetic. Other substances of unknown nature are formed in minute amounts.

The effect of its growth in milk is to produce a jellylike curd that has an agreeable odor and a mild acid taste. When the curdled milk is shaken, the curd becomes very finely divided and the milk assumes a creamy consistency. The milk fermented by this organism forms an appetizing and healthful food.

The second group is commonly known as *B. coli-aërogenes* group. The organisms are facultative and non-spore-forming. Some produce sufficient acid to curdle milk; others do not. The chief characteristic is the formation of a mixture of carbon-dioxide and hydrogen in greater or less abundance. The formation of gas in curdled milk causes an open or spongy curd. The milk in which these organisms have grown usually has a disagreeable taste and odor, for which reason they are dreaded by butter- and cheese-makers.

The organisms of the first group may be said to be desirable, since they protect the milk from the action of other less desirable forms and are of great value in dairy manufacturing. The coli-aërogenes organisms are undesirable from every point of view.

If the milk has been produced with due regard to cleanliness, the lactic bacteria will generally predominate, but if quantities of manure and dirt find their way into the milk, the colon group of bacteria will gain the ascendency. It is often asserted that milk fermented by the colon group of bacteria is unhealthful. Whether this is true may be questioned, but every consideration, both esthetic and economic, demands that the milk be produced under conditions that will render contamination as small as possible.

Only a portion of the sugar of the milk is fermented by these acid-forming bacteria. The casein and the ash constituents of the milk are able to unite with a certain amount of acid, but as soon as free acid appears in the milk the growth of these bacteria is checked. The amount of acid normally formed ranges from 0.8 to 1 per cent.

The acid-forming bacteria of milk are non-spore-bearing, a fortunate provision, since it permits of the use of the pasteurization process as an aid in the preservation of milk. Each of the groups is able to grow both in the absence and the presence of air—again a fortunate circumstance, otherwise the use of either group in dairy manufacturing would be impossible.

Milk forms an excellent example of the preservative action of acid against putrefaction. The increase of acid due to the lactic bacteria quickly inhibits the development of types capable of attacking the casein and albumen. As long as the milk remains sour, it is not subject to the action of putrefactive bacteria. But usually there appears on the surface of the sour, raw milk in the course of time a white mold, *Oïdium lactis,* which uses the acid by-products as food, gradually changing the reaction from acid to alkaline. Under these conditions the putrefactive bacteria that are always present are able to develop, and soon the milk is changed to a vile smelling mass. Milk forms an excellent example of the sequence of decomposition processes in nature where one type of life is dependent upon the by-products formed by another group of organisms.

Representatives of another group of acid-forming bacteria are found constantly in milk. The type of organism is usually called *Bact. Bulgaricum.* The members of this group are long rods which often occur in threads of considerable length. They do not form spores, are facultative, and are often classed as thermophilic, since they grow best at 110° to 120° F. The visible change that they produce in milk is identical with that produced by the lactic bacteria. The chemical change is also similar. These organisms form an exception to bacteria in general, in that they can grow in rather acid solutions. Owing to this property, they continue to grow after the lactic and colon organisms have ceased to grow. They can produce from 1 to 3 per cent. of acid in milk. They play no part in the souring of milk, since they grow very slowly at from 60° to 80° F.

These organisms play an important rôle in the ripening of certain varieties of cheese. They are also widely used in the preparation of fermented milks, either alone or with the lactic bacteria. Attention was first directed to them in this connection by the investigations of Metchnikoff in regard to the cause of senility. He believed it to be due to the gradual change in the intestinal flora from one that was primarily acid-forming to one in which putrefactive bacteria predominated. His idea was that this change could be prevented by the ingestion of the peculiar type of fermented milk used by the people of Bulgaria. Metchnikoff believed the Bulgars are characterized by a large number of people who attain an extreme age, a fact that he ascribed to the use of *yoghurt,* the name given to the fermented milk as it is prepared in Bulgaria.

The organism was isolated, and cultures soon distributed to all parts of the world. Their use is often recommended by physicians for cases of digestive troubles. It is not at all certain that this particular type of organism is more valuable in this regard than is the true lactic organism. In fact, most of the fermented milk, commercially prepared, is made by employing the two groups of organisms, which are grown separately, and then the fermented milks are mixed in the desired proportions. *Bact. Bulgaricum* gives to the fermented milk a smooth, creamy texture, which is sometimes difficult to secure with *Bact. lactis acidi* alone.

Buttermilk and cottage cheese are to be classed as types of fermented milk. Both form excellent examples of a material that has undergone fermentative changes, and yet is a desirable and a healthful food. Milk in the fermented form is widely used in the Southern States and in certain tropical regions where it would be difficult to keep it in the unfermented condition.

Sweet curdling of milk.—Sometimes milk becomes seeded with other organisms than the usual lactic type, and other fermentative changes are produced. A common type of change that is especially likely to occur in the absence of the lactic fermentation is the sweet curdling change in which the casein and albumen are acted upon, the former being precipitated in a manner similar to that caused by rennet. Since this change is occasioned by many spore-forming organisms, heated milks (sterilized or boiled) are peculiarly prone to undergo this change. In addition to the curdling enzymes analogous to rennet, digestive enzymes are also produced that are similar in their action to trypsin, which is found in the intestinal juices of all animals and which changes protein materials to soluble products. The bacterial trypsin gradually digests the curdled casein, so that the change is frequently called the digestive fermentation of milk. The digestive changes produced are very similar to those that take place in the decomposition of all proteins.

Butyric fermentation.—Those organisms that are able to ferment sugars with the formation of the volatile butyric acid are also found in milk, and at times, in the absence of the lactic acid bacteria, produce their characteristic fermentation, which is marked by the peculiar odor of the acid. Gas is also produced, and in its initial stages the fermentation may be mistaken for one due to the colon group of bacteria.

Slimy fermentations.—In sugar solutions fermentations are often noted that change the solution into a slimy or ropy liquid. In the manufacture of sugar the syrup may be changed into a mass almost jellylike in consistency, due to the growth of bacteria that possess a gelatinous capsule around the cell. In maple sap a ropy change is often noted, and the same is true of milk.

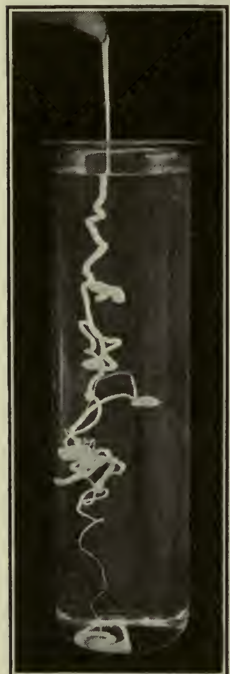

Fig. 40. Ropy Milk
It may be drawn out into long threads and does not mix with water when poured into it as does milk that has undergone a more normal type of decomposition

This abnormal change seems to be produced most frequently at low temperatures. In milk two types of organisms may be concerned. The Norwegians have long used a fermented milk of this type as a drink. This prepared milk, known as *taetem-jolk,* has the taste of ordinary sour milk, but the texture is more or less slimy, depending on how long the fermentation has been allowed to proceed. The organism *Bact. lactis longi,* used in its preparation, is a member of the *Bact. lactis acidi* group, all of which form a slight ropiness in milk. The slight increase in viscosity caused thereby is desirable in any fermented milk that is to be used as food, since such change prevents the settling of the curd and the appearance of the free whey, which imparts to the milk an unappetizing appearance.

The second group of bacteria that produce a ropy change in milk are aërobic, and therefore only the upper layers of the milk show an abnormal condition. It is most commonly noted in milk stored at rather low temperatures for a period of from twenty-four to thirty-six hours. Outbreaks of this trouble often cause extensive losses in the milk-distributing business. It seems probable that utensils, once infected, become a constant source of infection of milk. Remedial measures should always include the thorough scalding of the utensils. There is no reason to believe that the milk in which either of these groups of organisms has grown is in any way unhealthful.

Various other abnormal fermentations are sometimes noted in milk when the lactic bacteria are replaced by other groups. Many bacteria produce colored by-products, and when such organisms grow in milk to any extent a color may be imparted to the same. The appearance of red and blue milk is thus explained. Bitter milk may be due to the ingestion of feeds that contain a bitter principle, or to the production of soluble decomposition products by the putrefactive bacteria. Some acid-forming bacteria produce bitter flavors.

Alcoholic fermentation.—When fruits such as the grape and apple are pressed, and the juice allowed to undergo spontaneous decomposition, the sugar will be fermented with the production of alcohol and carbon-dioxide. This fermentation is due to the yeasts that are present on the fruit, having been carried there from the soil by dust and insects. Any rupture of the skin allows the juice to exude, and thus the growth of yeast on unpicked fruit is possible. The juice is more heavily seeded with bacteria and molds than it is with yeasts. The acidity of the juice is sufficiently high to prevent the growth of most bacteria; the molds do not find so favorable an environment as do the yeasts, and hence these organisms are certain to dominate the fermentation in the fruit juice.

The variety of yeast has much to do with the quality of the wine or cider that is secured. The sugar is not changed wholly into the two products named, but other substances are formed which influence the flavor of the product.

In the making of fermented and distilled liquors, such as beer and whisky, and in the manufacture of industrial alcohol, starch is used. This is changed into maltose by the enzymes of the malt, which is prepared by allowing barley to sprout. In this process the enzyme is formed in such abundance that a small amount of malt can be used to convert the starch of a much larger amount of grain into maltose, which is easily fermented by yeasts. The liquid to be fermented will not contain sufficient yeasts to insure rapid fermentation. It is also a favorable medium for many kinds of bacteria. In order to avoid trouble therefrom, it is necessary to heat the liquid to free it from bacteria, and then to seed it heavily with selected yeasts. The variety of organism that ferments the sugar will have much to do with the flavor of the finished product. This is of especial importance in the manufacture of beer. In the manufacture of distilled liquors and industrial alcohol, it is essential to employ a yeast that will be capable of producing large amounts of alcohol before it is inhibited thereby.

The growth of harmful types of bacteria in the material to be subjected to alcoholic fermentation may be prevented by inoculating it with certain lactic-acid-forming bacteria. The acid formed by them will not influence the growth of the yeast, and since but traces of volatile acid are formed, the quality of the distillate from the fermented liquor will not be unfavorably influenced by the acid formed in the fermented material.

The alcoholic fermentation does not occur normally in milk, because lactose, the sugar in milk, is not readily susceptible to fermentation. Also, the extent of seeding with yeasts is not sufficient to enable fermentative changes to develop rapidly. As a consequence, the activity of the yeasts is usually overshadowed by bacterial changes. While most yeasts are unable to act on milk-sugar, yet lactose-fermenting yeasts do occur not infrequently, and are often to be noted in milk if it is held for some time after it has become sour. The soured milk will contain an abundance of sugar, and the acid will prove no deterrent to yeast growth. Yeast development occurs in milk and cream held for considerable periods. Cream supplied to large centralized creameries often shows a yeasty fermentation. An alcoholic fermentation is also noted in cheese factories, especially in the whey-tanks, on account of the favorable opportunity for contamination and growth of yeasts.

When such fermented whey is returned to the farm in milk-cans and these are imperfectly washed, the fresh milk may become seeded to such an extent as to cause abnormal fermentations in the cheese, injuring its commercial value. This is particularly true of Swiss cheese, in which the development of acid in the process of making is not carried sufficiently far to transform all of the sugar into acid.

Certain kinds of fermented milks are also prepared by the use of lactose-fermenting yeasts. When raw milk is used, the milk will be subject to both the acid and the alcoholic fermentations, and the taste of the acid milk will be modified by that of the alcohol. Two of these fermented milks are widely used in eastern Europe and western Asia. *Koumiss* is prepared from mare's milk by the nomadic peoples of the Caucasus. The fresh milk is inoculated with a little of the previously fermented milk, which may be dried when it is desired to keep it for long periods. The drink contains about 2 per cent. of alcohol and 1 per cent. of acid. It has been introduced into western Europe and America because of its supposed therapeutic value in the treatment of tuberculosis, and for typhoid-fever convalescents. An artificial koumiss is sometimes prepared by adding cane-sugar to cow's milk and seeding it with ordinary yeast.

Kefir is another drink prepared from milk by inoculating the milk with Kefir grains, which consist of masses of yeasts and bacteria. The grains are dried and may be bought as articles of commerce. The composition of kefir is similar to that of koumiss, except that the content of alcohol is less. These drinks have been largely supplanted in this country by yoghurt, which is prepared by the use of the *Bact. Bulgaricum.*

Manufacture of vinegar.—Ethyl or grain alcohol, as it is often called to distinguish it from methyl or wood alcohol, furnishes a source of food and energy for a class of bacteria that are known as the acetic acid bacteria, since they oxidize alcohol to acetic acid. If cider, wine, or a similar liquid containing alcohol is exposed to the air, it soon becomes covered with a whitish or gray film, or membrane, and the alcohol is gradually changed to acetic acid. The term vinegar is applied to the product thus obtained. If the film, which consists of masses of bacteria, is allowed to remain undisturbed, the liquid remains clear. If it is disturbed, the particles of membrane sink to the bottom, and the surface soon becomes covered with a fresh film. The film, which is known as mother of vinegar, may increase in thickness, and finally present the appearance of a leathery mass. The acetic acid formation can go on only under aërobic conditions, since the organisms concerned in it must have access to the free oxygen of the air to convert the alcohol into vinegar. If the alcoholic liquid is kept in closed or completely filled containers, the process does not occur. In the presence of too large quantities of alcohol, above 14 per cent., the acetic bacteria can not grow, and the alcohol is not attacked.

The juice of most fruits contains sufficient sugar so that, after the juice has undergone the alcoholic fermentation, it can be used successfully for the preparation of vinegar. The quality of the vinegar will depend on the source of the juice. The vinegars made from wine and cider are most highly prized for table use where the vinegar is used as a condiment, since they contain many organic acids and esters, derived from the fruits and formed in the fermentation, not found in vinegars derived from other sources. The flavor of these vinegars is therefore not simply that of a solution of acetic acid. Most of the vinegar used in the preparation of pickles commercially is distilled vinegar in which the alcohol is obtained from the fermentation of hydrolyzed starch. This vinegar has no flavor other than that of the acetic acid. In the pickling industry the preservative action of the vinegar is the important thing, rather than its flavor.

On the farm the vinegar is most often prepared from apple cider. It is necessary that an abundant supply of air be furnished to the organism, and that the surface of the liquid be large in proportion to its volume. These conditions are secured when a barrel is used in which the alcoholic liquid is to be changed to a solution of acetic acid. The barrel should be filled from one half to two thirds full, and then placed on its side. The circulation of air through the barrel can be facilitated if openings are made in the barrel-heads just above the level of the liquid. To prevent the entrance of flies, these holes should be screened with thin cloth or with wire netting that has been varnished so that the iron will not be attacked by the acetic acid. The addition at the start of a small quantity of vinegar, or better some of the mother of vinegar, serves to seed the liquid with the necessary acetic bacteria. The fermentative process progresses rather slowly at first, but in a few months the vinegar will be ready for use. If it is desired to continue the process in the same barrel, fresh alcohol can be added through the bung-hole by means of a glass funnel to which a rubber tube is attached. This enables the alcohol to be added without disturbing the bacterial film on the surface.

Temperatures between 65° and 75° F. are most favorable for both alcoholic and acetic fermentation. At much lower temperatures the alcohol is produced so slowly that undesirable organisms have opportunity for growth. When the acetic fermentation is complete, it is desirable to protect the acid from the action of organisms that would destroy it. This can be done by storing it in completely filled and closed containers, for all harmful organism will be of the aërobic group.

Under the best of conditions the oxidation of the alcohol to acetic acid will require a long period in the method described, since the growth of the organism is confined to the surface of the liquid. In the manufacture of distilled vinegar, the oxidation process is hastened by providing a much extended surface for the growth of the bacteria and by providing for a constant supply of air to the organisms. These conditions are secured by filling large conical tanks with beech shavings that have been extracted with water and with vinegar. The tank has a false bottom containing numerous holes for the passage of air, which enters the tank through holes in its side below the false bottom.

The alcoholic liquid is sprinkled on the surface in such a way that it will be evenly distributed over the shavings, which soon become covered with bacterial growth. The fermentative process produces heat, and hence there is a constant current of air through the holes at the bottom of the cask. The air passes upward through the shavings and out at the top. In this manner the bacteria are provided with an abundant supply of air facilitating the oxidation process. As fresh food is constantly supplied and as the by-products of the fermentation are also constantly removed, the change goes on continuously and with rapidity.

It is essential that the alcoholic solution does not contain too much nitrogenous food, since this would so accelerate the growth of bacteria as to fill the interstices with bacteria and cause a consequent reduction of the flow of air through the shavings. It is essential that the mass of bacterial growth be kept in a healthy and active condition, so that the cells shall exert their oxidizing action rapidly. Usually the liquid is passed through a series of three tanks situated one above the other. By the time it flows from the last tank, the oxidation will be sufficiently complete.

Bread.—The making of bread represents another fermentation industry that is carried out in many homes. If flour, water, a small amount of salt, and fat, such as lard or butter, are mixed and baked, a dense hard mass is obtained, such as a cracker or unleavened bread. If, however, yeast is added to the mixture, and the dough placed under conditions that permit the yeast to grow, the sugar is changed into alcohol and carbon-dioxide. The dough made from such flours as wheat contains gluten, which imparts a plasticity to the mass. As the gas is formed, it is unable to escape readily from the dough, and the whole mass "rises," producing the "sponge" which characterizes all leavened breads. When the gas is heated in the baking process, further expansion occurs, and the loaf becomes light and porous. The yeast may be obtained by saving a piece of the dough from the previous baking, by the purchase of dried or compressed yeast, or by furnishing conditions favorable to the natural seeding of the materials.

The leaven most commonly used is compressed yeast. The yeast is secured by seeding a maltose solution, as in the manufacture of alcohol, with a pure culture of yeast. Air is passed through the solution for from six to eight hours. The thorough aëration results in an increased growth of yeast. The cells are removed by centrifugation, washed, and pressed.

In the baking industry large quantities of compressed yeast are added to the dough, and the rising takes place rapidly. If smaller amounts of yeast are used, as is usual in the home, a longer time must be allowed for the growth of the yeast and the formation of sufficient gas to produce the desired porosity in the bread. In this case the yeast is added to a thin mixture of flour and water, called the batter. After standing for a few hours in a warm place, additional flour is added, and the yeast uniformly incorporated with the dough by the kneading process. More or less bacterial growth takes place in the dough, depending on the length of time it is allowed to stand before baking. The bacteria have much to do with the flavors of the bread. If the development of the bacteria has been too great, the bread is likely to have an acid or sour taste.

If no yeast is added, but the mixture of flour and water is allowed to stand, gas will be produced by the gas-forming bacteria that are normally in the flour. The yeasts that are naturally present will also function. Such bread has a quite different flavor from the ordinary product, and is called *salt-rising* bread. The flavor is far from constant, since there is no control over the kind of organisms that grow in the mass. Studies made during recent years point to the use of pure cultures of bacteria in the manufacture of salt-rising bread, and to a better control of the flavor of the product.

Bread and other bakery goods are not likely to undergo decomposition changes to any great extent, as ordinarily they are consumed in a fresh state. If kept in a damp place, mold soon appears on the surface. In the baking process the vegetative bacteria are readily destroyed, but the spores resist. At times, especially in warm weather, bread and cake may undergo a slimy fermentation, due to the growth of the spores of certain kinds of bacteria that may be present in either the flour or the yeast.

CHAPTER XVII

THE RELATION OF MICROÖRGANISMS TO BUTTER AND CHEESE

The preparation of condensed milk and milk powder represents a method of conserving milk in a less bulky form than the original product, a great advantage when it is to be transported for long distances. The manufacture of butter represents a method of concentrating the butter fat of the milk, while in cheesemaking the fat and the casein, together with a small part of the milk serum, are concentrated.

It is essential that the butter and cheese shall possess certain physical and chemical properties. Most important among these properties is the flavor. The flavor of foods is very important, an agreeable odor and pleasant taste in any food making it much more appetizing, if not influencing its nutritive value. The use of condiments and flavoring substances of all kinds in many foods is evidence of the value of flavor in foods.

If cream is separated from sweet, fresh milk, and the remaining part of the milk serum is eliminated by the churning process, the butter thus obtained will be devoid of flavor, and, to those accustomed to butter made from cream that has been allowed to sour before being churned, it is not at all attractive. Sweet-cream butter is made in all the countries of southern Europe, while that from fermented cream is made in northern Europe, America, Australia, Asia, and South America. Sour or acid cream butter, therefore, represents the great mass of the butter supply of the world.

Acid-fermentation and flavor of butter.—As earlier set forth, two groups of acid-forming bacteria are concerned in the souring of milk. The lactic group tends to predominate in milk that is produced under clean conditions, since it grows more rapidly at ordinary temperatures than does the colon group, which, by reason of the products formed, injures the taste and odor of the milk, and hence the flavor of the butter. In the lactic fermentation not only is lactic acid produced, but also other acids, alcohols, and esters that impart to the sour milk and cream an agreeable odor and taste. Butter-fat has the power of absorbing some of these volatile substances, just as it will absorb the odors of fruits when kept in the same container with them. The difference in flavor between the sweet and ripened cream butter is, therefore, due to the absorption of some of these products during the ripening of the cream and the churning process. Cream that is allowed to sour spontaneously will usually contain many more lactic than colon organisms, and the butter made from it may be of very excellent quality.

As long as butter was made on the individual farm, no great need of control was felt; but as its manufacture became centralized in creameries, it became necessary to control not only the kind of organisms growing in the cream, but also the rate at which the fermentation should go on.

The bacterial content of cream, both qualitatively and quantitatively, will depend on the method by which the milk has been creamed. If it has been allowed to stand in shallow vessels at the fluctuating air temperatures and exposed to contamination from the air, it will contain greater numbers and more varied kinds of bacteria than if the creaming takes place at low temperatures and in covered containers. If the centrifugal separator is employed, the bacterial content of the cream will be similar in kind to that of milk.

Control of flavor.—The modern methods of butter-making are designed to give the maker control over the kinds of bacteria that are concerned in the fermentation of the cream. The first step in this direction was to add to the cream selected pure cultures of lactic bacteria that had been isolated from varied sources, and tested as to their favorable flavor-forming properties and their ability to grow at the ripening temperatures. The organisms are propagated in milk that has been heated sufficiently to destroy all of the non-spore-forming bacteria. This is then inoculated with the pure culture of lactic bacteria. If the butter-maker exercises care in the prevention of contamination and in the control of the temperature at which it is kept, this pure culture starter can be maintained for a long period of time. He is thus in a position to grow the desirable organism in any quantity, for addition to the cream that is to be fermented or ripened.

The lactic bacteria are quite resistant to desiccation. The commercial laboratories make use of this property, and market the pure cultures of the organism, not only in milk, but in a dried condition. These are usually prepared by adding the fermented milk to a relatively large amount of some inert substance, such as milk sugar or milk powder, and drying the mass at a low temperature. The organisms remain alive in this condition much longer than where they are exposed to their by-products, as in milk.

Pasteurization of cream.—If the starter is added to raw cream, its effect will depend on the number of bacteria present in the cream. If the cream is sweet and clean, the number of bacteria added in the starter will greatly exceed those naturally found in the cream, and the fermentation will be largely due to the added bacteria. If, however, the cream is nearly sour from the bacteria already present, the fermentation may not be much influenced by the starter.

In order to obtain the maximum effect and to give the organisms added in the starter a clear field, it is advisable to pasteurize the cream and then add the starter. In the pasteurizing process the acid-forming bacteria will be destroyed, and the entire fermentation will be controlled by the added bacteria. By the use of this method, the butter-maker has entire control over the flavor of the product, provided he is furnished with a good quality of raw material, *i. e.*, sweet, fresh, clean cream.

The ripening of the cream also aids in the churning process, causing the cream to churn more quickly, and diminishing the loss of butter-fat in the milk. The ripening of the cream also improves the keeping quality of the butter made from raw cream. It is probable that if the butter is made from pasteurized cream the sweet-cream butter has better keeping qualities. The pasteurization also destroys any pathogenic bacteria that may be present in the cream.

The control of the ripening of the cream is desirable from the standpoint of the rapidity with which the process goes on. The flavor of the product will depend on the degree of acidity developed in the cream, or, in other words, on the relative amounts of flavoring substances and butter-fat. If the cream is low in fat, the same amount of flavoring substances will impart to the butter a higher degree of flavor than if there had been twice as much fat in the cream. It is, therefore, desirable, from the standpoint of the control of the degree of flavor, to stop the development of acid when it has reached the proper point. Also from the standpoint of convenience it is desirable to have the cream ripened at that rate which will make possible the churning at about the same hour each day. This can be accomplished by varying the amount of starter, and by the control of the temperature at which the cream is kept.

Starters are rarely used on the farm. It is certain that a great improvement in farm butter could be made by a more perfect control of the ripening process, which could be easily attained by the use of starters. Small quantities of starters can be made in such vessels as fruit-jars or milk-bottles.

Flavor of butter substitutes.—Oleomargarin is made from fats that are devoid of butter flavor. If this product is to be sold as a butter substitute, the butter flavor must be developed. This is imparted to the product by churning the fats with sour milk. The flavor of the oleomargarin is thus identical in origin and nature with the flavor of butter. Renovated butter is made from a poor quality of butter, the flavor of which is of low grade or decidedly below standard. The fat used in its manufacture is melted, and the obnoxious flavors are removed by passing air through it, and by washing the fat. The desirable flavor is then imparted by churning the fat with some milk soured by pure cultures of bacteria. In the manufacture of oleomargarin and renovated butter, the most approved scientific methods are employed to impart to the otherwise neutral fats the characteristic flavor that is so much in demand in the butter market.

Decomposition of butter.—Butter deteriorates more or less rapidly, depending on the kinds of bacteria present in the cream and on the temperature at which it is stored. The best keeping butter is that made from sweet cream that has been pasteurized; the poorest keeping butter is that from a raw, sweet cream or from a sour cream that contains not only great numbers of undesirable flavor-forming bacteria, but yeasts and molds. If the butter is stored at ordinary temperatures, the development of undesirable flavors is rapid, while at the temperatures maintained in butter-storage rooms, below zero F., the changes are very slow. If the butter is kept in small packages, so that a considerable surface is exposed to the air, the spoiling will be more rapid than if the package is larger or if the butter is hermetically sealed.

Abnormal flavors in butter.—Various abnormal flavors are noted in butter. These may be due to feed, to absorption of odors by the milk or cream, or to bacterial by-products. The action of the bacteria may be indirect, as in the case of the "fishy" flavor in butter which is probably due to the presence of small quantities of iron or copper dissolved from utensils by the acid of the cream. The small amount of metal acts as a catalytic agent, accelerating to a marked degree some of the decomposition changes. All vessels should be well tinned, so that no solvent action can be exerted by the acid.

Cheese-making.—In the making of cheese it is necessary for the casein and the fat to be separated from the milk serum. This separation is accomplished by allowing the milk to sour, or by the addition of rennet to it. The latter is the method used in the making of all the varieties of cheese that are of much commercial importance. Cottage or sour-milk cheese is made by allowing the milk to undergo an acid fermentation. It is ready for use as soon as the whey has been drained from it, and represents a form of fermented milk.

The rennet cheese has no particular flavor at the time it is made, and moreover is rather indigestible. It is essential that the green cheese be allowed to undergo a ripening process in which are formed the peculiar flavors that characterize the various types. This process also renders the cheese more easily digested. Bacteria and other microorganisms function in the ripening of the different varieties of cheese, which vary greatly in texture and in flavor. The important commercial varieties of cheese are all made from the same raw materials, cow's milk, rennet, and salt; but variations in the methods of manufacture, and in the conditions under which the ripening takes place, permit the growth of different classes of bacteria in the different types of cheese.

The amount of whey allowed to remain in the cheese will cause a difference not only in moisture, but also in sugar, and hence in the acidity of the product, since all of the sugar will be fermented by the acid-forming bacteria of the milk, which in large part are retained in the curd. The type of cheese, such as the Camembert and Brie, in which a large amount of whey is left is called soft cheese; those from which the whey is more completely expressed, as represented by the Cheddar and Swiss types, are called hard cheese. The latter are usually made in considerably larger sizes, and in the case of the American or cheddar cheese may be made of any desired size. In the case of the soft cheese, the ripening is, in part, due to the action of organisms that grow on the surface of the cheese, and act on the curd by the secretion of soluble enzymes that diffuse into the cheese. If the cheese is too large, the outer layers will be ripe before the inner portions, and the softness makes the handling of large cheese impossible.

