Textbook of MICROBIOLOGY

---

by Kenneth L. Burdon, Ph.B., Sc.M., Ph.D.

PROFESSOR OF BACTERIOLOGY AND IMMUNOLOGY, BAYLOR UNIVERSITY COLLEGE OF MEDICINE, HOUSTON, TEXAS; CONSULTANT IN BACTERIOLOGY, METHODIST HOSPITAL, HOUSTON.

Third Edition, Revised

The Macmillan Company: New York

1947

Set up and electrotyped. Published, April, 1932.
Second Edition completely revised and reset, August, 1939.
Third Edition completely revised and reset, June, 1947.

PRINTED IN THE UNITED STATES OF AMERICA

PREFACE TO THE THIRD EDITION

In this thoroughly revised edition there has been a rearrangement of some of the chapters, and much of the text itself has been rewritten; otherwise, the general plan of the book remains the same.

As in the previous editions the student is introduced in the very early chapters to *all* the principal types of microorganisms—protozoa, fungi, spirochetes, rickettsiae, and filtrable viruses, as well as the ordinary bacteria—but the *detailed* description of microbes other than the bacteria is deferred to later chapters. Discussions of the special properties of fungi, and of the viruses, for example, are now placed just before consideration of the diseases with which each is concerned. This rearrangement is in conformity with the suggestion made by a number of friendly critics of the second edition. The methods of laboratory study, previously outlined in a separate section, or in an appendix, have been incorporated into the general body of the text. It is believed that these and other changes in the order of the subject matter will be welcomed. Instructors may now assign chapters for study in regular sequence, from first to last.

In order to incorporate the more significant new knowledge, many topics have been given somewhat fuller treatment than in earlier editions. Even so, the text has been kept as brief as it seemed possible to make it, while still giving something more than a simple listing of things, and something beyond a mere superficial and uncritical presentation. The purpose has been, as before, to write a clear, balanced account, which is comprehensive and scientifically sound, yet easily understandable, and of real usefulness to student nurses and others who are interested in the medical and public-health aspects of microbiology, and who approach this study for the first time.

Among the new features of this edition is the inclusion of more references to current bacteriological literature. Here limitations of space forbid the listing of more than a fraction of the original papers consulted in preparation of the book, and force the writer to make painful decisions in omitting many significant contributions. Those

references that are included have been chosen primarily for subject matter and teaching value, rather than for authorship. Full titles are given, and it is hoped that the mere reading of the titles will open some doors in the minds of the students.

Several new illustrations have been added which show the newer techniques, and give a glimpse of the fascinating revelations of the electron microscope. For generous assistance in securing many of these, acknowledgment is given to the several persons mentioned in the accompanying legends. Other new illustrations have been prepared by our medical artist, Miss Ella Mae Shackelford, and by our photographer, Mr. Carlyle G. Breckenridge, to whom I am indebted for most helpful cooperation.

I wish to thank the many friends, teachers and students who at one time or another have made constructive suggestions or criticisms during the preparation of this edition. My associates of the Baylor University College of Medicine, Dr. Preston E. Harrison, Dr. Edwin A. Johnson, Dr. Wilton M. Fisher, and Dr. James A. Greene, Dean of the Clinical Faculty, have helpfully read various portions of the manuscript. It is a particular pleasure to express my deep appreciation to Dr. Walter H. Moursund, Dean of the College of Medicine, for valuable specific criticisms, and for constant support throughout the work.

KENNETH L. BURDON

Houston, Texas

CONTENTS

PART ONE

Elements of Microbiology

PART TWO

Methods of Destroying Microorganisms and of Controlling the Spread of Communicable Diseases—Sources and Modes of Infection

PART ONE

ELEMENTS OF MICROBIOLOGY

CHAPTER I

SCOPE AND CHARACTER OF MICROBIOLOGY

The microbic world. The first thing to learn, in beginning the study of microbiology, is the existence of what may be called the *world of microbes.* We must realize that the familiar plants and animals are not the only living forms on the earth, but, on the contrary, there are all about us everywhere great numbers of very tiny living creatures, too small to be seen by the naked eye. We are ordinarily quite unconscious of their presence; if we wish to see them, it is necessary to use a microscope. They are appropriately called *microorganisms* or *microbes* (from the Greek *micros,* meaning small, and *bios,* meaning life), and may be defined as *living forms of microscopic or submicroscopic size.*

Microorganisms. Conspicuous among the microbes are those belonging to the group called **bacteria.** These are simple microscopic plants. The word *bacteria* comes from a Greek word meaning "a rod." Some, but not all, of the bacteria have the shape of little rods, or sticks. *Bacteria* is a plural word and is used in a collective sense to refer to the whole group. When we wish to speak of a single one of these microscopic organisms, we use the singular, *bacterium.*

The bacteria of simplest structure are called the **true bacteria.** Those of more complex make-up, resembling the common molds in some respects, are spoken of as the **mold-like higher bacteria.** In another group are included certain peculiar, flexible, spiral-shaped microbes, that seem to be more animal-like than plant-like, yet have basic properties in common with the true bacteria. These we call the **spirochetes.**

These various forms of the bacteria are so numerous and so widely distributed, and their activities are of such great practical importance, that they are often given the major share of attention in academic courses dealing with the microbes, and commonly the

entire study is called *bacteriology*. We are concerned not only with the bacteria, however, but with *all* the types of microbes. Hence, the title adopted for this book—*microbiology*—is a more accurate name for our study.

Besides the bacteria, there are four other important groups of microorganisms. These are: (1) the **protozoa,** (2) the **true fungi,** (3) the **rickettsiae,** and (4) the **filtrable viruses.**

The *protozoa* are relatively large and complex, animal-like organisms, the lowest forms of life classed in the animal kingdom. The special study of these forms is the science of *protozoology*.

The *true fungi* include yeasts, molds, and other microscopic plant-like forms of more complex nature than the true bacteria. The intimate study of the fungi is called *mycology*.

The *rickettsiae* are peculiar microbes smaller than most bacteria, but still visible under the microscope. They are so closely adapted to life within the tissues of their human and animal hosts that they cannot be cultivated in the laboratory on artificial, nonliving, food mixtures.

The *filtrable viruses* are the smallest of all the disease-producing agents. They are invisible by ordinary methods of microscopic examination (i.e., they are ultramicroscopic) and, like the rickettsiae, they refuse to grow in the laboratory on lifeless materials.

It is helpful to have in mind, at the beginning of our study, the seven groups of microorganisms we have mentioned: (1) *protozoa,* (2) *true fungi,* (3) *mold-like higher bacteria,* (4) *spirochetes,* (5) *true bacteria,* (6) *rickettsiae,* and (7) *filtrable viruses.*

The bacteria and other microorganisms are often called *germs.* It is permissible to employ this term in a popular sense when referring to *disease-producing* organisms, but it should not be used to include all varieties of microbes, because it implies that they are all harmful, and this is untrue. The fact is that *the great majority of microorganisms are entirely harmless* and have no connection whatever with disease. Many are very useful to man.

Scope of microbiology. Microbiology is the science which treats of the nature and activities of microorganisms.

Since the microbes are *living,* it follows that we are studying one branch of the great science of *Biology,* which includes all studies dealing with the nature of living matter and the character and functions of living things. The relation of microbiology to other biological sciences is indicated in Table I, p. 6.

So vast is now the accumulated knowledge about the microbes and their activities that no one microbiologist can claim to be intimately familiar with all aspects of the subject, nor could the whole of microbiology be taught in a single course, or described in a single textbook. As the outline shows, the science of bacteriology alone may be divided into several branches, or subsciences, each dealing with an important phase of bacterial activity. Similarly, protozoology and mycology might be subdivided into subsidiary branches of study.

In this book we are concerned largely with an introduction to the *medical* aspects of microbiology. The subject matter of medical microbiology includes not merely a description of the disease germs themselves, but also a study of the way in which these organisms cause disease, how we "catch" disease, how the germs injure our bodies, and how they pass from one person to another. Also, the defense the body puts up against the germs and the factors which explain resistance to disease are studied, and so important and complex is this special phase of the subject that it is given a name of its own—*immunology*. The study of medical microbiology and immunology furnishes the basic knowledge on which depend the practical methods used for the laboratory diagnosis and prevention of germ diseases. It therefore gives us a sound foundation for the intelligent promotion of both the individual and the public health.

Practical nature of microbiology. It will be evident from this brief outline that microbiology is a thoroughly practical subject. It includes a great deal more than a mere description of strange microscopic organisms. Of course, study of the form, structure, and mode of life of microorganisms must be included in order to understand them, but the subject is concerned principally with the things microbes *do,* and with the practical effects of their activities on human affairs. Many of the most familiar natural changes going on about us continually, such as the souring of milk, the spoiling of food, and the decomposition of the dead bodies of plants and animals, are brought about by bacteria or fungi. These microbes have a direct influence upon the fertility of the soil. They play an important part in various industrial processes, such as the manufacture of leather. Thus the dairyman, the agriculturalist, and the manufacturer are vitally interested in certain kinds of microbes, and for good practical reasons.

Table I. Relation of Microbiology to Other Biological Sciences—Branches of Bacteriology

- *Biology* (Study of living things)
  - *Zoology* (Study of animal life)
    - *Protozoology* (Study of protozoa,—microscopic animal-like forms)
  - *Microbiology* (Study of microscopic forms of life)
    - *Bacteriology* (Study of bacteria and related organisms, including the spirochetes and rickettsiae, and the filtrable viruses*)
      - *General Bacteriology* (Study of fundamental properties of bacteria)
      - *Agricultural Bacteriology* (Study of bacteria in relation to soil fertility and plant diseases)
      - *Food and Dairy Bacteriology* (Study of bacteria in relation to the preparation and preservation of food)
      - *Industrial Bacteriology* (Study of bacteria in relation to industrial processes)
      - *Medical Bacteriology* (Study of bacteria in relation to disease in animals and human beings). Includes study of:
        - (1) Pathogenic bacteria
        - (2) General factors influencing the start and course of germ diseases
        - (3) Resistance (or immunity) to disease (*Immunology*)
        - (4) Application of bacteriology and immunology to diagnosis, prevention, and specific treatment of disease—the basis for *public health work*
    - *Mycology* (Study of microscopic plants other than bacteria—yeasts, molds, and other fungi)
  - *Botany* (Study of plant life)

* Sometimes the study of viruses is regarded as a separate activity and is called *Virology*.

For the nurse, doctor, dentist, public-health worker, sanitary engineer, and others interested in medical matters, some knowledge of microbiology is essential because so many diseases are caused by germs. The nurse finds in microbiology the explanation of the rules of nursing technique which are taught in the hospital. Many procedures which form a part of routine nursing work are designed to destroy, exclude, or avoid germs, and only by understanding the nature of microbes can such techniques be carried out intelligently. When the student has acquired, through her study of microbiology, an acute consciousness of the almost universal presence of living microbes, she can realize that many apparently unnecessary details of nursing methods are in reality of the highest importance. They are designed to avoid or destroy invisible (and possibly dangerous) microbes, and are necessary for the protection of the nurse herself, as well as of the patient.

When the nurse is called upon to care for a patient with a germ disease, it is helpful to know something of the nature of the organisms which are responsible for the illness. And since most germ diseases are *communicable,* i.e., are capable of being transmitted more or less readily to other persons, the nurse has the grave responsibility of preventing their spread. She must know how the germs enter the body, and particularly how they leave the body and pass from person to person, and how to destroy them in the discharges from the patient. Otherwise she may carry the disease to other patients or contract it herself.

Nurses are responsible for the technique by which germs are kept out of wounds during operations and in the dressing room. Obviously, this exceedingly important part of nursing work could not be carried out safely by a person who does not have a clear understanding of how to avoid and destroy microorganisms.

Frequently the nurse is asked to help in collecting specimens of pus, sputum, etc., or to make smears or cultures from the patient, for purposes of laboratory diagnosis. The proper methods may be learned in microbiology.

The modern methods of preventing and treating germ diseases with vaccines and serums, the value of personal hygiene and sanitation in preventing disease, and other practical information may be obtained in the study of microbiology. This basic knowledge will enable the nurse to teach others how to avoid illness, and in this way she may make an important contribution to the public health.

REVIEW QUESTIONS—CHAPTER I

1. Define *microorganism, microbe, bacteria, bacterium, germ.*
2. Define *microbiology.* Why is this a better title for our study than *bacteriology*?
3. Name and characterize briefly the seven principal groups of microorganisms.
4. Define *biology, protozoology, mycology.*
5. Name five branches of bacteriology. Could protozoology and mycology be divided similarly into subsciences?
6. State briefly the nature of the subjects studied in general, agricultural, food-and-dairy, and industrial bacteriology.
7. What are the four topics included in medical bacteriology? Define *immunology.*
8. Explain why microbiology is a practical subject.
9. Give some reasons why a knowledge of microbiology is useful to a nurse.

CHAPTER II

EARLY HISTORY OF MICROBIOLOGY

Microbiology a comparatively new science. The study we now call microbiology had its beginnings only about sixty years ago. It would not be accurate to say that it was established at any particular date, but the year 1880 may be remembered as the time when the truly modern science was firmly founded. This means that microbiology is a field of comparatively recent development. Many persons still living can remember when germs were little known and their true importance was hardly suspected.

A great deal is still unknown. Our information about microbes is far from complete, but additions to our knowledge are being made continually, as the result of investigations going on all over the world. Especially rapid is the advance now being made in our information about filtrable viruses and rickettsiae—organisms that were little understood just a few years ago. Microbiology and Immunology are developing rapidly, and the student must expect to learn frequently of new discoveries and new ideas concerning germs and germ diseases.

Periods of development. Microbiology may be said to have had three periods of development: (1) *the period prior to 1865,* during which there was a slow accumulation of some facts about bacteria and other microbes and a great deal of more or less correct speculation about them, (2) *the period between 1865 and 1882,* when the foundations of the new science were securely laid, especially through the pioneer work of the French scientist Louis Pasteur, the German physician Robert Koch, and the English surgeon Joseph Lister, and (3) *the modern period from 1882 to the present day,* during which there has been a very rapid development of information about microbes and a truly revolutionary application of this knowledge to human affairs, especially in medical and public-health work.

THE BEGINNINGS OF MICROBIOLOGY
KNOWLEDGE OF MICROORGANISMS BEFORE 1865

Discovery of microbes. Antony van Leeuwenhoek (1632–1723). The existence of bacteria and other microorganisms was discovered almost two hundred years before anything approaching a science of microbiology was evolved. This discovery was made by Antony van Leeuwenhoek (pronounced "Lay-wen-hōōk"), a most remarkable man and a unique figure in the history of science (Frontispiece).

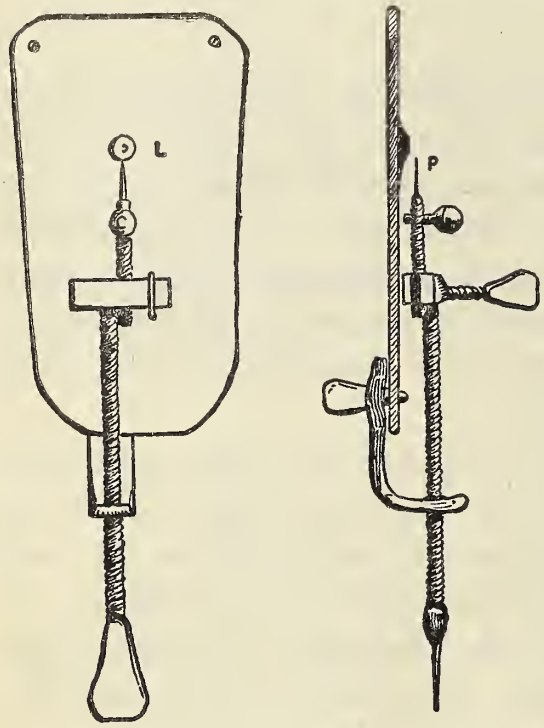


FIG. 1. Front and side view of one of Leeuwenhoek's microscopes. The single magnifying lens was fastened between two thin metal plates at L, and the object to be examined was mounted upon the adjustable pin at P. The object was viewed through the lens by holding the microscope very close to the eye.

Leeuwenhoek, a citizen of Delft, Holland, was not a man of great learning, but he was very ingenious. He became expert in the grinding of simple magnifying lenses. He made these lenses of very small bits of glass, polished them carefully, and mounted each separately between two brass, copper, silver, or gold plates, to which he fastened an adjustable holder for the object to be examined (Fig. 1). He constructed many of these "microscopes," each containing a single lens ground by himself. The best of his lenses magnified about 200 times and they were better than any of the magnifying devices which had been constructed up to his time. With these truly remarkable instruments, he diligently and patiently examined a great variety of natural objects, and described them in a series of letters to the Royal Society of London.

In 1674 he first saw "animalcules," which we now recognize as protozoa and bacteria, in rain water and in pepper infusions (he was trying to find out what made the pepper hot). Later he found them in the human mouth and in excreta. In his letter of September 17,

1683, he supplements his quaint descriptions of the "animalcules" in saliva with drawings which depict clearly the same types of bacteria we know today.

Early studies which helped to develop microbiology. By 1700, the existence of microorganisms was probably widely known, but the crude microscopes of that day did not permit much to be learned about them. There were no satisfactory methods for cultivating microbes artificially, or for separating one kind from another. Consequently, for nearly two hundred years following Leeuwenhoek's time, definite knowledge about them developed very slowly indeed, until the work of Pasteur and his immediate followers, beginning about 1860, led directly to the founding of the modern science. In the meantime, however, there were many who studied microbes, and these early studies helped to lay the foundation for modern microbiology.

There were four questions which interested the early workers. These were: (1) the classification of microorganisms and their relation to the larger and more familiar forms of life, (2) the origin of microbes and the truth or falsity of the theory of the spontaneous generation of living things, (3) the relation of microorganisms to fermentation and putrefaction, and (4) the relation of microbes to disease. Important steps in the development of knowledge of these topics up to 1865 are described in the following paragraphs.

Early classifications of bacteria. The first important attempt to classify bacteria was made by Ehrenberg in 1836. He distinguished between spherical, rod-shaped, and spiral-shaped bacteria, and was the first to use the terms "bacterium," "spirillum," and "spirochaeta" with reference to bacteria. These terms are used today, though not with quite the same meaning.

Ehrenberg and other workers of that time thought the bacteria belonged to the animal kingdom, as Leeuwenhoek himself had believed. It was not until about 1850 that several investigators placed them where they properly belong—in the plant kingdom. About this time, Naegeli pointed out that bacteria are colorless plants and are clearly different from green plants. He gave the scientific name of *Schizomycetes* to the class of microorganisms we commonly call bacteria. This term means "fission fungi" and is appropriate because bacteria multiply by a process called simple fission—the splitting of one organism into two.

The question of spontaneous generation and the origin of

microbes. One important question about bacteria and the other microbes was debated for more than a century. The question was, *What is the origin of microorganisms? Are they generated spontaneously from the materials on which they are found to be growing, or do they develop, like other kinds of living things, from preëxisting organisms of the same kind?* This question had to be answered before a science of bacteriology could be developed.

The debate centered about the origin of the bacteria which appeared in decomposing liquids, such as meat or vegetable infusions. The early investigators noticed that when a perfectly clear infusion was allowed to stand for a time in a warm place it became cloudy, and when the cloudy fluid was examined with the microscope, it was found to be teeming with living microorganisms. Nearly everyone believed that the microbes developed spontaneously from the liquid itself, that is, the living microorganisms were thought to be generated from the nonliving infusion. This belief was a natural one for that day, because the idea of the *spontaneous generation of life* was a very old one, and with respect to microscopic forms of life, at least, it had never been convincingly disproved. Aristotle and others of the ancient Greek writers taught that living forms develop from nonliving matter, and the belief was general throughout the Middle Ages. In the sixteenth century, the statement that mice could be created by placing some old linen and corn in a cupboard was believed in all seriousness. Later the absurdity of such an idea became apparent, and by the late seventeenth century the better-informed persons had ceased to believe that the larger and more familiar plants and animals could spring into being from lifeless matter. But when microbes became well known there were many who were ready to believe that these tiny living things must be spontaneously generated.

Experimental studies on spontaneous generation. In 1749, Needham described some experiments which were intended to prove the spontaneous generation of bacteria. He placed vegetable infusions in flasks, which he sealed. He then heated the infusions by immersing the flasks in boiling water for a short time. Despite this treatment, the fluid in most of his flasks became cloudy and filled with microorganisms in a few days. Needham thought he had killed all microbes originally in the infusions by the heating, and therefore believed that the growth which appeared in the fluid later must have been spontaneously generated.

But Spallanzani, in 1769, showed the error of this conclusion. He repeated Needham's experiments, but boiled his infusions for a longer period than Needham had done, and sealed the flasks in the flame. We now realize that, by the prolonged and vigorous boiling of his infusions, Spallanzani killed some resistant forms of microbes which had survived the relatively slight exposure to heat given them by Needham. These boiled infusions remained clear and contained no living organisms, so long as the flasks were completely sealed from the air. Spallanzani, therefore, really proved that spontaneous generation of microbes does not occur.

Appert, in 1810, demonstrated that if food were placed in proper containers and subjected to heat, and if then the container were hermetically sealed, the food would remain free from living microbes and would keep without spoiling. The success of this process of canning food was actually a proof that living things come only from living ancestors.

However, the believers in spontaneous generation were not convinced. They thought that Spallanzani and Appert had destroyed the necessary conditions for the generation of living microbes by excluding the oxygen of the air from their containers. This objection was answered by Schulze, in 1836, who showed that a boiled fluid, such as Spallanzani used, would not contain living bacteria, even if air were admitted to the flask, *provided the air was first freed of microbes* by passing it through strong sulphuric acid. Schwann, in 1837, had the same results when he passed the air through very hot tubes before admitting it to his flasks. In 1854, Schroeder and von Dusch demonstrated that boiled infusions would remain clear and free of microorganisms if the flasks were stoppered with plugs of cotton. Unchanged air or other gases could pass freely through the cotton plugs and, therefore, the fact that microbes were not generated from the boiled infusions could not be due to the lack of air (Fig. 2: A, B, C.).