Cheddar cheese.—In the making of cheddar cheese, the most common variety made in America, it is essential that the milk contain great numbers of lactic bacteria, since it is necessary that a relatively large amount of acid be formed during the making of the cheese. If the milk is too sweet, or, in other words, too low in bacteria, the maker adds a starter prepared from pure cultures of lactic bacteria, just as the butter-maker does for the control of the cream-ripening process. The action of the rennet that is used to curdle the casein is also facilitated by the acid reaction of the milk. Within a few minutes from the time the rennet is added the milk curdles, and as soon as it is properly set, it is cut into small pieces for the purpose of facilitating the expulsion of the whey. With the development of acid, a certain action is exerted on the casein, which causes the pieces of curd to adhere to each other, and to fuse into one mass under the influence of the pressure exerted in the press in which the curd is placed after the whey has been largely removed. Bacterial growth in the curd occurs rapidly, the colonies developing in a manner similar to those obtained in the plate cultures of the bacteriologist.

The rennet used also contains the digestive enzyme of the stomach juice, pepsin, which can exert its action only in the presence of an acid. In the stomach of an animal, the hydrochloric acid secreted by the stomach wall is the activating agent, while in the cheese the lactic acid formed in the acid fermentation of the sugar gives favorable conditions for action. The protein of the cheese is changed into soluble decomposition products similar to those formed in the stomach digestion of nitrogenous compounds. The acid reaction established by the lactic bacteria serves to protect the cheese from the action of the putrefactive bacteria that are always present, in the same manner as the acid of silage protects it against putrefaction. It is evident that without the lactic bacteria the cheese will either remain in the same condition in which it is when removed from the press, or else it will undergo putrefaction. It is also evident that without the bacteria the manufacture of cheese would be impossible.

Gassy cheese.—If the milk is handled in such a way as to allow the colon or gas-forming type of bacteria to overcome the usual lactic type, the quality of the cheese will be injured, owing not only to the impairment of the flavor by the by-products of these organisms, but the texture of the cheese is rendered open or porous by the development of gas which permeates the mass of the cheese.

The cheese-maker can not, of course, test the milk of the different patrons of his factory for gas-forming bacteria by the methods employed by the bacteriologist. He can, however, gain an idea of the quality of the raw product by making what is known as a curd test, in which a small portion of each patron's supply is curdled with rennet, after which the curd is broken up so as to expel the whey, which is then turned off. The small mass of curd is kept for several hours at temperatures that favor the growth of the gas-forming bacteria, 100° to 104° F. The quality of the milk is then determined by the appearance of the curd with reference to the presence of gas-holes; the flavor and odor are also noted. This rough qualitative test is of great service to the cheese-maker in detecting the quality of the raw material. All of the operations must be carried out with due regard to contamination from outside sources, since it is essential that the changes noted in the curd be caused only by the bacteria in the milk, and not by those introduced by the use of unclean utensils.

The growth of mold, which occurs readily on the surface of the cheese and which is objectionable on account of discoloring the surface, can be easily prevented by dipping the cheese in melted paraffin. The impervious layer thus formed excludes the air, thereby preventing the growth of mold spores.

Swiss cheese.—Swiss cheese is made from sweet milk that contains only small numbers of lactic bacteria. The rennet used to curdle the milk is obtained from natural "rennets," *i. e.*, portions of dried calves' stomachs, which are soaked in whey and kept at a temperature of from 80° to 95° F. for from twenty-four to thirty-six hours. The whey contains numerous lactic bacteria, and on the dried rennets there are always organisms of the *Bact, Bulgaricum* type. This serves as a starter to inoculate the

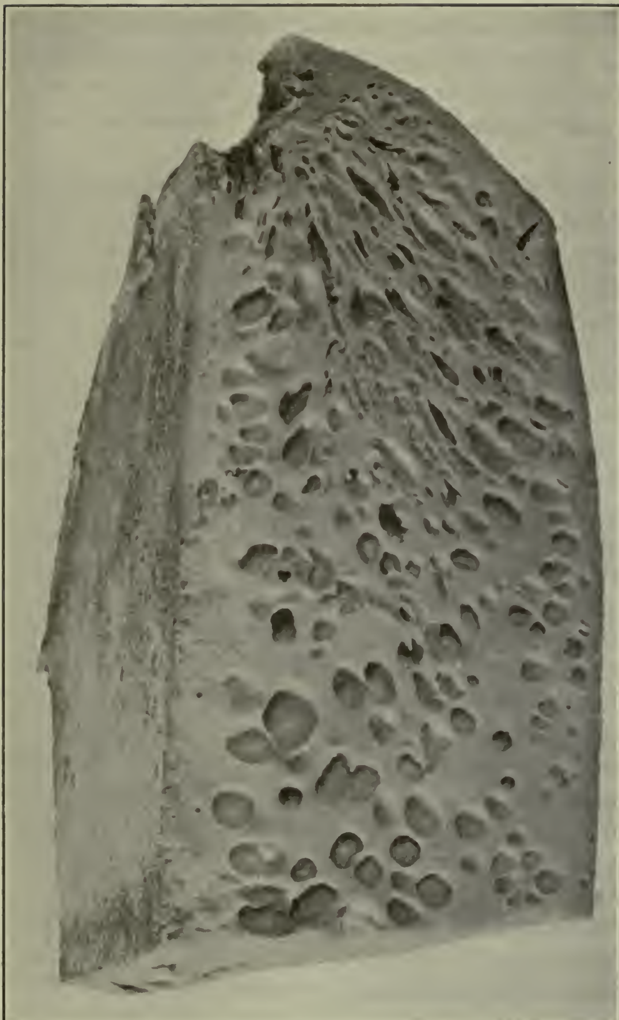

Fig. 41. Gassy Cheese

Such a cheese will have an undesirable flavor as with the gas are produced substances of objectionable odor and taste milk with bacteria capable of fermenting the sugar, and with others that produce propionic acid and carbon-dioxide. The milk is heated to about 135° F. and the curd is removed from the whey in one mass, so as not to allow it to become cool, which checks the growth of *Bact. Bulgaricum* in the curd.

At every step in the making of this and other kinds of cheese, the process is conducted in a manner that influences the growth of certain groups of organisms. Of course, these methods were primarily worked out entirely from the standpoint of experience; but more recently their relation to the action of certain bacterial groups has been more definitely traced.

In the making of cheddar cheese the salt is added to the finely cut curd before it is placed in the press, but in the case of Swiss cheese the salt is applied to the surface of the cheese. The most marked characteristic of Swiss cheese is the presence of gas-holes, ranging from the size of a cherry to that of a walnut, which are scattered quite uniformly through the interior of the cheese. These so called eyes are formed by the fermentation of the lactates with the formation of propionic acid and carbon-dioxide, the latter causing the holes in the plastic curd, while the acid influences the flavor of the cheese. These organisms can not grow in the presence of salt, and it is therefore essential that an opportunity first be given for their growth. Later the application of salt to the outside checks the development of these gas-forming organisms as the salt gradually penetrates the substance of the cheese.

Mold-ripened cheese.—Roquefort, a French cheese made from sheep's milk, Gorgonzola, an Italian cheese, and Stilton cheese, made in England, are illustrations of hard, firm cheese that contain molds. Not only does the presence of these molds confer a peculiar flavor and appearance on the cheese, but undoubtedly the ripening or digestive changes are influenced by these types of organisms. With the Roquefort type a green-spored mold, quite similar to the ordinary bread mold, is grown on rye bread, which, after drying, is powdered and the powder sprinkled over the curd before it is placed in the press. In order that the mold may have the necessary supply of air for the maturing of the spores, the cheese is pierced with many small holes. The green color of the mold imparts to the cheese a marbled appearance, and the peculiar flavor is due, at least in part, to the same factor. In the other varieties mentioned, the mold is not added intentionally, reliance being placed on the contamination in the factory during the process of making.

Soft cheese.—In the making of Camembert, a French cheese, the curd produced by rennet is not cut, but is placed in small molds to allow the whey to drain off. After removal from the press the cheeses are placed in a very moist room. The lactic fermentation goes on rapidly in the cheese, changing the curd to an acid mass that is favorable for the growth of molds. The characteristic mold of milk, *Oïdium lactis,* and a white-spored mold, related to the mold that grows in Roquefort cheese, are essential to the ripening and the development of the characteristic flavor. It is essential that a certain balance be maintained between the two types of molds, which can be accomplished only by regulation of the temperature and moisture conditions within certain limits. The inability of the maker to control these conditions makes the ripening a difficult problem, and a large portion of the cheese is of low value because of the non-development of the typical flavor.

Brie, Limburger, and brick cheese are other varieties of soft cheese that are made and ripened in a manner similar to Camembert.

The manufacture of cheese is an industry that is closely connected with the farm, and is an example of the value that microörganisms exert in the preparation of a valuable food product. It indicates how valuable the products of decomposition may be in imparting a desirable flavor to what would otherwise be a tasteless product.

CHAPTER XVIII

THE BACTERIOLOGICAL CONTROL OF FOODS

The various American governmental units, national, State, and municipal, are all expending much effort in attempting to control the quality of food supplies. While national activity is confined to foods embraced in interstate commerce, and in the main is concerned with those that are preserved, yet the interstate control of fresh meats is a large factor in governmental enterprise. As far as municipal control is concerned, the regulatory service includes in the main only fresh food products, and of these milk is of the most importance. With the recognition of the fact that milk is the chief food product in its relation to health, especially of children, much more attention has been given of late years to the formulation of sanitary rules than ever before. Even small cities and towns are now dealing in a direct way with dairymen, so that by far the larger part of milk supplies used for direct consumption now comes under some kind of supervision.

In order to have the milk reach the consumer in the city in an unchanged condition, the greatest care must be observed by all who handle it. The regulations that a modern city imposes on the milk dealer and on the producer are complex and cover every phase of its production and handling that can in any way affect the value of the milk as human food. A city can enforce its regulations by refusing to allow the sale of milk that has not been produced in conformity therewith. In order to determine whether the regulations are observed, two types of inspection are maintained: first, the examination of the milk in the city as to the number and kind of bacteria it contains; and, second, an inspection of the dairy farms as to the methods there used and to the health of the animals. A summary of the rules imposed by the city of New York follows. It will be noted that the rules are intended to force the production of a clean and healthful milk.

THE COWS

1. The cows must be kept clean, and manure must not be permitted to collect upon the tail, sides, udder, or belly of any milch-cow.

2. The cows should be groomed daily, and all collections of manure, mud, or other filth must not be allowed to remain upon their flanks, udders, or bellies during milking.

3. The clipping of long hairs from the udder and flanks of the cows is of assistance in preventing the collection of filth which may drop into the milk. The hair on the tails should be cut, so that the brush will be well above the ground.

4. The udders and teats of the cow should be thoroughly cleaned before milking; this to be done by thorough brushing and the use of a cloth and warm water.

5. To prevent the cows from lying down and getting dirty between cleaning and milking, a throat-latch of rope or chain should be fastened across the stanchions under the cow's neck.

6. Only feed that is of good quality, and only grain and coarse fodders that are free from dirt and mold, should be used. Distillery waste or any substance in a state of fermentation or putrefaction must not be fed.

7. Cows that are not in good flesh and condition should be immediately removed and their milk kept separate until their health has been passed upon by a veterinarian.

8. An examination by a veterinary surgeon should be made at least once a year.

THE STABLE

9. No stagnant water, hog-pen, privy, or uncovered cess-pool or manure pit should be maintained within one hundred feet of the cow stable.

10. The cow stable should be provided with some adequate means of ventilation, either by the construction of sufficient air-chutes extending from the room in which the cows are kept to the outside air, or by the installation of muslin stretched over the window openings.

11. Windows should be installed in the cow barn to provide sufficient light (2 square feet of window light to each 600 cubic feet of air space the minimum) and the window-panes be washed and kept clean.

12. There should be at least 600 cubic feet of air space for each cow.

13. Milch-cows should be kept in a place that is used for no other purpose.

14. Stable floors should be made water-tight, be properly graded and well drained, and be of some non-absorbent material. Cement or brick floors are the best, as they can be more easily kept clean than those of wood or earth.

15. The feeding-troughs and platforms should be well lighted and kept clean at all times.

16. The ceiling should be thoroughly swept down and kept free from hanging straw, dirt, and cobwebs.

17. The ceiling must be so constructed that dust and dirt therefrom shall not readily fall to the floor or into the milk. If the space over the cows is used for the storage of hay, the ceiling should be made tight to prevent chaff and dust from falling through.

18. The walls and ledges should be thoroughly swept

down and kept free from dust, dirt, manure, or cobwebs, and the floors and premises be kept free from dirt, rubbish, and decaying animal or vegetable matter at all times.

19. The cow beds should be so graded and kept that they will be clean and sanitary at all times.

20. Stables should be whitewashed at least twice a year, unless the walls are painted or are of smooth cement.

21. Manure must be removed from the stalls and gutters at least twice daily. This must not be done during milking, nor within one hour prior thereto.

22. Manure should be taken from the barn, preferably drawn to the field. When the weather is such that this can not be done, it should be stored not nearer than 200 feet from the stable, and the manure pile should be so located that the cows can not get at it.

23. The liquid matter should be absorbed and removed daily, and at no time be allowed to overflow or saturate the ground under or around the cow barn.

24. Manure gutters should be from six to eight inches deep, and constructed of concrete, stone, or some non-absorbent material.

25. The use of land plaster or lime is recommended upon the floors and gutters.

26. Only bedding that is clean, dry, and absorbent should be used, preferably sawdust, shavings, dried leaves, or straw. No horse manure should be used as bedding.

27. The flooring where the cows stand should be so constructed that all manure will drop into the gutter and not upon the floor itself.

28. The floor should be swept daily. This must not be done within one hour prior to milking-time.

29. If individual drinking basins are used for the cows, they should be frequently drained and cleaned.

30. All live stock other than cows should be excluded

from the room in which the milch-cows are kept. (Calf or bull pens may be allowed in the same room if kept in the same clean and sanitary manner as the cow beds.)

31. The barnyard should be well drained and dry, and should be sheltered as much as possible from the wind and cold. Manure should not be allowed to collect therein.

32. A suitable place in some separate building should be provided for the use of the cows when sick, and separate quarters must be provided for the cows when calving.

33. There should be no direct opening from any silo or grain pit into the room in which the milch-cows are kept.

THE MILK-HOUSE

34. A milk-house must be provided which is separated from the stable and dwelling. It should be located on elevated ground, with no hog-pen, privy, or manure pile within 100 feet.

35. It must be kept clean, and not used for any purpose except the handling of milk.

36. The milk-house should be provided with sufficient light and ventilation, with floors properly graded and made water-tight.

37. It should be provided with adjustable sashes to furnish sufficient light, and some proper method of ventilation should be installed.

38. The milk-house should be provided with an ample supply of clean water for cooling the milk, and if it is not a running supply, the water should be changed twice daily. Also a supply of clean ice should be provided to be used for cooling the milk to 50° F. within two hours after milking.

39. Suitable means should be provided within the milk-house to expose the milk-pails, cans, and utensils to the sun or to live steam.

40. Facilities consisting of wash-basins, soap, and towel

should be provided for the use of milkers before and during milking. In the summer months the milk-house should be properly screened to exclude flies.

THE MILKERS AND MILKING

41. Any person having any communicable or infectious disease, or one caring for persons having such disease, must not be allowed to handle the milk or milk utensils.

42. The hands of the milkers must be thoroughly washed with soap and water, and carefully dried on a clean towel, before milking.

43. Clean overalls and jumpers should be worn during the milking of the cows. They should be used for no other purpose, and when not in use should be kept in a clean place protected from dust.

44. The milker's hands and the teats of the cow should be kept dry during milking. The practice of moistening the hands with milk is to be condemned.

45. The milking-stools should be at all times kept clean. Iron stools are recommended.

46. The first streams from each teat should be rejected, as this foremilk contains more bacteria than the rest of the milk.

47. All milk drawn from the cows fifteen days before or five days after parturition should be rejected.

48. The pails in which the milk is drawn should have as small an opening at the top as can be used in milking, the top opening preferably not to exceed eight inches in diameter. This lessens the contamination by dust and dirt during milking.

49. The milking should be done rapidly and quietly, and the cows should be treated kindly.

50. Dry fodder should not be fed to the cows during or

just before milking, as dust therefrom may fall into the milk.

51. All milk utensils, including pails, cans, strainers, and dippers, must be kept thoroughly clean, and must be washed and scalded after each using; and all seams in these utensils should be cleaned, scraped, and soldered flush.

THE MILK

52. Milk from diseased cows must not be shipped.

53. The milk must not be in any way adulterated.

54. The milk as soon as drawn should be removed to the milk-house and immediately strained and cooled to the proper temperature.

55. All milk must be cooled to a temperature below 50° F. within two hours after being drawn, and kept thereafter below that until delivered to the creamery.

56. The milk should be strained into cans that are standing in ice water which reaches the neck of the can. The more rapidly the milk is cooled, the safer it is and the longer it will keep sweet. Ice should be used in cooling milk, as very few springs are cold enough for the purpose.

57. If separators are used, they should stand where the air is free from dust or odors, and on no account should they be used in the stable or out of doors.

58. Milk-strainers should be kept clean, scalded a second time just before using, and if cloth strainers are used, several of them should be provided, in order that they may be changed frequently during the straining of the milk.

59. The use of any preservative or coloring matter is adulteration, and its use by a producer or shipper will be a sufficient cause for the exclusion of his product from the city of New York.

WATER SUPPLY

60. The water supply used in the dairy and for washing utensils should be absolutely free from any contamination, sufficiently abundant for all purposes, and easy of access.

61. This supply should be protected against flood or surface drainage.

62. The privy should be located not nearer than 100 feet of the source of the water supply, or else be provided with a water-tight box that can be readily removed and cleaned, and so constructed that at no time will the contents overflow or saturate the surrounding ground.

63. The source of the water supply should be rendered safe against contamination by having no stable, barnyard, pile of manure, or other source of contamination located within 200 feet of it.

In order that the farm inspection will be as effective as possible, and to make the work of the several inspectors as uniform as may be, the dairies are scored. A copy of the score-card follows.

DEPARTMENT OF HEALTH

The City of New York

Division of General
Sanitary Inspecton **Dairy Report**

Inspection No.......Time......A. P. M. Date..........192..

1 **Dairyman** .... **Owner** ....
2 **P. O. Address** .... **P. O. Address**.......**State**......
3 **County** ....**State** .... **Party Interviewed** ....
4 Milk delivered to Creamery at..........Formerly at..........
5 Operated by ....Address.....................
6 Distance of farm from Creamery......Occupied farm since....
7 No. Cows........No. Milking.......No. Qts. Produced........
8 All persons in the households of those engaged in producing or handling milk are........free from all infectious disease. Weekly reports are........being filed.....................
9 Date and nature of last case on farm........................
10 **WATER SUPPLY** for utensils is from a...............located

............feet deep and apparently is............pure and wholesome............ State any possible contamination located within 200 feet of source of water supply or if water supply is not protected against surface drainage...............
....
....

11 Water supply on this farm analyzed......192.. Result......
12 Style of Cow Barn........Length........ft. Width.......ft. Height of ceiling......ft.
13 Dairy Rules of the Department of Health are...........posted ....
14 Dairy Herd examined by.............on..............192.. Report..........................

	EQUIPMENT	Perfect	Allow
15	**COW STABLE** is..........located on elevated ground with no stagnant water, hog-pen, privy, uncovered cesspool, or manure pit within 100 feet	 1	....
16	**FLOORS**, other than cow beds, are............ of concrete or some non-absorbent material...	2	
17	Floors are............properly graded and water-tight	2	......
18	**Cow beds are**......of concrete or planks laid on concrete	2	......
19	**DROPS** are....constructed of concrete, stone, or some non-absorbent material	2	......
20	Drops are.......water-tight and space beneath is clean and dry.........................	2	......
21	**CEILING** is constructed of........and is...... tight and dust-proof.....................	2	......
22	**WINDOWS** No. ......total square feet...... there is............2 square feet of window light for each 600 cu. ft. air space (1 sq. ft. per each 600 cu. ft —1)....................	2	......
23	**VENTILATION** consists of......sq. ft. muslin covered openings or......sq. ft. open chutes in ceiling or............which is sufficient 3, fair 2, poor 1, insufficient 0.............	3	......
24	**AIR SPACE** is........cu. ft. per cow (600 and over—3) (500 to 600—2) (400 to 500—1) (under 400—0)	3	......
25	**LIVE STOCK**, other than cows, are........ex-		

		Perfect	Allow
	EQUIPMENT—*Continued*		
	cluded from rooms in which milch-cows are kept	2	......
26	There is............direct opening from stable into silo or grain pit........................	1	......
27	Separate quarters are.............provided for cows when calving or sick..................	1	......
28	**COW-YARD** is............properly graded and drained	2	......
29	**WATER SUPPLY** for cows is..........unpolluted and plentiful	1	......
30	**MILK-HOUSE** has..........direct opening into cow-barn or other building.................	1	......
31	Milk-house has......sufficient light and ventilation	1	......
32	Floor is............properly graded and watertight	1	......
33	Milk-house is..........properly screened to exclude flies	1	......
34	**MILK PAILS** are..........of smoothly tinned metal in good repair	1	......
35	Milk-pails have.............all seams soldered flush	2	......
36	Milk-pails are...........of the small-mouthed design, top opening not exceeding 8 inches in diameter. Diameter	2	......
37	**Racks** are........provided to hold milk-pails and cans when not in use................	2	......
38	**Special milking suits** are......provided......	1	......
		40	......
	METHODS		
39	**STABLE INTERIOR** painted or whitewashed on...........which is satisfactory 3, fair 2, unsatisfactory 1, never 0..................	3	......
40	**FEEDING-TROUGHS**, platforms, or cribs are ......well lighted and clean...............	1	......
41	**Ceiling** is..........free from hanging straw, dirt, or cobwebs	3	......
42	**Window-panes** are............washed and kept clean	1	......

		Perfect	Allow
	METHODS—*Continued*		
43	**WALLS AND LEDGES** are..........free from dirt, dust, manure, or cobwebs..............	2	......
44	**FLOORS AND PREMISES** are........free from dirt, rubbish or decayed animal or vegetable matter	2	......
45	**COW BEDS** are........clean, dry, and no horse manure used thereon	2	......
46	**Manure** is............removed to field daily 4, to at least 100 feet from barn 2, stored less than 100 feet or where cows can get at it 0..	4	......
47	**Liquid Matter** is..........allowed to saturate ground under or around cow-barn...........	2	......
48	**Milking-stools** are...........clean...........	1	......
49	**Cow-yard** is...............clean and free from manure	2	......
50	**COWS** have.........been tuberculin tested and all tuberculous cows removed...............	7	......
51	Cows are...........all in good flesh and condition at time of inspection.................	2	......
52	Cows are............all free from clinging manure and dirt. (No dirty)................	4	......
53	**LONG HAIRS** are..........kept short on belly, flanks, udder, and tail.....................	1	......
54	**UDDER AND TEATS** of cow are............. thoroughly brushed and wiped with a clean damp cloth before milking..................	3	......
55	**ALL FEED** is........of good quality and distillery waste or any substance in a state of putrefaction is..........fed...............	2	......
56	**MILKING** is........done with dry hands.....	2	......
57	**FORE MILK** or first few streams from each teat is........discarded	2	......
58	**Clothing** of milkers is.........clean.........	1	......
59	Facilities for washing hands of milkers are.... provided in cow-barn or milk-house.........	2	......
60	**Milk** is strained at..........and..........in clean atmosphere	1	......
61	Milk is..........cooled within two hours after milking to 50 degrees F. 3, to 55 degrees F. 2, to 60 degrees F. 1.........................	3	......
62	Ice is........used for cooling milk...........	1	......
63	**MILK-HOUSE** is..........free from dirt, rub-		

	Perfect	Allow
bish, and all material not used in the handling and storing of milk......................	1	......
64 **Milk Utensils** are..........rinsed with cold water immediately after using and washed clean with hot water and washing solution...	2	......
65 Utensils are............sterilized by steam or boiling water after each using.............	2	......
66 **Privy** is...........in sanitary condition, with vault and seats...........covered and protected	1	......
	60	......

Remarks ....

Equipment 40 per cent. Score.................per cent.
Methods 60 per cent. Score...................per cent.
Perfect Dairy 100 per cent. Score.............per cent.

A copy of the completed report is left with the dairyman.

It is to be noted that, almost without exception, each of the foregoing rules has a direct effect in preventing the contamination of the milk with bacteria that injure its keeping quality, its taste and odor, or its healthfulness. A rigid adherence to the rules will insure the production of milk of high quality. The task of the inspector is to determine which producers are derelict in their methods, and then to aid them in improving their methods and equipment. It should be remembered that methods are of far greater importance in the production of good milk than equipment. Additional care may make up for the lack of suitable stables and utensils, but the most elaborate equipment is of no value unless supplemented by good methods.

It is also to be noted that the conditions outlined in the rules are nothing more than those any milk producer should be willing to establish of his own volition. Some may have but little influence on the quality of the milk produced, but are nevertheless to be classed as essential conditions in a plant producing human food. The dairy farm is a manufacturing plant, where vegetable matter, not available for human food, is changed by the dairy cow to the most valuable human food. The most important factor in the production of good milk is the dairyman and his conception of his duty to the people consuming his milk.

Before the farm inspection is carried out the creameries to which the milk is delivered by the farmers are inspected at the time the milk is being delivered. The temperature of the milk and its cleanliness are noted. In the creamery the straining, cooling, and handling of the milk are observed, as well as the washing of the milk-cans and other utensils, the construction and condition of the creamery, the opportunity for the water supply to become contaminated, and the presence of infectious diseases among the employes.

Grades of milk.—It has come to be recognized that milk varies in its quality, which is a complex property depending on many factors. It is recognized that it costs more to produce good milk than poor milk, and that the former is of more value to the consumer. The grading of milk has, therefore, been introduced, so that the consumer may know something of the quality of the milk he purchases. The following are the grades established by the State of New York:

All milk sold and offered for sale at retail, except milk sold or offered for sale as sour milk under its various designations, shall bear one of the designations provided in this regulation, which constitute the minimum requirements permitted in this State.

No term shall be used to designate the grade or quality of milk or cream which is sold or offered for sale, except:

"Certified"
"Grade A raw"
"Grade A pasteurized"
"Grade B raw"

"Grade B pasteurized"
"Grade C raw"
"Grade C pasteurized"

Certified.—No milk or cream shall be sold or offered for sale as "Certified" unless it conforms to the following requirements:

The dealer selling or delivering such milk or cream must hold a permit from the local health officer.

All cows producing such milk or cream must have been tested at least once during the previous year with tuberculin, and any cow reacting thereto must have been promptly excluded from the herd. The reports of such tuberculin tests must be filed with the local health officer and the milk commission of the county medical society in the municipality and county respectively in which such milk is delivered to the consumer.

Such milk must not at any time previous to delivery to the consumer contain more than 10,000 bacteria per cubic centimeter, and such cream not more than 50,000 bacteria per cubic centimeter.

Such milk and cream must be produced on farms which are duly scored on the score-card prescribed by the State Commissioner of Health, not less than 35 per cent. for equipment and not less than 55 per cent. for methods.

Such milk and cream must be delivered within thirty-six hours of the time of milking.

Such milk and cream must be delivered to consumers only in containers filled at the dairy or central bottling plant.

The caps must contain the word "Certified" and bear the certification of a milk commission appointed by the county medical society organized under and chartered by the medical society of the State of New York, and must also contain the name and address of the dairy as well as the date of milking.

Every employee before entering upon the performance of his duties shall be examined by a duly licensed physician, and the reports of such examination shall be sent to the milk commission certifying the milk from such dairy.

The milkers and all persons handling the milk must be provided with suits and caps of washable material which shall be worn while milking or handling the milk and shall not be worn at other times. When not in use these garments must be kept in a clean place free from dust. Not less than two clean suits and caps must be furnished weekly. The hands of the milkers must be washed with soap and hot water, and well dried with a clean towel, before milking.

Grade A raw.—No milk or cream shall be sold or offered for sale as "Grade A raw" unless it conforms to the following requirements:

The dealer selling or delivering such milk or cream must hold a permit from the local health officer.

All cows producing such milk or cream must have been tested at least once during the previous year with tuberculin, and any cow reacting thereto must have been promptly excluded from the herd.

Such milk must not at any time previous to delivery to the consumer contain more than 60,000 bacteria per cubic centimeter, and such cream not more than 300,000 bacteria per cubic centimeter.

Such milk and cream must be produced on farms which are duly scored on the score-card prescribed by the State Commissioner of Health not less than 25 per cent. for equipment, and not less than 50 per cent. for methods.

Such milk and cream must be delivered within thirty-six hours from the time of milking, unless a shorter time shall be prescribed by the local health authorities.

Such milk and cream must be delivered to consumers only in containers sealed at the dairy or a bottling plant. The caps or tags must be white and contain the term "Grade A raw" in large black type, and the name and address of the dealer.

Grade A pasteurized.—No milk or cream shall be sold or offered for sale as "Grade A pasteurized" unless it conforms to the following requirements:

The dealer selling or delivering such milk or cream must hold a permit from the local health officer.

All cows producing such milk or cream must be healthy as disclosed by an annual physical examination.

Such milk or cream before pasteurization must not contain more than 200,000 bacteria per cubic centimeter.

Such milk must not at any time after pasteurization and previous to delivery to the consumer contain more than 30,000 bacteria per cubic centimeter, and such cream not more than 150,000 bacteria per cubic centimeter.

Such milk and cream must be produced on farms which are duly scored on the score-card prescribed by the State Commissioner of Health not less than 25 per cent. for equipment and not less than 43 per cent. for methods.

Such milk and cream must be delivered within thirty-six hours after pasteurization, unless a shorter time shall be prescribed by the local authorities.

Such milk and cream must be delivered to consumers only in containers sealed at the dairy or at a bottling plant. The caps or tags must be white and contain the term "Grade A pasteurized" in large black type.

Grade B raw.—No milk or cream shall be sold or offered for sale as "Grade B raw" unless it conforms to the following requirements:

The dealer selling or delivering such milk or cream must hold a permit from the local health officer.

All cows producing such milk or cream must be healthy as disclosed by an annual physical examination.

Such milk must not at any time previous to delivery to the customer contain more than 200,000 bacteria per cubic centimeter, and such cream not more than 750,000 bacteria per cubic centimeter.

Such milk and cream must be produced on farms which are duly scored on the score-cards prescribed by the State Commissioner of Health not less than 23 per cent. for equipment and not less than 37 per cent. for methods.

Such milk and cream must be delivered within thirty-six hours from the time of milking, unless a shorter time shall be prescribed by the local health authorities.

The caps or tags on the containers must be white and contain the term "Grade B raw" in large, bright green type, and the name of the dealer.

Grade B pasteurized.—No milk or cream shall be sold or offered for sale as "Grade B pasteurized" unless it conforms to the following requirements:

The dealer selling or delivering such milk or cream must hold a permit from the local health officer.

All cows producing such milk or cream must be healthy as disclosed by an annual physical examination.

Such milk or cream before pasteurization must not contain more than 1,500,000 bacteria per cubic centimeter.

Such milk must not at any time after pasteurization and previous to delivery to the consumer contain more than 100,000 bacteria per cubic centimeter, and such cream not more than 500,000 bacteria per cubic centimeter.

Such milk and cream must be produced on farms which are duly scored on the score-card prescribed by the State Commissioner of Health not less than 20 per cent. for equipment and not less than 35 per cent. for methods.

Such milk must be delivered within thirty-six hours after pasteurization between April 1 and November 1 and within forty-eight hours after pasteurization between November 1 and April 1, and such cream within forty-eight hours after pasteurization, unless a shorter time is prescribed by the local health authorities.

The caps or tags on the containers must be white and contain the term "Grade B pasteurized" in large, bright green type, and the name of the dealer.

Grade C raw.—No milk or cream shall be sold or offered for sale as "Grade C raw" unless it conforms to the following requirements:

The dealer selling or delivering such milk or cream must hold a permit from the local health officer.

Such milk and cream must be produced on farms which are duly scored on the score-card prescribed by the State Commissioner of Health not less than 40 per cent.

Such milk and cream must be delivered within forty-eight hours from the time of milking, unless a shorter time shall be prescribed by the local health authorities.

The caps or tags affixed to the containers must be white and contain the term "Grade C raw" in large red type.

Grade C pasteurized.—No milk or cream shall be sold or offered for sale as "Grade C pasteurized" unless it conforms to the following requirements:

The dealer selling or delivering such milk or cream must hold a permit from the local health officer.

Such milk and cream must be produced on farms which are duly scored on the score-card prescribed by the State Commissioner of Health not less than 40 per cent.

Such milk and cream must be delivered within forty-eight hours after pasteurization, unless a shorter time shall be prescribed by the local health authorities.

The caps or tags affixed to the containers must be white and contain the term "Grade C pasteurized" in large red type.

It is to be noted that the grades of milk are based on the bacterial content of the milk and on the opportunity for the milk to become contaminated with pathogenic organisms. From the statements made in a previous chapter, it is evident that the number of bacteria in any sample of milk is dependent upon the original amount of contamination and the extent to which the introduced bacteria have grown. The latter is dependent on the age of the milk and the temperature at which it has been held. A high bacterial content is indicative of poor milk, while a low bacterial content can be obtained, in the case of raw milk, only where due attention is paid to cleanliness and cooling.

This relation between the quality of milk and its bacterial content has led many cities to adopt numerical bacterial standards, even when grades of milk have not been established. Boston requires that the milk shall not contain more than 500,000 bacteria per cubic centimeter; Rochester, New York, has a standard of 100,000 per cubic centimeter;

while Chicago requires that the milk on arrival in the city shall contain not more than 1,000,000 per cubic centimeter from May first to September thirtieth, and not more than 500,000 between October 1 and April 30. The sale of milk containing more than 3,000,000 bacteria per cubic centimeter is prohibited.

It has been urged that bacterial standards are not of value since the healthfulness of milk depends on the kind of bacteria present rather than on the number. It is well recognized that milk containing millions of acid-forming organisms, buttermilk, is a healthful food, while that containing many less bacteria may harbor some disease-producing organisms. It has been urged that a qualitative standard should supplant the quantitative. The consumer desires milk that has been produced under clean conditions and which has good keeping qualities. The harmless forms of bacteria exert the greatest influence on the keeping quality. Experience has shown that the quantitative examination of the milk supply as it comes from the farm is the most feasible method of determining, in the laboratory, whether the farmer has obeyed the rules with reference to cleanliness and cooling of the milk. The bacteriological examination also gives an indication as to whether the large number of bacteria is due to gross contamination of the milk with mud and manure, or actual growth of bacteria, as in old milk. In the latter case the ordinary acid-forming bacteria will usually predominate in the milk, while in the former the number of kinds of bacteria and the proportion between the kinds will be changed. It is, of course, evident that the quantitative standards should be applied with judgment.

It is also claimed that the delay in securing the results in the quantitative examination of milk is an objection to the bacterial standard, since the milk is consumed before the laboratory findings can be obtained. It is true that it does not protect the community as far as the particular sample is concerned, but it is also true that the examination is not made for the purpose of determining the condition of the particular sample, but rather to determine the methods that are employed on any particular farm. These do not vary widely from day to day. Thus if the bacterial content of a number of samples from a particular source is uniformly high, it is evident that conditions surrounding production need investigation.

If the milk is well cooled on the farm, and kept cold while being shipped, the growth of bacteria will be slow, and the condition of the milk, as far as keeping quality is concerned, much better than if less care is used. Some cities have temperature standards; New York requires that the milk shall be cooled to 50° F. on the farm, and shall not be above 50° F. on arrival in the city. Others require that it shall not be above 50° F. on delivery to the consumer.

Certified milk.—In many cities the medical societies have appointed milk commissions, which adopt rules and regulations concerning the production of milk that shall receive the certificate of the commission. Producers who desire to have their milk thus certified must satisfy the commission that they are able to conform to the rules. The commission appoints a physician to examine the personnel of the farm, a veterinarian to make frequent examinations of the herd, a chemist to examine the milk as to its content in fat and other solids, and a bacteriologist to determine the bacterial content of the milk. The rules are very stringent, and cover every point that may in any way influence the value of the milk as human food. In order to conform to these requirements, a heavy expenditure must be incurred, and the business must pay for such expert service—hence certified milk must be sold at high prices. This price makes it a special product, and its use is confined mainly to infant feeding.

The bacterial standard for certified milk is usually 10,000 bacteria per cubic centimeter. It is only by the exercise of the greatest care at every point that the bacterial content can be kept below this maximum.

The term "certified milk" has been registered by Mr. Francisco of New Jersey, who was the first to engage in the production of such milk under the direction of the Medical Milk Commission of Essex County, New Jersey. The use of the term is allowed when the milk is produced under the regulation of any medical milk commission.

Most certified milk is now produced on fancy dairy farms conducted by wealthy men. The barns and other equipment are the best that can be obtained, and the methods employed, as far as cleanliness is concerned, are extreme. In some of the dairies the bacterial content is reduced to a few hundred per cubic centimeter, or to that which is derived from the interior of the udder. Such milk will, when well refrigerated, keep for long periods of time. It is a not uncommon thing for such milk to keep perfectly sweet for from ten to fifteen days.

Pasteurization of market milk.—Milk may become contaminated with pathogenic bacteria from a multitude of sources, none of which can be guarded against perfectly. It is estimated that at least one person in each thousand is a "typhoid carrier." It is impossible to detect which individuals are a menace to their fellow men in this respect. Even when a typhoid carrier is detected, it is difficult to control his movements so that he will not present a danger to those with whom he may be, directly or indirectly, associated.

The larger cities have realized the impossibility of using the tuberculin test as a means of protection against bovine tuberculosis. The magnitude of the task of testing all of the milch-cows each year, the expense connected therewith, and the imperfection of the protection have led to the abandonment of the demand for the tuberculin testing of all cattle. The presence of dangerous cases of udder inflammation may never be recognized until the epidemic of septic sore throat is well under way.

Under the most ideal system of inspection, the safety of large supplies of milk can not be assured. The pasteurization of the milk offers an effective solution of this problem of healthfulness of market milk. The process is almost a necessity under modern conditions as a preservative measure. Without it the provisioning of our great cities would be most difficult, and in some cases impossible. For all of these reasons the introduction of the pasteurization process has been rapid, until more than 90 per cent. of the milk sold in large cities is now pasteurized.

As previously mentioned, heating causes certain changes in milk. In the treatment of market milk it is desirable to use as low temperatures as will suffice to destroy the disease-producing bacteria. It is fortunate that temperatures that will insure this result have little effect on the milk. The State of New York requires that the milk shall be subjected to a temperature of from 142° to 145° F. for not less than thirty minutes, and immediately cooled. The acid-forming bacteria are not completely destroyed, and the pasteurized milk as a rule will undergo the same type of fermentation as raw milk. It is, however, deemed essential that all pasteurized milk be sold as such; that it be delivered to the consumer within twenty-four hours after pasteurization; and that no milk be pasteurized a second time.

The continuous pasteurizing machines have the disadvantage that a small portion of the milk passes through so quickly that all pathogenic bacteria therein might not be destroyed (p. 181). This has led to the use of the "holding" process, in which the milk is heated to the desired temperature and then placed in tanks, where it remains at this temperature for any desired time. Every portion is thus treated in a uniform manner.

If the milk is bottled after pasteurization, there remains opportunity for reinfection, possibly with typhoid bacilli. Pasteurization in the final container, the bottle, is being recommended. This is possible only when a special bottle is used, having a metal cap lined with paper, or some other special appliance.

PART IV

TRANSMISSIBLE DISEASES

CHAPTER XIX

THE RELATION OF MICROÖRGANISMS TO DISEASES OF ANIMALS

Communicable diseases.—The diseases of animals may be divided into two classes: the organic or constitutional, which are due to the faulty operation of some organ; and the communicable, which are caused by the invasion of the body by some organism and the growth thereof with the formation of substances that have a harmful action on the body. The latter are termed *communicable* diseases, because the passage of the causal organism from the diseased to the healthy animal is sufficient to spread the trouble. The organic diseases can not be thus transmitted, for they are not due to the presence of a living organism in the body. The communicable diseases are also termed *infectious, contagious,* and *preventable,* since the prevention of their spread can be accomplished by stopping the transmission of the organism. In some instances the knowledge of the manner of transmission is so complete that if stockmen could be induced to put that knowledge into practice the diseases would soon disappear, while in other cases the information is not yet sufficiently complete to enable their spread to be prevented.

Such transmissible diseases as tuberculosis, contagious abortion, hog cholera, Texas fever, and glanders entail an enormous tax on the livestock industry of the world, as will appear in the discussion of the specific diseases. Since the prevention of disease is a problem that must always rest in the hands of the farmer himself, rather than in any professional aid he may employ, it is desirable that every stockman be acquainted with the salient facts concerning the more important of the transmissible diseases of domestic animals, just as everyone should know something of the important transmissible diseases of man, so that he may intelligently protect himself from them.

Infection.—In order to produce disease the organism must invade the body, must grow therein, and its by-products must exert an injurious effect on the body tissues. This sequence of events is known as infection. The severity of the attack of any communicable disease may vary from one animal to another, owing to the difference in resistance of the host and to a difference in the virulence of the organism, which may be defined as the power of the organism to multiply within the body and produce disease. Little is known of the conditions that increase or diminish the virulence of organisms in nature. The resistance of the host may be impaired by any condition that tends to weaken the body, such as fatigue, exposure to cold, heat, or dampness, improper diet, thirst, age, wounds, and other diseases. Neither the invading organism nor the host are to be considered as passive agents. The relation between them is a true struggle, a fight to the finish. In the struggle the host seeks to overcome the parasite by means that will be discussed later, and the organism protects itself in the progress of its growth and development. In so doing the perpetuation of the species is accomplished.

The portals of entry into the body are the broken skin, or an injured mucous membrane, the alimentary tract, the respiratory tract, the genital tract, and the conjunctiva, or the mucous membrane of the eye. Many organisms have specific, definite methods by which they enter the body of the host; for example, the hog cholera organism enters by way of the alimentary tract, while the tubercle bacillus enters in a variety of ways, as through wounds, by inhalation, or by ingestion. The tetanus or lockjaw bacillus is always introduced through wounds of the skin or of the mucous membrane.

The original or initial infection is called the primary infection, and may be followed by a second invasion with another kind of organism which may have been present in the body, but which was unable to multiply until the resistance was first lowered by the primary infection. Infection is usually due to a single specific organism, but some troubles are due to a mixed infection with two or more organisms. When the body has been weakened by organic diseases, it is sometimes more susceptible to invasion by certain disease-producing organisms, or the results of such invasion are more likely to be serious. The original trouble might have resulted in death, but the end is hastened by the terminal infection, as it is called.

The invading organisms injure the tissues by the production of poisonous substances known as *toxins.* In some cases the organisms grow in a limited area, and do not cause any great destruction of the tissue at the point of growth; but the toxin is so active that a minute quantity is sufficient to cause death. Such a disease is known as *toxemia,* in contradistinction to the *bacteremia* or *septicæmia,* in which the entire body is invaded by the organism, as in the case of anthrax. Examples of toxemias are lockjaw and diphtheria. In still other instances the invasive powers of the organisms are not great, but the tissue is destroyed at the point of growth, as in the case of the pus-producing organisms.

It is evident that the symptoms of any disease can not appear until the organism has had time to multiply and to form sufficient toxin to have a visible effect on the body of the animal. This period which may vary from a few days to several months is called the *period of incubation* of the disease. The changes in the various tissues due to the action of the organism are called the *lesions* of the disease. With some diseases they are very characteristic, in others not.

The external defenses of the body.—There are many means by which nature has sought to protect the body against the invasion of microörganisms. The surface of the body is covered by the skin, and all of the cavities of the body that are in contact with the exterior are provided with a mucous membrane. The bacteria normally gain entrance with but few exceptions through these protective membranes, only as they are injured. The mucous membranes are always bathed with the products of glandular activity, which possess a more or less marked germicidal or antiseptic action. By reason of this many of the organisms that come in contact with these fluids are thus destroyed. Wounds in the mouth and in the intestine must of necessity frequently occur, especially with animals that feed on coarse, dry fodder. Yet a harmful effect from such a source is rarely noted. The lungs and air passages are constantly exposed to dust laden with adherent bacteria. These foreign bodies are removed by the action of the cilia of the cells lining the air-passages. The hairlike appendages are constantly in motion and tend to move any foreign particle outward.

The internal defenses.—After the microörganisms have invaded the tissues, their development can not go on unhampered, for the body has a number of internal defenses that must be overcome before growth and disease production can occur. An animal is said to be *immune* to a disease when it resists the development of the organism, or is not injured by the poison that the organism produces. Various explanations have been offered to explain the immunity of animals. The white blood corpuscles possess amœboid properties, and are able to ingest solid bodies like the bacteria and digest them. This process is known as phagocytosis, and such devouring cells are called *phagocytes.* Whenever any portion of the body is invaded by a foreign agent, or when any abnormal condition arises, the phagocytes are attracted to this point as a result of a chemical stimulus. This causes them to accumulate at or near the point of invasion, where they soon engulf and destroy many of the invading organisms. If these white blood corpuscles are able to overcome the harmful bacteria, the initial infection may be rendered of no importance. Again, the organism may multiply and form poisonous products which may injure the body to the extent that death is caused, or, under the stimulus of these harmful products, the body cells may react and form substances that neutralize or nullify in some way the poisonous effects of the products of the organism. These antagonistic and protective products are diffused through the liquids of the body, especially in the blood serum and form the basis of the anti-serums that are used for protective purposes.

Immunity.—A specific disease may occur only in a single host species, as in hog cholera, or it may be capable of spreading throughout a variety of different animals. Blackleg affects only cattle and sheep, while the anthrax bacillus produces a characteristic disease in man as well as in many of the lower animals. Numerous diseases affecting man, such as typhoid fever, diphtheria, yellow fever, and cholera, are limited to this host alone. Other warm-blooded animals are naturally insusceptible to these maladies; they possess a natural immunity.

The term natural immunity is applied to that condition which enables an animal to resist the natural invasion by organisms that attack other varieties or species of animals. It is a condition that is present at birth, continues throughout life, and is transmitted to the offspring. A striking example of natural immunity is that of Algerian sheep to anthrax, while other varieties are very susceptible to this disease.

Immunity to a disease may be established in the individual after birth. It is then called acquired immunity. The lessened susceptibility to certain diseases with increasing age is an example, as is noted in measles, chickenpox, and whooping-cough in human beings, and with blackleg in cattle.

The immunity that is conferred by resisting successfully an attack of infectious disease is another example. Instances of this type of acquired immunity are noted in smallpox and yellow fever in man, in Texas fever in cattle, and in cholera in hogs. The period of persistence of the acquired immunity is variable, sometimes extending through the remainder of the life of the individual, or again persisting but a short time. A successful recovery from some diseases does not seem to convey any immunity against second attack. Acquired immunity may also be produced artificially in a number of ways which may be summarized as follows:

By inoculating the individual with such a small number of organisms that a fatal attack of the disease will not result. This method is used in the case of Texas fever.

By inoculating with an attenuated or weakened organism. This is practiced in anthrax, blackleg, rabies, and bubonic plague in man.

By inoculating with an organism that has been modified by passage through another species of animal. This method is illustrated by vaccine for smallpox.

By the injection of toxins. This is used in the immunization of animals against the virus of a disease for the purpose of securing antitoxins from their blood, as in the preparation of diphtheria antitoxin.

By the injection of antitoxins. These are used to protect against toxins and natural infection, as in the case of diphtheria.

By the injection of blood serum from immune or hyperimmune animals for preventive purposes. The serum used in the case of hog cholera is an example.

Active and Passive Immunity.—If the organism of the disease is concerned directly in the process of bringing about the production of the anti-bodies, the immunity is termed active. An immunity that is due to recovery from a natural attack is active, as is that produced by injecting the organism or its toxins into the body. Active immunity is slow in its development, is somewhat dangerous, and is always attended with some discomfort to the person or animal in which it is produced. It persists, as a rule, for a considerable period, varying from a few weeks to several years.

If the blood serum of an animal that has an active immunity to a disease is injected into an animal that is susceptible to the disease, an immunity is produced which is called passive immunity. It involves no activity of the tissues of the immunized animal. The passively immunized animal is simply the recipient of substances formed in the bodies of other animals and transferred to it. Passive immunity is rapidly produced, and is attended with little danger and discomfort. The period of protection is measured by a few days or weeks. The most extensive use of passive immunity is in hog cholera, tetanus, and diphtheria.

It is a well known fact that some outbreaks of a disease are very severe in that many of the infected die, while in another outbreak of the same disease practically all of the infected individuals recover. This can not be explained by the greater resistance of the second group over the first, but rather the explanation is to be sought in the diminished virulence of the organism. As has been stated, the causes that induce such changes in nature are not known.

The first effort to impart immunity by artificial means was by the intentional *inoculation* of individuals with material taken from mild cases of smallpox. The mild attack thus induced afforded protection to the individual against the more severe form of the disease. Later it was noted by Jenner, an English surgeon, that those individuals that had acquired cowpox by milking a cow suffering from this trouble were thereby protected against smallpox. Following this observation, the inoculation of human virus against smallpox was superseded by *vaccination* with material taken from the pustules of the animal disease, cowpox, or *vaccinia*, as it is called.

It is now known that the organism causing cowpox is a modified form of the smallpox virus. In some manner its residence in the body of cattle has so changed its properties that it is no longer able to produce a dangerous form of the disease in man, but it is able to stimulate the tissues to manufacture sufficient anti-bodies to protect the body for a number of years against a natural attack. The vaccine used for inoculation contains the virus of smallpox, the nature of which is unknown.

All vaccines that are used as a protective measure against any contagious disease contain the virus of the disease against which protection is sought. The virus may be virulent; it may be attenuated or weakened; or it may be dead. The degree of protection afforded by the vaccination process will depend on the extent to which the organism has been attenuated. Thus the protection afforded by "killed" organisms is not so great as when the weakened organisms are used. In the case of human vaccination, "killed" cultures are usually employed, because of the possibility that the virulence of the weakened organism may accidentally be regained. The organisms are killed in such a way as to destroy their reproductive power, but not to change them chemically to such an extent that when introduced into the body they will not stimulate the cells to the production of the anti-bodies. The manner in which the different vaccines are made will be discussed in the treatment of the specific diseases. Vaccines are used not only to prevent but also to cure disease.

In the production of passive immunity the anti-bodies are transferred from the body of the animal in which they have been actively formed to the animal to be protected. This transfer is accomplished by withdrawing a portion of the blood from the immune animal and injecting it into the animal that it is sought to protect. Since the blood serum is used, the term *protective serum* or *anti-serum* is often used. In many instances the anti-serum contains primarily a substance that neutralizes the toxin. The term antitoxin is therefore often used.

The blood of a hog that has recovered from hog cholera will contain sufficient anti-bodies to protect the individual against a subsequent attack, but not a sufficient amount so that the blood would bestow any marked degree of protection on another animal when inoculated with an amount that would be practicable to use. In order to make the method of practical value, the immune animal is forced to manufacture a larger amount of the anti-bodies than would normally be produced. An animal so treated is said to be *hyper-immunized*. In preparing hog-cholera serum, this is accomplished by injecting into the body of the immune hog a large quantity of blood from a hog that is already sick with hog cholera. The specific organism causing hog cholera is yet unknown, although it can be transferred by the use of blood from a sick hog. The introduction of a large quantity of the virus into the body of the immune hog causes the formation of an increased amount of the protective bodies. The immunizing process is thus repeated until the blood contains such a quantity of protective substances that, when transferred in practicable amounts, it imparts a considerable degree of immunity.

The disease virus is obtained from a sick hog by bleeding from the throat. This virulent blood is usually introduced into the blood vessels of the animal to be hyper-immunized, which, when its blood is sufficiently high in the protective bodies, is bled for the anti-serum by cutting off a piece of the tail. The animal can be bled several times in this way, a fresh cut being made each time until this appendage is too short for further use. The final bleeding is then made from the throat.

In the preparation of diphtheria antitoxin the horse is used to produce the anti-bodies. The animal is not susceptible to diphtheria; hence, the organisms themselves can not be employed to stimulate the production of anti-bodies. The horse is, however, susceptible to the toxin of the diphtheria organism. The organism is grown in the laboratory in beef broth, which is filtered through porcelain to remove all the bacteria, and gradually increasing doses of this filtrate are then injected into the body of the horse. At first only very small doses can be administered without killing the animal; but after recovery from the first injection, repeated doses of increasing amounts are applied, the effect of which is to produce the protective anti-bodies in the blood of the animal. The blood is then drawn from the jugular vein; it is allowed to coagulate in order to remove the clot, and the blood serum is used. Not only does this serum protect an individual from acquiring diphtheria by rendering him artificially immune, but it acts as a curative agent in neutralizing the poison of the disease if applied in the earlier stages of the disease.

The blood serum of animals hyper-immunized against hog cholera or diphtheria varies greatly in the amount of anti-bodies formed. Before it is used it is necessary to know something of the strength or potency of the serum, so that the proper quantity to be used in the animal to be protected may be determined. This is accomplished, in the case of hog cholera, by inoculating a number of young pigs with a definite quantity of the disease virus and a varying amount of the protective serum, noting the amount which is required to protect the animal against the artificial inoculation.

In the case of diphtheria and tetanus antitoxin a number of guinea-pigs are inoculated with a definite amount of toxin and with varying amounts of antitoxin. If the antitoxin administered to a particular individual is sufficient to neutralize the toxin, the animal remains alive. If the toxin is in excess, the animal dies.

Persistence of immunity.—Passive immunity, produced by the transfer of the anti-bodies, is always of short duration as compared with the active immunity produced by artificial means, while the active immunity produced as a result of the natural cause of the disease persists for a still longer period. The variation in time during which protection persists must be taken into account in the practical employment of serums and vaccines in the prevention of animal diseases.

Exit of organisms from body.—Almost without exception, the pathogenic organisms grow only in the bodies of susceptible animals. Their continued existence in nature is therefore dependent upon their expulsion from the diseased body, and the opportunity for introduction into a new susceptible host. The exits from the host by which organisms find their way to new hosts vary in the different diseases. In intestinal diseases, as hog cholera, the excreta serve as a mode of exit. In tuberculosis the secretions, as milk and saliva, function as carriers of contagion. In some diseases the blood from wounds caused by biting insects or the discharges from abscesses on the surface of the body serve as channels of transmission.

The transfer of the causal organisms from one animal to another may take place in a multitude of ways. In the same herd a healthy animal easily comes in direct contact with the infectious material from a diseased animal. The spread of the disease to other herds may take place through the transfer of an infected animal or of infectious material, such as milk or contaminated objects. The direct methods of transfer can be readily guarded against, but the more indirect modes of transmission are much more difficult to detect. Thus hog-cholera virus can be readily transferred by dogs, crows, or persons carrying the virus on their feet.

The opportunity for the transfer of organisms for any considerable distance is dependent on the resistance of the organism, which is largely determined by the fact as to whether it produces spores or not. Most of the non-spore-forming organisms can not persist for any long period outside the animal, since they succumb quite readily to such unfavorable environmental influences as drying, sunlight, and the action of saprophytic bacteria. The spore-forming organisms, on the other hand, can persist for long periods, owing to the resistance of the spores to all ordinary environmental conditions.

The prevention of the transmissible diseases involves the keeping of the causal organisms from coming in contact with healthy animals. This can be accomplished by isolation of diseased animals, by the disinfection of their secretions and the objects with which they have been in contact, and by the proper disposal of their bodies. A personal knowledge of the nature of the organism and of its method of distribution is the only thing that enables one to protect himself or his herds and flocks.

Necessity for correct diagnosis.—It is very essential that a correct diagnosis of any of the transmissible diseases be made, for the methods that will prove effective against one may have no effect against another. Especially is this true when serums or vaccines are to be used in preventing further spread, for these substances are specific in their action. The farmer must usually rely on the experienced veterinarian for a proper diagnosis of any transmissible disease, and the veterinarian is frequently forced to call to his aid the facilities of a bacteriological laboratory.

The use of drugs in the treatment of the transmissible diseases is usually without any curative effect. The farmer must exert himself to prevent the disease, and especially to prevent their introduction on his farm.