Spontaneous generation disproved by Pasteur and by Tyndall. The question of spontaneous generation was finally settled by the experiments conducted by Pasteur during the years 1860–1865, supplemented, a few years later, by those of Tyndall. Pasteur showed that living microbes are carried about in the air by particles of dust and that a cotton plug in the container serves as a filter, straining out these dust particles. Cotton plugs are now universally used in our tubes and flasks for this very purpose. Pasteur proved by

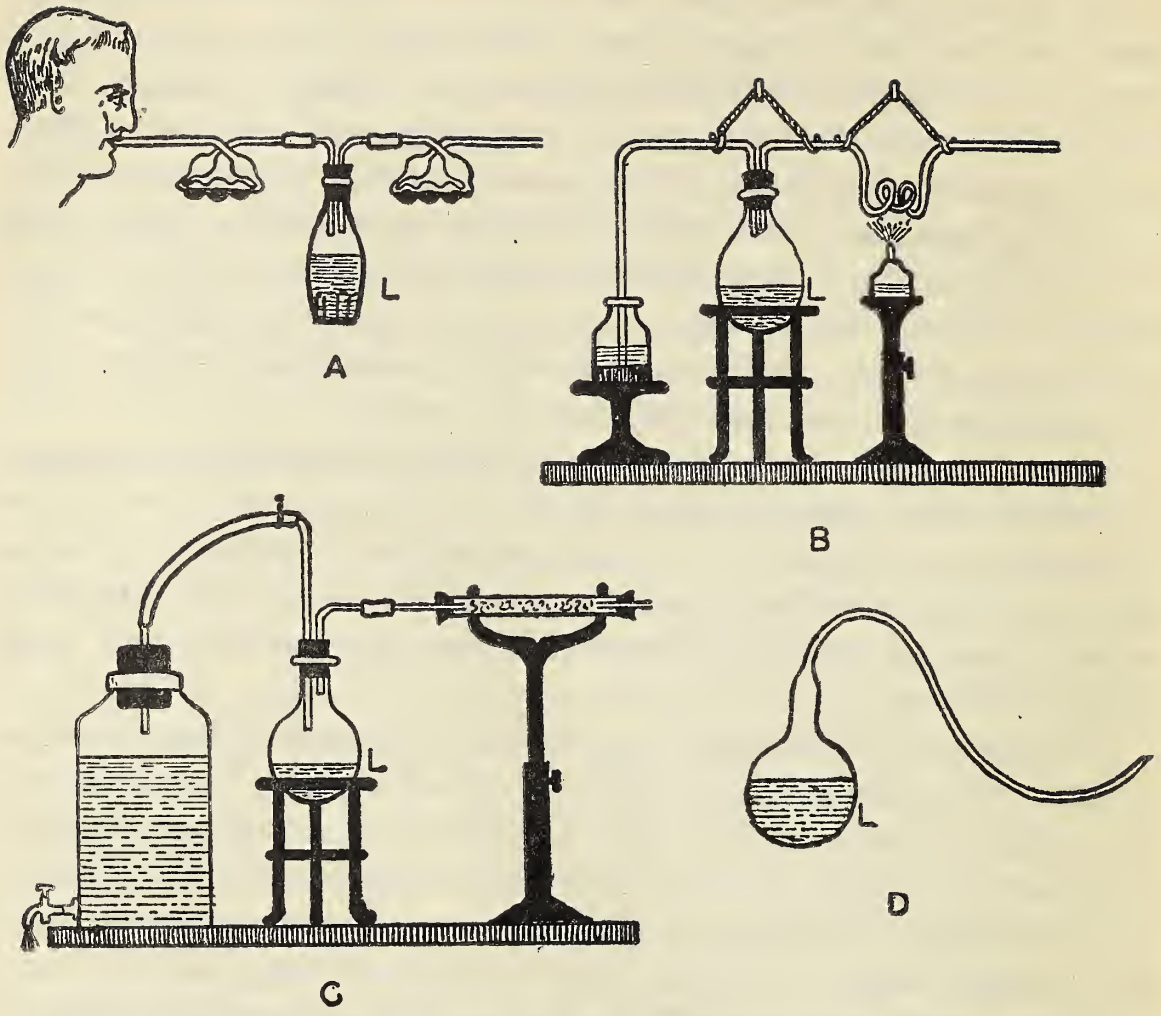


FIG. 2. Illustrating some of the principal experiments which helped to overthrow the belief in "spontaneous generation" of life. The first step in each experiment was to boil the infusions (L) very thoroughly, so as to destroy all living microbes in them. Air was then admitted to the flasks in order to satisfy the believers in spontaneous generation, who insisted that oxygen must be present for life to originate. But the air admitted to the infusions was first freed of living microbes in some way. Under these conditions the infusions remained perfectly clear; no microorganisms appeared in them, showing that living things could not generate spontaneously from the lifeless liquid.

A: Schulze's experiment. The set of bulbs next to the face contained alkali and the other set concentrated acid. Air was drawn in through the acid and thus freed of microbes before it reached the liquid. B: Schwann's experiment. In this case air could get into the flask only by passing through the coiled glass tube kept hot by the flame. C: Schroeder and Dusch's experiment. The aspirating bottle drew air into the flask through the tube containing cotton at the right. The cotton filtered out the microbes in the air, just as the cotton plugs now used in bacteriological culture tubes protect the culture from air contamination. D: one of Pasteur's U-neck flasks. Although this flask was freely open to the air, the infusion remained sterile so long as the microbe-bearing dust which collected in the U did not reach the liquid.

many ingenious experiments that an infusion which has been properly heated, so as to free it entirely of living microbes, will remain without life, even though open to the air, so long as dust is not permitted to enter. To contain his boiled infusions, he constructed flasks with a long neck bent into the shape of a "U" (Fig. 2: D). The end of the U-tube was left open, and dust could collect in the open arm of the tube; but so long as the flask remained upright and none of the dust was allowed to reach the liquid, no growth of microbes occurred.

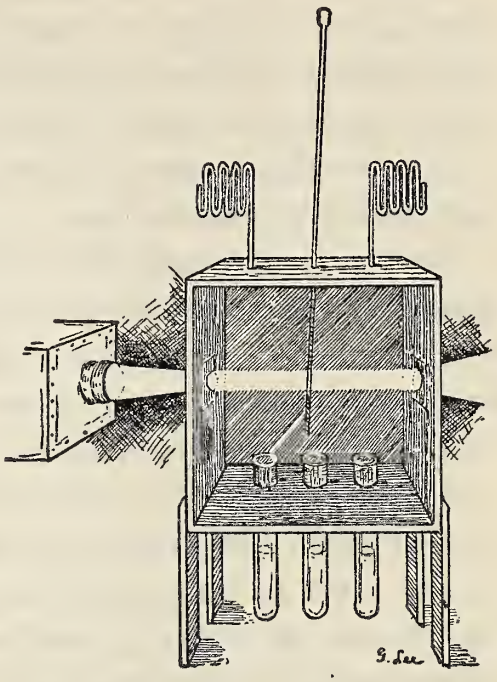


FIG. 3. Tyndall's dust-free chamber. The front side of the box, which has been removed to show the construction, contained a window through which the beam of light could be observed. The inner walls of the chamber were made sticky by coating them with glycerin, and the box was allowed to stand until the bright ray of light crossing the dark interior revealed no motes, that is, until all floating dust particles had settled out. A vegetable infusion, or similar clear fluid, was then introduced through the pipette into the open test tubes, and sterilized by heating the tubes in an oil bath. The convoluted tubing passing through the roof of the box permitted the entrance of air from without, but excluded dust. In these circumstances, the fluid in the test tubes remained sterile (free of all microbes) indefinitely.

It was, therefore, established beyond doubt that microorganisms do *not* generate spontaneously, but *always originate*—as do all other kinds of living things—*by reproduction from parent beings like themselves.*

The truth of this was demonstrated again, a few years later, by the work of John Tyndall (1820–1893), the English physicist, whose studies provided the final evidence that served to refute completely the ancient doctrine of spontaneous generation. Tyndall proved by a curious, but very accurate method, that microorganisms are carried on dust, and that this dust is the source of contamination of fluids exposed to the air. He showed that previously heated fluids (free of all microbes) may be kept in open vessels in a small chamber for long periods without showing any growth of microbes in them, *provided the air of the chamber is dustless;* i.e., free from all floating particles. This he determined by passing a strong beam of light through the sides of the chamber (Fig. 3),

and when no motes were thus made visible in the air, he found that fluids could be freely exposed without becoming contaminated.

Studies of fermentation. The souring of milk, the rising of bread dough, the making of alcoholic drinks from sweet fruit juices, and other kinds of fermentation have been known and utilized by peoples all over the world since the very earliest times, but it was not until after the discovery of microbes that the real nature of fermentation could be worked out.

Discovery of yeasts. The most familiar fermentations are brought about by the action of *yeasts,* which are simple microbes belonging to the fungi. Yeasts were seen in fermenting materials long before their significance was understood. It was not until 1837 that Latour and Schwann independently showed that yeasts are living organisms and that, as they grow in the sugar solution, they cause the formation of alcohol by the fermentation (decomposition) of sugars. Opposition to this view of fermentation was strong, however, and it remained for Pasteur to furnish convincing demonstration of the relation of microorganisms to fermentation.

Pasteur's work on fermentation. When Pasteur began his studies, the prevailing opinion regarding fermentation and putrefaction of organic matter was that the changes that go on in the decomposing material were due primarily to the action of the oxygen of the air, and the process was thought to be purely a chemical one. Pasteur was at this time a professor at the University of Lille, France, a town where the making of beers and wines—both products of alcoholic fermentation—was a business of much importance. He studied alcoholic fermentations thoroughly and showed that the changes which occurred in the fermenting matter, with the resulting formation of alcohol, were not simple chemical processes, but were, in fact, brought about by the action of living yeasts. He also demonstrated that other kinds of fermentation, which result in the formation of various *other* chemical products, are produced by bacteria or by other microbes, and that *each kind of microbe will bring about a characteristic type of fermentation.* His studies made it clear that the *decomposition of lifeless matter of all sorts is caused by the growth of microorganisms.*

Pasteurization. In connection with his studies of the making of wines and beers, Pasteur found that the so-called "diseases" of wine, which brought much loss to the makers, were caused by undesirable fermentations due to contamination of the original sweet juices by

certain kinds of microbes. He further made the important discovery that these undesirable fermentations could be prevented by heating the wine to 60°–65° C * (140°–150° F). This heating was sufficient to kill the contaminating organisms, and yet did not injure the quality of the wine. This process of heating has since been called *pasteurization,* and is of immense practical value as now used for destroying any disease germs that may be present in milk.

Early speculations and observations concerning the relation of microbes to disease. In the earliest medical literature there are vague expressions of the idea that invisible living creatures might be responsible for disease and for the spread of illness from one person to another. Such references are found, for example, in the writings of Aristotle (384 B.C.) and of a great Arabian physician, Rhazes (860–932 A.D.).

In the sixteenth century, the germ theory of disease began to assume more definite form. While common men clung to the ancient belief that disease was the result of a visitation of divine wrath, or held to the later idea that pestilence was the natural consequence of some cosmic upheaval—such as an earthquake or a volcanic eruption, or of some peculiarity of the weather, which somehow filled the air with a deadly miasma—a few acute observers expressed a view more nearly approaching reality.

In 1546, Fracastoro (1484–1553), a celebrated physician of Verona, published a treatise in which he clearly expressed the belief that invisible living organisms are able to cause disease, and transmit illness by direct or indirect contact from one person to another.

The discoveries of Leeuwenhoek (1674), already mentioned, demonstrated beyond question that microorganisms really do exist, and ideas concerning their relation to disease gradually became clarified as knowledge slowly accumulated. The belief that bacteria may be the immediate cause of disease was held by many, though denied by others. Among those who expressed this theory most clearly and forcibly was Anton von Plenciz (1705–1786), a prominent physician of Vienna. In 1762, he published a work in which he presented strong arguments for the theory that living agents were the cause of communicable diseases, and stated that a specific germ probably exists for each different disease.

During the first half of the nineteenth century, many investigators

---

* C indicates Centigrade temperature system; F indicates Fahrenheit system.

reported the occurrence of microorganisms of one kind or another in various disease conditions. But proof that these microbes were the actual causes of the diseases in which they were found was generally lacking. It must be remembered that microscopes were still imperfect, and that successful methods of studying microbes—especially the tiny bacteria—had not yet been worked out.

Jacob Henle and "Koch's postulates." In 1840, Jacob Henle (1809–1885), a German scientist, and an immediate predecessor of Pasteur and Koch, expressed the germ theory of disease almost exactly as we now conceive it, and also laid down certain necessary procedures which would have to be carried out in order to prove this theory. His argument is generally known today in the form in which it was stated later (1884) by Robert Koch, and these principles have become known as "Koch's laws" or "Koch's postulates."

These postulates declare that, before any organism can be accepted as the cause of a particular disease, all the following steps must be carried out: (1) The germ must be found in every case of the disease and not in healthy individuals; (2) It must be isolated from the patient and grown in the laboratory apart from all other organisms; (3) It must be inoculated by itself into healthy, susceptible animals and must then reproduce the disease; (4) The same organism must be found again in these inoculated animals and recovered in laboratory cultures.

No one was able to carry out these procedures in 1840, and Henle's admirable ideas had no proof behind them. It was the immortal work of Pasteur, Lister, and Koch that finally demonstrated the truth of the germ theory.

REFERENCES *

General historical accounts

BULLOCK, W.: "History of Bacteriology," in *A System of Bacteriology*. London: H. Maj. Stat. Off., 1930.

DEKRUIF, P.: *Microbe Hunters*. New York: Harcourt, Brace, and Co., 1926.

NEWSHOLME, SIR ARTHUR: *The Evolution of Preventive Medicine*. Baltimore: Williams and Wilkins Co., 1927.

* **Note to the student.** The original sources of information concerning microbiology consist in the published researches of scholars in the field. These are to be found in many different journals, monographs, and other scientific medical publications. The names of some of the principal journals and books will be found among the references given in this and later chapters.

The titles of papers written upon any one subject can be assembled in a short time by consulting the *Cumulative Index Medicus,* to be found in any medi-

Cohn, Ferdinand (1828–1898)

COHN, F.: "Bacteria; Smallest of Living Organisms (1872)," translation by Charles S. Dolley (1881), introduction by M. D. Leikind, *Bull. Hist. Med.* 1939, 7:49.

Fracastoro, Girolamo (1484–1553)

LONG, E. R., Editor: *Selected Readings in Pathology*. Baltimore: Thomas, 1929.

Leeuwenhoek, Antony van (1632–1723)

COHEN, B.: *The Leeuwenhoek Letter*. Baltimore: Soc. Am. Bacteriologists, 1937.

DOBELL, C.: *Antony van Leeuwenhoek and His Little Animals*. New York: Harcourt, Brace, and Co., 1932.

Pasteur, Louis (1822–1895)

FAULKNER, F. and ROBB, D. C.: Translation, "The Physiological Theory of Fermentation," by Louis Pasteur, *Harvard Classics* 38:289.

Spallanzani, Lazzaro (1729–1799)

ADAMS, A. E.: "Lazzaro Spallanzani (1729–1799)," *Scient. Monthly* 1929, 29:528.

Tyndall, John (1820–1893)

TYNDALL, J.: "The Optical Condition of the Atmosphere, in Its Bearings on Putrefaction and Infection" in *Fragments of Science*. New York: D. Appleton-Century Co., 5th Ed., 1880.

REVIEW QUESTIONS—CHAPTER II

1. About what year was microbiology established as an independent science?
2. What are the three periods of time into which the historical development of microbiology is naturally divided?
3. When and by whom were microorganisms first seen?
4. Describe the microscope made by Leeuwenhoek.
5. What four questions about microbes were studied by early investigators.
6. Who worked out the first important classification of bacteria, and when?
7. When were bacteria recognized as plants rather than animals? What is the scientific name for bacteria, and what is the meaning of the term?
8. What is meant by the theory of spontaneous generation? How did Needham try to prove this theory? What was wrong with his experiments?

---

cal library. The student should learn the use of this and other reference books in the libraries, and experience at first hand the pleasure of "looking up" the latest published work in a particular phase of microbiology. The habit of glancing through scientific journals, and noting the matters discussed, is recommended even for the beginning student, for this is perhaps the fastest way to make the strange new facts encountered in microbiology seem real, and to learn that the great practical value of this subject in connection with everyday problems, claimed for it by textbook and instructor, is no academic boast, but the simple truth.

9. Explain how the experiments of Spallanzani and Appert proved that spontaneous generation does not occur. What was the objection to these experiments on the part of the believers in spontaneous generation?
10. What did Schulze, Schwann, and Schroeder and von Dusch contribute toward settling the question?
11. How and when did Pasteur succeed in definitely disproving the theory of spontaneous generation? How did Tyndall confirm the conclusions of Pasteur?
12. What is meant by alcoholic fermentation? Who discovered yeasts and first showed their relation to fermentation? When?
13. What were the conclusions of Pasteur as to the cause of fermentation and putrefaction? What is the process called pasteurization?
14. Give examples, with dates, of men whose speculations concerning the relation of microorganisms to disease were close to the truth. Why was it impossible to prove the germ theory at that time?
15. What are "Koch's laws" or "postulates"?

CHAPTER III

LATER HISTORY OF MICROBIOLOGY

THE FOUNDING OF BACTERIOLOGY AND IMMUNOLOGY
1865–1882

In less than twenty years following 1865, more progress was made toward a true understanding of microorganisms and their relation to disease than had been accomplished in all the preceding time. The most important advances were made as a result of the work of the English surgeon Joseph Lister, the French scientist Louis Pasteur, who was truly the founder of modern bacteriology and immunology, and the German physician Robert Koch, who became the first master of the new science. The perfection of the modern compound microscope also contributed greatly to this rapid development.

Joseph Lister (1827–1912) and antiseptic surgery. Joseph Lister, born in England, April 5, 1827, the son of an eminent scholar, won his degree in medicine in 1852 (Fig. 4). He became professor of surgery at Glasgow and at Edinburgh, and later at King's College in London.

Lister made many contributions to surgery, but his greatest work was the conquest of wound infection. This work was begun while he was at Glasgow, during the years 1865–1869. His first papers on the subject were published in 1867. As a surgeon, he was stirred, as any other kind-hearted man must have been at that time, by the desire to prevent the severe and often fatal inflammations which so frequently followed operations and accidental wounds of any kind. He saw the similarity between the changes that go on in these inflamed wounds and the processes of putrefaction and fermentation which Pasteur had shown to be due to the growth of microorganisms, and he reasoned that microbes must be the cause of the inflammation in wounds. He saw that bacteria could be kept out of a wound if he would protect it by means of a dressing saturated with

a substance which would kill microbes. This was the basis of his antiseptic method.

For a germicidal substance, Lister chose carbolic acid, which was well known as a preservative. He began by applying carbolic-

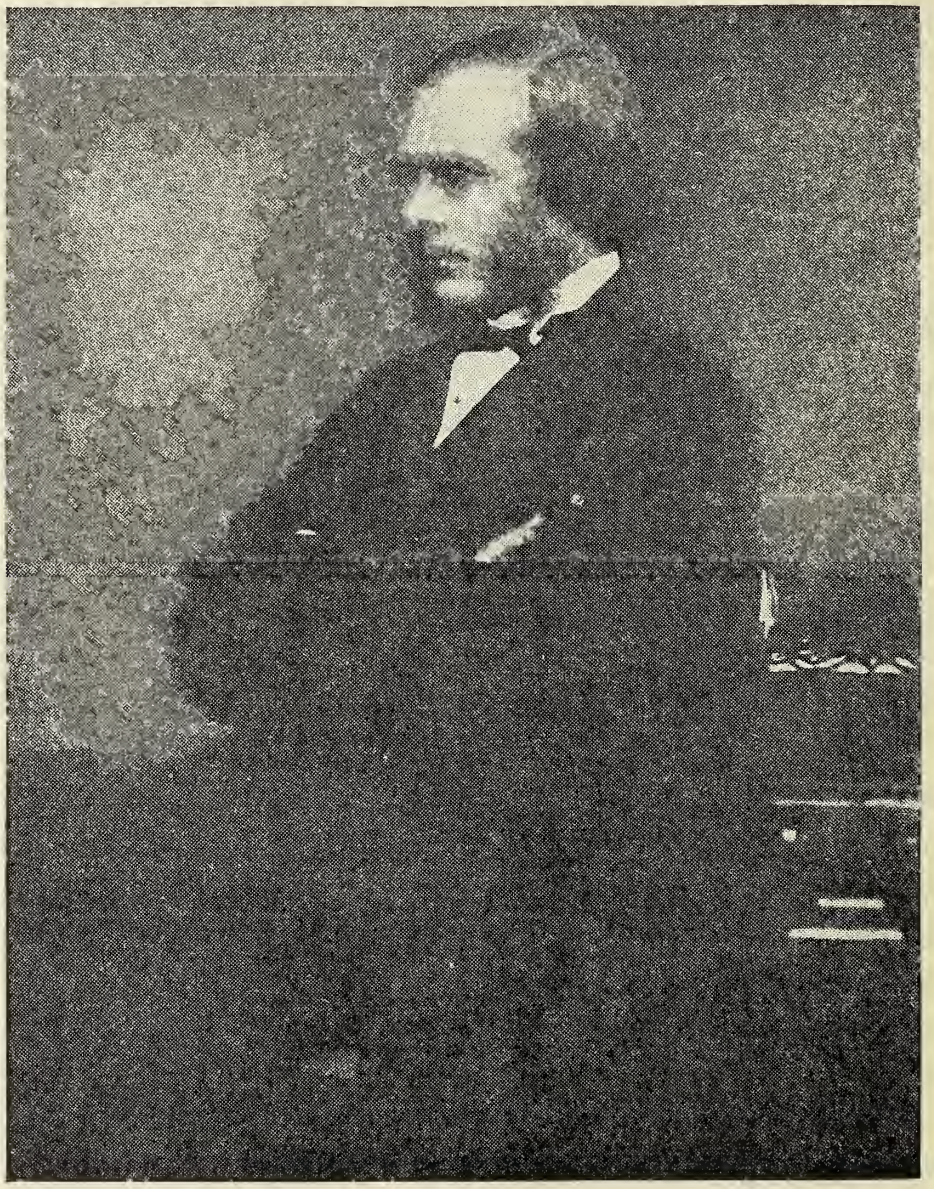


Fig. 4. Joseph Lister, 1827–1912.

acid dressings to compound-fracture wounds—the wounds made when broken bones protrude through the skin. Wounds of this type usually became badly inflamed, and frequently this condition led to the death of the patient. The success of the carbolic-acid dressing was immediate and astonishing; the wounds healed without the usual quantities of pus, and as readily as if there had been no break in the skin at all. Later, Lister applied his method to the treatment

of abscesses and to other kinds of accidental wounds. Finally, he developed a routine for the use of carbolic acid at operations, which became known as "Lister's antiseptic system." Today, the methods used are modified, and we employ not an antiseptic, but an *aseptic* system, in which instruments and other materials to be used at an operation are previously sterilized by heat. Nevertheless, the principle established by Lister is still adhered to, and it forms the very cornerstone of surgical and obstetrical practice. The principle is that *microorganisms must be kept out of wounds.* Lister suffered the criticism which seems to be the lot of all pioneers, but by 1875 he was internationally famous, and honored everywhere. He died February 10, 1912.