CHAPTER XX

ANTHRAX, BLACKLEG, HEMORRHAGIC SEPTICEMIA, AND CORN-STALK DISEASE

Anthrax.—The disease commonly known as anthrax is one of the most interesting of the transmissible diseases of man and the lower animals. The causal organism is large, and is found in great numbers in the tissues of the dead animal. It grows profusely on many kinds of culture media of both animal and vegetable origin. These facts led to its discovery and cultivation early in the development of bacteriology. The information gained from a study of this organism was of the greatest importance in the study of other and more obscure diseases.

In 1849 Pollender noted the organism in the blood of animals that had died from the disease. This observation was confirmed by others. Robert Koch, in 1876, cultivated the organism on artificial media. He proved that its artificial cultivation could be continued for long periods of time, and that on reintroduction into the body of a susceptible animal, a disease identical in symptoms and lesions with the naturally occurring cases would be produced. The causal relation of bacteria to the production of disease was thus proved where previously it had been suspected, but not established with certainty. In 1881 Pasteur published the results of his researches on a method of protecting animals against anthrax by vaccinating them with weakened cultures of the organism. This work was the starting-point for the development of vaccines and other biological products that have been of inestimable value in the prevention and cure of many transmissible diseases.

The organism.—The anthrax bacillus is one of the largest of the disease-producing bacteria. The rodlike cells have square-cut ends. In liquid media the cells appear in chains of great length, giving to the growth the appearance of a tangled mass of cotton fibers. The growth is rapid on all of the ordinary types of culture media. It grows under both aërobic and anaërobic conditions. In the presence of air, spores are formed quickly and in great abundance. The spores are not especially resistant to heat, being killed in a few moments at 100° C. They are, however, extremely resistant to desiccation, and also live for years in water. In the author's laboratory some spores were still alive after eighteen years' residence in a sample of water collected from a pond in a pasture in which a number of animals had died of anthrax. The rapidity and profuseness with which spores are produced when the vegetative cells are exposed to air is of the greatest importance in the distribution and persistence of the disease.

The disease is said to be the most widespread, geographically and zoölogically, of all the transmissible diseases. It has been present in Europe for hundreds of years. In 1613 it is asserted to have caused the death of fifty thousand people in southern Europe. In still earlier times the Arab physicians called it "Persian fire." As the civilization of Europe has spread to other lands, anthrax has been one of its gifts, until to-day no part of the world in which stock-raising is important is free from it. In this country it has been reported in many of the States.

The organism is one that is easily transported over long distances in time and space, due to its resistant spores, which are likely to be present on many articles of commerce, such as hair, wool, bristles, and hides. Many of the outbreaks in both animals and man in this country have been caused by animal products from the Orient and South America.

The disease is primarily one of cattle, sheep, goats, horses, and less frequently hogs. The other common domestic animals of our country, cats, dogs, and fowls, are relatively immune, acquiring the disease only when exposed to large doses of the organism, as is the case when meat from an anthrax carcass is eaten. Man is also susceptible to the disease. He is, however, more resistant to it than are the ruminants, both domestic and wild.

The disease is variously known in its several forms. The old English term for it was murrain. Splenic fever refers to the enlarged condition of the spleen, while malignant carbuncle refers to the appearance of large swellings on the surface of the body, a common manifestation of the disease in man when the organism has been introduced into wounds.

Infection.—In the case of cattle, sheep, and horses, the portal of entry is most frequently the alimentary tract. It is supposed that the compound stomach of the ruminating animal gives opportunity for the growth of the organism and for the more frequent infection of this type of animal. It seems probable that the organism can, in many cases pass through the uninjured wall of the intestine. It is certain that its entrance is made more easy and certain by the presence of wounds in any part of the alimentary tract. It is probable that abrasion of the mucous surfaces in the mouths of grazing animals or those fed on dry fodder readily permits of the entrance of the organism. The transference of the organism from infectious material and its introduction into the body may be accomplished by biting flies.

It is believed that this is the chief way in which the horses and mules of the plantations in the Mississippi delta region become infected. The infection may occur by grazing on infected pastures and by the use of contaminated dry fodder, the spore enabling the organism to persist on the dry material.

Before the development of the procedure for protective vaccination, the disease occurred yearly on many farms in France. These were known as "anthrax farms." The continued persistence of the organism in the soil of a contaminated field is usually ascribed to the resistant powers of the spores. It may be that growth may occur each summer in the soil, a new crop of spores being thus produced to favor the continued existence of the organisms. Its more frequent and constant appearance in stock pastured on low, moist land is evidence of the growth of the organism under these conditions. The contamination of stables, yards, or fields with anthrax bacilli is certain to make them unsafe for a number of years.

Symptoms.—The rapidity of progress of the disease in the individual has led to the division of the various cases into three types: the peracute, the acute, and the subacute. In the first the animal may show no visible symptoms until a few hours before death. Indeed, so rapid is the progress of the disease that the usual yield of milk may be obtained at one milking and death occur before the next milking-time. An artificially infected guinea-pig may show no visible symptoms two hours before death. The temperature may reach 106° F. in the absence of all other symptoms. With such a temperature the respiration will be increased and the heart-beats so pronounced that they may be heard. Later the animal becomes weak and stupid, and the temperature falls to subnormal.

In the acute type symptoms of nervousness are present, manifested by kicking and convulsions. The visible mucous membranes become bluish and the urine is often bloody. In the subacute type the duration of the disease is from one to seven days. Tumors or carbuncles are quite common. They usually appear on the shoulders and neck, and are due to the bruising of the parts, which injury gives rise to a collection of the bacilli within the blood-vessels of the parts, inducing inflammation, followed by the development of the tumor. Carbuncles may also be occasioned by infection through a wound.

The subacute type is the most common, and is the only form that can be treated. It is also the type noted in most isolated cases of anthrax. At the beginning of an outbreak the first animals lost usually show few or no symptoms. This rapid progress of the disease may be due to the lack of resistance of the animal. Such rapid progress of the disease often leads to suspicion of poisoning, or to death by lightning. Such conclusions as to the cause of death may lead to the careless disposal of the carcass, thus endangering human life as well as causing a widespread outbreak, not only on the farm but in the neighborhood. It is well to consider all cases of sudden death in an animal, especially when no definite cause can be given, as due to dangerous causes, and to act accordingly in the handling and disposal of the carcass. The slower process of the acute and subacute types of anthrax and the evident symptoms give greater opportunity for the recognition of the nature of the trouble. The mortality from the disease is from 70 to 80 per cent.

Lesions.—The lesions are usually so characteristic that they enable the recognition of the disease, or at least arouse suspicion as to its probable cause. The most marked is the dark blood, which may appear almost tar-like and which does not coagulate as does normal blood. The spleen, or milt, is usually greatly increased in size; it is dark red in color instead of the grayish color of the normal organ. The

Fig. 42. Anthrax

The spleen on the right is from a normal pig. A portion of the lower end has been torn off. The spleen on the left is from a pig of the same size as the first. Its enlarged condition is due to anthrax interior has a semi-liquid consistency due to the breaking down of its structure. Hemorrhages or areas in which the blood has passed from the blood-vessels into the tissues are often noted in the internal organs and on the membranes. Blood often issues from the natural openings of the body.

The carbuncles are at first hard, hot, and painful, but later, due to the death of the tissue at the center, the fever subsides, and no pain is evidenced by the animal when the carbuncle is opened. The exudate is tarlike in color and consistency. Gangrene, due to a secondary invasion of the abscess with putrefactive bacteria, is often noted. In the case of the hog, swelling of the tissues of the throat is commonly present. The carcass does not become rigid; bloating and decomposition occur more quickly than in the case of death from other causes.

The sudden death of cattle or sheep, accompanied by black non-coagulating blood and an enlarged darkened spleen, should always lead to a suspicion of anthrax. The absence of these typical lesions is not to be considered proof of the absence of anthrax.

The disease is a true septicemia in that the organisms are to be found in great numbers in every portion of the body at the time of death. The absolute diagnosis of the disease is made by finding the anthrax bacillus in the tissues, and, in some cases in which the post-mortem changes are not typical, this is the only way in which the diagnosis can be made with certainty.

Whenever an animal has died and anthrax is suspected, the temperature of each animal in the herd should be taken. All that show a temperature of 104° F. or above should be allowed to remain in the infected quarters; the remainder of the animals should be at once removed.

Before the transmissible nature of the disease was recognized, extensive epidemics were common. Prompt separation of the animals, and care in the disposal of the carcass and in disinfection will do much to prevent continued loss. It has been shown that the loss of one or two animals from a herd by anthrax is a more common occurrence than is a more extensive outbreak. Carelessness, however, may result in great losses.

Vaccination.—The development of a protective treatment against anthrax was one of the great gifts of Pasteur to the world. Starting from observations he had made on chicken cholera, he devised ways of attenuating the anthrax organism by growing it at a temperature above its optimum. The longer the organism was grown at this unfavorable temperature, the less virulent it became. The weakened organism was unable to produce a serious form of the disease when introduced into the tissues of a susceptible animal. It did, however, grow, and cause the animal to produce substances that protected it against a natural attack of the disease. The original method of Pasteur was to apply the vaccine in two doses at an interval of ten days. The vaccines were standardized by injecting them into small animals. The first vaccine should possess such a degree of virulence that it would kill white mice, but not guinea-pigs; the second should kill the latter animal, but not rabbits. This mode of treatment caused the loss of about 1 per cent. of the treated animals. It served to reduce the losses in cattle and sheep, especially in France, to a marked extent. The immunity thus conferred is active in form, persisting for about one year.

It is evident that difficulties are encountered in the preparation of the vaccine. If it is weakened too much, it will give but a slight degree of protection. If, on the other hand, it is not weakened sufficiently, the animals with a low degree of resistance will be unable to withstand the effects of the vaccine and will die. The vaccines also deteriorate rapidly. Their use under carefully controlled conditions is successful. Such conditions were obtained in France both as to preparation and use. Under commercial conditions in this country, the Pasteur type of vaccine has not been an unqualified success.

Recently the simultaneous application of the protective serum and vaccine has been introduced. A horse is immunized by the injection of gradually increasing doses of a virulent culture of anthrax. It is possible so to accustom the animal to the organism that 500 cubic centimeters of a highly virulent culture can be given at one time, or more that ten thousand times as much as would have killed the animal at first. The blood of the horse will have a high content of protective bodies. The animal to be protected is injected with a quantity of serum on one side of the body and with a small quantity of a somewhat weakened culture on the other. The United States Bureau of Animal Industry has prepared a spore vaccine for use with serum. The vaccine is very permanent, due to the resistance of the spores. But one treatment is necessary, and, it is claimed, no losses are occasioned by the treatment. The treatment can be used only on non-infected animals. In the vaccination of a herd in which the disease has appeared, only animals with normal temperatures should be vaccinated. The other animals should receive only the serum, which is a curative as well as a preventive agent. The stock-owner should not rely on vaccination alone to prevent the spread of the disease, but should use all possible precautions to limit the distribution of the organism, especially in the disposal of carcasses.

Disposal of carcasses.—In the unopened carcass the organism cannot form spores, owing to the lack of oxygen; but in the discharges from the nose and rectum, and in any blood that may be brought in contact with the air in making a post-mortem examination, spore formation readily occurs. In the vegetative form the organisms are easily killed; but the spores are exceedingly resistant, so that every precaution should be taken to prevent the blood from coming in contact with the air. The carcass should not be skinned or opened, unless it is necessary for the purpose of establishing the cause of death. If it is not possible to destroy the carcass at the place where death occurred, it should be removed by placing it on a stone-boat instead of dragging it on the ground. The opening of the carcass should take place only at the point where its ultimate disposal is to be carried out, which, if possible, should be by burning rather than burying. If the carcass is buried, it should be covered with quick-lime in a spot where there will be no danger of the carcass being washed out. It is well to fence in the spot to keep out dogs and stock. In the unopened carcass the vegetative organisms rapidly die owing to putrefactive processes. The great danger in anthrax lies in the non-recognition of the first case and in the careless disposal of the carcass.

Anthrax of man.—Man is less susceptible to anthrax than are cattle and sheep. In the disposal of anthrax cadavers, especially when the disease is not recognized and the hide is removed, there is great danger of infection through wounds on the hands or those inflicted during the process of skinning. In case infection occurs, the trouble is likely to be localized in the form of a carbuncle at the point of entry. In the handling of infected material such as hides, wool and bristles, the workers are likely to become infected, especially when the material is handled in a dry condition, so that the spores of the anthrax bacilli may be taken into the lungs or into the alimentary tract. Both respiration and ingestion anthrax are usually fatal.

Importance of correct diagnosis.—There are a number of diseases that are frequently mistaken for anthrax, and, since the advent of vaccines for the prevention of some of these diseases, it is much more important than formerly that a correct diagnosis be made. In cases that show typical lesions there is little danger of confusion; but cases occur that are more or less atypical, and frequently a bacteriological examination of the tissues is necessary to determine the cause of the trouble with accuracy. For this purpose samples of the tissues must be forwarded to some laboratory for examination. Such laboratories are maintained by many States in connection with the colleges of agriculture.

The tissues should be so packed that they will not be a source of danger to those who must handle them in transportation. The tissue should be placed in a jar that is closed so tightly that no liquid can escape. The jar should be packed in a mixture of sawdust and cracked ice and sent at once to the laboratory. No disinfectant should be added if a bacteriological examination is desired; the putrefactive processes must be delayed as much as possible by the maintenance of a low temperature. In order to avoid the dangers due to the sending of the moist tissues, some of the blood may be placed in a concave piece of glass, such as a fruit-jar cover, and allowed to dry.

Every animal that dies from an unknown cause should be subjected to a post-mortem examination by a competent person. In making such examination, it is well to proceed on the supposition that the cause of death is due to an organism dangerous to man. The hands should be examined for wounds, and in all cases it is well to coat the hands with grease before beginning the examination. It should also be remembered that the cause of the death may be traceable to something that can be communicated to other animals of the herd, and the examination should not be made until the carcass has been brought to the place of final disposal. Such simple precautions may be cheap insurance against future trouble.

Blackleg.—A disease frequently mistaken for anthrax is that commonly called blackleg or symptomatic anthrax. Before the causal organisms of the two diseases were discovered, it was thought that blackleg was a special form of anthrax. The disease is also known under the names quarter-ill or quarter-evil. The causal organism is called *Bacillus Chauvei.* It is a large rod which grows only under anaërobic conditions. It produces spores in the tissues of the unopened animal, a property that differentiates it sharply from the anthrax bacillus.

Blackleg is found in all parts of the world, from the tropical regions to the northernmost limits at which cattle are kept. In the United States it is most common in the great grazing States of the Southwest. There are infected localities in many of the States east of the Mississippi River. The disease has often been mistaken for poisoning in localities in which it was not known to occur. It has been probably the most important disease in the great beef-producing districts of this country. Recently, means of prevention have been so effectively used that the disease has greatly decreased in importance.

Cattle between the ages of six and eighteen months are virtually the only ones that are susceptible to the disease. It represents one of the few cases of age immunity to a disease noted in the lower animals.

A great increase in blackleg followed the introduction of the improved breeds of beef cattle on to the ranges of the Southwest. It is believed that the thinner the skin, the more likely is the animal to acquire infection.

Again, animals that are gaining in flesh rapidly and that are taking little exercise seem to be the most susceptible.

It is believed that this increased susceptibility is due to the accumulation of lactic acid in the tissue. The effect of lactic acid in increasing the ability of the organism to produce disease can be shown by injecting a weakened culture that has been treated with lactic acid. The mixture will produce a fatal attack, while the weakened culture alone would not harm the animal.

It is believed that the organism does not pass directly from one animal to another by contact, but that the infection results from a common source, the soil. It is generally conceded that the organism is introduced into the system through puncture wounds caused by thorns, spines, and grass burs, and that to take effect the organism must penetrate the subcutaneous tissue, a fact that explains the greater ease with which thin-skinned animals become infected. It is a disease primarily of the pasture; and hence appears in the Northern States only in the summer, but farther South it occurs during the entire year. It affects sheep and goats less frequently than cattle.

Symptoms.—The symptoms of the disease are usually so characteristic that there is little likelihood of its being confounded with other causes of death. The general symptoms are high fever and loss of appetite. The mucous membranes are at first dark red, changing in the course of a few hours to a dirty leaden or purplish color.

The tumors, which appear most commonly in the thigh or shoulder, are the most characteristic lesion of the disease. They may be present on the neck, chest, flank, or rump—indeed, on any part of the body except below the knee or hock joints. The tumors are at first small and painful; they increase rapidly in size. The lymph-glands in the vicinity of the tumors become swollen. The tumor is cool to the touch and painless in the center. The skin covering it is dry and parchment-like. The lack of sensation at the center of the tumor is due to the death of the tissue. Gas is formed in the tissue to such an extent that the muscle fibers are blown apart. Pressure on the tumor causes the gas to flow through the spaces with a rustling sound, which gives rise to the German term for the disease, *Rauschbrand*. No pain is evidenced by the animal when the tumor is opened, for the tissues at the center are dead. The exudate is dark in color, and has an odor of rancid butter, due to the presence of butyric acid. The exudate is frothy on account of its gas content. The dark color and location of the tumors have given rise to the common name of the disease, blackleg.

The gas formation continues after death, often greatly distending the carcass. The affected tissues do not decompose nearly so quickly as the non-affected areas of the body. A blood-colored frothy discharge flows from the nostrils and anus. The blood is normal in color and in coagulating properties. The spleen is normal. These conditions, together with the gas formation in the tumors, something lacking in anthrax tumors, serve to differentiate blackleg from anthrax. The disease is almost always fatal. Treatment is of little if any value.

The stockman must seek to limit the spread of the disease by hygienic and preventive measures. Care should be used in the disposal of carcasses in order not to contaminate the soil with the resistant spores, which are abundant in the exudate and in the diseased tissue. Cremation of the carcass is advisable whenever possible. Deep burial is a substitute. There is no danger of transmission to man.

Vaccination.—The vaccination against blackleg, first used in France, has been of great value to the stock interests of this country. Before the introduction of the protective treatment in the Southwest, the losses amounted to 10 per cent. of the annual calf crop. These losses were reduced to one-half of one per cent. by the use of the vaccine distributed by the Bureau of Animal Industry of the United States Department of Agriculture.

The vaccine is made by inoculating an animal with the blackleg bacillus. After death the affected muscular tissue is removed and reduced to a pulp, which is then squeezed through a cloth. The juice is dried quickly at 95° F. The cake thus obtained may be stored for long periods, since it contains the resistant spores of the organism.

When it is desired to prepare vaccine, the dried material is mixed with water and heated to from 212° F. to 219° F. for seven hours. This treatment weakens the spores to such an extent that a fatal form of the disease is not produced when a suspension of the material obtained after heating is injected into animals. But one treatment is necessary. The fact that only animals between the ages of six and eighteen months are susceptible to blackleg, and the further fact that vaccination will protect during the susceptible period have made the preventive treatment a great practical success. More than 25,000,000 doses have been distributed by the government to stock-raisers.

Hemorrhagic septicemia.—Sudden death with no well defined symptom is likely to be ascribed to poison or lightning. The next most common cause to which sudden death in cattle and sheep is ascribed is anthrax. As has been pointed out, the use of specific biological products in prevention necessitates the correct diagnosis of the first cases of death in a herd, in order that effective means of control may be employed. Another disease, commonly known as hemorrhagic septicemia from the fact that the causal organisms produce reddened congested areas in the tissues, is not infrequently confounded with the above causes of death.

The organism causing the disease is one of a large group producing a number of diseases in different animals.

Among the most important are chicken cholera, swine plague, and bubonic or Asiatic plague in man. In cattle, deer, and related animals, outbreaks of varying intensity occur. The disease is found in all parts of the world. In the United States it has been most frequent in the upper Mississippi Valley. The discussion in question will be limited to the disease in cattle.

The normal habitat of the organism is unknown. It has been thought by some to be a saprophytic organism, which, under unknown conditions, may suddenly become virulent, and thus cause an epidemic that usually disappears as quickly and as mysteriously as it appears. The rapidity of its appearance and the suddenness with which the animals die, together with the helplessness of the owner to combat it, make it a disease much to be dreaded. Frequently the animals die without showing previous symptoms of illness. In less acute types of the disease, weakness of the limbs may be noted. Recovery is rare.

The manner in which the organism enters the body is unknown, as is also the method of transmission from one animal to another.

On post-mortem examination reddish spots varying from a pinhead to several inches in diameter are found beneath the skin. Hemorrhagic areas are usually present on the heart, stomach, and intestines. The blood is red and coagulates in a normal manner. The spleen is normal. It is frequently mistaken for anthrax on account of the suddenness with which death occurs. In atypical cases an examination of the blood for the specific organism is necessary to confirm the diagnosis.

A method of vaccination has been devised. The causal organism is grown in broth. The cultures are heated to 55° C. (131° F.) for thirty minutes in order to destroy the life of the cells. The low degree of heat does not change them chemically to such an extent that they are unable to stimulate the vaccinated animal to produce bodies that protect from a natural attack. The term "bacterine" is applied to killed cultures of bacteria used for protective purposes. The vaccine used to protect against typhoid fever is of this nature. The value of the vaccine in preventing hemorrhagic septicemia is not fully established. In small herds the isolation of the healthy animals from those that show any symptoms is preferable to vaccination. The healthy animals should be removed to a fresh pasture or meadow and staked out so that no contact between the animals can take place. The carcasses should be disposed of with the same care as in the case of anthrax. If animals die in the stable, the litter should be burned, the stable cleaned and disinfected. The organisms are easily destroyed, since they do not form spores; consequently there is no danger that they will persist in yards and stables, as in the case of anthrax.

Corn-stalk disease.—In those sections of the country in which it is the custom to harvest the ear corn in the row, and then turn the stock into the standing fodder, a disease known as corn-stalk disease is sometimes encountered. The trouble appears soon after the cattle or horses are turned into the field (four to ten days) and runs a rapid course.

On account of the suddenness with which death occurs, and the large losses that follow in a short time, it is often mistaken for a contagious disease, especially for anthrax, blackleg, or hemorrhagic septicemia. It is important, however, to differentiate the trouble, which is probably physiological, from those diseases that are caused by specific organisms. The differentiation can be made by the lack of abnormal changes in the tissues, and the relation of the appearance of the disease to the period of admission of the animals to the fields.

CHAPTER XXI

TUBERCULOSIS

Tuberculosis is one of the most important communicable diseases of both man and domestic animals. In the latter it is of both economic and sanitary importance, since, as noted in the spread of diseases by means of foods, a portion of the tuberculosis occurring in man is due to the organism from bovine sources. Statistics show that one seventh of all deaths of human beings are due to tuberculosis, and that one third of the mortality occurring between the ages of twenty and forty-five, the productive period of life, is caused by the tubercle bacillus.

Animals affected.—All of the domestic animals may be affected, but the disease is most prevalent among cattle, hogs and fowls. The other domestic animals are rarely affected. Besides the domestic animals, a large number of wild animals are also susceptible. There is probably little or no opportunity for wild animals to come in contact with the organism in nature, but when placed in captivity where there is opportunity for infection, the disease makes rapid strides. It is the chief cause of death of monkeys, caged animals, and birds in zoölogical gardens. In the London zoölogical garden 30 per cent. of the birds that died were found to have tuberculosis. A disease caused by an organism belonging to the same group as the tubercle bacillus produces what has been termed tuberculosis in some of the cold-blooded animals.

Distribution.—Within very recent times the commerce in domestic animals and their products and in cultivated plants has increased in a most remarkable manner, due to the development of methods of transportation. From northwestern Europe the improved breeds of cattle, sheep, horses, and hogs have been shipped to all parts of the world, and with them have been carried the diseases with which they were affected. Many countries into which such diseases were thus introduced had been previously free from them, and could have been kept so if there had been sufficient knowledge concerning the nature of these communicable diseases, their detection and mode of dissemination. This knowledge, in many instances, was acquired only after the harm had been done. The United States is still free from some of the communicable diseases that are a cause of great loss to the farmers of Europe, and it behooves us to use all possible precautions to prevent their introduction into this country.

The islands of Jersey and Guernsey are the only important breeding centers that are free from tuberculosis, and this condition has resulted from a rigidly enforced rule that no live cattle should be imported on to these islands. The percentage of animals affected with the disease ranges from over 50 in some of the German states, as Saxony, to less than 5 in our Western and Southern States. The disease is not widespread in those sections in which cattle-raising is not important and into which the improved breeds have not been introduced in considerable numbers. In England it is asserted that less than 5 per cent. of the milking herds of Shorthorn, Ayrshire, and Jersey cattle are free from tuberculosis, while in Wisconsin, one of the most important dairy States in this country, not over one third of the herds contain any tubercular animals. In the Eastern States, in which dairying has been longer established, the percentage of affected herds is higher.

The rapid spread of the disease within recent years is shown in the following figures, which indicate the percentage of animals found to be tubercular on slaughter.

		Cattle %	Calves %	Sheep %	Hogs %	Horses %
Bavaria	1898	5.07	0.05	....	0.35	....
	1906	10.31	0.28	0.12	1.40	0.11
Prussia	1898	16.09	0.15	0.11	2.32	0.12
	1906	23.40	0.33	0.17	2.96	0.16
Saxony	1898	30.46	0.24	0.09	3.16	0.16
	1906	37.58	0.50	0.09	5.07	0.30

The only figures available as to the spread of the disease in this country are those collected by the Bureau of Animal Industry in the meat inspection service which is maintained in all the larger packing-houses of the country. The following table presents the data with reference to the percentage of animals found tubercular on post-mortem examination.

	Cattle %	Swine %
1907	0.4	1.5
1908	0.9	2.0
1909	1.3	2.4
1910	1.5	2.7
1911	1.7	3.7
1912	2.1	4.6
1913	2.1	5.6
1914	2.1	6.6
1915	2.3	7.6
1916	2.6	7.2
1917	2.3	9.8
1918	2.0	7.0

These figures show that tuberculosis has increased in this country rapidly in recent years.

The disease is most common in the pure bred herds—not because these animals are more susceptible than grades, but because the opportunity for infection in pure bred herds has been much greater because of the interchange through purchase. For the same reason, large herds are more likely to be tubercular than small herds. The disease is one that spreads by direct contact among cattle kept out of doors almost as rapidly as in the case of those that are stabled for a large part of the year.

Different names are applied to the various manifestations of the disease in man. *Consumption* and *phthisis* refer to tuberculosis of the lungs; *scrofula* to tuberculosis of the glands of the neck; *lupus* to that of the skin; and *joint disease* to that of the joints. In cattle, *grapes* and *pearl* disease, terms sometimes used by butchers, refer to tuberculosis of the serous membranes.

The tubercle bacillus.—The tubercle bacillus is a slender rod that is a member of the acid-fast group, which includes the organism causing leprosy and that producing Johne's disease in cattle, and also a number of non-pathogenic bacilli, which are found in manure and on plants. The term acid-fast refers to the fact that the organism, when stained, retains the dye when treated with dilute solutions of mineral acids, while other bacteria are decolorized at once. This property of the tubercle bacillus is used for its detection in sputum, milk, and tissues.

The tubercle bacillus does not produce spores. It is, however, resistant to desiccation and other physical and chemical factors, a fact of importance in the spread of the disease. It grows very slowly on all kinds of culture media, and probably in the animal body, a fact of significance in explaining the extended period of incubation of the disease, which is from a few weeks to several months in length. In cultures the tubercle bacillus grows only on the surface of the medium.

Infection.—The organisms enter the body through either the respiratory or the alimentary tract. The importance of these portals of entrance varies in different animals. It is probable that the bovine becomes easily infected in either of these ways, while in the case of the hog the infection under natural conditions is by way of the alimentary tract. The guinea-pig, one of the most susceptible animals, acquires the disease with ease by the inhalation of the bacilli, while it is very resistant to infection through the alimentary tract.

Lesions.—The lymph-glands, which are widely distributed in the body, are to be looked upon as filters, and tend to remove any foreign solid particles, such as tubercle bacilli, that have entered the lymph or blood circulation. The bacilli are most likely to lodge in those glands that are in close proximity to the point of invasion. Thus, the neck glands are most frequently diseased in the hog, and the glands near the lungs and intestines in the bovine. The lungs, liver, and spleen are also frequently tubercular. The organs mentioned are those to which the greatest attention should be paid in making a post-mortem examination. Any organ may be tubercular, such as the heart, brain, udder, and neighboring lymph-glands, the joints, bones, and infrequently the lymph-glands located in the muscles.

The bacilli collect in some of these organs and grow slowly. Their by-products exert a stimulus on the body cells in the immediate vicinity, causing the formation of the characteristic tubercles or nodules. The tubercle is most evident when located on one of the smooth serous membranes of the body, producing that type of disease known as *pearl* disease or *grapes*. The cells at the center of the tubercle are soon killed by the poison elaborated by the bacilli. As the area of the dead tissues continues to increase, the tubercles may become confluent, and form tubercular abscesses of varying sizes. Ultimately these ab-

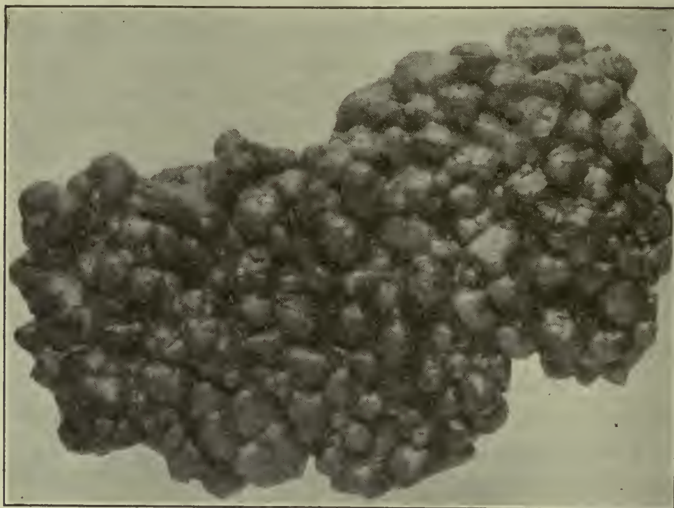

Fig. 43. Tubercular Omentum
In a healthy animal this is a smooth thin membrane. Grapes is the term commonly applied to this form of disease by the butcher

scesses break and discharge their contents into the air-passages of the lungs, the milk-ducts, the bile-ducts, or into some other opening that will enable the bacilli to escape from the body. This condition is known as "open" tuberculosis, as opposed to the "closed" form where the tubercles do not break down.

The tubercular animal is a source of danger to others only as it is eliminating the organisms from the body. If the disease has not reached the open stage, or if the lesions are found in parts of the body that have no exterior opening, the animal can not be dangerous to others or to the human beings consuming the milk. It is impossible to foretell when the closed form of the disease will change to the open, as it is certain to do if the disease progresses. Hence every affected animal must be considered a potential source of danger to the herd and to public health.

The tubercles vary in size from a pinhead to abscesses as

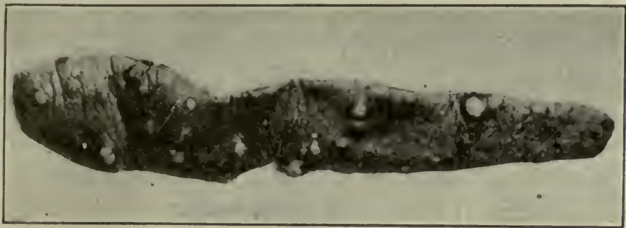

Fig. 44. A Tubercular Spleen
This organ shows a number of tubercles. It is an example of tuberculosis in hogs due to the feeding of infected milk

large as the closed fist. The small tubercles are usually of a light pearly gray color throughout, or they may show a yellowish area at the center, composed of dead tissue. The larger tubercles and abscesses may be filled with creamy pus, or with hard, gritty yellow material due to the deposition of lime salts. The tubercle is then said to be *calcified,* and its contents have the appearance of corn meal.

The lungs of a healthy animal are light pink in color and spongy in texture; in the tubercular organ the firm, hard tubercles may be felt upon pressure, or they may even be raised above the surface of the lung. As has been stated, the disease is readily recognized in the liver and spleen by the sharp contrast between the yellow affected areas and the surrounding healthy tissue.

Tubercular organs and glands are usually increased in size in comparison with the healthy tissue. In the case of a tubercular udder the disease is usually confined to a single quarter and the affected part may be much enlarged.

There is no fever or pain in the tubercular udder, as in the case of acute inflammations.

Distribution of the tubercle bacillus.—The organisms are eliminated in the sputum discharged from the mouth in the act of coughing, in the feces, and to some extent in the milk. In the stable the material from the digestive tract becomes dry, and the dust therefrom with the adherent tubercle bacilli may be drawn into the air-passages of healthy animals. The fodder or water may be contaminated with infectious material, or the transmission of the organism may be direct, through the diseased animal licking herself and then being licked by a healthy animal. The milk becomes infected through the introduction of manure and dust, and also from tubercular udders. The feeding of such milk to calves and hogs readily serves to infect them. Hogs also acquire the disease from following cattle in the feed lot or from manure. The reproductive organs are rarely diseased, and calves from tubercular dams are usually healthy.

The spread of the disease in the herd may be rapid or slow, the determining factor being the prevalence of cases of open tuberculosis. It is a popular view that the disease will not spread among cattle kept out of doors or in stables well ventilated and lighted. Experience shows these views to be wrong, and that when open cases, or spreaders, as they are called, which give out large numbers of the organisms, are present, the disease will spread rapidly under conditions ideal in other respects.

Infection of the herd.—The infection of the herd takes place through the introduction of a tubercular animal, or through the use of contaminated products, such as the milk. In the more acute communicable diseases, the transmission of the organism from place to place may be accomplished by the transportation of contaminated objects, but in the case

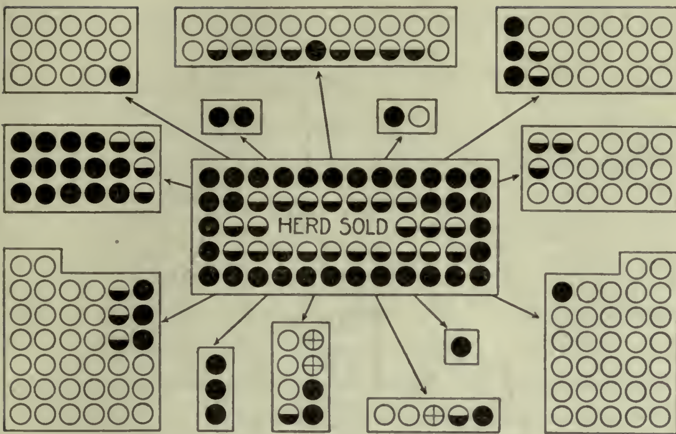

Fig. 45. Buying Tuberculosis

The bounded area in the center of the diagram represents a herd that was dispersed by auction sale. Dots signify tubercular animals and half black circles healthy animals. The healthy animals in the herds into which the dispersed animals went are shown by circles. The crossed circles indicate originally tubercular animals in these herds. Note the number of herds that were infected from the one herd

of tuberculosis it is certain that this is of no importance, and attention should be focused on the animals purchased and the dairy products fed. The introduction of the disease in the latter manner is easily guarded against by requiring that the by-products of creameries and cheese factories be properly heated before being returned to the farm.

The rapid introduction of the farm-separator has resulted in the practical abolition of the whole milk creamery, and this is solving the problem of the spread of tuberculosis by mixed skim milk. The return to the farms of unheated whey is certainly an important factor in the spread of tuberculosis and other milk-borne diseases of animals. New York, Wisconsin, and some other States require the heating of all creamery and cheese factory by-products before they are returned to the farms. Both farmers and operators

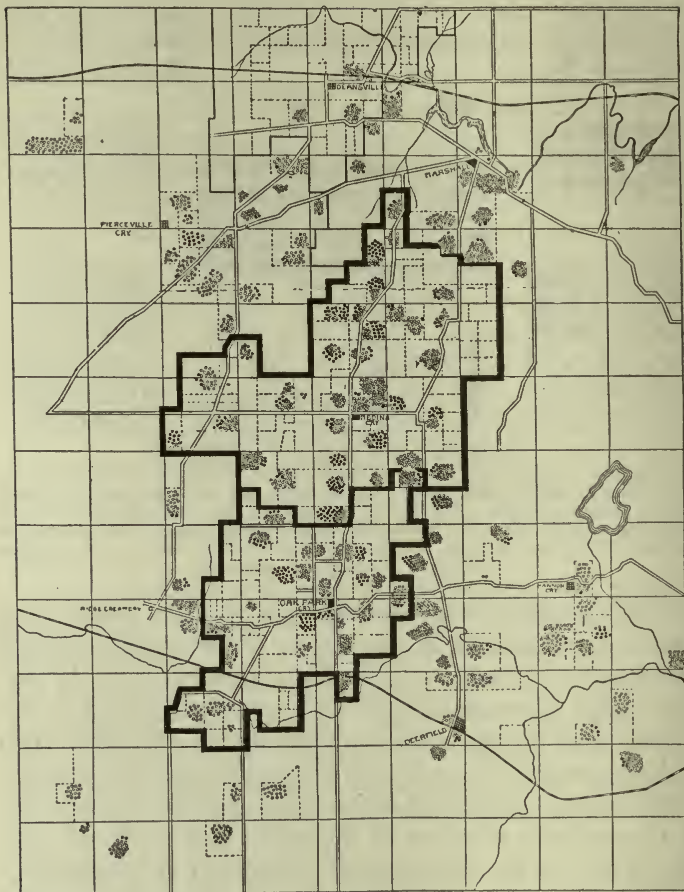

Fig. 46. Tuberculosis Spread by Creamery Byproducts

The tubercular animals are represented by dots, healthy animals by circles. In the two bounded areas thirty per cent. of the cattle were diseased. Outside of these districts but five per cent. were tubercular. The feeding of mixed creamery skim milk was the principal cause of the conditions within the bounded areas should welcome such a regulation, for the effect of its enforcement is to prevent the spread of disease and to improve the quality of milk supplied by the farmer to the operator.

The prevention of the introduction of incipiently diseased animals into the herd is a far more difficult problem. The history of the disease in the individual animal usually shows a slow but not a continually progressive development. Periods of development are followed by periods of dormancy, in which neither the animal nor the parasite is active in its attack on the other. The organisms remain alive in the tissues, and during periods of diminished vitality in the animal may find opportunity for renewed action, causing a rapid progress of the disease. In man recovery from infection is the rule rather than the exception, while in cattle recovery is probably so rare that it can not be considered as a factor in the fight against the disease. Fortunately, the period of latency may cover several years, and so permit of its detection in the earlier stages of the disease before the infected animal becomes an element of danger.

Detection of tuberculosis.—In the early stages of the disease it is impossible to detect it by a physical examination alone. Not until the disease has made sufficient progress to affect in considerable degree the general health of the animal are the symptoms apparent, even to an experienced person. In the last stages the animal becomes emaciated, the hair rough, the eyes sunken, and the head often extended. The appetite may remain good, but food seems to have no effect. If the lungs are involved, coughing may be noted, especially after the animal has been forced to violent exercise. In the case of glands that can be examined in the living animal, such as those of the neck and udder, an enlarged condition should always arouse suspicion. It is impossible for the average farmer or veterinarian to tell

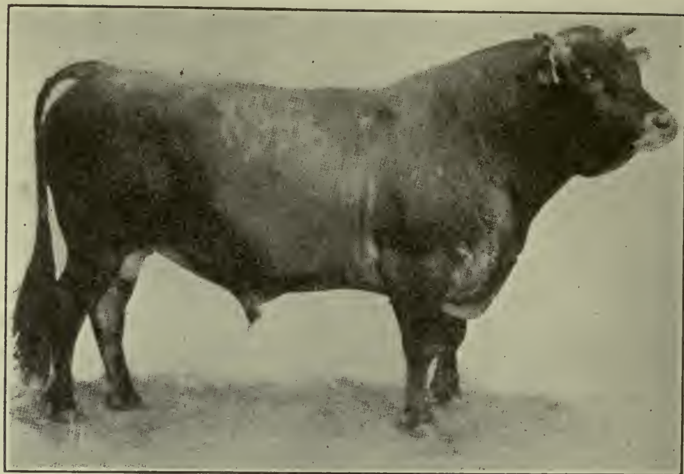

Fig. 47. A Tubercular Animal

The animal showed no physical symptoms although it was eliminating the disease-producing organisms and was thus serving to infect the remainder of the herd

from a physical examination alone whether an animal has tuberculosis or not, or to determine the stage of the disease. An animal may be apparently in perfect health, and yet be dangerous to other animals because of the elimination of tubercle bacilli.

Many of the open cases can be detected on physical examination by veterinarians highly trained in physical diagnosis, especially when aided by a microscopic examination of the excretions. The physical diagnosis and detection of the open cases form the basis of the Ostertag method of eliminating the disease, as practised in Germany. This is applied especially where the percentage of diseased animals is so high that the removal of the tubercular animals becomes an economic impossibility. The method has not been used for a long enough period to give indications as to its final success. In many sections of the United States it would not place an impossible burden on the dairy industry if all tubercular animals were removed from the herds. In order to detect the disease in the herd, and to determine

Fig. 48. A Tubercular Animal

An advanced case of tuberculosis. Such an animal is termed a "piner" or a "canner"

the real condition of animals to be introduced into the herd, a more effective method of detecting the disease than physical diagnosis must be employed.

The diagnosis of the disease can be made with the greatest certainty by applying what is known as the tuberculin test. Tuberculin was originally devised by Robert Koch as a cure for tuberculosis. Its use in the case of cattle is of much value in indicating whether they are affected in any degree with the tubercle bacillus. If any tubercular tissue exists, the introduction of tuberculin causes a rise of temperature which can be easily determined by thermometer readings before and after the subcutaneous injection of the tuberculin.

In making tuberculin, the tubercle bacillus is grown in beef broth to which glycerine has been added. After the maximum growth has been obtained the cultures are heated to kill the bacilli and extract all of their soluble cell products. This extract is concentrated to a definite volume, and then filtered through porcelain filters to remove all the dead organisms. It is, of course, impossible for the tuberculin to produce tuberculosis, as has often been stated by those ignorant of its nature. When this material is brought in contact with the tissues of a healthy animal, no effect is to be noted, while in the case of a tubercular animal, an effect varying with the method of application is produced, because the tissues of the diseased animal are supersensitive to the tuberculin.

There are at present three methods of applying tuberculin: to the eye, when it is known as the ophthalmic test; into the skin, when it is called the intradermal test; and beneath the skin, as it was originally used, when it is referred to as the subcutaneous or thermal test. In the former a drop of tuberculin is placed on the eyeball. A more or less marked inflammation results in a few hours in the case of a tubercular animal. If the tuberculin is introduced between the layers of the skin, a swelling results which is more extensive and persists for a longer period in a tubercular than in a healthy animal. The subcutaneous test is the more reliable and used far more extensively than the others, although it takes a considerably longer time to produce the desired reaction. In addition to the temporary fever produced in a reacting animal by the subcutaneous injection of tuberculin, a constitutional reaction is frequently noted, as is indicated by loss of appetite, increased respiration, diarrhea, and a local swelling at the point of injection. The thermal reaction is, however, most constant, and since it is easily detected by taking the tem-

Fig. 49. Injecting Tuberculin
The diagnostic agent is injected beneath the skin of the neck or shoulder

perature, it is the characteristic on which the greatest reliance is placed.

Details of the tuberculin test.—In testing an animal, a series of temperatures should be taken before the tuberculin is injected, to determine the normal range, and to note especially whether any abnormal condition obtains. The temperature is taken by means of a clinical thermometer inserted in the rectum. The temperature of the bovine varies considerably in different animals, and even in the same animal at different times of the day. The temperature of healthy milch-cows may range from 100° F. to over 102° F. The temperature of calves and fat stock is usually higher, while that of aged or weak animals is lower. The variations that may be noted in a well kept, healthy animal are illustrated in the following table, in which are also given the pulse and number of respirations a minute. Exercise, excitement, and hot weather tend to increase the temperature. The drinking of large quantities of cold water lowers the temperature for some hours.

Temperature, Rate of Pulse, and Respirations per Minute

	Cow No. 1			Cow No. 2		
	Temperature	Pulse	Resp.	Temperature	Pulse	Resp.
10 A. M...	99.5° F.	66	18	98.6° F.	60	15
12 M......	100.8	54	15	99.4	54	15
2 P. M...	101.6	48	15	100.2	54	18
4 P. M...	103.0	66	24	102.7	72	24
6 P. M...	103.1	57	18	103.0	60	27
8 P. M...	103	56	16	102.0	60	14
10 P. M...	102	60	20	102.0	50	18
12 P. M...	102.5	56	16	101.6	54	20
2 A. M...	102.4	64	18	102.2	58	18
4 A. M...	102.2	54	24	101.5	60	24
6 A. M...	101.8	60	18	102.2	60	20
8 A. M...	102.5	56	16	103.2	60	18

The temperature should be taken four times at two-hour intervals before the injection of the tuberculin. The injection is made by means of a hypodermic syringe, usually just back or in front of the shoulder. It may be injected wherever the skin is thin and loose. The needle is inserted through a fold in the skin. Care should be taken in inserting the needle to see that it penetrates through the skin but not into the deeper lying muscular tissue. Before the syringe is used it should be sterilized by placing it in cold water and bringing the water to the boiling-point.

The dose of tuberculin varies, depending on the degree of concentration. The usual strength employed requires 2 cubic centimeters to 1,000 pounds live weight. It is desirable that the animals tested shall be in a normal condition; hence, the injection of the tuberculin should be preceded

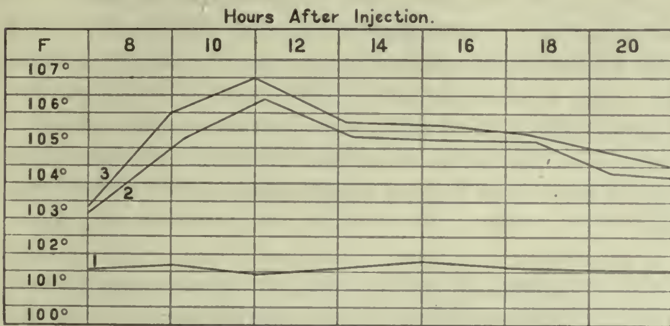

Fig. 50. Reaction Curves in the Tuberculin Test

Curve 1 represents the temperature of a healthy animal after the injection of tuberculin. Curves 2 and 3 represent the temperatures of tubercular animals following the administration of tuberculin

by a careful examination of each animal. Any animals showing abnormal temperatures should not be injected. The normal physiological functions, such as calving, œstrum, or "heat," may or may not affect the temperature. Complete notes should be made as to the condition of the animals, so that these facts may be considered in the interpretation of the records. A negative reaction in the case of a cow in heat or that has recently calved is as reliable as on any animal, while a positive reaction may not be so reliable.

Since it takes a number of hours to produce the febrile reaction in the case of an affected animal, it is not necessary to take temperatures until eight to ten hours after the injection of the tuberculin. On account of the length of this period, it is most convenient to inject the tuberculin in the evening. Four or five temperature readings should be taken at two-hour intervals until there is a marked and permanent decline toward the normal. In the case of a positive reaction, the temperature usually begins to rise from 10 to 14 hours after injection, reaches a maximum in from 12 to 16 hours, and then declines rapidly. The maximum temperature may reach from 105° to 107° F., or three to five degrees above the average normal temperature. The reaction is considered positive when the highest temperature after the injection is at least two degrees above the average normal before injection, or is one and five tenths degrees above the highest temperature noted before injection.

It is often a question of judgment as to whether a reaction is positive or not, especially in those cases in which the increase just reaches the standards assumed. In those cases, all circumstantial features, including especially the character of the temperature curve, must be taken into consideration. In no case should the decision of such doubtful cases be left in the hands of persons who are not thoroughly familiar with such work. The limitations of the test must be recognized, and the results must always be interpreted with judgment.

In making the test, conditions should be kept as nearly normal as possible. The herd should be kept quiet, fed, and watered as usual, unless the manner of watering would permit them to drink large quantities of cold water, which might vitiate the results by excessive reduction of temperature. Preferably water should be given in small quantities, if it is cold.

Animals that show a doubtful reaction should be retested after sixty days. If the retest is made at an earlier time, it is likely to be less reliable, owing to the effect of the first dose of tuberculin. On the retest a triple dose of tuberculin should be used, and the temperatures after the injection should be begun by the fourth hour, since the reaction is likely to appear earlier than in the original test.

Animals recently infected but not yet containing diseased tissues, *i. e.*, those in the incubation stage or those in which the disease is dormant, do not as a rule react to the test, while those in which the disease is far advanced often fail to react because they are already saturated with the tuberculin naturally formed as a result of disease.

The disease in the latter can be recognized by a physical examination; in the former only by a repetition of the test at intervals so as to determine its presence before it has made such headway as to make the animal a source of danger.

The purely mechanical part of the test can be carried out by any intelligent farmer. He should learn how to read the thermometer accurately and acquaint himself with all the details of the test. No farmer need neglect the testing of his herd because of the inability to obtain a veterinarian, or on account of expense. The advantage of being able to test one's own herd is great, since retests can be made on single animals as the occasion seems to warrant, and all animals purchased can be tested before they are placed with the herd.

Freeing the herd from tuberculosis.—The methods to be followed depend on the value of the animals, and the extent of the disease in the herd. If but few animals are found to be infected, the cheapest and most effective way is to remove them. If a larger portion is diseased, and especially when the animals are valuable for breeding purposes, the herd may be separated into healthy and reacting sections, which should be kept in separate quarters and pastures. The calves from tubercular animals should be removed at birth, placed with the healthy portion of the herd, and fed on the milk of healthy animals, or on that of the tubercular animals after proper sterilization. In almost all cases, calves so treated can be raised to maturity in a healthy condition. As the herd is built up in this way, the old reacting animals can be discarded; by this means the valuable characteristics of the particular family can be transmitted through the progeny. This method of "weeding out" the disease, instead of "stamping" it out by wholesale slaughter, is known as the Bang system, and has been widely used in Denmark, where the percentage of affected animals is so high that immediate slaughter would be almost prohibitive.

If 50 per cent. or more of the animals react to the test, it is advisable to consider the entire herd as infected, and not attempt to eradicate by separation and slaughter, but to build up the healthy herd by the progeny alone. This plan is recommended because it has been found by experience that when so large a part of the herd reacts to the test, most of the remaining animals will react later.

Many of the States require the removal of known tubercular animals from dairy herds. In most cases the State bears a part of the loss by compensating the owner in part for diseased or reacting animals. The reacting cattle are usually slaughtered at some abattoir in which federal inspectors are stationed. The meat of reacting animals may or may not be passed as fit for food, depending upon the extent of the lesions of the disease. It was formerly the custom to destroy all carcasses of reacting animals, but this economic loss is no longer sanctioned. In the incipient stages of the disease the affected parts are usually confined to definite organs, and if those are removed, no danger exists in the use of meat that has passed federal inspection. This salvage has reduced to a marked extent the cost of the eradication of tuberculosis.

The basis for the compensation of stockmen by the State is the sanitary importance of the disease, and the necessity of safeguarding the public welfare as well as the economic relations. In determining this amount, the proportion paid by the State should not be too large or otherwise there is danger that the public treasury will be made the victim of unscrupulous design.

Farmers do not appreciate the losses that are occasioned by this disease, as its course is slow and insidious. If it ran as rapid a course as the more acute communicable diseases, its importance would be more appreciated. With the development of an unthrifty condition, the farmer is apt to dispose of the animal to someone else, which simply perpetuates the disease in another herd. Many are sold as "canners." If the time ever comes when the losses due to the condemnation of carcasses because of tuberculosis is placed on the producer instead of on the consumer of meat, farmers will be forced to the necessity of eradicating this plague to save themselves from such economic losses as now obtain. The rapid spread of the disease, especially in hogs, makes this problem already a factor of considerable importance in the price paid for swine.

Vaccination.—Many attempts have been made to use vaccines in the prevention of tuberculosis, but without success. Some of the methods employed have imparted a certain degree of immunity; but, because of the fact that the protection persisted for only a few months, and it could be bestowed only on young animals, the methods have not been a practical success.

Prevention.—There is no doubt that a breeding herd free from tuberculosis is a most valuable asset, and one that will increase in value as people become more alive to the economic importance of the disease, and seek to purchase only sound cattle. At present it is difficult, if not impossible, to avoid the introduction of tuberculosis into a herd where considerable numbers of cattle are purchased. The widespread distribution of the disease, even though sparingly present in any particular herd, always raises the question as to whether any animal purchased from such a herd may not be in the incipient but non-reacting stage. The limitations of the tuberculin test are such that it will not enable one to recognize *every case,* no matter in what stage the disease may be. Individual animals may show a plainly negative reaction to the test at the time of purchase because of the dormant form of the disease, because the period of incubation has not been completed, or because of dishonest practices by the seller. These animals, if maintained in the herd, are quite certain in time to develop open tuberculosis, and be a source of loss if their condition is not detected.

The only effective way to prevent the introduction of the disease is to purchase only from herds that are known to be *absolutely free* from tuberculosis. It is certain that loss can be largely avoided by the testing of all animals at the time of purchase, by keeping the animals separate from the herd for at least three months with a retest at the end of this period, and by the annual testing of the entire herd. In this way, the use of tuberculin serves as a cheap kind of stock insurance, and should be maintained regularly as an annual duty in the herd. Where such vigilance is practised, little danger from the disease need be feared.

Tuberculosis of hogs.—As has been stated, the infection of the hog takes place with the greatest ease by way of the alimentary tract, hence the lesions are most likely to be found in the glands of the throat, or in the mesenteric glands. The bacilli are almost entirely of bovine origin, the hog coming in contact with them in the manure, or in skim milk and whey. Due to the fact that hogs are not kept for long periods of time, there is little opportunity for the disease to make such progress as to permit the animal to eliminate the bacilli; hence there is probably little, if any, direct infection from one hog to another.

The disease can be detected in the living animal most easily by the intradermal tuberculin test, which avoids the factor that makes the thermal tuberculin test unsatisfactory —namely, the rapid and wide variation in temperature of the hog.

Avian tuberculosis.—It is certain that tuberculosis has made rapid progress in barnyard fowl in this country in

Fig. 51. Avian Tuberculosis

A section through the breast of a healthy bird and a section through that of a bird that died from tuberculosis. On account of the extreme emaciation the disease is frequently called "going light"

recent years, due undoubtedly to the commerce in breeding birds. In many sections of the country it is becoming of great economic importance, in some instances causing the death of 50 per cent. of the flock in a year.

The disease is characterized by the extreme emaciation of the bird in the last stages, and by paleness of the comb and wattles. Lameness is also an indication, since the joints are often affected. These manifestations of the disease have given rise to the terms *going light* and *rheumatism.* Unlike mammalian tuberculosis, post-mortem changes of the disease are found chiefly in the abdominal cavity. The liver is often dotted with yellow necrotic areas, which has caused the expression *spotted liver* to be commonly used.

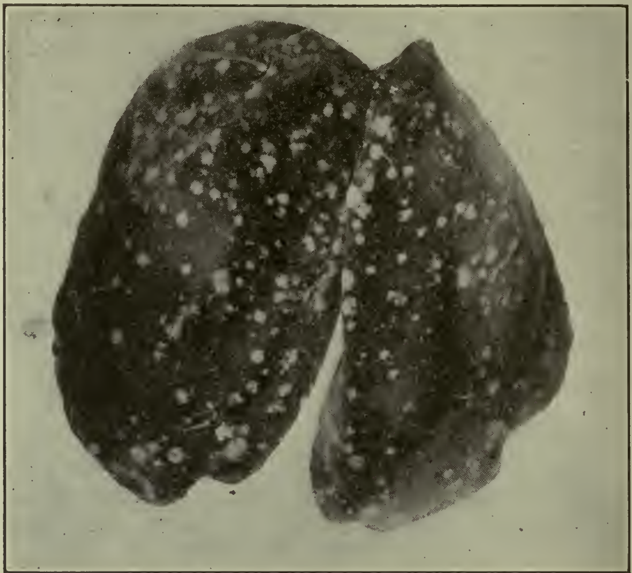

Fig. 52. Avian Tuberculosis

A tubercular liver. The organ is greatly enlarged. The yellow areas give rise to one of the common names applied to the disease, spotted liver

This organ is often much increased in size. The spleen is usually enlarged and frequently abnormal in shape. On section, the normal tissue may be found to be almost wholly destroyed. The intestinal wall may be studded with tubercles of varying size. In the more advanced cases the lesions will be found in other organs, such as the kidneys, lungs, and ovaries.

The bacilli are eliminated in the excreta, and infection is by way of the alimentary tract. The disease is spread from flock to flock by the sale of tubercular birds, and possibly by the sale of eggs, which, when the ovaries are tubercular, may contain the organisms. The chicks hatched from the eggs may develop the disease, a case of hereditary transmission.