The result of the introduction of antiseptic methods into surgery and obstetrics was the seemingly miraculous disappearance of those serious complications which had previously accompanied all types of wounds. Thus, countless lives were saved and untold suffering was prevented. Aseptic methods together with ether anesthesia (introduced by Morton in the United States in 1846), have made possible the wonderful benefits of modern surgery. The work of Lister also powerfully stimulated the further study of germ diseases.

Louis Pasteur (1822–1895). Louis Pasteur (Fig. 5) was born in the village of Dole, France, December 27, 1822, the son of humble parents. His father was a tanner. The boy began life with no special advantages, but became the most prominent man of his day, and is universally recognized as a genius whose brilliant achievements place him in the front rank of great men and whose influence is still felt in many fields of science. He was originally trained as a chemist, and at the age of twenty-five had already distinguished himself in this work. His discoveries revolutionized medical practice, although he never studied medicine. In 1847, he was graduated with a doctorate in chemistry from the Normal School in Paris and taught at Strassburg and Lille before returning to Paris, where he became a professor at the Ecole des Beaux Arts and later (1867) at the Sorbonne. In 1888, in recognition of his incomparable achievements, a special laboratory, called the Pasteur Institute, was built for him in Paris by popular subscription. Acclaimed the world over for his epoch-making discoveries, Pasteur died in Paris, September 28, 1895.

Principal contributions of Pasteur. *Early studies.* We have already described how Pasteur disproved the theory of spontaneous

generation and demonstrated the true nature of fermentation. His studies in fermentation led him to the study of disease, first in animals, then in human beings.

Work on silkworm disease and anthrax. In 1865, he was asked to attempt to find the cause of a disease called pébrine, which was threatening to ruin the business of raising silkworms, an important industry in southern France. Pasteur succeeded in demonstrating that this silkworm disease is caused by a microscopic germ—a protozoon—and showed that the infection could be eliminated by choosing for breeding only those worms which were free of the parasite. This discovery was one more step toward the establishment of the truth of the germ theory of disease.

But the conclusive proof was furnished by the work of Pasteur and Koch with anthrax—a common cattle and sheep disease which occasionally occurs in human beings. Rayer and Davaine, as early as 1850, and Pollender, in 1855, had seen little rods in the blood of animals dead of anthrax, and Davaine in 1863 reproduced the disease in healthy animals by inoculating them with blood containing these rods. It was left to Koch and Pasteur to demonstrate fully that the rods were bacteria and the cause of anthrax (1877). Pasteur grew the anthrax organisms by themselves in sterilized yeast water and kept them in the laboratory for several months, transferring them frequently to new culture fluid, in which they multiplied readily, and showed that these cultures would always cause anthrax when inoculated into healthy animals. There could be nothing in the cultures but the bacteria themselves which could possibly be responsible for the production of the disease.

Koch, working independently in Germany about the same time, also cultivated the anthrax germs in pure culture, and showed that they produced disease in laboratory animals. He studied the growth and development of the organisms under the microscope, and for the first time demonstrated the real nature of the resistant bodies, called spores, which the bacilli form. This careful work, taken with that of Pasteur, completed the proof that anthrax is caused by this organism, and this organism alone. Anthrax was, therefore, the first disease of animals and man which was proved to be caused by a particular bacterium.

Development of vaccines for anthrax and rabies. A contribution of still greater importance was made by Pasteur when he discovered and applied the principle of *protective inoculation or vaccination*

against disease. In 1880, he was working on a malady of fowls, called chicken cholera. He found that pure cultures of the germ of this disease which had been kept in the laboratory for some time would not kill his animals as fresh cultures did, but would merely cause a

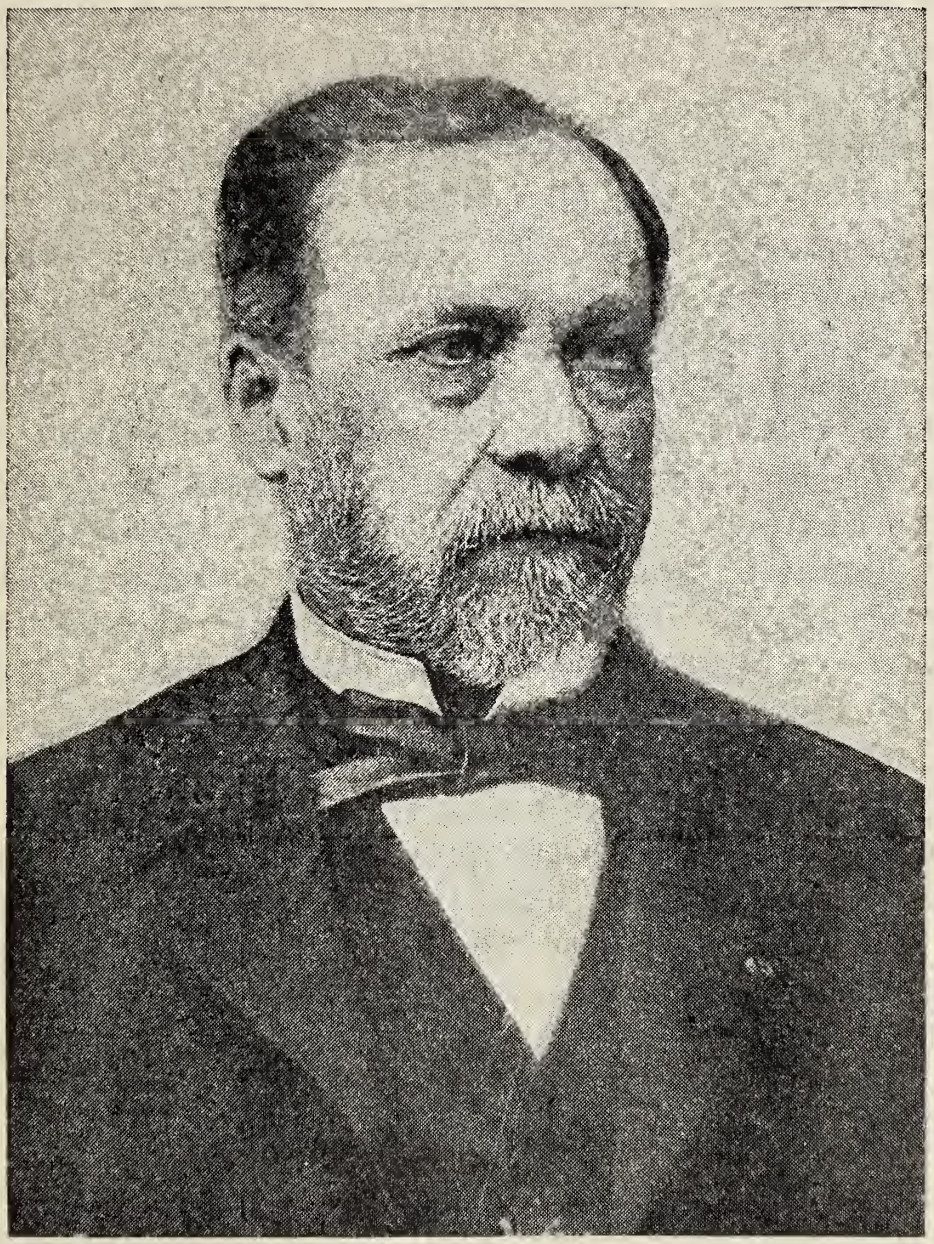


FIG. 5. Louis Pasteur, 1822–1895.

passing illness from which the chickens recovered. Then he discovered that *the animals that had recovered from a previous inoculation of a weakened germ were immune,* and did not succumb to the disease when inoculated with a fresh culture. Pasteur immediately perceived that here might be a practical method of preventing disease. He saw that it might be possible to make individuals re-

sistant by inoculating them with the weakened (and therefore harmless) germs of a particular disease.

The idea of protective inoculation was not entirely new, but the principle was not fully understood before Pasteur. One very important application of the idea was already well known—the method of vaccination against smallpox with the virus of cowpox. This great discovery had been made by the English physician Edward Jenner in 1798.

Pasteur first applied the principle of protective inoculation to the prevention of anthrax. He succeeded in making animals immune by a series of inoculations with anthrax germs whose disease-producing power had been so reduced that the injections caused no harm to the animals. The preparation of anthrax organisms used for these inoculations was called *anthrax vaccine.**

In 1881, before a large audience of friends and critics, Pasteur demonstrated the value of his method. He had 50 sheep, 25 of which he had previously inoculated with his vaccine containing weakened anthrax germs; the other 25 were untreated. In the presence of his visitors, he inoculated all 50 animals with a fresh culture of anthrax germs and invited everyone to return in two days to observe the result. When the company assembled again at the appointed time, everybody was moved to the most profound admiration, for all the vaccinated animals were perfectly well, while, of the unvaccinated animals, 22 were already dead and the other three were dying. This method of protecting sheep and cattle from anthrax by use of a vaccine was soon tried out on a large scale, and it is still used all over the world with great success.

The crowning achievement of Pasteur was the successful application of this principle of vaccination to the prevention of rabies, or hydrophobia, in human beings (1885). He did not find the germ of this disease under his microscope—we now know that the causative agent is an invisible virus—but he was able to propagate it by artificial inoculation into the brains of dogs and rabbits. Finally he developed a system of vaccination with the weakened virus which

* The term "vaccine" had previously been applied only to the cowpox virus used by Jenner for immunization against smallpox. *Vacca* in Latin means *cow,* hence cowpox virus was called "vaccine virus," and Jenner's method of protective inoculation against smallpox was properly called *vaccination.* Pasteur, wishing to honor Jenner, deliberately extended the use of the term "vaccine" to indicate any preparation used like "vaccine virus" for immunization, and the word *vaccination* thus came to have the general meaning it has today.

prevents the development of this fatal disease if the inoculations are given soon after the bite of the rabid animal. Now this life-saving "Pasteur treatment," or a modification of it, is used everywhere for the prevention of rabies in man.

Perfection of the compound microscope. The first microscopes really worthy of the name were those made by Leeuwenhoek about 1675. These were *simple microscopes,* that is, they contained only *one lens,* or magnifying glass. They permitted Leeuwenhoek to see some of the larger microorganisms. But bacteriology and other modern microscopical sciences depended for their development upon the perfection of *compound microscopes,* having *several lenses arranged in combination* and capable of very high magnification.

Early compound microscopes. The earliest compound microscopes were manufactured many years before the time of Leeuwenhoek. As early as 1600, a compound microscope was invented by a spectacle-maker named Zacharias Jansen, in Holland. The Italian scientist Galileo, famous for his improvement of the telescope, also made a compound microscope about 1610, and it was to one of Galileo's instruments that the name microscope was first applied (1625). The early instruments were exceedingly crude, judged by the standards of today. They were really small inverted telescopes intended for observation of minute objects by reflected light. It was not until the end of the seventeenth century that microscopes were made to observe *transparent* objects by transmitted light, as in the modern instrument.

During the eighteenth century, the microscope was gradually improved by various workers in England, France, Italy, Germany, and America, but for most of this time it was scarcely more than a kind of scientific toy, distorting more than it magnified. About 1830, important advances were made in the construction of the optical parts of the instrument, largely as the result of the principles worked out by Joseph Jackson Lister, father of the surgeon, Joseph Lister. But it was not until about 1880 that the microscope was converted into the beautiful instrument of precision and power that we have today.

The modern microscope. We owe the excellence of the modern microscope almost entirely to the work of one man, Ernest Abbe (1840–1905). Abbe was a German physicist who became connected in 1866 with the firm of Zeiss, manufacturer of microscopes in Germany. He made fundamental improvements in the entire optical

system of the microscope, including the introduction of superior oil-immersion objectives, and the Abbe substage condenser. The perfection of the compound microscope, with its powerful lenses magnifying one thousand times, was the one thing necessary to assure the rapid development of the new science of bacteriology.

Robert Koch (1843–1910). Robert Koch was born December 11, 1843, in Germany (Fig. 6). In 1866, he took his degree in medicine and began a general practice in a small country town. He became much interested in microscopical studies, and soon these absorbed most of his time. On April 22, 1876, Koch addressed to Ferdinand Cohn, professor of botany at Breslau, the nearest university town, a letter in which he reported that he had made a study of the anthrax germ. Cohn invited the unknown country physician, Koch, to Breslau, and gave him the opportunity of demonstrating his specimens and methods. His studies were so complete and well ordered that they made a profound impression, at once marking him as a new leader in bacteriology. The work on anthrax was published the same year, and soon Koch's name became well known throughout the medical world. In 1880, Koch was called to the Imperial Health Department at Berlin, and later became Professor of Hygiene, and Director of the Institute for Infective Diseases. Many years of his later life he spent abroad in the study of cholera, malaria, and other diseases. He died May 27, 1910.

Principal contributions of Koch. *Studies on anthrax; improved methods.* Aside from his work with the anthrax germ, already mentioned, Koch is especially noted as the first to master the technical difficulties in the study of microorganisms. It was Koch who improved and simplified methods of studying bacteria, and thus made it possible for persons of ordinary skill to obtain satisfactory results. This improvement of methods is the greatest single factor which accounts for the phenomenally rapid development of bacteriology during and since Koch's time.

Koch introduced the method of making smears of bacteria on glass slides, and of staining them with the anilin dyes, first used a few years earlier by Ehrlich and Weigert. He also was the first to employ in bacteriological work the improved compound microscope of Abbe.

Plate method for isolating pure cultures. In 1881 Koch devised a method of growing bacteria in a solid culture medium made with gelatin, and introduced his plate method of securing pure cultures.

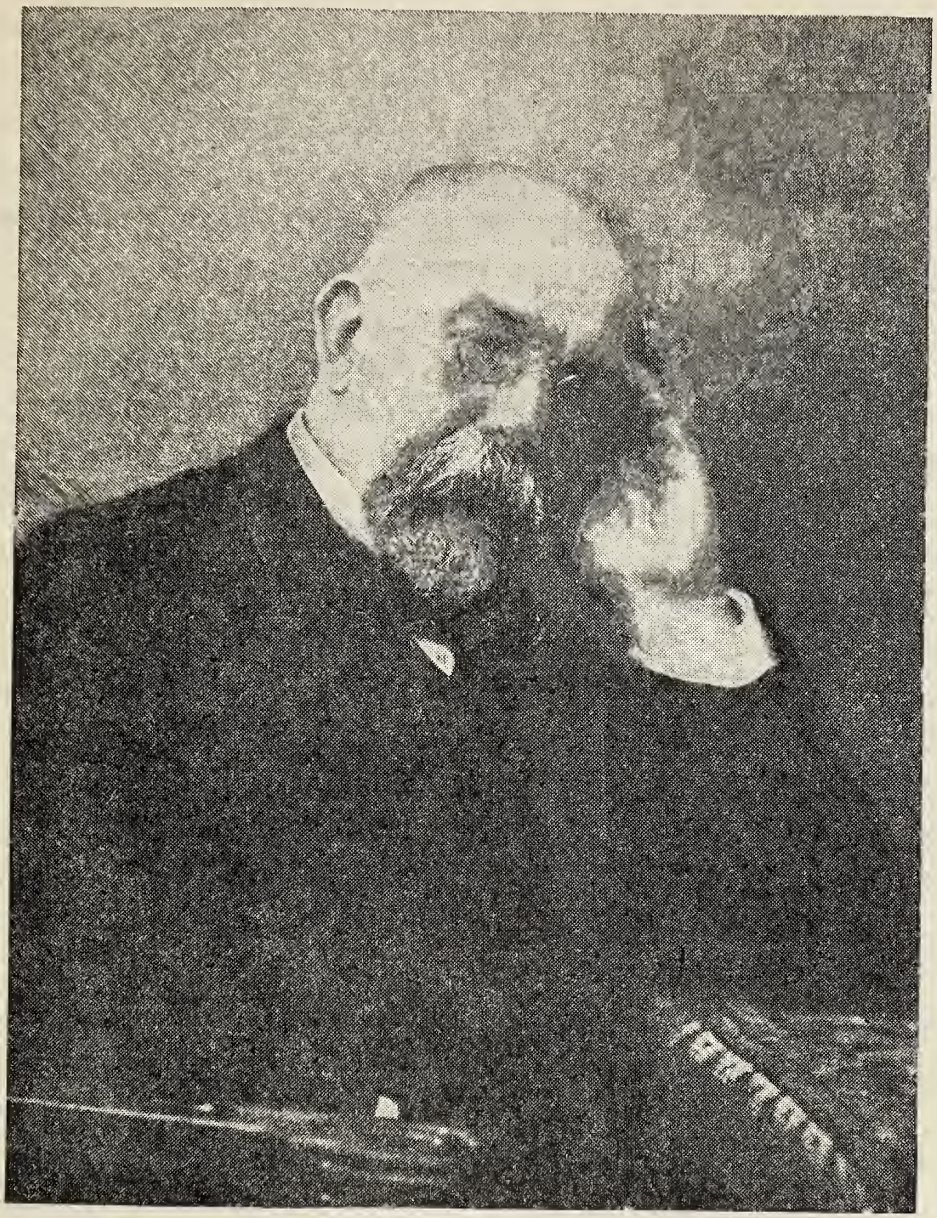


FIG. 6. Robert Koch, 1843–1910.

The use of a liquefiable solid medium was a new idea. Vegetable infusions and other liquids had been used almost exclusively up to this time. It was naturally very difficult to separate one kind of bacteria from a mixture of organisms in a liquid medium, and to get it growing by itself in a pure culture. Koch's solid-culture-medium plate method made the task of securing pure cultures comparatively simple and certain, and immensely facilitated the study of individual kinds of bacteria.

He inoculated the gelatin with a mixture of bacteria after the medium had been melted by warming, then poured the inoculated medium upon a cold, sterilized glass plate to harden. Wherever

the bacteria were caught in the solidified medium, they multiplied and soon formed a mass, called a *colony,* visible to the naked eye. If the organisms originally inoculated were well separated from one another in the medium, the colonies they formed would be pure, that is, the colonies would contain only one kind of bacterium. To secure a pure culture it was only necessary, then, to pick out such a colony with a sterile wire and transfer it to a tube of fresh sterile medium.

This method of obtaining pure cultures is essentially that used today, except that *agar* is used instead of gelatin for this purpose, and for our "plate" cultures an especially devised glass dish is employed, called the *Petri dish,* introduced by Petri in 1887.

Discovery of the tubercle bacillus and other disease germs. These new and better methods permitted Koch and his followers to discover the germs of several important diseases almost at once. Koch himself described several of the organisms found in wound infections (1878), and the spirillum of cholera in 1883.

In 1882, Koch startled the world when he announced the discovery of the germ of tuberculosis. He was able to demonstrate in a most convincing manner that all forms of this dread disease are due to the same cause—the tubercle bacillus (*Mycobacterium tuberculosis*). He described a special staining method for detection of this germ, and grew the organism in pure cultures in the laboratory. He showed that animals would develop tuberculosis when inoculated with pure cultures of this organism, and he recovered the identical germs from the diseased tissues of the animals. In his full report on these studies (1884), Koch expounded the postulates or laws by which an organism may be proved to be the cause of a particular disease, and he showed how he had fulfilled these laws in his own studies on the causation of tuberculosis. This brilliant work of Koch gave mankind for the first time a true understanding of the cause of one of the most common and deadly of human ills, and furnished the rational basis for practical preventive measures. It is a shining landmark in the history of medicine.

Koch made one other important contribution when he discovered *tuberculin,* a substance in cultures of tubercle bacilli that causes a specific reaction when injected into a tuberculous individual. He advocated use of tuberculin in the treatment of tuberculosis. This was a grievous error; nevertheless his work demonstrated basic features of immunity in this disease.

THE MODERN PERIOD, 1882 TO THE PRESENT DAY

The great contribution of Pasteur and Koch to the development of bacteriology and immunology cannot be measured merely by the discoveries they themselves were able to make. Their influence in stimulating and directing the work of others must be counted as of nearly equal importance. About each of these leaders there developed a group of younger workers, and in America as well as in Europe many able investigators began to study bacteria intensively. One of the pioneer American bacteriologists in the medical field was George M. Sternberg (1838–1915), an Army physician who became Surgeon General. Other early American bacteriologists who have attained world-wide fame are William H. Welch (1850–1934) of Johns Hopkins University, and Theobald Smith (1859–1934) of the Rockefeller Institute (Fig. 7).