Tuberculosis can be detected with some degree of certainty by injecting a small amount of tuberculin prepared by the use of the avian tubercle bacillus. It is preferable to avoid the purchase of stock from suspected flocks, and to get rid of the entire flock when the disease has made its appearance, rather than to attempt to eradicate it in other ways.

There is no reason to believe the disease is of sanitary importance. It may have other economic aspects than those mentioned, for it has been shown that the organism is capable of producing a non-progressive form of the disease in hogs which may cause the rejection of certain parts by the meat inspectors. To avoid such trouble and to prevent the spread of the disease in the flock, all dead birds should be buried, so that they can not be eaten by hogs or birds.

Differential diagnosis.—There are a number of diseases that may be mistaken for tuberculosis in domestic animals. Actinomycosis may produce nodules in the udder that resemble tubercular nodules. Sheep are sometimes affected by an intestinal disease known as *nodular disease*, which to the uninitiated might be thought to be tuberculosis, but is really caused by a parasitic worm which burrows into the wall of the intestine, forming a greenish-colored nodule about the size of a pea.

Johne's disease.—As has been mentioned, there are a number of acid-fast bacilli other than the tubercle bacillus. The disease, known as Johne's disease, is becoming of considerable economic importance in cattle. It is a very slowly progressing disease, and until recently no method of detecting it was available other than by the symptoms, the most marked of which are intermittent diarrhea and progressive emaciation. The symptoms become apparent only after the disease has made such progress that the organisms are being eliminated from the affected animal.

The isolation and cultivation of the causal organism in pure culture has made it possible to prepare a product comparable to tuberculin in its manner of preparation and use. This diagnostic agent is injected into the blood stream, and causes a thermal reaction in the case of infected animals. Its use has been limited. It can not be said at this time whether it will be possible to free a herd from the disease through the detection of the infection in the individual animal before the organisms are eliminated.

CHAPTER XXII

TEXAS FEVER, CONTAGIOUS ABORTION, AND FOOT-AND-MOUTH DISEASE

Communicable diseases of both man and the lower animals are caused not only by microörganisms usually classed as plants, but by microscopic animals, the protozoa. Malaria and sleeping-sickness are among the important human diseases caused by protozoa, and Texas fever is the most important protozoal animal disease.

The pathogenic protozoa may be found in the intestines, and are then eliminated in the feces, from which there is opportunity for them to find their way into the bodies of healthy animals. Another class of protozoa are to be found in the blood, and are transmitted from one animal to another by biting insects. The anopheles mosquito is responsible for the transmission of the malarial organism, and one of the cattle ticks for the transmission of the Texas fever organism. In the case of diseases transmitted by insects, there is opportunity for widespread distribution, while in the case of the diseases caused by intestinal forms, the chance of the organism gaining entrance to the tissues of a susceptible host is much smaller. The fight against the spread of the insect-borne protozoal diseases is a fight against the transmitting insect, the tick in the case of Texas fever.

Texas fever.—The parasite is found in the red blood-cells, which are destroyed by it, and the red coloring matter, the hemoglobin, is set free to be eliminated from the body in the urine, to which it imparts a red or black color.

The disease is often called *tick fever* because of the method of transmission, and *red* or *black water* from the dark urine. Of the ticks that are to be found on cattle, but one, *Margaropus annulatus,* is concerned in the transmission. The female tick that is infected with the parasitic organism drops off the host animal to lay the eggs on the ground, where they hatch in from thirteen days to six weeks, depending on the temperature. If the young seed ticks come in contact with an animal, they attach themselves to the inside of the thighs and flanks, along the belly and brisket, inside the fore legs, and around the base of the tail. They remain attached to the animal with which they come in contact, living on the blood.

The protozoa causing the disease pass from the female into the egg, and thence into the seed tick, which infects the host animal. If the host is immune, no harm results; but if the animal is susceptible, a fatal form of the disease is usually produced. Young animals are not very susceptible to the disease. When raised in contact with the ticks, they gradually become infected with the protozoa during the time when the immunity is so high that a fatal form of the disease is not occasioned, and a permanent immunity is produced by the mild attack. The loss from the disease is not so much from death of animals as from the fact that cattle can not be sent from infected regions to free areas. Thus Southern cattle can not be shipped to the Northern cattle markets, except under certain restrictions that add to the cost of marketing. In the markets the cattle from infected regions are sold at a lower price than the same grade from a non-infected territory. The ticks are also a constant drain on the vitality of the animals, diminishing the milk production and rate of growth. The susceptibility of cattle from free territory is so great that they can not be taken into infected territory without immunization. This fact has limited the improvement of the Southern cattle by the introduction of pure bred animals from the North.

Eradication.—It is, of course, impossible to allow the free shipment of tick-infested cattle into the Northern States, where they would be brought in contact with susceptible animals. Before the nature of the disease was recognized, such shipments made in the summer caused great losses in Illinois and Indiana. In 1891 the Texas-fever line was established, marking the boundary between the sections free from the transmitting tick and those in which it is still present. The line is not a fixed one, but changes from year to year as the work in tick eradication progresses. The methods formerly used to free the cattle from ticks were based on the life history of the insect. The female deposits her eggs on the ground. The shortest period required for the eggs to hatch is twenty days. The young ticks that do not succeed in attaching themselves to an animal die from starvation. The period required to insure the destruction of the ticks by starvation is dependent on moisture and temperature conditions. In general, moisture and cold prolong while dryness and heat shorten the period the ticks will live on a pasture. This period is important, since one of the ways of freeing cattle from ticks is to place them on one pasture for a short period, then on another, and not return them to the first until starvation has destroyed the ticks.

By a proper rotation on tick-free pastures the cattle can be freed from ticks. The cattle must not be allowed to remain on one pasture longer than twenty days, since this is the shortest time in which the young ticks appear after the females have dropped from the cattle to lay the eggs. This method is a long and somewhat uncertain one to employ when large areas are to be made free from the tick. It has been largely supplanted by dipping the animals in a solution of sodium arsenite, which destroys the tick without injuring the animal. The animals are dipped every two weeks from March to November. If the work is carefully and systematically done, the area in which all animals have been dipped will be free from the tick and will no longer be

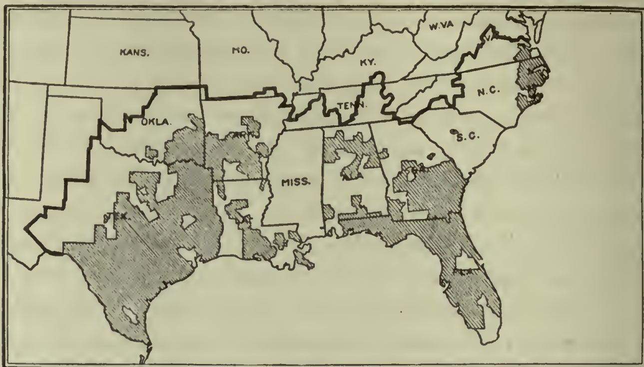

Fig. 53. Texas Fever

The heavy black line bounds the area in which the disease-transmitting tick was present in 1906. The white areas below this line have been freed from the tick and the disease between 1906 and 1918

under the burdensome regulations that so hamper the tick-infested areas.

The total area rendered free from the tick since the beginning of the work in 1906 to December, 1918, embraced 458,529 square miles. It seems reasonably certain that within a few years Texas fever will be eradicated from this country. The work of eradication should progress rapidly when the stockmen realize the great economic advantages that will accrue to the industry from the eradication. Texas fever is a disease the eradication of which simply awaits the practical application of knowledge already acquired; while, in the case of other important communicable diseases, the information is not yet sufficiently complete, so that effective measures can be carried out, or else the nature of the diseases is such that there seems little hope of getting rid of them in the near future.

Immunization.—The young animal is not very susceptible to Texas fever, and can be readily immunized by introducing into the blood a small number of the causal organisms that will not produce a fatal form of the disease, but will cause a sufficient degree of immunity to protect against natural infection. The requisite number of organisms may be introduced by transferring blood from an infected animal to the animal to be immunized. The amount of the blood to be thus transferred depends on the susceptibility of the animal. If an old animal is to be immunized, one cubic centimeter of the blood is used, but if a yearling is to be treated, three cubic centimeters of the blood may be employed without danger.

Again, the tick itself may be allowed to infect the animal, the number of organisms introduced being governed by the number of ticks placed on the animal. The method of immunization with blood is very successful, about 3 per cent. being lost by vaccination and 7 per cent. by subsequent infection. The 10 per cent. loss, when compared with the 90 per cent. loss that resulted when non-immunized cattle were placed on tick-infested pastures, is a measure of the success of the treatment.

Certain cattle, as those of India, are resistant to the disease, and attempts have been made to breed strains that possess such an immunity.

Contagious abortion.—The term *abortion* signifies the premature discharge of the fetus. If the abortion occurs late in the gestation period, so that the young may live, it is often termed premature birth; there is, however, no essential difference, as far as the cause is concerned, between abortion early in the gestation period and that which occurs later.

Causes of abortion.—The expulsion of the fetus may be due to slipping, injury by another animal, or other mechanical causes, and to feeds that have a specific action on the pregnant animal, such as grains and fodders that contain large amounts of smut or ergot. It is quite certain, however, that abortion as it is observed in cattle is almost wholly due to the invasion of the animal by a specific organism which may pass from animal to animal, producing what is commonly known as *contagious abortion*. The disease is of the greatest economic importance in cattle. A similar trouble, caused, however, by a different organism, is noted in mares, and a third organism is responsible for abortion in sheep.

The disease as it appears in cattle has spread rapidly in recent years, owing to the great increase in the sale of breeding animals. It is now rare to find a herd of any considerable size that has an entirely clean record. The losses it occasions are felt especially by the breeder who relies on the progeny of his herd for a large share of his returns, much more than by the farmer who is primarily interested in the production of milk. In the beef districts the disease, of course, becomes of major importance to the farmer.

The loss is not wholly confined to that incurred from the death of the calf, for if the abortion occurs early in the gestation period, the animal will rarely prove a profitable producer of milk during that lactation period. If the abortion occurs late in the period, the flow of milk will be normal. In economic importance the disease ranks with tuberculosis, Texas fever, and hog cholera. Against these the farmer has much hope of making a successful fight with the knowledge that is already available, but against contagious abortion the case is far less hopeful.

The cause of bovine abortion is a small bacillus that does not form spores and is relatively non-resistant to disinfectants. It is called *B. abortus,* or the Bang organism, after the Danish veterinarian who first discovered it. The organism has been found widely distributed in all parts of the world, and there is no doubt concerning its causal relation to the bovine form of the disease.

Nature of the disease.—The disease is one that has a specific action on the pregnant animal, causing an inflammation of the uterus, with injury to the fetus or causing its death. The general health of the animal is not affected to any noticeable extent. The organism is eliminated at the time of abortion, and for an indefinite period thereafter in the discharges from the uterus. It has also been found in the milk.

The organism may be present for months before abortion actually occurs, indicating that it is to be looked upon as a chronic disease. Again, an animal may continue to eliminate the abortion bacilli in the milk years after the last abortion occurred or when no abortion has been known to occur. The infection of an animal does not necessarily produce abortion, since this will depend on the extent of injury to the fetus. The infected animals that do not abort may be as dangerous to healthy animals as those that have aborted. The fact that some unsuspected animals may thus act as bacillus carriers makes it an especially difficult disease to control in a herd.

The natural manner of infection is not known with certainty. It has been shown by experimental methods that the infection may occur through the genital passages. It is probable that, if infection occurs in this manner, it must take place before or very shortly after conception, as, after the uterus is closed, no invasion can occur through the blood. It is believed by many that the male is one of the common agencies in the transmission of the organism from the infected to the healthy animal. It has also been shown that infection may occur through the alimentary tract by the ingestion of contaminated food. By some it is thought that this is the most important if not the sole way by which the organism enters the body under natural conditions. Infection in this manner may occur after conception. It will be evident that if the disease is introduced into a herd, there will always be ample opportunity for it to spread, whatever the method by which the organism invades the individual animal, since the stable and fodder is certain to be contaminated with the organism.

The disease may be introduced into the herd by the purchase of an infected animal, and probably by the feeding of mixed creamery and cheese factory by-products. While this last method of spread has not been proved, the presence of the organism in the milk of a considerable proportion of the infected animals, and the fact that infection can occur by way of the alimentary tract, would lead to the conclusion that this may be one method by which the disease is being distributed from farm to farm. This method of distribution can easily be avoided by pasteurizing the factory by-products, a method equally effective against both tuberculosis and foot-and-mouth disease.

Detection of the diseased animal.—Among the antibodies formed by the cells of an infected animal are substances to which the term *agglutinin* has been applied. These substances possess the property of causing the bacteria producing the disease to clump or to come together in large aggregates. The clumping of the cells may be determined by examining the solution under the microscope or by the unaided eye.

The actual tests are made by preparing a uniform suspension of the cells of the causal organism in a salt solution. A small quantity of blood is drawn from each animal to be tested, the blood is allowed to clot, and the serum removed, which is then added in varying proportions to the suspension of bacterial cells. If the blood is free from the specific agglutinin produced by the animal on invasion by the organism of abortion, the cells remain in suspension. If the specific agglutinin is present, the cells clump and the masses soon settle, leaving a clear liquid. The agglutinin can be developed only as the host has been invaded by the organism. A positive test does not imply that the animal at the moment the blood was drawn was harboring the organism, for the anti-bodies persist long after the organism has disappeared from the body. Similar tests are used in the detection of typhoid fever in man, glanders in horses, and white diarrhea in chickens.

A test for another of the anti-bodies is also used in the detection of animals that are or have been affected with *B. abortus.* The test is so complicated that it can not be described here. It is similar to the Wassermann test used for the detection of syphilis in humans. It is also known as the complement fixation test. It involves the use of the blood corpuscles or serum from three different species of animals other than the bovine animal that is being tested. The organism causing the disease is also used. It is an example of the progress that has been made in the detection of the most minute quantities of specific substances of unknown nature formed by the body cells of an animal invaded by a parasitic organism.

The stock-owner must rely for protection very largely on the information he can secure as to the health of the herd from which he intends to purchase.

Control and prevention.—It is not usual for an animal to abort more than once or twice, and such condition is frequently interpreted as an indication that a certain degree of immunity has developed as a result of the earlier attack. It seems probable that this is not the case, but that there is an age immunity, since the greatest number of abortions occur with heifers during the first and second pregnancies. It is not good practice to sell aborting animals with the hope of getting rid of the disease, since it will rarely if ever succeed. The replenishing of the herd by purchase will serve to continue the trouble, either by the addition of healthy animals to become infected, or by the introduction of new centers of infection.

Many treatments have been devised and recommended for the prevention and cure of abortion. For example, the internal administration of carbolic acid, both with the feed and by hypodermic injection, has been widely used. There is little reason to believe that it has any favorable effect. The use of vaccines has been attempted, but without success. It seems evident that the breeder and farmer must rely entirely on sanitary precautions to prevent the spread of the disease. He must seek to destroy the organism in the infectious material discharged by the animal. The dead fetus and also the afterbirth should be buried; the contaminated litter should be destroyed; and the aborting animals should be flushed out with a 0.5 per cent. solution of some of the soapy, coal-tar disinfectants, such as lysol, or with a 0.5 per cent. Lugol's solution, which consists of one part of iodine and two parts of potassium iodide dissolved in three hundred parts of water. The solution should be warmed to 100° F. The treatment should be continued daily until no discharge is to be noted. This procedure will not only serve to limit the distribution of the organism, but will be of some service in the prevention of sterility, which is a frequent sequence of abortion, and an important factor in its economic importance. It is also well to disinfect the bull after each service.

A separate maternity stall should be provided, to which all animals that are to calve at the normal time should be removed. This stall should be kept clean, and supplied with an abundance of clean bedding. Animals that show signs of aborting should not be placed in this stall, but removed from the stables to a separate building.

Foot-and-mouth disease.—There are a number of transmissible diseases of domestic animals prevalent in Europe that are not found in this country. Among them are contagious pleuro-pneumonia of cattle, hog erysipelas, and foot-and-mouth disease, which affects the cloven-hoofed animals and man. All domestic animals imported from Europe must be kept in quarantine for a considerable period in order that there may be time for them to develop symptoms of any disease with which they may be infected, and to permit of a detailed examination as to their health. These precautions are taken primarily to prevent the introduction of diseases that are not known in this country. Even with all precaution, there is always opportunity for some of these diseases to be introduced and to spread rapidly. Foot-and-mouth disease forms a striking example. There have been six outbreaks of this disease, as follows: 1870, 1880, 1884, 1902–3, 1908–9, 1914–15. The disease has, however, been eradicated at each appearance. The method followed has been to slaughter not only the diseased animals, but all other susceptible animals on the farms on which the outbreaks occurred. In 1902–3 4,461 animals were killed; in 1908–9 3,636 animals were slaughtered. The cost of the eradication of each of the outbreaks in 1902–3 and 1908–9 was approximately $300,000. Not a large amount to pay as insurance of the entire stock industry of this country against this disease for ten years.

The disease is like other acute communicable diseases in that it has an ebb and flow. These waves occur at intervals of several years. The reason for this variation in severity is not known. In Germany, after a period in which the disease was not especially important, it began its ravages, and in 1911, 3,000,000 cattle were affected, 1,000,000 sheep, and 2,500,000 hogs. The loss from death of animals is not great, only about 1 per cent. The economic losses are due to the loss of flesh, to diminished milk production, or to loss of reproductive power. It has been estimated that for cattle this loss ranges from seven to twenty dollars a head, and proportionately less for smaller animals. It is thus clear that the disease imposes a great economic burden upon stockmen, and if by the expenditure of reasonable sums the country can be protected from it, it is certainly wise to spend the money.

The disease presents an excellent example of the influence of modern commercial conditions on the rate of spread. The infection of the great shipping-yards is an especially important factor in the distribution. The outbreak of foot-and-mouth disease of 1908–09 was due to the importation from Japan of vaccine virus that was used in one of the vaccine establishments in Pennsylvania. Some of the virus was sent to a Detroit establishment that rented calves from a dealer for the manufacture of smallpox vaccine. After the animals had been used for this purpose they were returned to the dealer and resold by him to farmers.

Bovine animals inoculated with the mixed virus of cow-pox and foot-and-mouth disease develop symptoms of cow-pox alone, but when brought in contact with healthy animals the virus of foot-and-mouth disease may spread from the animals that show no symptoms. The calves in question were placed in pens in the Detroit stockyards, and from there distributed as shown in the diagram. Four days later a shipment of cattle was placed in the same yards, a portion of the shipment was reshipped to Buffalo, and from there a number of animals were sent to two towns in Pennsylvania in the neighborhood of which outbreaks of the

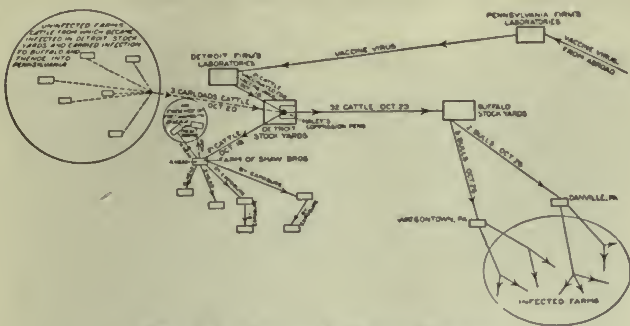

Fig. 54. Foot-and-Mouth Disease
The rapid spread of disease is made possible by modern commercial conditions

disease occurred as illustrated in the diagram. In ten days the disease had traveled hundreds of miles. The rapid and effective work of government officials saved the country at that time from the permanent introduction of the disease.

The outbreak of 1914–15 was of unknown origin. It was first noted in Michigan on October 15, 1914. Within approximately one month it had invaded nineteen States from Washington in the West to Massachusetts in the East. Some weeks later three additional States were invaded by the disease. This unparalleled rapidity of spread was due to the fact that the great stockyards of Chicago became infected early in the outbreak. The stream of animals into the stockyards is continued by a much smaller stream of animals leaving the yards to be sent to all parts of the country. No more ideal way of spreading an acute disease could be devised, and no more marked example of the rôle of modern commerce in the distribution of disease has ever been offered than the outbreak of the foot-and-mouth disease in 1914–15.

The number of animals slaughtered was 152,157 in 3,021 herds. The total loss occasioned by the outbreak amounted to $5,619,346. The greater part of this sum was paid to

Fig. 55. Foot-and-Mouth Disease
Sharply delimited eroded areas on the tongue form one of the characteristic lesions of the disease

farmers to recompense them for the animals slaughtered. This amount, large as it may seem, is not much to pay to insure the United States against the ravages of a disease that caused an estimated loss of $5,000,000 to the stock industry of England in one year, 1883.

Nature of the disease.—The causal organism is an ultramicroscopic one. In from three to six days after the animal is exposed to the infection, the disease makes its appearance. The onset of the trouble is marked by chills, followed by fever which may cause the temperature to rise as high as 106° F. In one or two days blisters or vesicles about the size of a hemp-seed or a pea are to be noted on the mucous

Fig. 56. Foot-and-Mouth Disease
Ulcers between the toes are also characteristic of this disease

membranes of the mouth, tongue, and gums. The vesicles are filled with a yellowish, watery liquid in which the causal organism is present. Similar eruptions appear on the feet between the digits and above the coronet. They may also appear on the udder and teats. The milking process ruptures the vesicles, and the organism finds its way into the milk, by which it spreads from one animal to another and from farm to farm when mixed cheese factory or creamery products are fed. The vesicles increase in size until they reach the diameter of a dime or even larger. They rupture soon after their appearance, leaving reddened sensitive spots or erosions behind. Food is refused, and a ropy saliva drools from the mouth. The soreness of the feet often renders it impossible for the animal to stand.

The disease spreads with great rapidity in the herd. The chance that all of the herd will acquire it has led to the inoculation of the animals by the transfer of some of the saliva from the diseased to the healthy animals, with the idea of shortening the period of trouble in the herd. In cases of doubt as to the nature of the disease, the inoculation of a calf should give definite information, since the inoculation should result in the characteristic vesicles in from twenty-four to seventy-two hours.

There are a number of diseases that somewhat resemble foot-and-mouth disease. Cowpox forms similar vesicles, but the inoculation does not result in symptoms of fever and eruption for at least ten days. In mycotic stomatitis, or inflammation of the membranes of the mouth, the entire mouth cavity is inflamed and vesicles are rare; if present, they do not increase in size. The thin skin between the toes may be inflamed, but the vesicles do not appear, nor is the udder affected. The disease does not spread and the inoculation of calves is not successful. In foot-rot the inflammation of the foot is general, and the mouth remains unaffected. In ergotism, or trouble due to the eating of too great quantities of smut, the mouth is not affected, and the tissue changes are to be noted at the tips of the ears, end of the tail, and upon the lower part of the legs.

Foot-and-mouth disease is transmitted to man by the use of infected milk. It causes eruptions in the mouth and on the fingers, but is seldom fatal, except in the case of weakened children. In man it is known as aphthous fever.

CHAPTER XXIII

RABIES AND ACTINOMYCOSIS

Rabies.—As has been shown, the losses from Texas fever are needless, since ways are known by which it can be completely eradicated. Rabies is a disease that likewise could be made to disappear, if simple procedures that could easily be carried out were enforced.

The disease is primarily one of the flesh-eating animals, but is transmissible to all mammals through the bite of a rabid animal. In reality it is transmitted almost wholly by dogs, since the dog is the only animal that is allowed to run about freely. It is found in most parts of the world; Australia and England are the only countries that are known to be free from it, and they are kept so through the rigid enforcement of wise quarantine regulations with reference to the importation of dogs. In the United States it has spread rapidly in the last few years, until at present it is found from the Atlantic to the Pacific. In 1908 it is known to have caused the death of 111 people. It is also of considerable economic importance because of the loss of domestic animals. The sanitary and economic aspects of the disease are small when compared with some others, but all loss is so unnecessary that it seems advisable to discuss the disease in some detail, especially since there are so many misconceptions concerning it.

The virus, of unknown nature, is known to be present in the saliva, the vitreous humor of the eye, lymph, milk, urine, and the peripheral nervous system. The presence of the virus in the saliva is the explanation of the transmission by the bite of an infected animal. Many times the saliva is so completely removed from the teeth of the rabid animal that none of the organisms is introduced into the wound. Especially is this likely to be true when the wound is inflicted through the clothing, through the coat of a long-haired dog, or through the wool of a sheep. This is one of the reasons why only a small proportion of the human beings and animals that are bitten by rabid animals develop the disease, even though no protective treatment is employed.

The virus is known to be present in the saliva from two to five days before the symptoms of the disease are evident. The wounds in which the infection is most likely to be of a serious nature are those inflicted on the head and face rather than on the extremities. The virus develops in the nerves, and is more likely to establish itself in tissues that are rich in nerves than in those deficient in these structures. The extent of the bites is also an important factor in determining whether infection is to occur, since the amount of virus introduced will be in proportion to the number of bites inflicted. The tissues seem to have the power of destroying a limited number of the organisms. Again, if the wounds are such that bleeding is marked, there will be a tendency for the organisms to be washed out. The deep puncture wounds are likely to be more serious than a tear in the flesh.

It is commonly believed that there is a seasonal distribution of rabies, that it is more common during the so-called dog days of late summer. There is little or no basis of fact for this belief. There is, however, more opportunity for the rabid dog to come in contact with human beings and with animals in the summer than in the colder months when there is less out-of-door life. The regulations that require the muzzling of dogs for a few weeks in the summer have no justification unless the period is extended to include the entire year.

The incubation period varies considerably in length, depending upon the location and extent of the bites. The symptoms are not apparent until the central nervous system is involved. The rapidity with which this will occur is dependent on the distance of the bite from the brain. The extent of the bite is also of importance in determining the rate at which the disease progresses. The average periods of incubation are as follows:

Man	40 days
Dog	21–48 days
Horses	28–56 days
Cats	14–28 days
Pigs	14–21 days
Sheep	21–28 days

The virus may remain dormant after its introduction into the tissues for a varying period of time, thus delaying the development of the symptoms for months and possibly years after the bite is inflicted.

Symptoms.—Rabies is generally divided into two forms, furious and dumb. In the first the animal is irritable and bites nearly every object with which it comes in contact; in the second the muscles of its jaws are paralyzed early in the attack, and, being unable to bite, the animal remains more quiet. The two forms of the disease represent the extremes, and all gradations are to be noted between them. The saliva from a case of dumb rabies is just as dangerous as that from a case of the furious type. The furious type passes into the dumb type as the disease progresses, owing to the paralysis of the muscles.

The furious type is marked by symptoms of nervousness. The animal may be more restless or more affectionate than usual, seeking to lick the hand or face. If an abrasion of the skin is present on the part licked, inoculation with the virus may result. More frequently the dog seeks to be alone, but it does not remain quiet; it often acts as if it were being annoyed by something, snapping and howling at some imaginary object. The animal often leaves home, and on its journeyings is likely to infect animals and human beings. It does not usually go out of its way to bite. There is ample opportunity for other dogs to be bitten by it, because of the canine custom of seeking to extend acquaintances.

Frothing at the mouth often occurs, and the voice is more of a howl than a bark, due to the affected muscles of the throat. The rabid animal has no fear of water, as is expressed by the common name of the disease, hydrophobia. Attempts to drink cause a paroxysm of the affected muscles of the throat. The animal can not swallow. The paralysis of the muscles gradually extends itself, and death finally brings relief. Much the same symptoms are noted in man.

In the dumb type the nervous symptoms are lacking and the paralysis appears very early in the course of the disease. It may easily be mistaken for choking. The furious form is the usual type noted in the case of man and in most animals other than the rabbit, which is used extensively in the detection and prevention of the disease.

Diagnosis.—It was stated that only about 10 per cent. of human beings bitten by known rabid animals develop the disease, owing to the non-introduction of the organism into the tissues, or to the destruction of the organisms by the tissues. Death from rabies presents a series of horrible symptoms, and if a person is severely bitten, the preventive treatment should be applied without delay. It is, however, highly advantageous to know for a certainty whether the dog that has inflicted a wound is actually rabid or not.

The quickest way to determine with certainty the nature of the trouble is to confine the dog and note the symptoms. The disease is invariably fatal, and death is commonly preceded by a definite sequence of symptoms, so that there can be no mistake in the diagnosis. The diagnosis should be made as quickly as possible, for if preventive treatment is to be applied, it must be administered without delay, since it is of no avail if not begun until the symptoms appear. If it is impossible to secure and confine the dog alive, the diagnosis can be made by a microscopic examination of the brain. The head should be removed, packed in ice and sawdust, and sent to the laboratory that is maintained by most States and large cities for such work. In killing the dog, care should be taken not to injure the brain or spinal cord.

Preventive treatment.—The treatment used to prevent the development of the disease in persons bitten by a rabid animal was one of the triumphs of Pasteur. The organism found in ordinary cases of rabies can be increased in virulence for rabbits by passing it through a series of animals. The so-called *fixed* virus will kill a rabbit in seven days, when introduced beneath the membranes of the brain. If the cord is removed from the animal immediately after death and allowed to dry over caustic potash, the virus becomes attenuated, the extent of the attenuation depending on the length of the drying process. The preventive treatment consists in giving hypodermic injections of a suspension of the dried cord. The first injections contain material that has been dried for about two weeks, while the subsequent injections are made with material that has been dried for a shorter period, until at last an injection of the fresh cord can be given without danger. Injections are made for a period of twenty-one days. Of all the persons known to have been bitten by rabid animals, to which the preventive treatment has been administered at the Pasteur Institute in Paris, but one-half of one per cent. have died of rabies, while the mortality records of those that did not receive the treatment are approximately ten per cent.

Wounds inflicted by a dog known to be rabid, or suspected of the disease, should be cauterized as soon as possible by the application of concentrated nitric acid, strong carbolic acid, or by a hot iron when the chemical agents are not available. This strenuous treatment will destroy the tissue about the wound, together with the virus that has been introduced by the animal. The quicker the cauterization is carried out, the more effective it will be. The cauterization, if it does not completely destroy the virus in the tissue, will prolong the period of incubation and thus give a better opportunity for a careful diagnosis to be made and for the preventive treatment to be applied in time to be successful.

The treatment was first applied to a human being by Pasteur in 1886. Between 1886 and 1917, 48,107 people were treated at the Pasteur Institute in Paris. From experience it is known that at least 10 per cent. of this number would have died from rabies if the protective inoculation had not been administered. Actually but 137 of this great number died from rabies. Pasteur has to his credit the saving in his own laboratory of 4,673 people from a most horrible death. Similar laboratories were soon established in all parts of the world. The total number of people saved from rabies reaches many thousands.

Eradication.—Since the disease is transmitted almost entirely by the dog, it could be prevented and eradicated by keeping all dogs that are allowed their freedom muzzled at all times. The effect of such measures is shown by the history of rabies in England. The following figures represent the number of reports of the disease:

1895	672 cases
1898	17 cases
1901	1 cases
1903–7	0 cases

This reduction was due to the enforcement of muzzling laws after 1896. Rabies was reintroduced into England in 1919 by aviators who were able to disregard the quarantine regulations. The muzzling regulations that have been passed by governing bodies in this country are rarely, if ever, enforced in an effective manner, because many people believe it cruel to muzzle a dog. It is certain that the disease could be eradicated in a short time if its transmission could be prevented. It is also certain that the muzzling of dogs for a short time would be much more humane than to have hundreds of them, as well as other animals and people, dying from rabies each year.

Actinomycosis.—Actinomycosis, or *lumpy jaw,* as it is more commonly called, is primarily a disease of cattle, although horses, sheep, hogs, and dogs may be affected. Man is also subject to the disease. The causal organism is usually classed as one of the higher bacteria. In the tissues the growth is often in starlike clusters. This appearance gave rise to the name actinomycosis. The term *Actinomycetes* is applied to the group of which the organism is a member. In this country the disease is not nearly so widespread as in some other sections of the world. It has been found in about one out of sixteen hundred animals killed in this country. Tuberculosis is many times more prevalent and more important in every way; and yet, in many places, because of the appearance of the disease on the surface of the body, actinomycosis has made more of an impression on the popular mind than has tuberculosis.

The disease is not one that spreads from animal to animal, and hence, like tetanus, can not be classed as a contagious disease. It is believed that the organism grows on certain plants, as barley, and is introduced into the tissues by the barley awns. It may also enter through a wound in the mouth or through a hollow tooth, and possibly may be inhaled. It is rarely fatal, and when death is produced,

Fig. 57. Actinomycosis
The jaw bone has become spongy and a portion of the teeth have been lost due to the ravages of the disease

it is due to mechanical causes, such as the interference with breathing or swallowing, or to the weakening of a blood-vessel by the constantly growing fungus.

Symptoms.—The first symptom when the disease is located in the throat, as is most common, is a slight swelling, which gradually increases in size and is hard and dense. It undergoes disintegration at the center, and may discharge a thick yellow pus. The opening may heal over, only to break out again. The opening by which the content of the abscess finds its way to the outside may be on the surface of the body or in the mouth or throat. The sore at the point of discharge may become very large and have the appearance of a head of cauliflower. The growth of the tumor may continue for years. The tongue may be involved, in which case the disease is often given the name *wooden tongue*. The organism may invade the bony part of the jaw, causing it to become spongy and enlarged. This permits the teeth to become loose, so that some of them may fall out. The internal organs may be invaded by the organism. In the lungs, nodules similar to the nodules found in tuberculosis of the lungs may be formed under the stimulus of the fungus. These vary in size from mere specks to that of a pea. The spleen, liver, and udder may contain actinomycotic nodules.

The organism occurs in masses in the pus discharged from the nodules. Because of the color of the organism, these masses of growth, which can be seen by the unaided eye, are often called *sulphur granules*.

Treatment.—The disease is one of the few of those due to the invasion of the tissues by a parasitic organism that responds to treatment with drugs, the most successful of which is potassium iodide given in water as a drench. The dose is from one to two and one-half drams a day. The administration of this amount of the drug can not be continued for any length of time without producing in the animal the effect known as iodism. This causes the eyes to run, the skin to become dry and rough, and a loss of appetite. When these evidences of the drug become apparent, its use should be discontinued for a few days, and resumed later. In the case of milch-cows, the milk should be discarded, as the drug is excreted through this channel The drug may also cause abortion. All animals do not react favorably to the drug. Where beneficial results are obtained, treatment should be successful in from three to six weeks.

Man does not acquire the disease from cattle, but he becomes infected in the same manner as cattle, *viz.*, through wounds in the mouth. The meat of affected animals can be used as food, if the disease is localized.

CHAPTER XXIV

GLANDERS AND TETANUS

The most important transmissible disease affecting the horse and closely related animals is glanders, or farcy, as it is often called. The disease affects primarily horses, mules, and asses, but dogs and cats may acquire it by feeding on the carcasses of glandered animals. The disease is also transmissible to man, and usually results fatally.

Distribution.—Glanders is found in nearly all parts of the world; Australia and New Zealand are the only large areas of any importance free from it. Great numbers of horses have been congregated from varied sources for war purposes, and have been transported to other lands, thus spreading the disease. It is asserted that glanders was introduced into Mexico at the time of the Mexican war by the American cavalry. During and after the Civil War, the distribution was very rapid in this country, owing to the sale of horses and mules by the government.

At the present time glanders is most prevalent where large numbers of horses are brought together, as in lumber camps, on the ranges, and in the great stables maintained in cities. Constant change is going on in such stables, and every horse purchased may serve to introduce the disease, unless precautions are taken to determine the health of the animal before it is allowed to come in contact with healthy animals. The number of horses purchased by farmers is comparatively small, and, unless the farmer buys range animals, or those that have been in use by the large stables, little risk of acquiring glanders is encountered. Public stables and public watering-troughs are undoubtedly agents in the spread of the trouble. It is considered a wise pre-

Fig. 58. Glanders

Running sores on a swollen leg are often noted with this disease

After Reynolds.

caution not to make use of public watering-troughs, but to employ a pail.

Symptoms.—In some respects the disease reminds one of tuberculosis in that an animal may have it for a long time, and yet remain in good flesh and be able to stand a considerable amount of work. In other words, many glandered horses have an economic value, and yet are a constant source of danger to other animals with which they come in contact. It is thus considered wise that all glandered animals be destroyed, and that the owner be compensated by the State for the protection of the industry in general. It is through the purchase of such chronic cases that the disease may be introduced on the farm.

The disease primarily affects the membranes lining the

Fig. 59. Glanders

Healed sores on the nose may be present in cases of chronic glanders

After Reynolds.

nasal passages, and one of the most characteristic symptoms is the discharge of a sticky fluid, sometimes streaked with blood, from one or both nostrils. Small nodules may form on the upper part of the nasal septum. The nodules, which are translucent and grayish in color, may break and form ulcers, which destroy the surrounding tissue to a greater or less extent, and may even cause a perforation of the nasal septum. Similar nodules may be found in the lungs, and less often in the liver and kidneys.

In glanders of the skin, or farcy, nodules are found in the skin and the underlying tissues. These nodules are usually called farcy buds. They vary in size from a hemp-seed to that of an egg. These nodules break and form running sores on the surface of the body, the discharge being yellow and sticky. The sores thus formed often heal and leave marked scars on the head and legs, in which places they are most common.

The acute form of the disease is common in the mule and ass, but is rare in the horse. Death often takes place in from two to four weeks, although the disease may become chronic and the animal live for a number of years. Treatment is of little avail. Great precaution should be exercised in the care of glandered animals, since if any of the infectious material is introduced into the eyes or nose, or comes in contact with a wound, infection of the human being is likely to occur. The manifestations of glanders in man are quite similar to those noted in the case of the horse.

Detection.—Glanders is often easily recognized by the characteristic lesions in the nasal passages or by the farcy buds. When the disease can not be recognized by physical examination, recourse must be had to some other method of diagnosis. The most common method is to apply the mallein test, which is very similar to the tuberculin test in the nature and manner of application. Mallein is prepared by growing the glanders organism in glycerin broth. The culture is then killed by heating, and the dead cells removed by filtration. The mallein is injected beneath the skin, and a series of temperature readings is made both before and after the application of the mallein.

A few hours after the introduction of the mallein there appears at the point of injection a swelling which, in the glandered horse, is hot and painful and continues to increase in size for from twenty-four to thirty-six hours. The swelling persists for several days, disappearing in from eight to ten days. At the time the swelling is most prominent, the diseased animal appears dull, breathes rapidly, and has a poor appetite. In the case of a healthy horse, the swelling is small and disappears in twenty-four hours, and no signs of illness are to be noted following the injection of the mallein. The constitutional reaction in the diseased animal is accompanied by an increase in the temperature ranging from two to two and five tenths degrees. The increase begins about eight hours after the injection and reaches the maximum in from ten to fifteen hours. The fever persists for from twenty-four to forty-eight hours, instead of only a few hours, as in the tuberculin test. In the healthy horse there is no appreciable rise in temperature.

The test is not so accurate a method of diagnosing glanders as is tuberculin for tuberculosis, for some glandered horses do not react to the test; but a positive reaction is looked upon as proof of the diseased condition of the animal. Other tests are also employed, in which the blood is examined for certain of the anti-bodies that will be produced under the stimulus of the glanders bacillus. These methods can be carried out only in the laboratory.

The farmer must seek to protect himself by the purchase of animals from known healthy sources, and by care in preventing his animals from coming in contact with infectious material in public places. The organism does not form spores and hence is not especially resistant.

Tetanus.—Tetanus, or lockjaw, is an example of a toxemia. It is also an example of a disease caused by a microorganism that is not transmitted from one animal to another under natural conditions. *B. tetani* is widely distributed in soil, and is found in the large intestine of horses and cattle. Heavily manured soils and those with a high content of organic matter seem to contain the bacilli in greater numbers than soils lower in humus. The bacillus is an anaërobe that forms very resistant spores. The toxin it produces is one of the most poisonous substances known, when it is introduced into the tissues of an animal. A man weighing one hundred and seventy-five pounds will be killed by 0.23 of a milligram. Toxins have been made of such potency that 0.00,000,002 gram is fatal to a white mouse. An organism capable of producing such a substance does not need to grow extensively in the body of an animal in order to cause injury.

The tetanus bacillus is usually introduced into the tissues through a puncture wound by some object that carries the infective material into the deeper lying tissues. Puncture wounds made by rusty, dirty nails are most dangerous. Horses, especially, become infected in this manner. Puncture wounds bleed but little, and therefore the foreign matter is not likely to be washed out by the blood; nor is it easy to cleanse the wound by washing, as may be done with a more superficial abrasion. The infection may occur in such operations as docking, castration, and through the umbilical cord of foals. The more frequent occurrence of the disease in horses, as compared to other domestic animals, is apparently due to the greater susceptibility of the horse to the toxin. It is estimated that the horse is twelve times as sensitive as the mouse and 360,000 times as sensitive as the fowl to this toxin.

In man a large proportion of the disease is due to the wounds produced by Fourth of July accidents. The filling of many forms of fireworks is earth, which may contain the extremely resistant spores of the tetanus bacillus. Some portion of the filling may be blown into the skin by the premature discharge of a firecracker or some other form of fireworks.

Symptoms.—The organism grows only at the point at which it was introduced into the tissues, and only to a small extent even there. In fact, so little evidence of its growth is shown by the tissues that it is sometimes impossible to determine the point of infection. The organism produces its powerful toxin, which is absorbed and which has a specific action on the nerves, resulting in muscular contractions in various parts of the body. The tetanic spasms usually begin in the muscles of the head and neck, extending from these parts to the muscles of the throat, trunk, and extremities. In the head, the muscles of mastication are first attacked, giving rise to the disease commonly known as lockjaw. In the horse, the muscles of the tail may be the first to show the spasmodic contractions.

The duration of the disease in the horse may be a few days, or it may continue for several weeks. In cattle the disease is usually less rapid, but rarely runs longer than two weeks. Tetanus is usually fatal in sheep, and about 75 per cent. of the horses affected die. In man the disease manifests itself in much the same manner as in the lower animals. It was a very common disease in the early months of the Great War. In certain portions of the Western front, the soil apparently contained many tetanus bacilli. The contamination of wounds with the soil presented ample opportunity for the tetanus organism to enter. Later in the struggle, one of the first treatments each wounded man received was a dose of tetanus antitoxin.

Preventive measures.—A preventive and, to some extent, a curative treatment has been developed in the tetanus antitoxin. This antitoxin is comparable to the preventive serum used in hog cholera, and the diphtheria antitoxin used so widely in human medicine.

In the preparation of the antitoxin, it is necessary to force a susceptible animal, like the horse, to produce in its blood a quantity of the protective substances, so that the blood can be drawn and the serum obtained. In producing the serum, the animal is hyper-immunized by the addition of repeated doses of the toxin or poison produced by the organism, beginning with very minute doses and gradually increasing them. This treatment with constantly increasing doses of the toxin is continued until the body of the horse has produced a large quantity of the substance that will neutralize the toxin. Fortunately, the body can produce an amount of the protective substances in excess of that which is necessary to render harmless the toxin introduced.

If some of the blood of the hyper-immunized animal is carried to an animal that is just beginning to show symptoms of tetanus, the antitoxin will be ready to neutralize the poison as it is formed by the growth of the organisms. It will tide the body of the diseased animal over the period of danger, and give it time to protect itself by the manufacture of its own antitoxin.

The transfer of the protective substances is accomplished by drawing a small portion of the blood, allowing it to clot, and using the clear serum, which formerly represented the commercial product. Ways have now been found by which the protective substances can be concentrated by chemical means, a distinct advantage, since it avoids the introduction of large quantities of liquid into the animal to be protected.

The protective serum is expensive, and hence is used only on valuable animals. Its widest use is in the prevention of the disease in man. The immunity thus produced is passive and persists for only a short time.

CHAPTER XXV

HOG CHOLERA

THE most important communicable disease of hogs is known as hog cholera, supposed to have been introduced from Europe in breeding animals. The first outbreak in this country of which record exists is that which occurred in Ohio in 1833. Since that time the disease has spread to all parts of the country. In the corn-growing States the losses occasioned by it are enormous. In the interval from 1894 to 1912 only eleven of the 92 counties of Indiana lost less than 5 per cent. yearly of the annual hog crop, 38 lost between 5 and 10 per cent., 30 between 10 and 15 per cent., 12 between 15 and 20 per cent., and one county more than 20 per cent. It is estimated that 85 per cent. of the losses incurred in the hog industry are due to this disease. From these figures it is apparent that hog cholera places an enormous tax on the swine industry of the country.

This disease, like foot-and-mouth disease, is one that presents high and low tides. A widespread outbreak occurred in 1886-7, another in 1894 and in the years thereafter, and still another began about 1911 and continued for several years. Its gradual spread from south to north is shown in the following figures, which present the percentage of the annual hog crop lost through cholera:

	1912	1913
Iowa	16	25.5
Minnesota	5.5	21.4
Nebraska	11	17.5
South Dakota	3.8	23.0
North Dakota	2	7.5

The disease is due to an ultramicroscopic organism that gains entrance to the body by way of the digestive tract or through the broken skin. The causal organism is eliminated from the body in the feces and urine. All breeds of hogs are susceptible to the disease. It has been claimed by some that the mule-footed hogs would not acquire it, but experience has shown this statement to have no basis of fact.

Symptoms.—The disease may appear as a typical blood-poisoning or septicemia, as an intestinal infection, as a lung trouble, or in any combination of the three. It was formerly supposed that there was more than one disease that affected hogs; but, as methods of prevention have been devised, it has been found that all respond to the same treatment and hence must be caused by the same organism. The symptoms vary with the different manifestations of the disease.

The first hogs to die in any outbreak do so after having shown signs of illness a short time. It will usually be observed that the sick hogs fail to eat, are affected with chills, and huddle together in the pens to keep warm. They stand with back arched and with the hind feet close together or crossed. They show stiffness of the muscles and joints, and may stagger and fall from weakness. The skin of ears, nose, abdomen, and that inside the thighs may be reddened. The early stages are marked by constipation, followed by a profuse diarrhea in which the feces have an offensive odor. If the lungs are affected, a hacking cough is noted and an increased respiration. The eyelids are often stuck together by a purulent discharge. The temperature is increased, reaching from 104° to 109° F.

If the attack is of longer duration, as in the chronic form, there is more marked evidence of digestive disturbances. Animals with chronic cholera become emaciated, the hair may drop out, and even portions of the skin may die and slough off. As a rule, they do not become profitable feeding animals even after recovery.

It is sometimes difficult or impossible to determine from the symptoms alone whether hog cholera is present in a

Fig. 60. Hog Cholera

Hemorrhages on and in the kidney are one of the most characteristic lesions

herd. A careful post-mortem examination of the dead animals is necessary in order to make a conclusive diagnosis. This examination should be made preferably on the carcass of a sick animal that has been killed, or on one that has just died. If the examination is delayed for a number of hours, it is likely to be of little service in making a diagnosis because of post-mortem changes.

Lesions.—The extent of the changes in the organs will depend on the length of the attack. If the animal died of acute hog cholera, the lesions will not, as a rule, be so marked as in the more chronic form. The color of the skin should be noted. Red or purplish blotches are significant. The abdominal and lung cavities should be carefully opened and the following organs examined. The kidneys in the acute cases are likely to be darker than nor-

Fig. 61. Hog Cholera
Button ulcers on the intestinal wall are frequently noted in cases of chronic cholera

mal, and to show small, red spots which impart to the organ a "turkey-egg" appearance. The spleen or milt is usually enlarged, dark, and soft; the liver is normal in appearance; and the membranes of the abdominal cavity, the stomach, and the small intestines may show red areas, as if blood had been spattered on them. It will be found impossible to remove the blood by washing, showing it to be in the tissues rather than on them. The hemorrhages are to be found in many different parts of the body, and may vary in size from the pinpoint spots noted in the kidneys to areas of considerable size. The lungs may or may not be affected. If they are, the hemorrhages are present and portions of the lung tissue may be consolidated instead of being soft and filled with air. The surface of the heart may show the red blotches. In the acute cases the inner lining of the large intestine is frequently found to be blood-stained, and the feces may be bloody. In the more chronic cases the most characteristic lesions of the disease are found in the large intestine, the so-called *button ulcers,* which are round, hard, and yellowish with a dark center. They are distinctly raised above the surrounding healthy surface of the intestine. In size they vary from a small point to the size of a twenty-five cent piece. The finding of such ulcers is to be considered as a positive indication of hog cholera, and it is the only lesion that can be regarded as absolutely diagnostic.

The lymph-glands in various parts of the carcass are found upon section to be enlarged and reddened.

A number of causes may produce symptoms that may be mistaken for cholera. Pneumonia due to exposure, dust, or lung-worms is sometimes confounded with cholera. Improper feeding may cause intestinal disturbances. Slops containing alkalies, such as soap powders, are often a source of trouble to garbage-fed hogs.

Prevention.—Since but little can be done to cure the disease after it has made its appearance in the individual animal, the farmer must direct his efforts to the prevention of the disease. It should be remembered that the organism is eliminated from the body of the affected animal in the urine and feces, and that it is present in all the tissues of the body. An animal that has recovered from the disease may still harbor the organisms in its body and eliminate them. With these facts in mind, the farmer can outline his plan for the protection of his herd. No animal should be purchased from a herd in which cholera has been present during the previous year, nor from a herd that has been subjected to the preventive treatment in which the virus has been employed within six weeks. Animals purchased should be kept in quarantine for four weeks and then allowed to come in contact with a small number of the herd. If these exposed animals remain healthy after two or three weeks' exposure, it is safe to place the purchased animals with the herd. It is not essential that a rigid quarantine be established in the case of the purchased animals, for the prevention of intimate contact will usually suffice.

The method of keeping hogs in separate houses rather than in one large house has many advantages, one being that if cholera breaks out in one of the yards, it can often be prevented from spreading to the remaining sections of the herd.

Hogs that have been shown at fairs are likely to be exposed to infection, not only at the place of exhibition but also during shipment. Care should be taken in allowing such animals to mingle with the herd, until time has shown the animals to be free from infection.

The virus of hog cholera can be carried on objects from one farm to another. It is probable that this is one of the chief ways in which the disease progresses in any locality into which it has been introduced. The farmer should remember that any object transferred from an infected farm to his own may serve to carry the infection. The visiting of the hog-yards in which an outbreak has made its appearance, the transfer of tools or wagons, or of animals such as dogs or cows, are ways in which the disease spreads. It seems probable that, if the farmer takes care of those factors that he can control, he will have little trouble with those he can not control.

The herd should be kept in as healthy a condition as possible by providing clean, well ventilated pens, clean feeding-troughs, and proper feed, since anything that tends to weaken the animal makes it more likely to acquire hog cholera in case the organisms are taken into the body. It is generally believed that the feeding of new corn produces the disease. The feeding of large quantities of new corn may produce digestive troubles and make the animal more susceptible to cholera, but it can not cause hog cholera.

If cholera makes its appearance in the herd, all the healthy animals should be removed at once to another field. One can not rely on the appearance of the animals to tell whether they are infected or not. A much more reliable way is to take the temperature of each animal, and to retain all showing any fever in the yard with the diseased animals. The normal temperature of grown animals ranges from 101° to 103° F. In young animals the temperature will run somewhat higher, but in separating the herd all animals having a temperature of more than 103.5° should be considered as infected.

The carcasses of hogs that have died should be burned if possible. If this can not be done, they should be buried deeply. All infected litter should be burned. The careless disposal of carcasses is one of the chief ways of spreading and perpetuating the disease.

The hogs should be pastured in fields that do not border on the road and that are not traversed by streams, since infection may be introduced in either of these ways.

The most effective way of protecting the herd against the disease is to apply the preventive treatment described later. Many cures for hog cholera have been proposed and are widely advertised. It is certain, however, that no treatment other than the administering of the protective serum is of any value.

Protective treatment.—A hog that has recovered from a natural attack acquires an immunity to the disease, due to the presence in the blood of protective substances that have been formed under the stimulus of the disease-producing organism. The amount of protective bodies that are thus produced as a result of an attack of the disease is not sufficiently great so that the blood can be drawn and introduced into the body of another animal for the purpose of imparting immunity. If, however, such an immune animal is injected with large quantities of the blood of a hog that is ill with the disease, the stimulus imparted by the introduction of the virus will cause the animal to form additional protective bodies, so that it will be practicable to draw the blood and use it in protecting other animals.

The hogs thus treated are said to be hyper-immunized. The protective serum is secured by bleeding the immunized hog. This is done by cutting off the tip of the tail. About five or six cubic centimeters of blood to one pound of body weight is drawn. This process is repeated three times, at weekly intervals. The animal is then given another injection of the virulent blood, is then bled twice from the tail, and after the usual interval is bled from the throat. The blood is beaten with a wire as soon as drawn from the animal to remove the fibrin and prevent clotting. One half of one per cent. of carbolic acid is added as a preservative.

Before the serum is used in the field it is necessary to determine its protective power. This is done by injecting varying amounts into susceptible pigs that are inoculated at the same time with some virulent blood. In this manner it can be determined how much must be used in actual work to protect an animal. It will be seen that the preparation of the serum is expensive because of the large number of hogs that must be used and the labor involved. Many of the States have established laboratories for the preparation of the serum.

By the use of the serum alone a passive immunity is produced that will protect the animal from a serious infection for from six to ten weeks. If a small quantity of virulent blood is introduced into the animal at the same time the serum is injected, active immunity will be produced, which will generally protect the animal for life. The introduction of the virus at the same time as the anti-serum may result occasionally in a fatal case of cholera. In order to avoid this risk, the protective serum may be injected, and about a week or ten days later the virus may be given, together with a second dose of the anti-serum. The first method is known as the *serum-alone* method, the second as the *simultaneous* method, and the last as the *double* or *combination* method. Each has its advantages and disadvantages which must be considered in determining which to apply. The serum-alone is safe, but protects for only a short time, unless the animals come in contact with infectious material soon after treatment, in which case the results are substantially the same as those obtained in the simultaneous treatment. The method is of small value in the protection of breeding animals. It does allow the farmer to protect his herd for a short time when the danger of infection is great.

In the simultaneous method, some of the treated animals may die from cholera, because not sufficient serum was used to protect against the virus administered. The animals in which acute cholera is thus produced may serve as centers of infection from which the disease may spread to other herds. This danger has led many to advise against the use of the simultaneous method. In herds in which the disease already exists, only the serum should be used. The combination method avoids the danger of the simultaneous treatment, since rarely are any animals lost by cholera due to the treatment. It is more expensive, since serum must be given twice.

Breeding herds should be protected by the use of the combination method, even if cholera is not present in the vicinity, because it enables the breeder to send, without danger, breeding hogs into infected districts and to fairs.

Application of the serum.—The serum may be applied by the farmer himself, but if the virus is to be used, as is the case in the simultaneous or combination methods, a veterinarian should be employed, since the virus is dangerous material and, if handled by those who do not appreciate its nature, trouble may result. The animals should receive a light laxative diet for a day or so before being treated, and should be kept in clean, dry quarters.

Small hogs are usually injected in the arm-pit. The animal may be held on its back between two round fence-posts joined together by cleats. Larger animals may be snubbed to a post by a rope around the upper jaw, and the serum injected in the fold of loose skin at the side of the neck. The needle of the hypodermic syringe should be thrust deep into the tissue, not simply through the skin as when tuberculin is applied. If the infection of the animal with organisms that will cause inflammation and abscesses is to be avoided, it is necessary to see that the syringe is sterilized before use, by placing it in cold water and bringing it to the boiling-point. If the syringe has leather washers on the plunger, its sterilization must be accomplished by the use of chemical disinfectants, since boiling would destroy the leather. The skin at the point where the injection is to be made should be scrubbed with a stiff brush, warm water, and soap; then rinsed with some water that has been boiled and allowed to cool. The skin is then treated with a 4 per cent. solution of carbolic acid or tincture of iodine. Care should be exercised to keep everything clean during the process.

The doses of serum are as follows:

Weight of animal	When virus is used	No virus
0–20 lbs.	15 cc.	10 cc.
20–50 lbs.	25 cc.	20 cc.
50–75 lbs.	35 cc.	25 cc.
75–100 lbs.	40 cc.	30 cc.
100–150 lbs.	50 cc.	35 cc.
150–200 lbs.	55 cc.	40 cc.
200–300 lbs.	65 cc.	45 cc.
300–400 lbs.	85 cc.	65 cc.
400–600 lbs.	100 cc.	85 cc.

The larger quantity of serum is used with the virus in order to protect the animal against the virus, which by itself would cause death. The virus is usually applied at some other point than the serum, as beneath the skin at the center of the space between the fore legs when the serum is applied in the arm-pit.