Within twenty years following 1882, all but a few of the disease-producing bacteria, protozoa, and fungi we now know were identified. Many of the most important discoveries were made by associates or pupils of Pasteur or Koch. By 1910 other types of infectious agents—spirochetes, rickettsiae, and filtrable viruses—had been recognized. It was also early in this period that the fundamental observations and studies were made which founded the now complex subject of *immunology*. This rapidly increasing store of knowledge was soon applied to the solution of many of the practical problems of the farmer and the dairyman, and with particularly marked success to the diagnosis, prevention, and specific treatment of infectious diseases.

Discovery of important disease germs. It was natural that the larger organisms should be discovered first. It is not surprising to learn, then, that some of the important fungi and protozoa were recognized years before the modern period of microbiology began. For example, several of the types of fungi responsible for ringworm of the skin were described before 1850; the protozoan (ameba) causing amebic dysentery was discovered in 1875, and the germ of malaria (another kind of protozoan) was first seen by Laveran in 1880. With the development of better technical methods, under the influence of Koch, there ensued a "golden age of discovery"—through the 28 years from 1882 to 1910. More disease-producing protozoa and fungi were soon found, and there was an especially

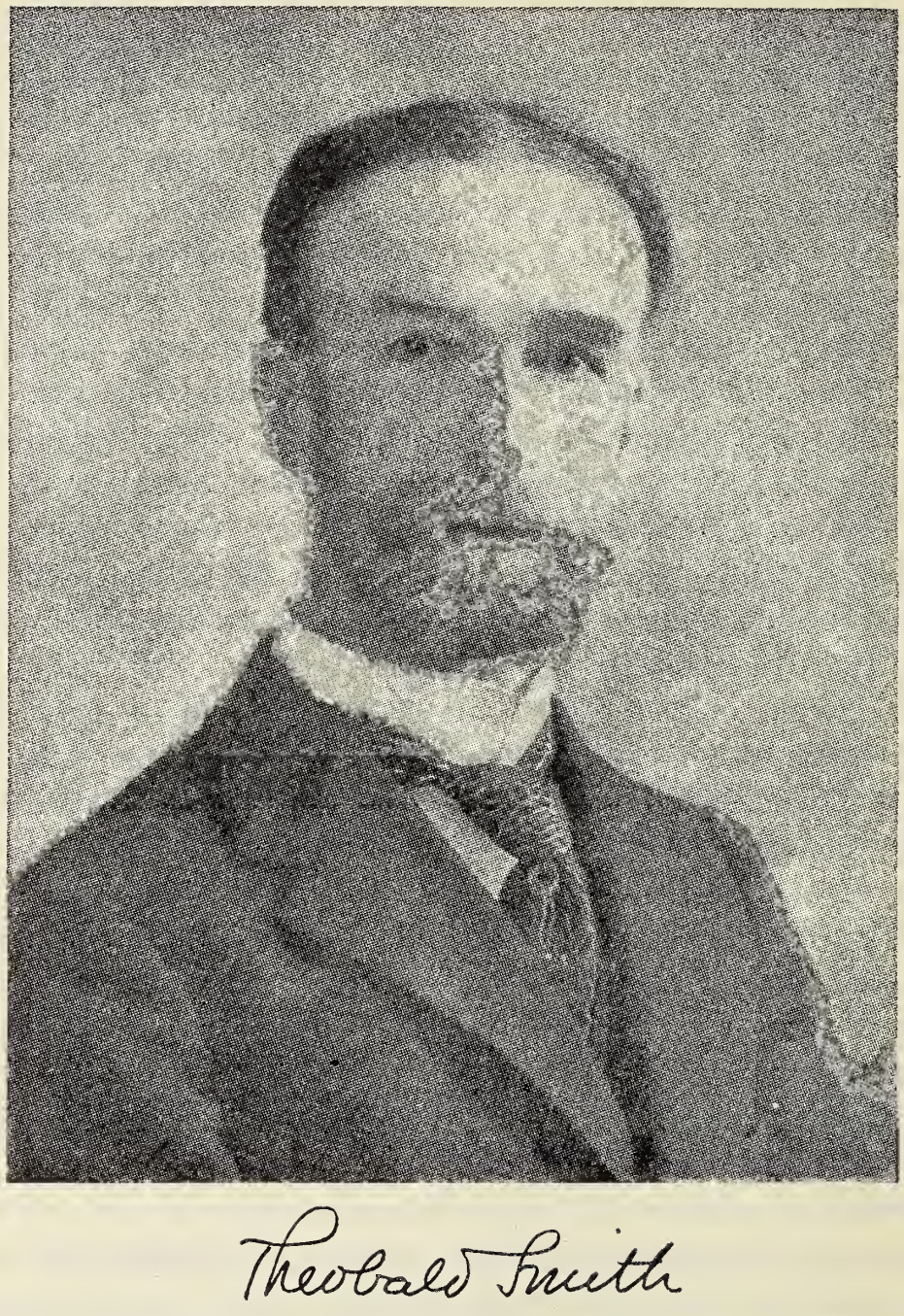


FIG. 7. Theobald Smith, 1859–1934. This splendid portrait, with autograph, of the great American bacteriologist, is reproduced through the courtesy of the late Professor Simon Henry Gage, Cornell University.

rapid advance in the recognition of the bacteria that cause common diseases.

In this book approximately 126 infectious agents pathogenic for man are now mentioned,—60 bacteria, 19 fungi, 14 protozoa, 6 rickettsiae, and 27 viruses.

The following list shows when and by whom some of the more important disease-producing bacteria were discovered:

Discovery of Some Important Pathogenic Bacteria

Actinomyces bovis, mold-like "higher" bacterium causing *actinomycosis*. Langenbeck, 1845; Hartz and Bollinger, 1877.

Borrelia recurrentis, spirochete of *relapsing fever*. Obermeier, 1873.

Bacillus anthracis, germ of *anthrax*. Rayer and Davaine, 1850; Pollender, 1855. Proved to be cause of anthrax independently by Koch and Pasteur, 1877.

Mycobacterium leprae, germ of *leprosy*. Hansen, 1874.

Gonococcus (Neisseria gonorrheae), germ of *gonorrhea*. Neisser, 1879; Bumm, 1885.

Pneumococcus (Diplococcus pneumoniae), germ of *lobar pneumonia*. Pasteur, Sternberg, 1880.

Eberthella typhosa, germ of *typhoid fever*. Eberth, 1880; Gaffky, 1884.

Staphylococcus, germ causing *abscesses*. Pasteur, 1880; Ogston, 1881; Rosenbach, 1884.

Streptococcus, germ causing *acute infections of various tissues, including septicemia and erysipelas*. Koch, Ogston, 1882; Fehleisen, 1883; Rosenbach, 1884.

Mycobacterium tuberculosis, germ of *tuberculosis*. Koch, 1882.

Malleomyces mallei, germ of *glanders*. Loeffler and Schütz, 1882.

Vibrio cholerae, germ of *Asiatic cholera*. Koch, 1883.

Corynebacterium diphtheriae, germ of *diphtheria*. Klebs, 1883; Loeffler, 1884.

Clostridium tetani, germ of *tetanus*. Nicolaier, 1884; Kitasato, 1889.

Meningococcus (Neisseria meningitidis), germ of *epidemic meningitis*. Weichselbaum, 1887.

Clostridium perfringens or welchii, principal germ in cases of *gas gangrene*. Welch and Nuttall, 1892.

Pasteurella pestis, germ of *plague*. Kitasato, Yersin, 1894.

Brucella abortus, one of the germs causing *undulant fever* (*brucellosis*). Bang, 1895.

Shigella dysenteriae (Shiga), a germ causing *bacillary dysentery*. Shiga, 1897.

Shigella paradysenteriae (Flexner), another variety of *dysentery* germ. Flexner, 1900.

Treponema pallidum, the spirochete causing *syphilis.* Schaudinn and Hoffman, 1905.

Hemophilus pertussis, germ of *whooping cough.* Bordet and Gengou, 1906.

Bacterium tularense, germ of *tularemia.* McCoy and Chapin, 1910.

Streptococcus, germ of *scarlet fever.* Savchenko, 1905; Gabritschewsky, 1907; Dick and Dick, 1923.

Discovery of filtrable viruses. Early in the period of most active discovery, investigators attempted to separate bacteria from the culture fluids in which they were growing by filtration, in order to study the properties of the filtrates. Chamberland, in 1884, devised

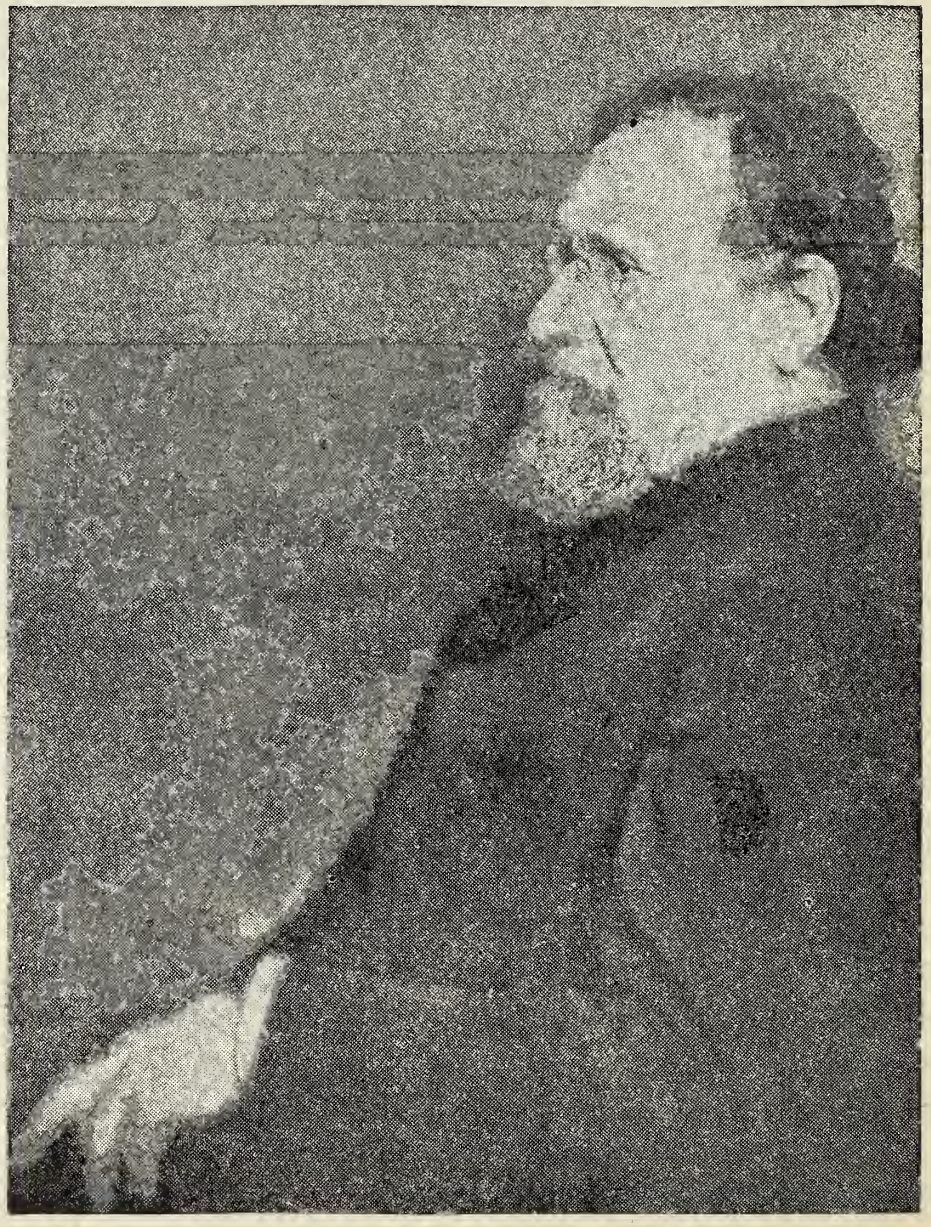


FIG. 8. Elie Metchnikoff, 1845–1916.

a cup of unglazed porcelain which proved to be the first successful bacterial filter. When a broth culture in which bacteria were suspended was passed through this filter all the organisms were held back, and the filtrate was clear and sterile. Later, other kinds of filters were developed, such as the widely used Berkefeld type, made of diatomaceous earth. Study of the filtrates obtained from cultures of various bacteria soon led to a most important finding—some of these organisms, notably the germs of tetanus and of diphtheria, were shown to produce powerful poisons (*toxins*) which appeared in the sterile filtrate.

Then came the unexpected discovery that the actual causative agent of some of the communicable diseases will pass through a bacterial filter in invisible form. In 1892, Iwanowski proved that the mosaic disease of tobacco leaves could be transferred to healthy young plants by the *filtered* juice from the diseased leaves, though this filtrate contained no microbes recognizable under the microscope. In 1898, Loeffler and Frosch discovered that foot and mouth disease is also caused by a filtrable, invisible "virus." Soon, many other diseases of this kind were recognized in both animals and man.

Among important human diseases found to be caused by viruses are *measles, mumps, influenza, smallpox, rabies, poliomyelitis, encephalitis,* and *yellow fever.*

Discovery of rickettsiae. Still another class of infectious agents was discovered by Ricketts in 1909–1910, when he discerned extremely minute bacteria-like bodies in the blood of patients ill with Rocky Mountain spotted fever and typhus fever, and in the lice that had fed on typhus patients. These bodies, now called *rickettsiae,* have since been proved to be living organisms. They are set apart from other organisms, in a class by themselves, because, although they are visible under the microscope, they will not grow in artificial media like the ordinary bacteria.

Rocky Mountain spotted fever and *endemic typhus fever* are the principal diseases caused by rickettsiae in the United States.

Modification of Koch's postulates. The investigators who made these discoveries were not always able to carry out completely Koch's rules for proving the relation of the organisms they studied to the causation of the disease in which they found them, largely for the reason that it was often difficult, and in some cases impossible, to reproduce typical human diseases in laboratory animals.

However, evidence of a new kind was used. It was found that the blood of a diseased individual will act in a definite way upon the germ causing the disease and, when properly tested, will act upon

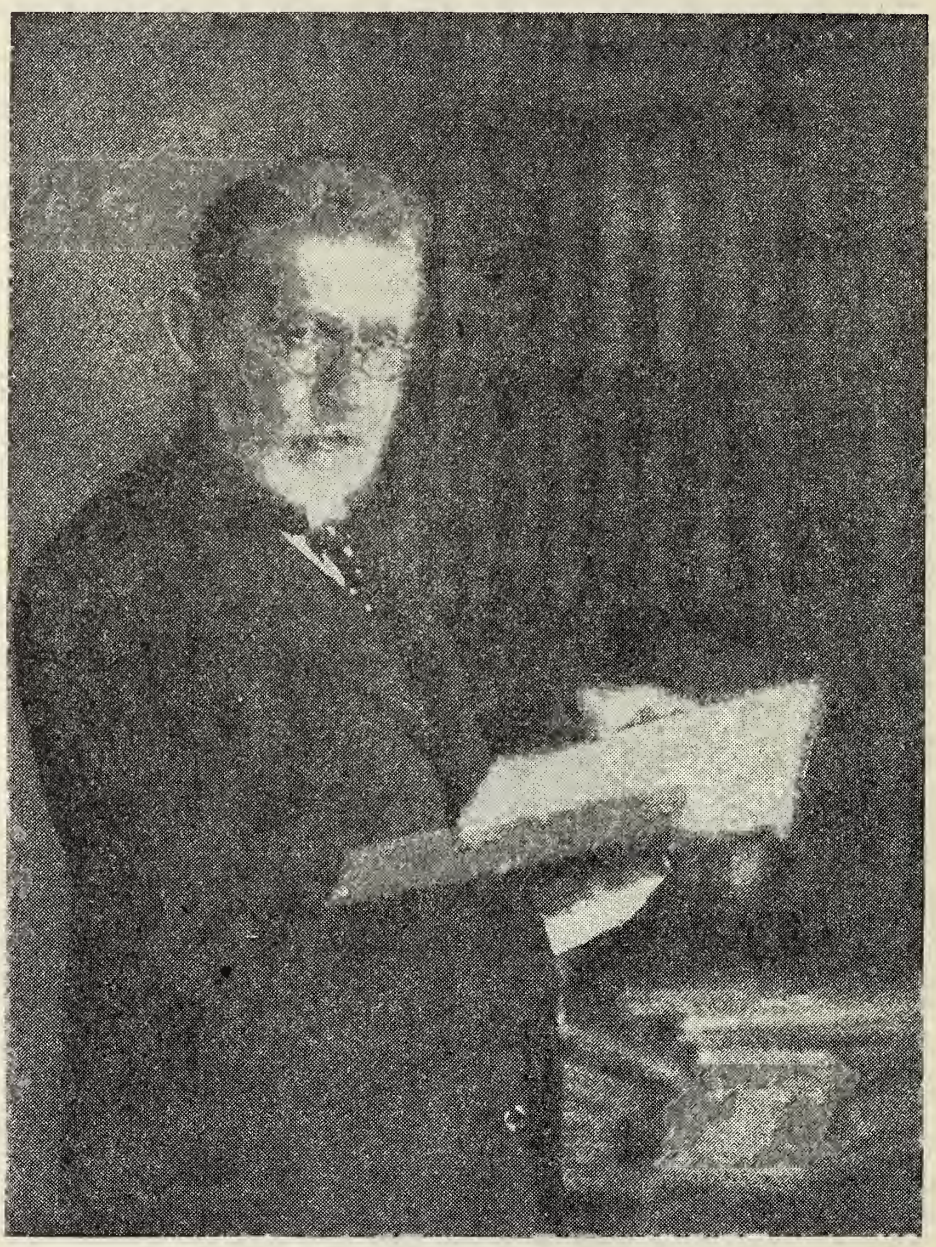


Fig. 9. Paul Ehrlich, 1854–1915.

this germ alone. Thus the guilty organism could be identified by tests of the patient's blood. These *blood tests* will be explained in our later discussions of immunity.

On one other point the original postulates of Koch have been modified. These postulates state that a microbe cannot be regarded as the cause of a disease if it is found in healthy persons. We now know that this is a mistaken idea. Diphtheria germs, for example,

may be found in the throat of a healthy person, and yet these same organisms may cause typical diphtheria if they lodge in the throat of another, more susceptible individual. A person who harbors germs in such a way that they may be transmitted to other persons but is himself without symptoms of illness, is called a *carrier.*

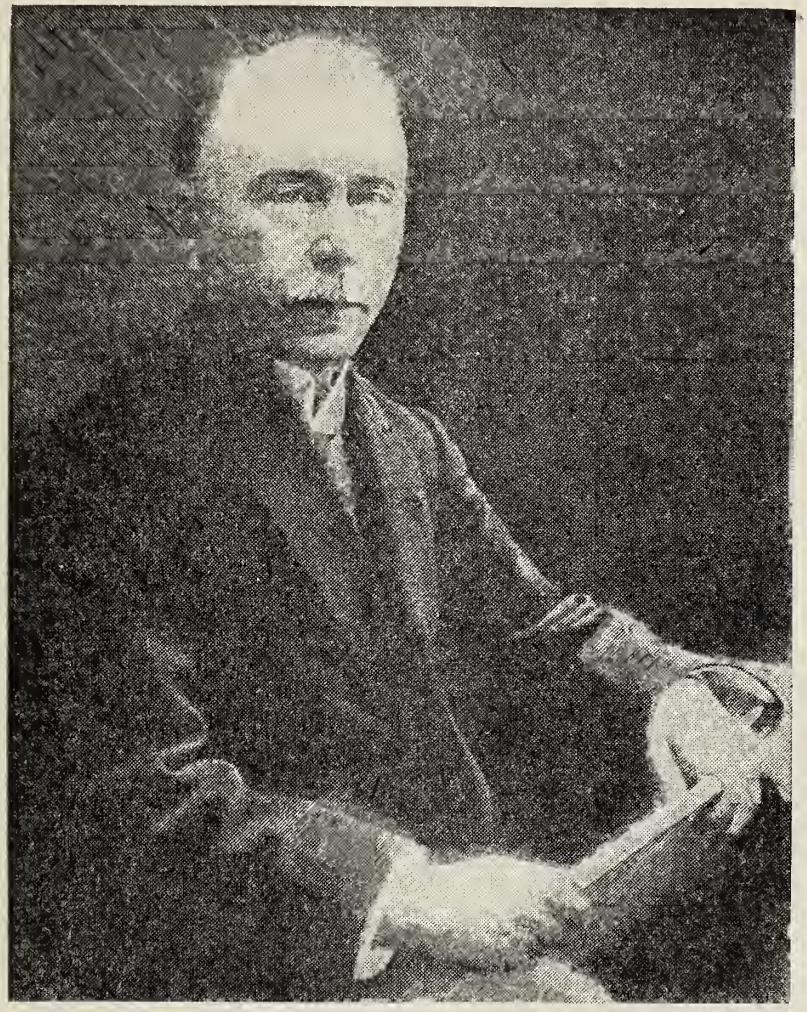


FIG. 10. Jules Bordet (1871—). Reproduced from Gay's *Agents of Disease and Host Resistance,* 1935. (Courtesy of Chas. C Thomas, Publisher, Springfield, Illinois.)

Development of Immunology. The growth of knowledge about resistance to germ diseases, and about practical immunological methods, was as rapid as the discoveries about the microbes themselves, but this is too long a story to be related in detail here. Among the more important advances, however, the following may be listed:

(1) The demonstration by Metchnikoff (1822–1884) (Fig. 8) of the *essential rôle played by the white cells (leucocytes) of the blood, and by other body cells,* in defending vital tissues against disease germs.

(2) The *discovery* of diphtheria and tetanus *antitoxins* by Kitasato and Behring in 1890.

(3) The studies of Ehrlich (Fig. 9), Bordet (Fig. 10), and many others, of the special properties of the blood of immune individuals, and particularly of the action of specific, so-called *antibodies* in the blood serum.

(4) The development of valuable *diagnostic blood tests,* such as the Wassermann test for syphilis and the Widal test for typhoid fever.

(5) The introduction of successful *vaccines* for the prevention of typhoid fever, diphtheria, and some other diseases.

(6) The recognition of the human *blood groups* by Landsteiner (Fig. 11) in 1901, which led to the universal practice of "matching" the blood of patient and donor before transfusions.

(7) The investigations by Rosenau and Anderson (1907) and others of the phenomenon of *hypersensitiveness.*

This last-mentioned work gave us an insight into the nature of a whole group of common ills, examples of which are hay fever and asthma, that are caused not by microbes, but by sensitiveness toward plant pollens or other foreign matter.

Applications of microbiology and immunology in public-health work. The influence of these many discoveries upon public-health work, and upon concepts and practices of personal hygiene, has been truly revolutionary. Before the rise of microbiology, diseases were thought to be carried by some mysterious effluvium which polluted the atmosphere, and against which we could have no protection. Epidemics of contagious diseases were thought to arise under the influence of the stars, or were attributed to storm or earthquake. The source of disease was always thought of as existing *outside* the bodies of men and animals, and filth of any kind was especially feared.

Microbiology, however, has taught that the diseases that pass from man to man, or from animals to man, are caused by certain particular microorganisms which live and grow in the bodies of men and animals, and which, with rare exceptions, do not have any natural life anywhere else, except sometimes in insect carriers of the germs. The secretions and excretions of individuals who are sick, and sometimes of individuals who are well, contain these organisms, and it is when these germ-laden materials are transferred more or less directly from one individual to another that disease spreads.

This knowledge has made clear the value of the isolation of the patient (or carrier of the organism) and prompt destruction of the germs in the discharges from the bodies of these persons.

Exact knowledge of the germs concerned has made possible the

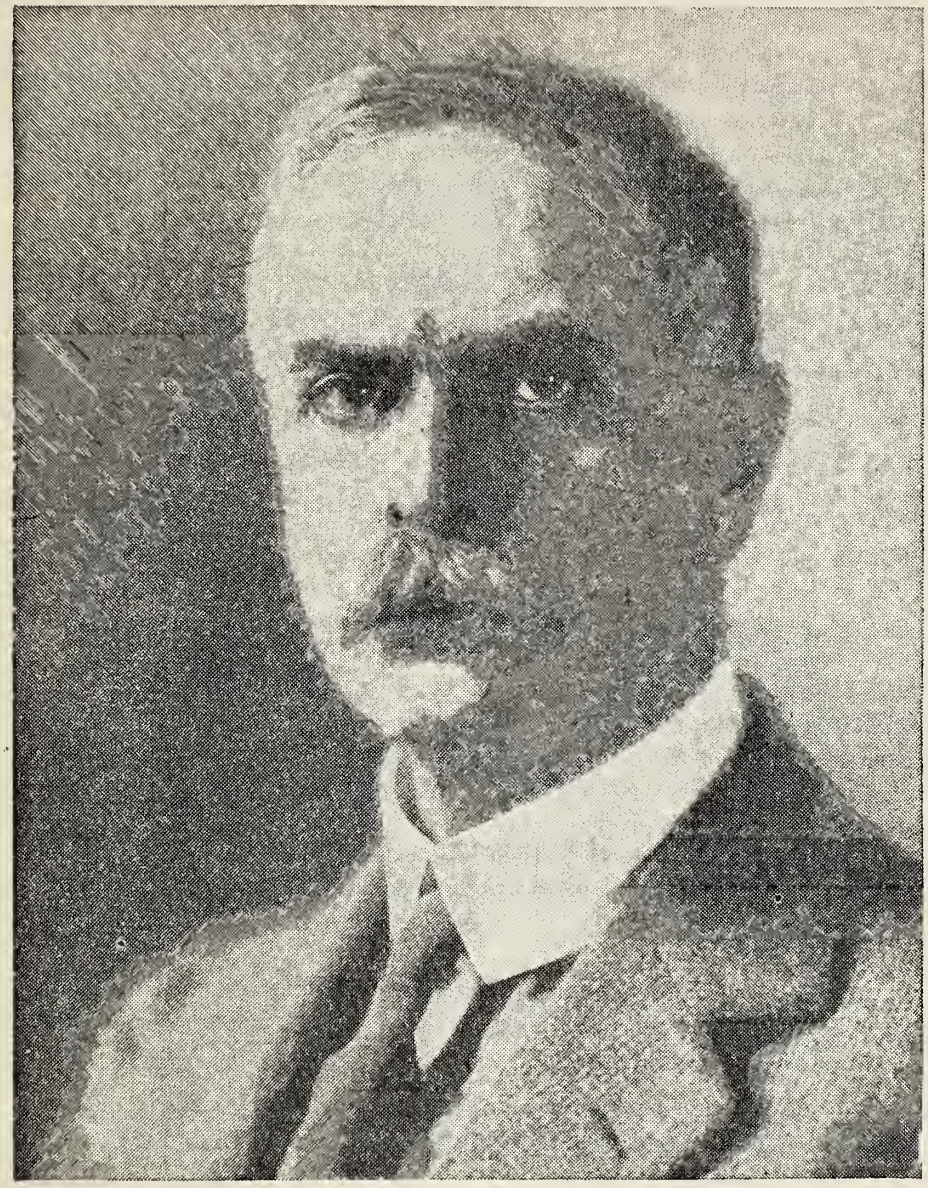


FIG. 11. Karl Landsteiner (1868–1943). (Courtesy of Dr. F. L. Campbell, Editor, *Scientific Monthly.*)

development of accurate laboratory tests for the diagnosis of disease, and has led to the establishment of diagnostic laboratories everywhere.

Microbiology has revealed the parts played by soil, water, sewage, milk, and insects in the transfer of disease germs, and special methods of protection have been developed. Moreover, the study of microbiology has taught scientists not only how to grow germs, but

how to exclude and avoid them, and so has furnished a rational basis for personal hygiene.

Immunologists have developed successful methods for increasing the resistance of individuals to a number of common diseases by the inoculation with vaccines or the blood serum of immunized animals.

Recently, marked advances have been made in *chemotherapy,* i.e., in the development and practical use of germ-inhibiting and germ-destroying chemicals, such as the various sulfonamide drugs and penicillin, for the treatment of infectious diseases.

Microbiology has thus become a complicated science, continually growing in scope and complexity as new facts emerge from research laboratories with bewildering rapidity. Also its usefulness increases, and it may be predicted confidently that the very real benefits to human welfare which have followed practical applications in the past, will be duplicated many times over as knowledge advances in the future.

REFERENCES

Ehrlich, Paul (1854–1915)
MARQUARDT, M.: "Paul Ehrlich," *Lancet,* 1935, 2:449.

Jenner, Edward (1749–1823)
JENNER, E.: "Three Original Publications on Vaccination Against Smallpox," *Harvard Classics* 38:149.

Koch, Robert (1843–1910)
BROWN, L.: "Robert Koch 1843–1910), An American Tribute," *An. Med. Hist.,* 1935, 7:99, 292, 385.

Landsteiner, Karl (1868–1943)
HEIDELBERGER, MICHAEL: "Karl Landsteiner," *Science,* 1943, 98:233.
"Karl Landsteiner, 1868–1943," *J. Immun.,* 1944, 48:1.

Lister, Joseph (1827–1912)
GODLEE, R. J.: *Lord Lister.* London: Macmillan & Co., 1918.
KEEN, W. W.: "Before and After Lister," *Science,* 1915, 41:845.
LISTER, J.: "On Antiseptic Principle of Practice of Surgery," *Harvard Classics,* 38:269.

Metchnikoff, Elie (1845–1916)
METCHNIKOFF, O.: *Life of Elie Metchnikoff (1845–1916).* New York: Houghton Mifflin Co., 1921.

The Microscope
FORD, W. W.: "Development of Our Early Knowledge Concerning Magnification," *Science,* 1934, 79:578.
GAGE, S. H.: *The Microscope.* Ithaca, N. Y.: Comstock Co., 17th Ed., 1941.

Pasteur, Louis (1822–1895)
DUCLAUX, E.: *Pasteur: History of a Mind,* Tr. by E. F. Smith and F. Hedges. Philadelphia: Saunders, 1920.
ROUX, P. P. E.: "Medical Work of Pasteur," Tr. by E. F. Smith, *Scient. Monthly,* 1925, 21:365.

VALLERY-RADOT, R.: *The Life of Pasteur,* Tr. by Devonshire, New York: Garden City Pub. Co., 1926.

Smith, Theobald (1859–1934)

GAGE, S. H.: "Theobald Smith, Investigator and Man," *Science,* 1926, 84:117.

Sternberg, George M. (1838–1915)

STERNBERG, M. L.: *George Miller Sternberg, Biography.* Chicago: Am. Med. Association, 1920.

Welch, William H. (1850–1934)

KAGAN, S. R.: "Dr. William Henry Welch (1850–1934)," *Med. Rec.,* 1938, 147:267, 317.

REVIEW QUESTIONS—CHAPTER III

1. Outline briefly the life of Joseph Lister.
2. Describe what Lister did to prevent the inflammation of wounds. What is the principle behind his antiseptic methods? What is the importance of Lister's work in the history of medicine?
3. Outline briefly the life of Louis Pasteur.
4. Describe Pasteur's work on silkworm disease.
5. Describe Pasteur's studies of the cause of anthrax. What importance has this work?
6. How did Pasteur discover the principle of protective inoculation (or vaccination) against disease?
7. Who had already applied this principle for protection against smallpox, and when? Explain the origin and present meaning of the terms vaccine, vaccination.
8. What did Pasteur's "anthrax vaccine" contain? Explain why the germs in the vaccine were weakened. How did Pasteur demonstrate the value of the vaccine for the prevention of anthrax?
9. For what other disease did Pasteur successfully develop a system of protective inoculations?
10. Define: (*a*) simple microscope, (*b*) compound microscope.
11. Who made the first good simple microscopes?
12. Outline the story of the invention and improvement of the compound microscope, up to 1880.
13. What did Ernest Abbe contribute to the perfection of the modern microscope?
14. Outline briefly the life of Robert Koch.
15. What was Koch's contribution to the study of anthrax?
16. How did Koch improve microscopical methods?
17. Describe Koch's plate method of isolating pure cultures. Explain why the introduction of liquefiable solid culture media and the plate method was an important advance in bacteriology.
18. Name important disease germs which Koch discovered.
19. Describe Koch's work on tuberculosis, and discuss its importance.

20. In addition to their own discoveries, what contribution did Pasteur and Koch make to the advancement of bacteriology.
21. Name three early American bacteriologists.
22. Name the bacteria causing some important diseases, and tell when and by whom the germs were discovered.
23. Describe the discovery of filtrable viruses. Name several virus diseases of human beings.
24. Who discovered the rickettsiae, and when? What two human diseases are caused by these organisms in the United States?
25. How and why have Koch's laws been modified?
26. Mention seven important practical advances in the development of immunology.
27. Mention five ways in which microbiology has been of help in public-health work. What is the promise of chemotherapy?

CHAPTER IV

MICROORGANISMS: THEIR CLASSIFICATION AND THEIR PLACE IN NATURE

It is somewhat difficult to realize, until one gets used to the idea, that the extremely tiny microscopic creatures to be studied in microbiology are *living,* independent organisms. They possess all the essential properties of life, though in elemental form, and all their activities and characteristics result from the fact that they are *alive.*

Most microbes unicellular (one-celled) organisms. One of the most outstanding peculiarities of living substance is revealed when we examine its intimate structure under a microscope. No matter how diverse the outward form may be, we find that the bodies of all plants and animals are made up of well-defined microscopic units called *cells.* Each cell is a microscopic mass of living protoplasm, enclosed within a more or less rigid cell wall which is relatively thick in most plant cells and very thin in most animal cells. Usually the cell-substance (cytoplasm) contains a deep-staining central body called the nucleus.

The larger, more complex living things—plants or animals—are many-celled or *multicellular organisms.* They are composed of great numbers of cells which are combined into various *tissues,* each made up of cells of a different type, such as muscle cell, nerve cell, bone cell, etc. These tissues are combined to form the various *organs* of the body: the muscles, brain, lungs, liver, heart, and the rest.

But the bacteria and most of the other microorganisms are one-celled or *unicellular organisms.* They occur as single cells, each cell being an independent individual, having within itself all the properties of life.

Distribution of microbes; their place in nature. The multitude of tiny creatures making up the microscopic world show all imaginable degrees of complexity in structure and mode of life. The microorganisms probably represent the oldest class of living things.

It is not surprising, then, to find among them a great diversity of forms, and examples of adaptation to existence in almost every conceivable sort of environment.

Microbes of some kind may be found in every spot where life is possible. These ordinarily invisible organisms are abundant not only in the soil, in natural waters, and on vegetation everywhere in nature, but also in the dust of our rooms, in the air (where they are carried about on particles of dust), and in food and water. Moreover, great numbers of them live constantly on the skin and other body surfaces, and in the intestinal canal, of healthy men and animals.

Many microbes are capable of existing in resting, seed-like forms, which are highly resistant to drying, moderate heat, and other unfavorable conditions. It is these resistant forms (called *spores* or *cysts*) that survive in soil, dust, and air, as well as in situations where circumstances are unsuitable for growth and multiplication. When these forms reach a favorable environment, however, they germinate at once into active, growing organisms.

Obviously, not all these universally distributed microorganisms are capable of causing disease. As a matter of fact, the disease-producing microbes are decidedly in the minority. The one place in which microbes are *not* naturally to be found is within the healthy tissues of the living body. Among the great hosts of microscopic creatures, there are only about a hundred common varieties that are able to grow in living tissues, or otherwise damage them. The great majority of microbes perform functions which are useful to man, and some, as we shall describe, play an indispensable rôle in the economy of nature.

Some of the microbes now existing, such as certain bacteria found in soil and in water, appear to represent extremely simple and primitive forms of life. They seem to have retained the characteristics of those earliest forms of microbic life that must have been abundant on the earth long before higher plants and animals evolved. Other present-day species, however, especially those microbes causing communicable diseases, can hardly be regarded as primitive in a strict sense, for they now show extraordinary adaptations to a particular mode of life in association with particular living hosts, which must be the result of a long evolutionary development.

Size and form. As we have already learned, the seven principal groups of microorganisms are called: (1) *protozoa,* (2) *true fungi,*

(3) *mold-like higher bacteria,* (4) *spirochetes,* (5) *true bacteria,* (6) *rickettsiae,* and (7) *filtrable viruses.* An idea of the size and form of these different kinds of microbes (except the viruses, which we do not attempt to picture) may be gained from an inspection of Fig. 12. Here are illustrated some typical members of each of the classes and subgroups.

Individual microbes are so small that the highest powers of the microscope, giving magnifications of about 1,000 times, are required for their study, and their minute size can be expressed accurately only by the use of a special unit of measurement. This unit is the *micron* (μ); plural, *micra.* One micron is equivalent to *1/1,000 of a millimeter,* or about 1/25,000 of an inch. The drawings in Fig. 12 are made in proportion to the scale in micra shown at the bottom of the page.

Criteria for the classification and naming of microorganisms. Criteria for grouping and naming the microbes are found by studying their differences and similarities in form and size, in natural distribution, in food requirements and metabolic activities, in general physiological characteristics, in methods of reproduction, in disease-producing power (if any), and in the specific response (antibody formation) which they induce when they are used to immunize experimental animals. On the basis of the observed properties, taxonomists attempt to classify microorganisms just as the larger plants and animals are classified, deciding first which organisms belong in the *plant kingdom,* and which in the *animal kingdom,* and then further dividing and subdividing into groups in the usual sequence: (1) *Phylum,* (2) *Class,* (3) *Order,* (4) *Family,* (5) *Tribe,* (6) *Genus,* and (7) *Species.*

The scientific name of any individual microorganism is written in Latin and italicized, and is made up of two elements: (1) the name of the *genus,* spelled with a *capital* letter, followed by (2) the name of the *species,* always spelled, whether derived from a proper name or not, with a *small* letter. Thus, for example, we have such names as *Bacillus subtilis, Bacillus anthracis, Staphylococcus albus, Staphylococcus aureus,* and *Rickettsia prowazeki.* In each case, the first word gives the genus to which the organism belongs, the second gives the species name.

Difficulties in systematic classification. The recognition of certain typical representatives of each of the main groups of microbes is easy. Many difficulties are encountered, however, in arriving at a

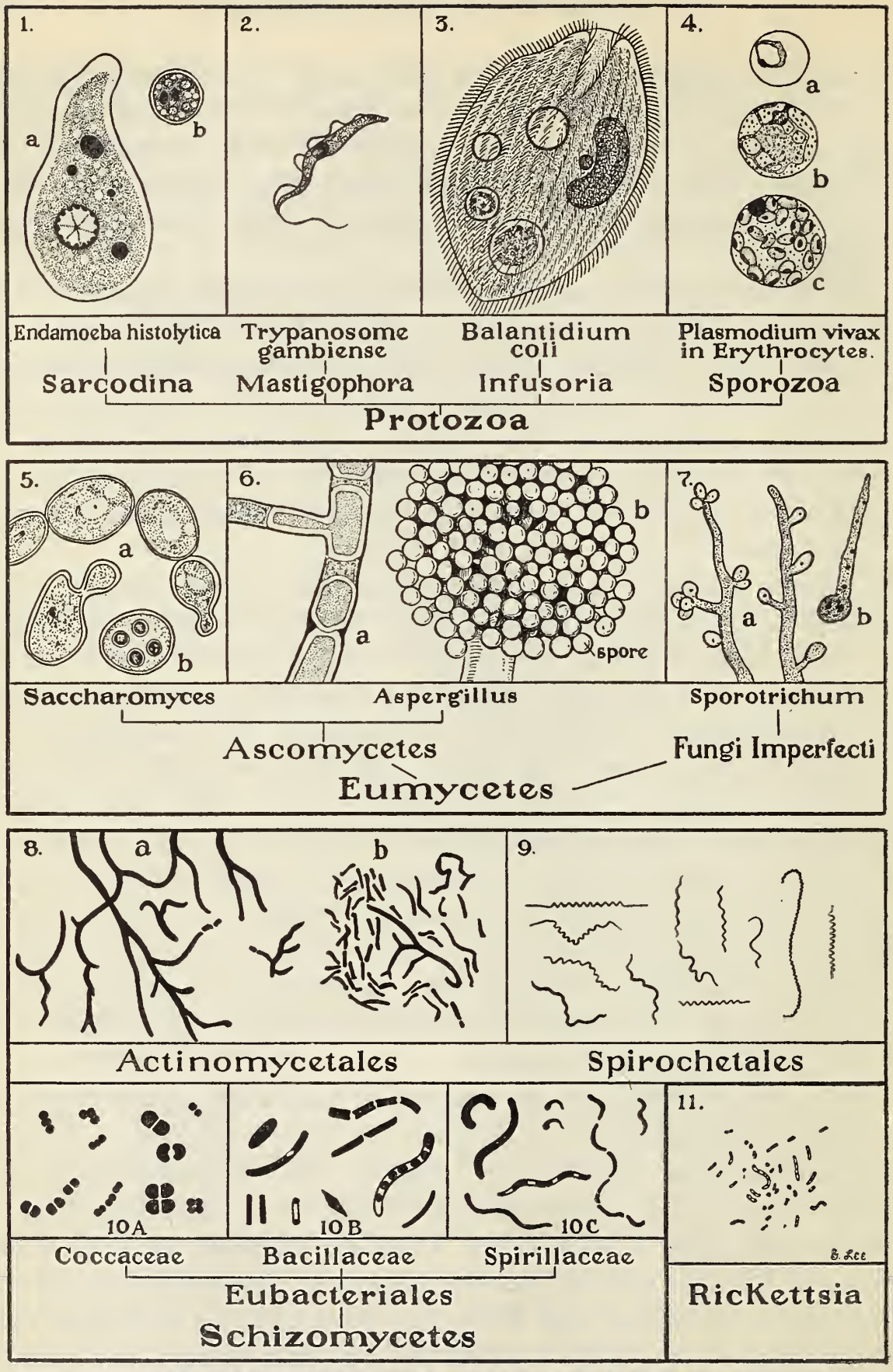


Fig. 12. Legend is on opposite page.

complete and logical classification of *all* the microbes. Their minute size and close similarity of form make it impossible to differentiate many kinds from related organisms by their morphology alone, and often no distinctive physiological characteristics can be demonstrated. It must be realized that all the microbes are to some extent interrelated, and Nature has not arranged them in sharply defined types for the convenience of the student, but instead has permitted the survival of numerous organisms which appear to be intermediate or transitional forms between the main groups.

Are microorganisms plants or animals? Even the first necessary step of placing each kind of microscopic life in either the plant or the animal kingdom is not as easy as it might seem at first thought. Differences between the simple plants and the simple animals are not very clear-cut, and, when we come to such lowly creatures as microorganisms, there is no rule by which to place them. There is no single characteristic which clearly separates the simplest animals from the simplest plants. The bacteria and other microorganisms really stand at the border line between the plant and animal kingdoms, and many microbes possess in some degree both plant-like and animal-like characteristics.

However, certain of the larger organisms, for example the amebae and the paramecium commonly studied in courses in general biology, have so many properties similar to those of the higher animals that they belong unquestionably in the animal kingdom. These microbes, together with the trypanosomes and other flagellated organisms, and forms that live, like the malaria parasites, in the cells of infected hosts, comprise the phylum *Protozoa* (first or simplest animals).