For a few days after the serum is administered the feed should be reduced to about one half the normal amount, gradually increasing until at the fourth week the full feed may be given. When only the serum is given, there should be little or no reaction. With the double or the simultaneous treatment in six to ten days after the injection, the reaction fever sets in and the temperature may rise to 106° F. The animals may lose appetite, have chills, and present the symptoms of a mild case of hog cholera. The more susceptible animals may die from the effects of the virus. The hogs that show symptoms may eliminate the virus, and be the starting-point of an outbreak of cholera in case they come in contact with susceptible animals.

The results that have been obtained with the serum have been such as to recommend its use. When applied in herds in which the disease had already made its appearance, more than 80 per cent. of the animals were saved, while the treatment applied before the infection of the herd took place has protected more than 90 per cent. of the animals against infection.

There seems to be little doubt but that any farmer or breeder can protect his herd against loss from cholera by the consistent and careful use of the protective serum and the virus of the disease. It is a matter of some expense, and the farmer must weigh the cost of the insurance against the probable loss from cholera before deciding whether or not to apply the treatment.

CHAPTER XXVI

DISEASES OF FOWLS

The transmissible diseases of fowls inflict a heavy tax on the poultry-raiser and the general farmer. Present knowledge concerning many of these diseases is far from complete, and in many cases so fragmentary that no definite plan for the eradication and prevention can be devised other than the customary plan applicable in most cases of transmissible diseases, *viz.*, removal of affected individuals, destruction of carcasses, and general cleanliness and disinfection.

Chicken cholera.—Chickens, like swine, are subject to dietary disorders which may often simulate a true contagious disease in the rapidity with which it appears in the flock and in its high mortality. Cholera is a term applied to many of such disorders that are not produced by a specific organism. The true chicken cholera is rare in this country, and is due to the invasion of the body by a specific form of bacteria.

Symptoms.—The urates, that part of the excrement excreted by the kidneys, in the case of healthy birds are pure white in color. In birds affected with cholera the urates are yellow, often a bright yellow, and sometimes a bright green. This change in color is not proof of the presence of cholera, but is a valuable indication of the disease. Diarrhea is usually present. The sick bird leaves the flock, becomes weak and drowsy, acts dumpish, and the feathers are roughened. Intense thirst is noted, the appetite is poor, and the crop remains distended with food. There is a rapid loss of flesh. The disease makes rapid progress in the flock, because of the short period of incubation, from one to three days. Most of the affected birds die in a short time of an acute form of the disease; others may have a chronic type; recovery is rare.

On post-mortem examination the digestive organs will be found to be inflamed, and the liver is usually enlarged and softened. The presence of cholera can, however, be established only by a bacteriological examination of the blood, which will be found to contain great numbers of the causal organisms. The disease is a true septicemia. The organism enters the body by the ingestion of contaminated food or water, which may become contaminated with the excrement of the affected birds or the material that drops from the beak. The extensive lesions in the intestine allow the excrement to become mixed with manure.

The disease may be introduced into the flock by the purchase of a bird with a chronic form of the disease, or by doves and wild birds that fly from farm to farm.

Prevention.—Nothing can be done for the birds that are infected. All efforts must be concentrated in preventing the spread of the disease. It should be remembered that every drop of blood contains great numbers of the causal organisms, and that if any portion of the carcass is consumed by well birds, they are certain to become infected. It is advisable to kill the birds that show any symptoms of disease. This should be done in such a way that no blood is drawn. The dead fowls should be promptly disposed of; the feed and water troughs should be thoroughly disinfected, as also the roosting houses. If possible, the still healthy birds should be removed to fresh, uncontaminated grounds. The causal organism does not produce spores and will not persist long outside the body of the bird. It is considered safe to bring new stock on the place after the expiration of two weeks, provided the house and other contaminated objects have been thoroughly disinfected.

Fowl typhoid.—This disease is often mistaken for chicken cholera. It is, however, produced by a different organism. The disease is less rapid in its progress in the individual bird than is cholera. The diarrhea so characteristic of cholera is absent, and the intestines are pale instead of deep red, as in cholera; the contents are normal in consistency, while in cholera the intestinal contents are liquid and blood-stained. The blood is free from the organisms. It is not especially important that a correct diagnosis be made as to which of these diseases is present in the flock, since identical methods of prevention should be employed with either. Cleanliness should be the chief reliance of the poultryman against these diseases.

Roup.—Roup, or diphtheria of fowls, is considered the most important transmissible disease affecting the barnyard fowl of this country. It occurs in turkeys, ducks, pigeons, and pheasants, as well as chickens. The cause of roup has not been discovered, and it is not certain whether chickenpox and canker are different diseases from roup or different manifestations of the same disease.

It has sometimes been considered that this disease has some relation to diphtheria in man. There is no reason for such belief other than that in certain forms of roup there may be formed a membrane similar to the membrane noted in diphtheria. The first symptom of roup is a watery discharge from the nostrils and often from the eyes. The bird becomes dumpish; the breathing is often noisy, due to the obstruction of the air-passages by the exudate. The fowl may be able to breathe only by opening the beak. Sneezing is frequent. The eyes may be covered with a dry discharge, or they may be forced from the sockets by the accumulation of cheesy matter in the sockets. There may be found in the mouth and throat patches of grayish-yellow exudate or membranes. Death is often occasioned through suffocation due to the closing of the throat by the membrane. The swelling of the head caused by the accumulation of the exudate in the various cavities of the head has given rise to the term *swell-head.*

It is considered that potassium permanganate is helpful

Fig. 62. Roup
The eye is swollen and filled with exudate as is the mouth

both as a preventive and for the treatment of affected birds. It may be added to the drinking water in sufficient quantities to impart a pink color to the water. The heads of the affected birds may be dipped in a 1 or 2 per cent. solution of the same substance.

Roup is to be differentiated from simple catarrh, which closely resembles the human trouble known as cold-in-the-head. Simple catarrh is caused by exposure to dampness, cold winds, and by improper ventilation of the houses. It has been shown experimentally that it is impossible to produce roup in these ways. There seems to be no doubt, however, that birds suffering from catarrh are susceptible to roup.

Roup may be carried from flock to flock by the transfer of birds with a mild form of disease. Fowls should not be purchased from infected flocks, and it is well to place in quarantine for some days new birds or those that have been at shows before placing them with the flock. Any bird showing any discharge from the mouth or eyes should be removed at once from the flock.

White diarrhea.—Of the diseases affecting young chicks, white diarrhea is the most important. It is probable that more than one trouble has been classed under this name. The white diarrhea of young chicks, caused by *B. pullorum*, is the most important. This disease offers an example of hereditary transmission of disease. It has been shown that the ovaries of the hen may be affected, and that the ova contain the organism. The young chick becomes infected from the yolk sac. Some of the females that survive continue to harbor the germ and become bacillus-carriers. The adult females may become infected by contact with other infected adults or by infected litter. They may then become bacillus-carriers. The infection is in all probability acquired through the mouth.

The economic importance of the disease is occasioned by its effect on young chicks. The greatest danger of infection is during the first forty-eight hours. The danger of infection is very slight after four days.

The affected chicks appear stupid and remain under the hover or hen much of the time. The feathers become rough, and the wings droop. There is constant loss of weight. The chicks eat little and appear unable to pick up their food. A whitish discharge from the vent soon makes its appearance. The discharge may be creamy or sometimes mixed with brown, and it is more or less sticky or glairy. In many cases it clings so closely to the down as to close up the vent. Many of the chicks peep constantly or utter a shrill cry, apparently of pain, when attempting to void the excrement. The abdomen is enlarged and protrudes to the rear. The post-mortem examination shows no marked lesions. The organs are all pale; the alimentary tract is usually empty except for some slimy fluid.

The prevention of the disease must rest on the non-introduction of bacillus-carriers in the purchase of breeding stock, and by the purchase of eggs and young chicks from flocks that are known to be free from the disease. The widespread infection of breeding birds is shown by the fact that, in a flock in which the losses of the young chicks had been excessive, more than 80 per cent. of the laying hens were shown to have diseased ovaries. The chicks that recover from the infection do not, as a rule, grow as rapidly as do non-infected birds.

It has been shown conclusively that the feeding of sour milk to young chicks is of value in preventing the spread of the disease. The dishes in which the milk is kept should be cleaned daily and a fresh supply of milk provided.

The incubators and brooders should be thoroughly disinfected after each hatch, and extreme cleanliness should be practised in all regards in the handling of young chicks.

CHAPTER XXVII

BACTERIAL DISEASES OF PLANTS

The most important transmissible diseases of animals are those caused by bacteria rather than by the other groups of microörganisms such as molds and yeasts, while in the plant kingdom the reverse is true. The molds are by nature better fitted to penetrate into the tissues of the plant than are the bacteria.

Just as the increased commerce in animals has hastened and accentuated the spread of the transmissible diseases of animals, the increased sale of seeds and plants of all kinds and their shipment from one part of the country to another has led to the rapid spread of both bacterial and fungus plant diseases. At the present time about forty bacterial diseases of plants have been described. A few are widespread and are certain to come to the notice of every one engaged in farming, and are of great economic importance.

The bacterial diseases of plants may be divided into four classes, depending on the manner in which they affect the plant: the blights, the rots, the wilts, and the galls. In the first the tissue is killed by the organism, but it is not decomposed as in the rots, in which the tissue is not only killed but decomposed; while in the wilts the passage of water to some portion of the plant is interfered with, and hence death of the affected tissues soon ensues.

The complicated questions that arise in connection with the immunity against bacterial diseases of animals do not occur in the bacterial diseases of plants. There is a difference in the susceptibility of different plants to the same organism. Efforts are being made to increase and extend this natural immunity. Much more can be done in an experimental way in the breeding of resistant varieties of plants than can be done with animals.

Pear blight.—The most important of the blights is that which affects the pear and apple, and to a lesser extent the quince, apricot, and plum. It was first observed in 1780 in the Hudson River valley, and as orcharding has spread

Fig. 63. Pear Blight

Normal fruit is shown on the right and diseased fruit on the left. In the center one of the pears is healthy, the other is affected

westward, the disease has developed, until it is now found in all parts of this country and Canada. In Colorado it found such favorable conditions that it has caused the abandonment of commercial pear-growing. It has also caused great losses in California. As far as our present knowledge goes, the blight is of American origin and is confined to North America.

The disease is readily recognized by the fact that the young twigs appear to have been injured by fire. This condition has given rise to the common name of *fire blight.* The leaves of the affected parts turn brown or black, and cling to the diseased twigs long after the other leaves have fallen. The twigs show a blackened and shriveled bark. The bacteria enter the tissues through the blossoms, being carried from flower to flower by bees and other insects. The immature fruit shows the disease by turning dark and gradually drying up. From the flowers the germs find their way into the cambium, or growing layer immediately below the bark. The diseased condition develops backward in the twig at the rate of an inch or more a day. The blackening of the bark does not occur as fast as the infection spreads, so that infected tissue is always found several inches in advance of any outward signs. As the season progresses the tissue becomes harder and less favorable for the growth of the organism, and by the middle of the summer the progress of the disease has ceased.

The infection may also occur through wounds in older tissue. It often reaches the large limbs and even the trunk, in which case it is known as body blight. The bacteria pass the winter in the blighted parts. These hold-over bacteria become active with the increased sap flow in the spring, and soon spread to the healthy bark, where they multiply so rapidly that at the time the blossoms open the bacterial growth oozes from the cracks in the diseased bark. Insects attracted to this material become contaminated and thus carry the organisms to the blossoms. The bacteria multiply rapidly in the nectar of the flowers, and thus infection is carried from the old wood to the new. It is not rare to see a tree with almost every new twig showing symptoms of the disease through its blackened leaves.

Since the bacteria are protected by the bark, nothing can be applied to the tree that will destroy the organisms; hence this trouble does not lend itself to such treatments as are found effective in combating fungous diseases, in which the causal organism is found on the surface of the plant. The only known method of control is by the removal of all diseased branches as the symptoms appear. The cut should be made twelve to fifteen inches below the last visible sign of the disease. The diseased wood should be burned. Care should be exercised not to spread the infection through the pruning-knife or saw.

Cabbage rot.—Of the class of bacterial diseases known as the rots, the black rot of the cabbage and related plants is most common and important. The first symptom is the appearance of yellow or brown areas near the margin of the leaf. The organism enters the tissues through the water pores on the edge of the leaf, and spreads along the ribs of the leaf, ultimately reaching the main stem of the plant in many cases. It causes the death and softening of the tissues. Invasion by other forms readily occurs in the broken-down tissue, with the result that the entire head ultimately is destroyed. The affected ribs are blackened, and on cutting across the infected stem one can see the blackened ends of the fibrous strands (the fibro-vascular bundles).

The organisms may also enter the plant through wounds on the roots, such as are made when the young plants are transplanted. It has been shown that the organisms may be on the seed, and thus the soil of the seed-bed infected with the organisms, which await a favorable opportunity to penetrate the plant.

Preventive measures must consist of disinfection of the seed and rotation of crops.

Rots caused by other bacteria occur in carrots, sugar-beets, muskmelons, and hyacinths.

Wilts.—The wilts of the cucumber, muskmelon, squash, and pumpkin are widespread in the eastern half of the United States. The disease is characterized by a wilting of the vine, without any visible external cause. The leaves and runners wilt suddenly, as if from a lack of water or from too hot a sun. The whole plant may wilt, or only one runner. The disease is caused by an organism, the growth of which fills the water-ducts with a viscid material that prevents the rise of the water, and wilting follows. If the cut ends of a vine are rubbed together gently and drawn apart slowly, the viscid matter will string out for some distance.

The infection is supposed to occur through wounds inflicted by insects, such as the striped cucumber-beetle and the common squash-bug.

A similar wilt affects the egg-plant, tomato, Irish potato, and tobacco.

Galls or tumors.—Another class of plant diseases is marked by the formation of galls and tumors. Crown gall is the most important, and is peculiar by reason of the great number of plants that are susceptible to the attacks of the organism. The apple, peach, plum, prune, apricot, cherry, grape, raspberry, blackberry, rose, English walnut, chestnut, almond, white poplar, hop, sugar-beet, potato, tomato, tobacco and Paris daisy are susceptible. The greatest economic losses are in connection with fruit trees and shrubs. It has also attracted attention because some of the manifestations of the disease are very similar to cancer in human beings. The presence of the causal organism stimulates the surrounding plant tissue to continued and persistent growth, which results in the formation of excrescences or tumors of varying size.

CHAPTER XXVIII

DISINFECTION

In the discussion of various diseases, it has been shown that there are a number of ways by which the causal organisms are eliminated from the body of the diseased animal. For each disease the manner of elimination is more or less characteristic. In some it is true that the organisms may be discharged in a number of ways, as in the case of tuberculosis, in which the organisms are to be found in the sputum, the feces, the milk when the udder is involved, and in the discharge from the genital passages when the reproductive organs are affected. In the case of Texas fever the causal organism is able to leave the body only as the blood is drawn, and under natural conditions the transmission of the organism from animal to animal is due entirely to the bite of a specific insect, one of the cattle ticks.

It has also been shown that the organisms vary greatly in their resistance to environment. Those that produce spores are, as a rule, resistant to all agencies, and persist for long periods outside the body in the dormant form. The non-spore-forming organisms differ greatly in resistance, some resisting certain agencies almost as long as the spore-bearing organisms, while others are so sensitive that the disease produced by them can be transmitted from animal to animal only by the most intimate contact. It is fortunate that none of the important transmissible diseases in man is due to spore-bearing organisms, and but two of the diseases affecting animals, *viz.*, anthrax and blackleg. If it were otherwise, the difficulties in combating the transmissible diseases would be greatly increased.

The control of infectious diseases rests on the prevention of the passage of the causal organism from diseased to healthy animals. This is accomplished in part by the isolation of diseased animals, thus preventing contact with non-infected animals. The federal and State quarantines, and those established by other agencies, seek to prevent the spread of disease in this way. Another phase in the prevention of disease is the destruction of the organisms in the material discharged from the body of the affected animal by the use of some physical or chemical agent before any healthy animal has opportunity to come in contact with the infectious material. This method is being used with the greatest success in the prevention of human diseases. It is evident that, before it can be applied, definite knowledge must be obtained of the nature and resistant powers of the organism, and the ways in which it is eliminated from the body; otherwise all efforts are likely to be unsuccessful, for, to secure effective results, every organism must be destroyed.

In the case of typhoid fever it is easy to treat all of the infectious discharges of the patient so as to prevent the spread of the disease. Indeed, the *concurrent* disinfection or the immediate treatment of all infectious material as soon as it leaves the body of the patient is so successful that practically all of the transmissible diseases of man are now treated in the same wards in some of the great hospitals of the world, without the various diseases spreading from one patient to another. The former plan was to pay little attention to the treatment of the discharges, but to attempt to destroy the organisms in the room and on the objects with which the patient had been in contact after death or recovery had taken place. This is called *terminal* disinfection, and represents that which must be employed in the control of animal diseases, together with isolation of the affected animals. In other words, the farmer will find it necessary to destroy the disease-producing organisms in the stables in which diseased animals have been quartered before the stables are used for healthy stock.

Natural agencies.—The two agencies of nature that destroy many disease-producing organisms are drying and light. The disease-producing organisms vary widely in their resistance to desiccation. Most of the non-spore-producing types endure drying for only a short time, and it is certain that this is one of nature's most effective ways of destroying and limiting the spread of harmful organisms.

The direct rays of the sun exert a powerful germicidal effect, and are able within a comparatively short time to destroy not only the vegetative cells of the organisms but many of the spores. The action of light as a purifying agent is, however, often overestimated. It is effective only when the direct rays of the sun strike the unprotected organism. The action of diffuse daylight is so small as to have no practical importance, and when the organisms are covered with a layer of dust, or are embedded in manure or other material, the action of sunlight is of no importance.

It is certain that ample provision should be made for light in our houses and stables, but not with the idea that disease-producing organisms shall be destroyed, but rather to render ourselves and our domestic animals more resistant in case they are brought in contact with infectious material.

Heat is another physical agent that can be used in the destruction of organisms. It is applied either in the form of dry heat, or as steam or hot water, depending on the material to be treated. Here again the resistant powers of the organism must be considered in determining the exposure that must be used to be effective. All small objects of wood or iron, and all clothing, can be most easily rendered harmless by boiling. In the control of the transmissible disease of human beings, this process is of the greatest importance. The use of dry heat is limited, except when material is to be destroyed by burning, a method that should be widely used in the disposal of carcasses of animals and litter.

Chemical disinfectants.—A large number of chemicals have an injurious action on microörganisms. It is customary to divide them into two classes, which differ in the intensity of action. Those that have a relatively weak action and tend to prevent the growth of organisms rather than to destroy them are termed *antiseptics,* or *preservatives* when used in foods. Those that have a more pronounced action and destroy the organisms are called *disinfectants.* It will be apparent that when a disinfectant is present in small amounts, it will have an antiseptic action, since cessation of growth will always precede death of the organism; but certain of the antiseptics can not exert a disinfecting action, even if used in concentrated form. For example, boric acid is but slightly soluble in water, and in a saturated solution exerts an antiseptic action; but, owing to its limited solubility, it can never be classed as a disinfectant. Some of the disinfectants have the power of overcoming offensive odors by combining with specific substances, and are often called *deodorants.* Some deodorants have little or no disinfecting action, and of course are of little or no value, since they do not act on the source of the trouble. Again, some chemicals used as disinfectants have an injurious action on insects, such as lice on animals.

In the use of these agents the farmer should first have in mind what he desires to accomplish, and then choose the agent that is most likely to be effective as a disinfectant, a deodorant, or an insecticide. He must also have information as to the resisting power of the organisms he is attempting to kill, and as to the effectiveness of the disinfecting agent, otherwise his work is likely to be of no avail. For example, the use of carbolic acid as a protection against hog cholera is of little or no value, since it is known that the organism will live for months in the presence of one half of one per cent. of this substance which is so effective in the destruction of many disease-producing organisms.

Each class of disinfectants has its advantages and disadvantages. A particular class can be used to advantage under certain conditions, while, under other conditions, its use may be of little value.

Lime.—In the manufacture of lime, the limestone, which is a carbonate of lime, is heated to drive off the carbon-dioxide, forming calcium oxide, which on exposure to the air gradually combines with the carbon-dioxide in the air to form the carbonate again, or *air-slaked* lime. If the quick or stone lime, as it is often called, is treated with six parts of water to ten of lime, a dry white powder will be obtained, called hydrate of lime or water-slaked lime. The water-slaked lime resembles air-slaked lime in appearance, but not in composition, as can be determined by placing a little of each on the tongue. The air-slaked lime has a chalky feel and taste, while the water-slaked causes the tongue to burn. It has caustic and disinfecting properties, while the air-slaked has no value whatever as a disinfectant.

Lime is one of the best disinfectants that can be used on the farm for many purposes. The dry powder, produced when a proper amount of water is added to the lime, can be used or it can be applied to the walls and ceilings in the form of whitewash. The whitewash has a germicidal action, as well as a mechanical incrusting effect, thus placing the organisms under such conditions that they can not exist for any length of time. It makes the stables lighter and cleaner than would otherwise be the case. If the whitewash is prepared from good lime and properly applied, it is probably as effective a disinfecting agent as can be used to advantage in ordinary stable disinfection. It can be made more effective by the addition of carbolic acid or chloride of lime.

In all disinfecting processes it is essential to use plenty of the agent. The cheapness of lime is thus an advantage, as is the ease with which it can be procured. It is also to be recommended when carcasses of animals that have died from transmissible diseases must be buried instead of being burned. In such cases the carcass should be well covered with lime, and then the dirt returned to the excavation.

Carbolic acid.—Carbolic acid is prepared from coal-tar and is sold in the form of white crystals which melt below the boiling-point of water, and which remain liquid on the addition of 5 per cent. of water to the melted acid. It is used as a disinfectant in solutions containing from 1 to 5 per cent. of the pure acid. Its action is not greatly retarded by the presence of organic matter, as is the case with so many other disinfectants; hence it can be used for the disinfection of feces from typhoid patients, and the treatment of sputum of tubercular people.

Carbolic acid, or phenol as it is often called, does not find wide use on the farm, because of its expense, its corrosive action on the skin, and its poisonous properties.

Coal-tar disinfectants.—After the carbolic acid has been removed from coal-tar, the residue still contains substances that have a disinfecting action. From this residue there is now prepared a great number of disinfectants commonly known as *coal-tar* or *cresol* disinfectants, a term that appears in the name of many of the proprietary compounds, such as kresol, kreso, kresolig. The value of these com pounds varies widely. Some are about equal to carbolic acid, while others are ten times as effective. The great majority are from two to three times as effective as phenol and are used in solutions containing from 1 to 3 per cent. of the agent. They are less corrosive and less poisonous than phenol, and can be used as a dip for the destruction of insects on animals. They are usually composed of the creosote oil and soap, and when mixed with water form a milky emulsion that is very permanent. They can be employed in widely varying concentrations, and their action is, as a rule, not greatly impaired by the presence of organic matter. Only soft or rain water should be used to dilute the coal-tar disinfectants, because the salts present in hard water may materially reduce the disinfecting action. They are undoubtedly the best class of disinfectants for common use on the farm, especially in the treatment of animals.

Formaldehyde.—This important disinfectant is sold in the form of a 40 per cent. solution of the gas dissolved in water, and is usually called *formalin.* Its widest use on the farm is in the disinfection of closed spaces by setting free the gas from the liquid, as will be described later, and for the destruction of smut on seed grains and scab on seed potatoes.

Corrosive sublimate.—This compound, frequently known as *bichloride of mercury,* is one of the strongest disinfectants known. Its disadvantages are its poisonous properties and its greatly decreased action in the presence of organic matter such as manure. It also has a corrosive action on metals, and is irritating to the tissues. It can be used to advantage in many places as a wash or as a spray on walls. For this purpose it is used in a one to one thousand solution, or one ounce of the salt to eight gallons of water.

Sulphur.—When sulphur is burned, irritating fumes are formed. When moisture is present in the air, sulphurous acid is formed which possesses a considerable disinfecting action. It is used in the place of formaldehyde as a gaseous disinfectant for such closed spaces as refrigerators and other rooms in which the bleaching action of the sulphur will be of no importance. It can not, as a rule, be used in furnished rooms because of this property, which formaldehyde does not possess.

Calcium hypochlorite.—*Bleaching powder* or *calcium hypochlorite* has long been used as a disinfectant and deodorant. Under certain conditions it is one of the most effective that can be employed, as for example in the treatment of water and sewage. Many cities draw their water supplies from sources that may become contaminated with typhoid bacilli. It has been found that the addition of minute quantities of bleaching powder is sufficient to destroy the typhoid organism.

Calcium hypochlorite finds its widest use as a deodorant for use in cellars, privies, and similar places. For these purposes the dry powder is usually employed.

It can be used for the treatment of cisterns in which a large amount of organic matter has been carried by the wash from the roofs. During the warm weather the decomposition of the organic matter may be so marked as to impart a disagreeable odor to the water, which can be overcome by the addition of a solution of bleaching powder. The amount to be added will depend on the quantity of organic matter in the water. It should be added in small quantities, as an excess will impart the characteristic odor of the hypochlorite to the water.

Ferrous sulphate and copper sulphate.—These substances, commonly known as *green* and *blue vitriol,* have been widely used as disinfectants in the past.

It is now known that they are almost worthless and should be discarded in favor of some one of the efficient disinfectants.

Disinfection.—The choice of a disinfectant for any particular purpose will depend on the conditions under which it is to be used. If the room to be treated is so constructed that it can be made tight enough to retain the gas for some hours, the use of formalin or sulphur is to be recommended. The gas penetrates to every portion of the room, into cracks and crevices into which it is difficult to force a liquid. Gas will not easily penetrate layers of clothing and bedding, so that the treatment of a living-room or bedroom with a gaseous disinfectant will often not accomplish what many conceive it will do. For the treatment of surfaces to which a liquid can not be applied it is of the greatest value.

The room to be treated should be made as tight as possible by pasting paper over the window and door cracks. The gas can be liberated from the liquid by the use of permanganate of potash. One pound of formalin and one half pound of permanganate will be needed for each thousand cubic feet to be treated. The permanganate is placed in a large pail and the formalin poured over it. A violent chemical action results, and a portion of the formaldehyde is set free. The room should be warm and the air moist to obtain the best results. The room should be opened for twenty-four hours. The gas has no harmful action on objects except those of delicate leather.

If sulphur is used, five pounds must be employed for each thousand cubic feet. The sulphur should be placed in an iron vessel, which should be set in a pan of water so that the heat will evaporate a portion of the water, for it is essential to have a considerable amount of moisture in the air if the sulphur is to prove effective. The powdered sulphur is ignited by making a depression in the center of the pile and adding a small amount of kerosene, which is ignited. The room should remain closed for twenty-four hours.

Stable disinfection.—In the treatment of stables and other places in which there is likely to be a large amount of material, such as manure that contains the harmful organism, the first step in the disinfecting process should be the thorough cleaning of the stable walls and floor, in order to remove as much as possible of the infectious material and allow the disinfectant to come in contact with the bare surface of the walls. Dried manure is not easily penetrated by the liquids, and the organic matter is likely to combine with the disinfectant used and thus reduce its action. The thorough cleaning will remove most of the organisms. All loose woodwork such as box mangers, etc., should be removed. The walls should be moistened with a solution of corrosive sublimate so as to prevent dust in the subsequent cleaning. The walls and floors should be scraped clean, and the material removed and all litter burned, not thrown into the yard, where animals may have access to it.

The disinfectant should be applied with a spray-pump that will enable one to reach all parts and to force it into the cracks. If whitewash is used, it should be strained and made thin enough not to clog the pump. The mangers should be well scrubbed with a solution of lye and then with water. A half-hearted job of disinfection is no better than none at all. It gives a fancied but no real security against a recurrence of the disease.

The disinfection of yards is something that can not be done under ordinary conditions. If a small yard is infected, a liberal sprinkling with dry, water-slaked lime is the best that can be accomplished. The disinfection of fields is impossible. Small areas may be limed or burned over, but neither of these methods is likely to be effective in the case of spore-bearing organisms, and all other forms will soon die. One must rely on natural agencies for the destruction of pathogenic organisms that have been brought in contact with the soil of yards or fields. It should be remembered that the pathogenic organisms do not find conditions for growth in the soil, and hence their destruction is only a question of time, and with all except the spore-forming bacteria the time will be relatively short. Burning over a pasture may be resorted to in order to get rid of vegetable growth and give a better chance for sunlight to exert its influence.

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