FIG. 12. Types of microorganisms, drawn to scale. The *Eumycetes* are the true fungi; the *Actinomycetales* are the mold-like higher bacteria; the *Spirochetales* are the spirochetes; and the *Eubacteriales* are the true bacteria. 1a, the trophozoite or growing form; 1b, the cyst; 4a, early signet-ring form of the protozoan within the red blood cell; 4b, later stage in the development of the parasite, accompanied by enlargement and stippling of the erythrocyte; 4c, final stage just before the cell bursts releasing the many small forms of the parasite (merozoites), which may then enter other red blood cells, thus continuing the asexual cycle in the human blood; 5a, budding of yeast cells; 5b, an ascus containing ascospores; 6a, portion of a branching filament, and 6b, part of the "head," or fruiting body, of a common mold; 7a, the mature fungus with conidia (reproductive spores) developing from the filaments; 7b, a new filament growing out from a spore; 8a, filamentous growth of *Actinomyces;* 8b, fragmentation of the filaments into bacteria-like rods; 10a, b, c, typical forms of the common bacteria.

All the other microorganisms are most appropriately classed in the *plant kingdom.* This decision is based principally on the fact that, while, like higher animals, the typical protozoa receive food particles into the interior of their bodies, assimilate the nutritious portions, and extrude the residue, the other microbes are nourished, like plants, by absorption through their cell walls of food substances in solution.

A useful classification. Despite the difficulties, separation of the multitudinous types of microbes into the chief groups and subgroups has been accomplished to the satisfaction of most scientists and, although no one scheme is free of all fault, it is possible to arrange the principal groups into a useful working classification, and to trace with some certainty their relationship to one another. This has been done in the accompanying chart (Table II).

In this chart, the *Protozoa* are lettered in italics and enclosed in a box to remind the student that these microbes alone belong to the animal kingdom, whereas all the others are subdivisions of the plant kingdom.

CLASSIFICATION ACCORDING TO DISTRIBUTION AND ACTIVITIES

Saprophytic and parasitic microbes. Among all these many kinds of microorganisms, two groups may be recognized on the basis of their distribution in nature and the character of their food requirements: (1) saprophytes, and (2) parasites.

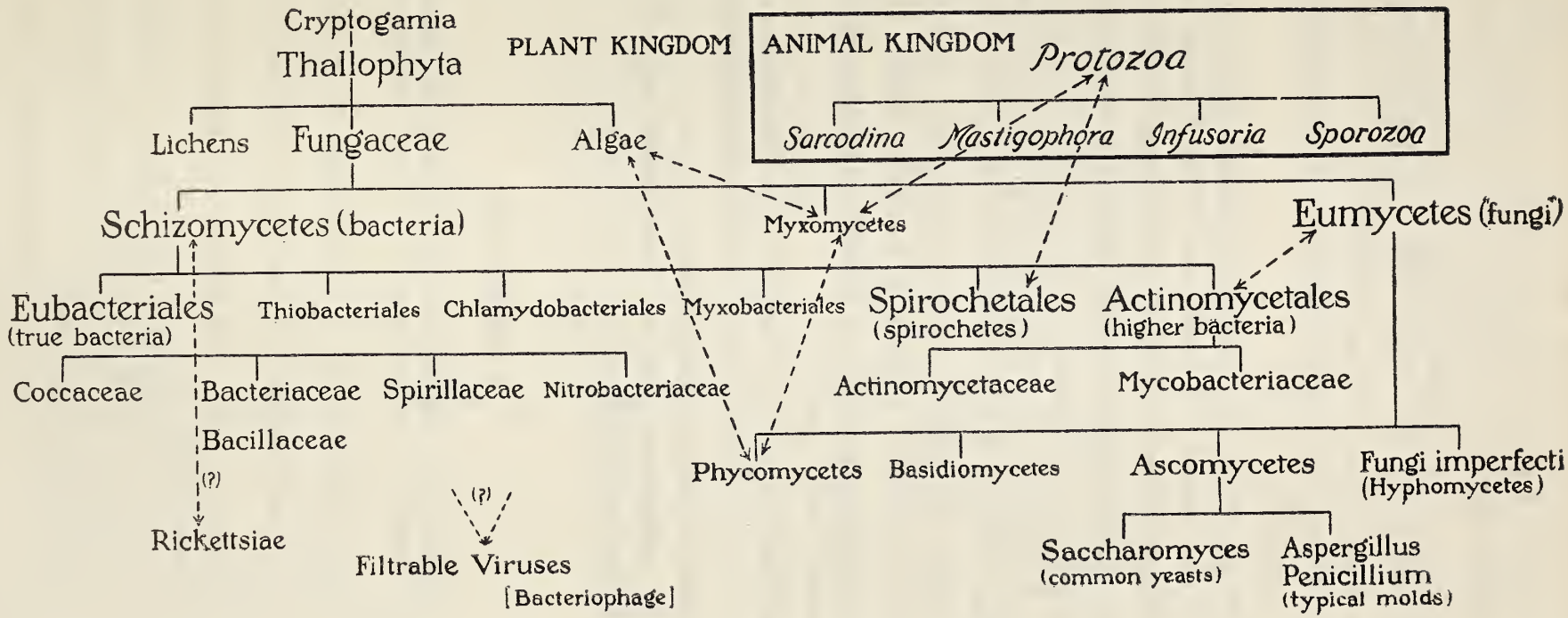
Saprophytes ("rot-plants") are so called because the most conspicuous result of their growth is the rotting or decomposition of organic matter. Organisms of this class are *free-living* varieties, widely distributed in the soil, in natural waters, and elsewhere throughout nature, and *their food is nonliving matter.*

Parasites are adapted to living on or in the living bodies of higher plants, animals, or human beings. Their most conspicuous property is their dependence upon other living things; *their food is derived more or less directly from living matter.*

Pathogenic and nonpathogenic microbes. Forms of parasitism. It is important to remember that *almost all the saprophytic organisms and many parasitic microbes are entirely harmless* (nonpathogenic) and have nothing to do with the production of disease.

Many of the bacteria and other kinds of microbes found con-

TABLE II. Relationships Among the Principal Groups of Microorganisms



stantly inhabiting the mucous membranes or other body surfaces are truly parasitic, for they are dependent for existence upon their relationship with their living host, and yet they are never responsible for disease. These harmless parasites are referred to as *commensals,* a word meaning "those who partake of the same food, or eat at the same table." Commensalism is an association in which the microorganism, i.e., the commensal, derives benefit, but neither injures nor benefits the host.

Most of the *pathogenic* (disease-producing) organisms, especially those causing the naturally communicable diseases, are adapted to a rather strict parasitic life. The pathogenic microbes are parasitic organisms which not only can live upon the *surface* of the body, as the nonpathogenic organisms do, but also can multiply *within the tissues* of the body. In most cases, they grow in the body fluids outside the tissue cells, and may be said to be *extracellular,* but some important germs, including all the rickettsiae and filtrable viruses, penetrate the body cells, assume a position inside the cell, and develop there. Organisms of this latter type are therefore truly described as *intracellular parasites.*

Infection. When microorganisms enter the body tissues and cause injury, we say an *infection* has occurred; the individual is *infected,* and suffers from an *infectious disease.*

Most of the infectious diseases are rightly regarded as examples of a special form of parasitism.

REFERENCES

Bergey, D. H., Breed, R. S., Murray, E. G. D. and Hitchens, A. P.: *Bergey's Manual of Determinative Bacteriology.* Baltimore: Williams and Wilkins Co., 5th Ed., 1939.

Buchanan, E. D. and Buchanan, R. E.: *Bacteriology.* New York: The Macmillan Co., 4th Ed., 1938.

Frobisher, M., Jr.: *Fundamentals of Bacteriology.* Philadelphia: W. B. Saunders Co., 3rd Ed., 1944.

Gay, F. P. and Associates: *Agents of Disease and Host Resistance.* Springfield, Ill.: Chas. C Thomas, 1935.

Henrici, A. T.: *Biology of Bacteria; Introduction to General Microbiology.* Boston: D. C. Heath and Co., 2nd Ed., 1939.

Jordan, E. O. and Burrows, W.: *Textbook of Bacteriology.* Philadelphia: W. B. Saunders Co., 14th Ed., Revised, 1945.

Kolle, W. and Hetsch, H.: *Experimental Bacteriology* (2 vols.). New York: The Macmillan Co.

Park, W. H. and Williams, A. W.: *Pathogenic Microorganisms.* Philadelphia: Lea and Febiger, 11th Ed., 1939.

STANIER, R. Y. and VAN NIEL, C. B.: "Main Outlines of Bacterial Classification," *J. Bact.*, 1941, 42:437.

TANNER, F. W.: *Bacteriology*, New York: John Wiley and Sons, 3rd Ed., 1937.

TOPLEY, W. W. C. and WILSON, G. S.: *Principles of Bacteriology and Immunity*. Baltimore: William Wood & Co., 2nd Ed., 1936.

ZINSSER, H. and BAYNE-JONES, S.: *Textbook of Bacteriology*. New York: D. Appleton-Century Co., 8th Ed., 1939.

REVIEW QUESTIONS—CHAPTER IV

1. What is meant by: (*a*) multicellular organisms, (*b*) unicellular organisms? Give examples.
2. Discuss the distribution of microorganisms, and their place in nature.
3. Name the seven principal groups of microorganisms.
4. What unit of measurement is used to express the size of microbes? Compare the different types of microbes, as to size.
5. What are the main groups and subgroups into which living organisms are classified?
6. What constitutes the scientific name of an organism? How is it properly written?
7. What are the *Protozoa*? Why are all the microbes, except the protozoa, classed in the plant kingdom?
8. What are the two great classes of microbes on the basis of distribution in nature and food requirements?
9. Define *saprophytic, parasitic, pathogenic, nonpathogenic microbes.* What is meant by *commensalism, intracellular parasite, infection*?
10. Give examples to illustrate the meaning of all these terms. In general, what proportion of microbes is harmful?

CHAPTER V

OUTSTANDING PROPERTIES OF THE PRINCIPAL KINDS OF MICROBES

No matter what we may choose to examine—whether it be milk or other foodstuff, water from some pond or river, soil, dust from the air, excreta, or material taken from the body surfaces of living animals or men—we always find present a teeming population of active microorganisms. In almost every case, we find not only bacteria, but representatives of several of the other classes of microbes mentioned in the preceding chapter. In the following sections, the chief characteristics of these various kinds of microbes will be outlined.

PROTOZOA

The *Protozoa* are unicellular organisms, the lowest forms of life classified in the animal kingdom. Their single-celled form distinguishes them from the *Metazoa*—the larger, multicellular animals.

Free-living and parasitic protozoa. Two great groups among the protozoa may be recognized: (1) the *free-living* (saprozoic) forms, and (2) the *parasitic* forms. Harmless, free-living protozoa, like the common amebae and paramecia, are found everywhere in nature. Parasitic protozoa live in association with practically every kind of living animal, and with man; most of them cause no harm to their hosts, but some are responsible for serious diseases. The parasitic species can be subdivided, therefore, into (a) the harmless kinds, called *commensals,* and (b) the frankly disease-producing (*pathogenic*) kinds. Protozoa of the commensal type are commonly found, for example, in the intestinal contents of men and animals. The pathogenic kinds include the germs of malaria, amebic dysentery, and other important infections (Chapter XLII).

Morphology and physiology. Some of the outstanding morphological features of the protozoa are illustrated in Fig. 13. Among

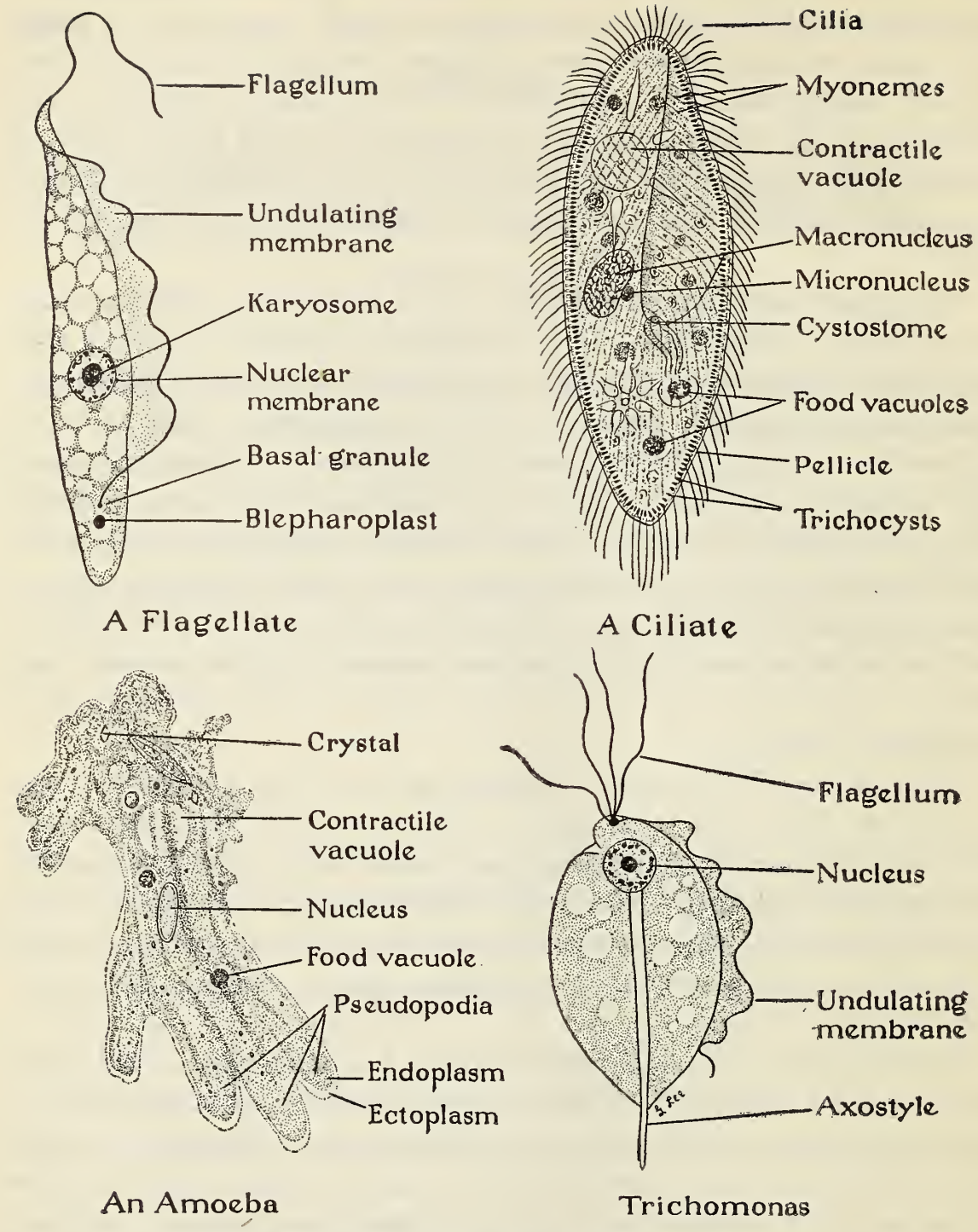
these creatures are to be found the largest and most complicated of all single-celled organisms. Although the whole organism is a single cell, many protozoa have a most complex internal structure, including an arrangement of organelles for the capture, digestion, and storage of food, for the excretion of liquid and solid wastes, for protection and support of the body, and for reproduction. These organelles function like the organs of higher animals (Figs. 12, 13, 14).

The great majority of protozoa, both free-living and parasitic, are distinctly animal-like in their nutritional habits, since they take into their bodies, often through a specialized mouth, solid food particles, digest these and then cast out the indigestible residue. There are some exceptions to this general rule, however, notably the parasitic protozoa of the class *Sporozoa,* which are nourished only by direct absorption of soluble food materials through their cell walls. Despite their specialized physiological needs, many protozoa, including pathogenic varieties, may be grown in test tubes in the laboratory on media containing blood and other complex protein substances, similar to the concoctions used for the cultivation of pathogenic bacteria.

Encystment. The wide occurrence of free-living protozoa is due in part to their ability to *encyst,* that is, to form resistant cysts. Most of the parasitic protozoa can encyst when their immediate environment is drying up or otherwise becoming unfavorable. Cysts of the intestinal protozoa form in the lower intestine and are voided in the feces. Fecal material thus becomes the source of infection for new hosts.

Reproduction. *Asexual reproduction.* A simple form of multiplication, seen for example in the paramecia and other ciliates and in amebae, consists of a *simple fission* of the nuclei followed by a splitting of the cytoplasm across the short axis into two approximately equal parts. In another form of the reproductive process, common in flagellates, the nucleus first undergoes more or less complicated changes, similar to the mitosis which regularly occurs when cells of higher plants and animals multiply, then the organism splits into two parts by *longitudinal division* of the cytoplasm.

In *Sporozoa* the characteristic method of reproduction is by *multiple division* (sporulation). The mature protozoan becomes divided, throughout, into a number of daughter cells, each with its own nucleus, thus forming a mass of new cells, which finally separate



Diagrams Illustrating some of the Morphological Characteristics of the Protozoa

FIG. 13. Morphological features of the Protozoa. (A ciliate, redrawn and modified from Wenyon's *Protozoology* (2 vols.). Wm. Wood and Company, New York, 1926; other figures, redrawn and modified from Kudo's *Protozoology,* Chas. C Thomas, Springfield, Illinois, 1939.)

and develop individually. When this process of multiple division is entirely asexual, and occurs without preliminary formation of a cyst, it is called *schizogony,* and the new cells are called *merozoites.* A similar process occurring within a cyst, and (usually) after the encysted female cell has been fertilized by previous union with a male cell, is called *sporogony,* and the new cells are called *sporozoites* (Fig. 142).

Sexual reproduction; life cycles. At some time in the development of practically all varieties of protozoa, the asexual processes of multiplication mentioned above are interrupted, and sexual phenomena intervene. Sexual activities among the protozoa involve the coming together of individuals of the same species in such a manner that nuclear material is exchanged or fused.

When this union is temporary it is called *conjugation.* The individuals concerned, after attaching themselves to each other for a time, during which nuclear matter is exchanged, separate completely and each then continues to grow and multiply independently.

When the union is permanent, and the substance of the two cells becomes fused, this is called *copulation.* The individuals taking part here are sometimes morphologically alike, but more commonly they differ in size and form in a manner comparable to the spermatozoon and ovum of the multicellular animals. In the highest development of this sexual type of multiplication the entire process is amazingly similar to reproduction among higher animals.

The series of changes a protozoan undergoes from one act of fertilization to another constitutes the *life cycle* of that organism. Many parasitic protozoa pass through most complicated life cycles, during which they may assume quite different forms, and multiply in different ways. Many pathogenic species pass the sexual part of their cycle exclusively in one host, and the asexual part in another host. For example, the malaria parasites develop, in the sexual phase of their cycle, only in the body of the malaria-carrying *Anopheles* mosquitoes, where they multiply by sporogony; but within the red blood cells of their human hosts, they multiply only asexually, by schizogony (Fig. 142).

Classes of protozoa. As we have indicated in Table II, there are four main classes of protozoa. These are:

(1) **Sarcodina** ("flesh-like" organisms), the most important subgroup of which is the **Amebae.** These exist as naked globules of protoplasm, and

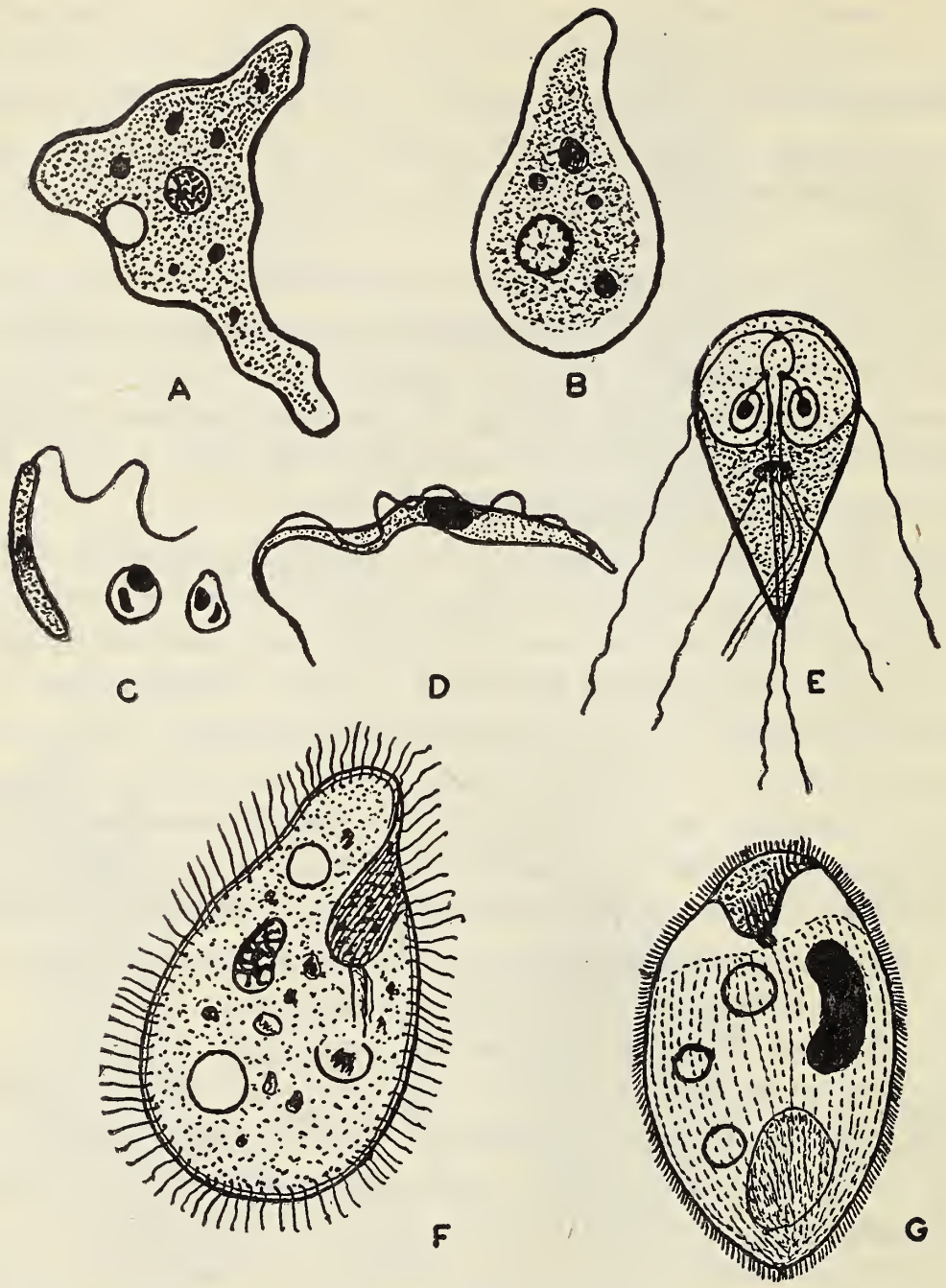


FIG. 14. Various protozoa. A: a harmless ameba; B: *Endameba histolytica,* the cause of amebic dysentery; C: forms of *Leishmania,* a kind of protozoan causing sores in the skin. D: a *trypanosome,* a flagellated protozoan of the type causing African sleeping sickness; E: *Giardia lamblia,* a common intestinal parasite of man; F: *Colpoda,* a harmless ciliated protozoan common in nature; G: *Balantidium coli,* a ciliate sometimes found in the intestine of man. (Figures D, E, and G redrawn from Hegner, Cort and Root's *Outline of Medical Zoology,* Macmillan Company, New York, 1927.)

are characterized by the use of *pseudopodia* as organs of locomotion and for the capture of food.

(2) **Mastigophora** ("whip-bearing" forms), called the **Flagellates.** These swim about by means of long whip-like projections from the cyptoplasm, called *flagella.*

(3) **Infusoria,** also called the **Ciliates.** These have the most elaborate internal structure of all protozoa. They move about actively, propelled by the sweeping movement of innumerable short and delicate hair-like processes, called *cilia,* on their surface.

(4) **Sporozoa.** These protozoa have no motor organs. They are relatively small, and they all live as *parasites* in the cells or tissues of animals. At some stage in their life cycle they reproduce by the formation of multiple *spores* (merozoites or sporozoites).

Amebae. An ameba is a truly primitive microscopic animal of very simple make-up. Its single-cell body is a tiny mass of living matter without special internal structures, except a nucleus. In the cytoplasm, however, there are numerous vacuoles and various other inclusions (Figs. 13 and 14).

Amebae are characterized by their curious method of locomotion. They continually send out, now from one and then from another part of their surface, blunt projections called *pseudopods* ("false feet"). Some of the semi-fluid cytoplasm of the ameba *flows* into each new pseudopod, while the older pseudopods go back into the general mass. Thus the animal moves about, in slow and irregular fashion, never progressing long in any one direction. We can now understand the appropriateness of the term *ameba,* which is derived from a Greek word meaning *change.*

The flowing method of locomotion is seen not only in the amebae, but in certain cells which make up the bodies of higher plants and animals. For example, the white blood cells (leukocytes) of the human blood are able to move about in this way. This kind of movement is so characteristic of the amebae that, wherever observed, it is referred to as *ameboid motion.*

Free-living amebae, such as *Ameba proteus,* reach large proportions, some having a diameter of about 600μ (Figs. 13 and 14). These organisms may be found in infusions made with dead leaves or grass, or in the material scooped from the bottom of shallow, quiet ponds.

Parasitic amebae are regularly found living in association with animals of all kinds, and also with man. Most of them are harmless commensals, deriving benefit to themselves from the association,

but neither injuring nor benefiting their hosts. Examples of harmless species in human beings are the *Endameba gingivalis,* an ameba that is occasionally found in the tartar about the teeth, and *Endameba coli,* a common commensal in the intestine. The latter species is important in medicine because it may be confused with *Endameba histolytica,* the ameba that causes amebic dysentery and other forms of amebiasis in human beings. This disease is so important that we give it special consideration in Chapter XLII.

Flagellates. This class of protozoa, known also as the *Mastigophora* ("whip-bearing" forms), swim about by means of long, delicate processes called *flagella,* extending from the cell surface. By the whip-like lashing of these flagella the organisms are propelled along, usually in a rather slow and irregular fashion. In addition to flagella, some species possess an *undulating membrane* which also assists in locomotion. There are numerous distinct varieties of flagellates, showing great differences in size and details of structure. Each kind has a definite and characteristic shape, usually more or less oval, and it is always the front end that bears the flagella.

Among the free-living flagellates are some common forms that are distinctly plant-like, since they contain chlorophyll and make their own food by photosynthesis. Examples are *Euglena* and *Volvox* (Fig. 15). These organisms sometimes grow so abundantly in the water of reservoirs that the oily secretions from their bodies add a disagreeable odor to the drinking water.

Other kinds of flagellates, whether free-living or parasitic, are animal-like, capturing and ingesting their food in the fashion of typical protozoa.

Parasitic flagellates include several species that are found in the digestive tract or on the genitalia of man and animals. The best known of these belong to the genera *Giardia* and *Trichomonas* (Figs. 13 and 14, Chapter XLII).

The most important *pathogenic* flagellates are the *Trypanosomes* and the *Leishmania* (Fig. 14). These organisms are responsible for African sleeping sickness and other serious and common tropical diseases (Chapter XLII).

Infusoria. This class of protozoa is appropriately named, for there are a great number of free-living species, and various representatives of the group are always to be found in conspicuous numbers in the infusions of hay, dead leaves and grass, and similar materials generally used for laboratory study. These organisms are also called

Ciliates, since they are all characterized by the possession of short, hair-like processes on their body surfaces, known as *cilia.* The sweeping movement of these cilia propels the organism rapidly and smoothly through liquids.

The ciliates have the most elaborate internal structure of all protozoa. There are two kinds of nuclei, a large one which apparently directs the general activities of the cell, and a small nucleus which seems to be concerned largely with control of reproductive processes. Although the whole organism is a single cell, we find within it a remarkable system of miniature organelles which function, like the mouth, stomach, and other organs of higher animals, for the capture and ingestion of food, for the excretion of waste, and in reproduction and other vital activities (Fig. 13).

Among the best-known of the *free-living Infusoria* are the several varieties of paramecia, for example, *Paramecium caudatum* (Fig. 13). Other kinds, likely to be seen in infusions, include *Colpoda, Didinium, Euplotes,* and *Stylonichia* (Fig. 15).

The only species of ciliate that is at all common as a parasite in human beings is *Balantidium coli* (Fig. 14). This organism is of medical importance, since it may be responsible for a mild dysentery (Chapter XLII).

Sporozoa. All the members of this class are relatively small, nonmotile organisms, adapted to an exclusively parasitic life. Many have complicated life-cycles, involving existence in two quite different hosts in succession. They all form reproductive spores at some time in their development—hence the name.

In practically every type of animal, parasites of the class *Sporozoa* may be found, living in the blood cells, or other tissues, or in body cavities. Some produce no evident illness in their hosts, while others are responsible for some of the most severe and widespread of protozoan diseases (Fig. 12:4).

By far the most important pathogenic varieties are the parasites of human *malaria.* (Similar organisms infect monkeys and birds.)

The species that cause human malaria are called *Plasmodium vivax, Plasmodium malariae, Plasmodium falciparum,* and *Plasmodium ovale.* They undergo an asexual development in the red blood cells of their human hosts, and a sexual transformation in the digestive tract of mosquitoes of the genus *Anopheles* (Fig. 142).

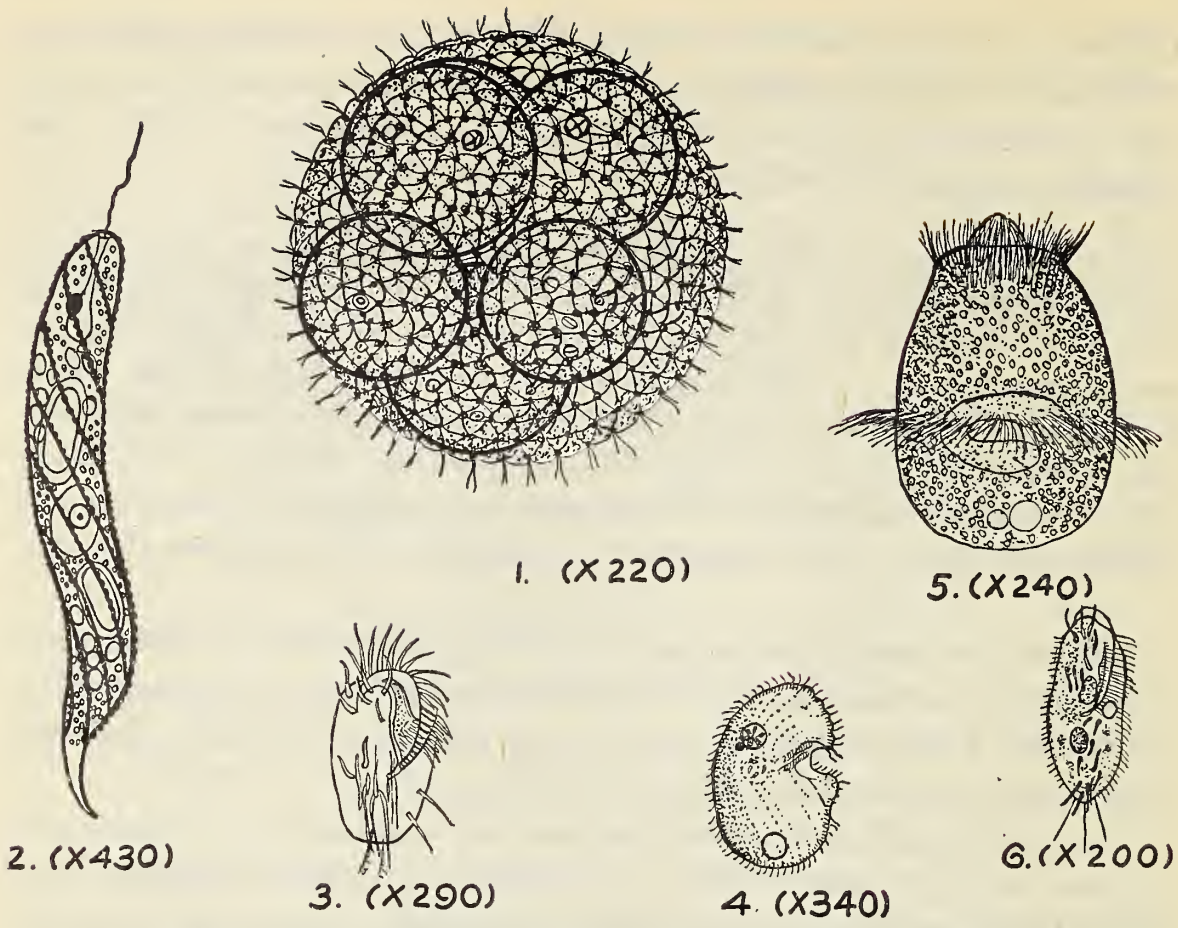


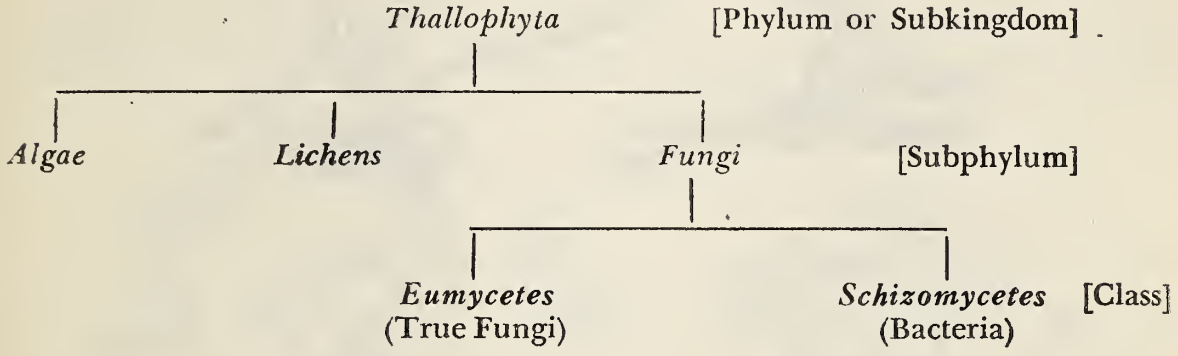
FIG. 15. Representative species of some varieties of protozoa (other than amebae and paramecia) that may be seen in fresh-water ponds, or in infusions of hay, dead leaves, or grass. The relative sizes of the different organisms may be judged by the magnifications given.

1. *Volvox aureus.* Drawing shows a typical colony; such a colony may have a diameter of 500–800μ. It consists of thousands of flagellated, pear-shaped organisms, each 5–9μ in diameter, embedded in a gelatinous matrix. The colony is green, because of the presence of green chromatophores (chlorophyll bodies). 2. *Euglena spirogyra.* One of the numerous species of *Euglena,* common in stagnant water. These organisms may form a green film on the surface of the water, or green spots on the bottom of a pool, because their bodies contain numerous green chromatophores. This species is a sluggishly motile form, about 100μ long. 3. *Euplotes patella.* This ovoid-shaped protozoan is 80–150μ long. The various species of *Euplotes* are Ciliates, but instead of numerous fine cilia, a smaller number of heavier appendages called *cirri* (representing fused and modified cilia) are present. 4. *Colpoda cuculus.* This actively motile organism is the most common ciliated protozoan in ordinary hay infusions. It is about 80μ long and has 8–10 indentations on its anterior edge. 5. *Didinium nasutum.* A common species of these remarkable, barrel-shaped ciliates. The ovoid body, which is highly granulated, has two girdles of cilia, and is 80–200μ long. These organisms are predatory, feeding on other ciliates, especially Paramecium. 6. *Stylonychia notophora.* Members of this genus of protozoa move along a surface as if walking on their large ventral cirri. This species is about 125μ long. (Drawn by E. M. Shackelford, after Kudo's *Protozoology,* Chas. C Thomas, Publisher, Springfield, Illinois, 3rd Ed., 1946.)

THE PLANT-LIKE MICROBES: BACTERIA AND TRUE FUNGI

The two great Classes among the plant-like microbes are the *Bacteria* (*Schizomycetes*), and the *True Fungi* (*Eumycetes*). The following diagram shows how these main groups fit into the plant kingdom:

Table III. The Classes of Plant-like Microbes



The phylum or subkingdom of *Thallophyta* contains those primitive plants that consist merely of a plant body (called a *thallus*), without differentiation into leaves, stems, or roots. The *Thallophyta* are divided into two main groups: (1) the *Algae,* which are provided with chlorophyll, like the familiar, higher green plants, and (2) the *Fungi,* which are colorless and devoid of chlorophyll.

Lichens are peculiar vegetations consisting of algae and fungi living together in a symbiotic relationship. They are commonly seen as scale-like growths covering tree trunks or rocks.

The most familiar *Algae* are those tiny green or brown plants which are often seen forming a scum over the surface of a quiet pond. Many of the algae are microscopic and are shaped very much like some of the bacteria. They are considerably larger, however, and are colored green, like the familiar plants, whereas bacteria are colorless. The seaweeds are a form of algae.

To the *Fungi* belong all the many varieties of lowly vegetable organisms which lack chlorophyll. Some of these plants, such as the common mushroom, are large, and visible to the naked eye. The majority are microscopic, however, and among them are the microorganisms in which we have the greatest interest; namely, the *true fungi* and the *bacteria.*

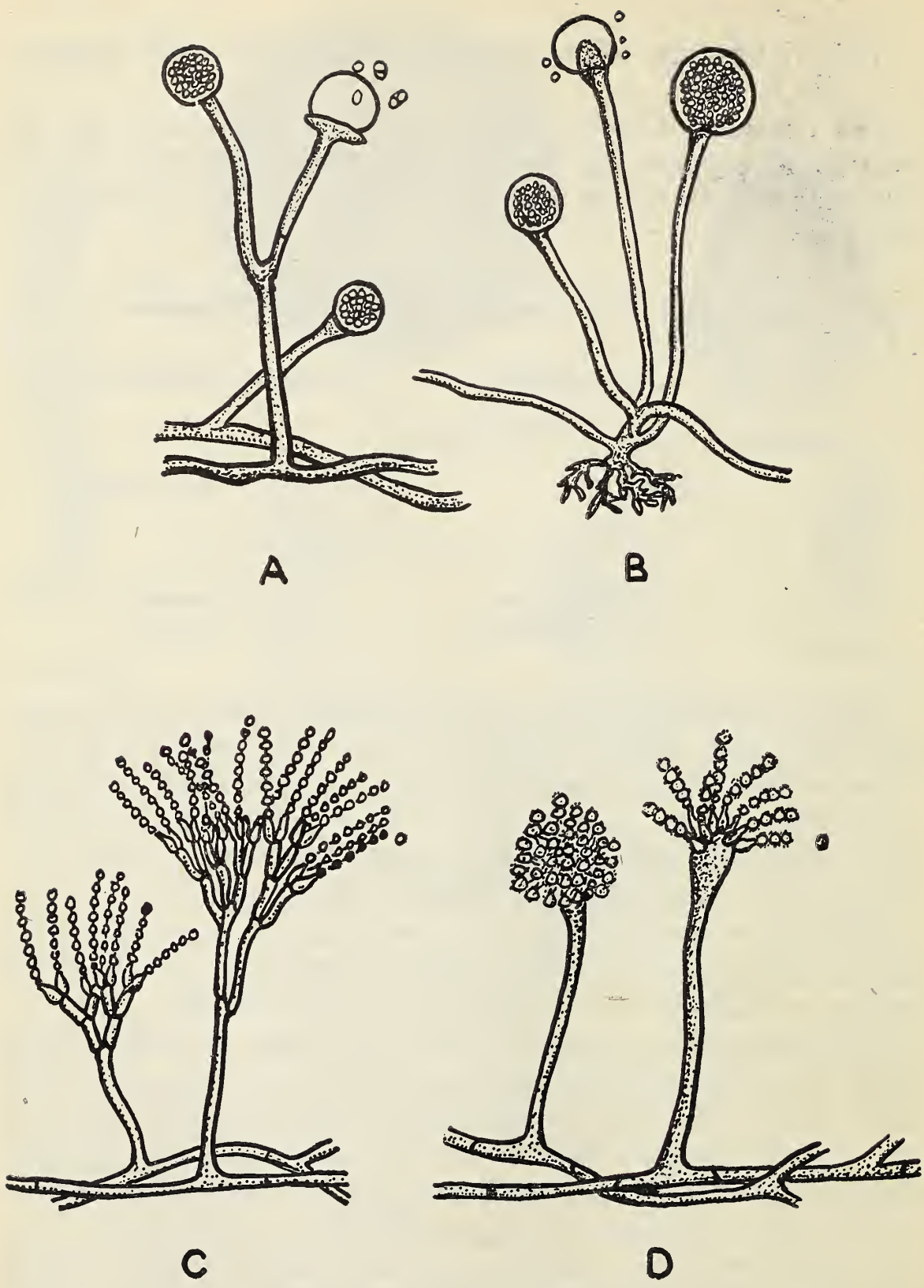


FIG. 16. The fruiting bodies (structures for formation of asexual reproductive spores) in common molds. A, *Mucor;* B, *Rhizopus;* C, *Penicillium;* D, *Aspergillus.*

TRUE FUNGI (EUMYCETES)

The *Eumycetes* (see Table II) comprise the class of microbes ordinarily referred to when we use the term *fungi* without qualification. Included are the organisms generally called the *yeasts* and the *molds,* as well as another large group, known as the *fungi imperfecti.* Detailed study of these microbes is the science of *Mycology.* A disease caused by a true fungus is called a *mycosis,* or a *mycotic disease;* also a *fungous disease.*

Fungi of many different kinds are widely disseminated. The majority are saprophytic, and only a few are adapted to a fully

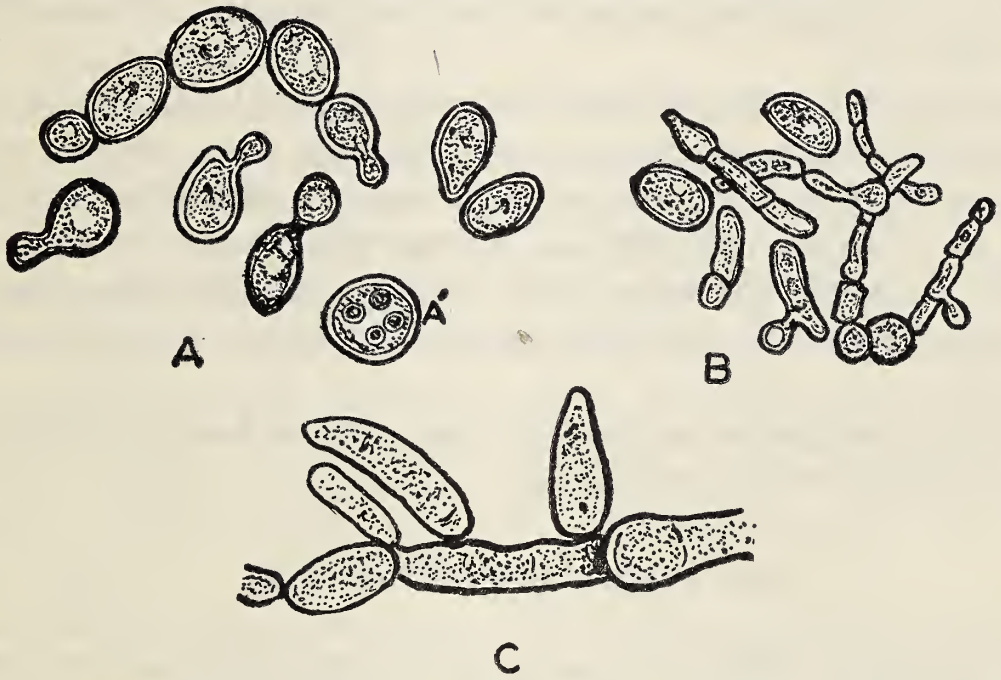


FIG. 17. Yeasts. A: Common harmless yeast (*Saccharomyces*), showing budding. A': an ascospore, a special reproductive body sometimes formed by true yeasts. B: *Blastomyces,* a pathogenic yeast. C: *Monilia,* a yeast-like fungus causing thrush. (Figure C drawn from a photograph in Zinsser and Bayne-Jones' *Textbook of Bacteriology,* D. Appleton-Century Company, New York, 8th Ed., 1939.)

parasitic existence. Even so, fungous infections of higher plants and animals are common, and in human beings diseases caused by fungi are among the most troublesome of all afflictions. On the other hand, among the saprophytic fungi are some of the most genuinely useful microbes known to man, such as the bread and wine yeasts, and others used in industrial processes.

In their systematic classification of the fungi, mycologists do not recognize "yeasts" or "molds" as distinctly separated botanical

groups. Everyone, however, knows what is meant by a *mold,* in the popular sense of the term. Common molds are the most familiar of all the microbes to the layman. Who has not seen moldy food, moldy grain? To the naked eye, molds may appear like a tuft of cotton, or a sooty black patch, or a fuzzy or powdery mass, variously colored. Most often we find them growing on old moist bread, jellies, fruits, or other foods, or appearing as contaminants in laboratory culture media. There is a characteristic "moldy" smell. Under the microscope, the growth is seen to be made up of a network of branched threads, called the *mycelium,* and usually there are also present small round bodies which are the reproductive elements or *spores.* This filamentous web-like structure, and the abundant spores, are outstanding morphological characteristics of these organisms (Fig. 16).

In contrast to the somewhat complicated make-up of these familiar molds, the common *yeasts,* such as those in the ordinary yeast cake, are fungi of much simpler form, for each individual organism exists as a single, independent, microscopic, round or oval cell. Unlike the molds, the yeasts never develop branched filaments, and they characteristically *multiply* not by free spores, but *by budding* (Fig. 17).

Further description of the true fungi and of fungous diseases is given in Chapters XL and XLI.

BACTERIA (SCHIZOMYCETES)

All the forms of bacteria, including the spirochetes and the mold-like higher bacteria, as well as the true bacteria, are included in the Class *Schizomycetes* (See Table III). This word means literally, "fission-fungi," and is appropriate because all bacteria multiply by a process called *simple fission*—a splitting of an organism across its short axis to make two new organisms.

True bacteria (Eubacteriales). Of all the microbes, the *true bacteria* are the most numerous in kind, the most conspicuous, and the best known. The majority of the harmless organisms that surround us everywhere, as well as the germs of important diseases, belong here.

A primary subdivision of the whole group is made on the basis of the characteristic shape of the bacterial cells. Those shaped like a ball, or sphere, are called **cocci** (singular *coccus*); the rod-shaped

or cylindrical bacteria are called **bacilli** (singular *bacillus*); while the spiral forms are called **spirilla** (singular *spirillum*) (Fig. 12, 10: A, B, C). These organisms are obviously much simpler in structure than the protozoa, yeasts, and molds. One important difference is that bacteria do not ordinarily contain a morphologically distinct nucleus.

Mold-like higher bacteria (Actinomycetales). Typical organisms among the more mold-like of the so-called *higher bacteria* (*Actinomycetaceae*) are those in the genus *Actinomyces.* These forms cause the disease called actinomycosis in cattle and in human beings. They grow as branched threads, like the true molds, but the individual filaments are much finer, and there is a marked tendency for the filaments to fragment into short, rod-like elements, resembling the ordinary bacteria (Fig. 18).

In the family *Mycobacteriaceae* are placed certain organisms which are much less mold-like than the *Actinomyces,* but which are somewhat more complicated in structure or mode of life than the true bacteria. The limits of this special group cannot be sharply defined, and the classification of a bacterium in this family, rather than with the ordinary *Eubacteriales,* is a merely arbitrary matter. It is generally agreed, however, to place here the *acid-fast bacteria* (including the germs of tuberculosis), because of their peculiar staining qualities and growth characteristics, and to classify here, also, for similar reasons, the organisms of diphtheria, and a few other kinds.

Spirochetes (Spirochetales). This order of microbes, although classed as a subdivision of the *Schizomycetes,* differs materially from all the ordinary bacteria, and indeed has little resemblance to any other type of microbe. The spirochetes are curious organisms, suggesting microscopic snakes. They are delicate, flexible, filamentous forms, having the general shape of a more or less tightly coiled spring, or a corkscrew (Figs. 12:9 and 19). Both free-living and parasitic species exist. Among the latter are several harmless varieties, found normally in the mouth, on the genitals, and in the intestinal tract of human beings and animals. The medical importance of the spirochetes is attested by the fact that among the pathogenic species are the organisms responsible for relapsing fever, infectious jaundice, and syphilis.

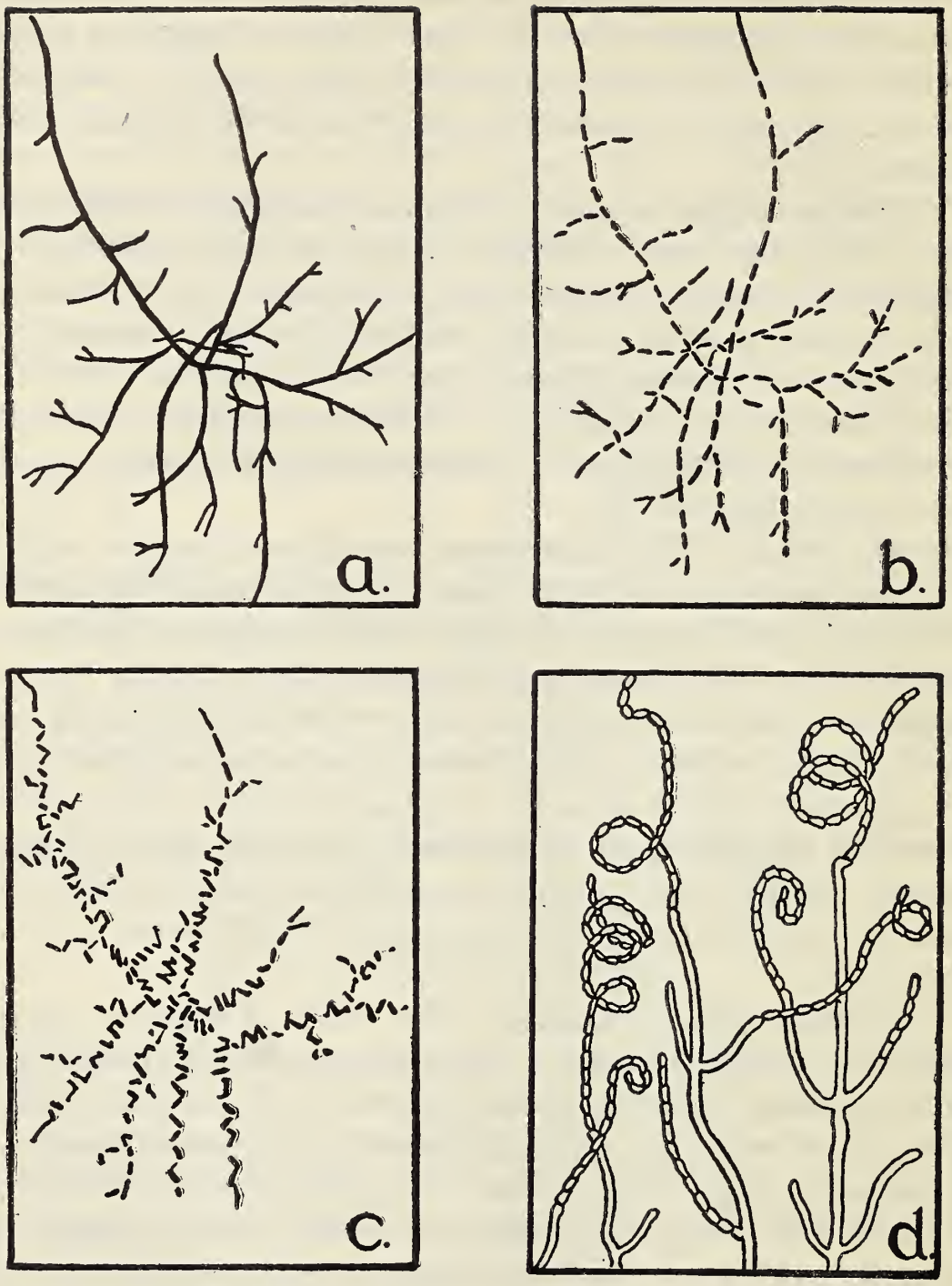


FIG. 18. *Actinomyces:* a, b, c, stages in the development of a pathogenic species, showing fragmentation of the mycelium, b, and the subsequent shift in the position of the bacteria-like fragments so that they lie more or less parallel, resembling diphtheroidal bacilli, c; d, the twisted, terminal, conidia-bearing filaments of a saprophytic *Actinomyces*. (Redrawn after Henrici's *Molds, Yeasts and Actinomycetes,* John Wiley & Sons, New York, 1930.)

RICKETTSIAE

The precise place to which these microbes should be assigned in any scheme of classification cannot be definitely stated. In appearance, the rickettsiae suggest very small bacteria, and it is generally

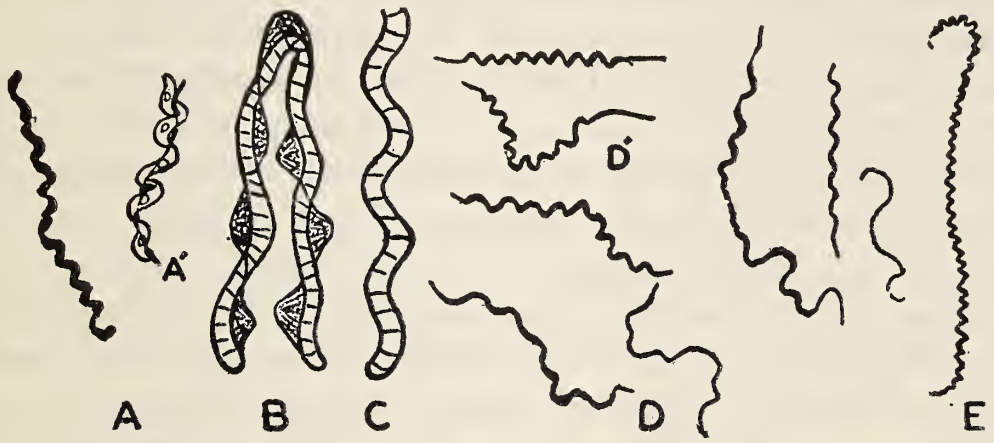


FIG. 19. Spirochetes. A: *Spirocheta;* A': detail showing the curious spiral structure of these organisms; B: *Cristispira,* showing the spiral crest running along the body; C: portion of a *Saprospira;* D: forms of *Treponema;* D': the syphilis spirochete, *Treponema pallidum;* E: *Leptospira.*

believed that they may have been derived originally from ordinary bacteria. They are now recognized, however, as a special class of microorganism, because of a number of peculiarities.

The rickettsiae have many shapes, but look mostly like tiny rods (Fig. 12:11). They show, in smears, a characteristic pale staining. Forms in infected tissues are just within the limits of microscopic vision, having a length of about 0.6μ and a diameter of only about 0.3μ. Unlike the true bacteria, they fail to grow in the laboratory on lifeless artificial culture media. (They can be cultivated, however, in the presence of living tissue, as in the yolk sac of fertile hens' eggs.) Lice, fleas, or blood-sucking insects or arthropods of some other kind act as the living carriers of the pathogenic rickettsiae from host to host in the case of all the rickettsial diseases. Finally, the most important characteristic of the rickettsiae is their habit of growing as *intracellular parasites.* In the arthropod carriers, and in the human victims of rickettsial infections, the organisms multiply *within the cytoplasm,* or *nucleus,* of the living body cells.

The most serious rickettsial diseases are *typhus* and the various forms of *spotted fever* (Chapter XXXVII).

FILTRABLE VIRUSES

The viruses are similar in some respects to the rickettsiae just described, but smaller, so that they are invisible by ordinary methods of microscopic observation. They pass more or less readily, through filters which hold back rickettsiae and ordinary bacteria, and therefore are said to be *filtrable.* Like the pathogenic rickettsiae, the viruses refuse to grow in the laboratory on the usual artificial culture media, but they can be cultivated successfully in special preparations containing susceptible living tissue. They are sometimes grown in *tissue cultures,* that is, in test-tube cultures of human or animal tissue-cells, but more commonly in *egg cultures,* where they may be made to multiply on the fetal membranes of the developing chick embryo (Figs. 116, 117, and 118). Many viruses are maintained for laboratory study by repeated passage through susceptible experimental animals, such as white mice. To an even greater extent than the rickettsiae, the viruses are dependent for multiplication upon an intimate association with living cells, and they act like perfect examples of strict *intracellular parasites.*

There are many different viruses, and each is a distinct entity having a characteristic disease-producing effect in some plant or animal, or in man. Each kind exists as an ultramicroscopic particle of definite size that reproduces itself. The sizes of viruses are expressed not in micra (μ), but in thousandths of a micron, or millimicra ($m\mu$). The smallest viruses, which include those causing poliomyelitis and yellow fever, are only slightly larger than some protein molecules; e.g., those of egg albumin. Viruses of medium size, such as the influenza virus, have been estimated to be about 100 $m\mu$ in diameter, while the largest viruses, typified by those of smallpox and psittacosis, approach the dimensions (250–300 $m\mu$) of the rickettsiae (Fig. 120).

There is strong evidence that some of these elusive disease-producing agents are nothing more nor less than living microbes of ultramicroscopic size—*midget microbes* adapted to an exclusively intracellular life. Other viruses, however, seem to lack the usual attributes of life, and appear like inanimate protein substances.

Whatever their real nature may be, it would be hard to overemphasize the importance of the viruses. They are responsible for a long list of common and serious maladies in plants, in animals, and

Table IV. Some Outstanding Properties of the Principal Microbes

KIND	DESCRIPTION	PRINCIPAL GROUPS	IMPORTANT GENERA	COMMENT
(1) Protozoa	Microscopic animal-like microorganisms, the lowest forms of life classed in the Animal Kingdom.	Amebae	Ameba Endameba	*Ameba proteus* = common free-living form *Endameba coli* = commensal in intestine. *Endameba histolytica* = cause of amebiasis
		Flagellates	Trichomonas, Giardia; Trypanosoma, Leishmania	Commensals in intestine, vagina; potentially pathogenic Pathogenic, cause African sleeping sickness, other tropical diseases
		Ciliates	Paramecium Balantidium	Common free-living forms *Balantidium coli* = cause of mild dysentery in man
		Sporozoa	Plasmodium	4 species cause malaria in man
(2) True Fungi (Eumycetes)	Microscopic plant-like organisms, generally larger and more complex than the bacteria, including the common molds and yeasts, and fungi imperfecti.	Phycomycetes	Mucor, Rhizopus	Common harmless molds
		Basidiomycetes		Mushrooms, etc. Plant parasites
		Ascomycetes	Aspergillus Penicillium Saccharomyces	Common molds Common yeasts
		Fungi imperfecti	Blastomyces, etc.	Numerous varieties include most of the pathogenic fungi
Bacteria (Schizomycetes) (3) Mold-like Higher Bacteria (Actinomycetales)	Microorganisms resembling the true mold fungi, but also closely related to the true bacteria.	family Actinomycetaceae	Actinomyces	*Actinomyces hominis* = cause of actinomycosis in man
		family Mycobacteriaceae	Mycobacterium Corynebacterium	Acid-fast bacteria, including germ of tuberculosis Includes germ of diphtheria
(4) Spirochetes (Spirochetales)	Flexible, spiral-shaped microbes, protozoa-like in appearance and habits, but classified as "higher bacteria."	Saprophytic species	Spirocheta, etc.	Free-living forms in water, etc.
		Parasitic (Commensals or Pathogens)	Borrelia Treponema Leptospira	*Treponema vincenti*, etc., found in normal mouth, genitalia, intestine *Borrelia recurrentis*, etc. = cause of relapsing fever *Treponema pallidum* = cause of syphilis *Leptospira icterohemorrhagiae* = cause of Weil's disease
(5) True Bacteria (Eubacteriales)	Simplest of the plant-like microbes, the most numerous and the best-known.	Cocci	Staphylococci Streptococci, etc.	Common bacteria, some pathogenic, some not. Pathogenic species cause most of familiar infectious diseases, such as boils, sore throats, scarlet fever, typhoid fever, tetanus, etc., etc.
		Bacilli	Bacillus Escherichia Clostridium, etc., etc.	
		Spirilla	Vibrio, Spirillum	
(6) Rickettsiae	Peculiar microbes smaller than most bacteria but still visible under ordinary microscope, grow intracellularly in certain insects and arthropods, and in human and animal hosts; not cultivable on ordinary laboratory media.	Typhus group	Rickettsia	*Rickettsia prowazeki* = cause of typhus
		Spotted-fever group		*Rickettsia rickettsi* = cause of Rocky Mountain spotted fever
(7) Viruses	Large group of disease-producing agents, invisible by ordinary methods of microscopic examination; pass filters that hold back rickettsiae and bacteria; act like intracellular parasites; not cultivable on ordinary laboratory media.	Causing generalized infections, skin lesions, infections of respiratory tract, central nervous system. Bacteriophage are viruses acting on bacteria.		Smallpox, chicken pox, influenza, measles, mumps, yellow fever, rabies, encephalitis, poliomyelitis, etc., etc.

in human beings. It seems probable that no form of life is exempt from virus diseases. Even bacterial cells are subject to injury and lysis (dissolution) by filterable agents known as *bacteriophages.* Human virus infections include diseases like smallpox and chickenpox, which are characterized by lesions in the skin; others, like measles and influenza, which primarily affect the respiratory tract; and still others, like rabies, encephalitis, and poliomyelitis, which attack the central nervous system.

Further description of the viruses and the virus diseases is given in Chapters XXXVII and XXXIX.

A summary of some of the chief points about the microorganisms discussed in this chapter is presented in Table IV.

REFERENCES

Fungi

CONANT, N. F., MARTIN, D. L., SMITH, D. T., BAKER, R. D. and CALLAWAY, J. L.: *Manual of Clinical Mycology.* Philadelphia: W. B. Saunders Co., 1944.

HENRICI, A. T.: *Molds, Yeasts and Actinomyces.* New York: J. Wiley & Sons, 1930.

———— "The Yeasts," *Bact. Rev.,* 1941, 5:97.

THOM, C.: *The Penicillia.* Baltimore: Williams & Wilkins Co., 1930.

THOM, C. and ROPER, K. B.: *A Manual of the Aspergilli.* Baltimore: Williams & Wilkins Co., 1945.

Protozoa

BUCHSBAUM, R.: *Animals Without Backbones.* Chicago: Univ. of Chicago Press, 1938.

CHANDLER, A. C.: *Introduction to Parasitology.* New York: J. Wiley & Sons, 7th Ed., 1944.

HEGNER, R. W., ROOT, F. M., AUGUSTINE, D. L. and HUFF, C. G.: *Parasitology.* New York: D. Appleton-Century Co., 1938.

KUDO, R. R.: *Protozoology.* Springfield, Ill.: Chas. C Thomas, 3rd Ed., 1946.

WENYON, C. M.: *Protozoology* (2 vols.). Baltimore: William Wood & Co., 1926.

Rickettsiae

DYER, R. E.: "The Rickettsial Diseases," *J. A. M. A.,* 1944, 124:165.

PINKERTON, H.: "The Pathogenic Rickettsiae, with Particular Reference to Their Nature, Biologic Properties, and Classification," *Bact. Rev.,* 1942, 6:37.

Virus and Rickettsial Diseases: A symposium. Cambridge: Harvard Univ. Press, 1940.

Spirochetes

NOGUCHI, H.: *The Spirochetes. Newer Knowledge of Bacteriology and Immunology* (Page 452). Chicago: Univ. of Chicago Press, 1928.

ROSEBURY, T.: "A Lecture on the Spirochetes," *J. Lab. & Clin. Med.,* Aug. 1942, 27:1470.

Viruses and Bacteriophages

BRONFENBRENNER, J.: *The Bacteriophage. Newer Knowledge of Bacteriology and Immunology* (Page 525). Chicago: Univ. of Chicago Press, 1928.

LAIDLAW, P. P.: *Virus Diseases and Viruses.* New York: The Macmillan Co., 1939.

MUDD, S. and ANDERSON, T. F.: "Pathogenic Bacteria, Rickettsias and Viruses as Shown by the Electron Microscope—I. Morphology," *J. A. M. A.*, Oct. 28, 1944, 126:561.

RIVERS, T. M.: *Viruses and Virus Diseases.* Stanford University, California: Stanford Univ. Press, 1939.

RIVERS, T. M. and OTHERS: *Virus Diseases.* Ithaca, N. Y.: Cornell Univ. Press, 1943.

VAN ROOYEN, C. E. and RHODES, A. J.: *Virus Diseases of Man.* London: Oxford Univ. Press, 1940.

REVIEW QUESTIONS—CHAPTER V

1. Distinguish between *Protozoa* and *Metazoa.* Explain the meaning of the following terms used in describing protozoa: *free-living, parasitic, commensal, pathogenic.*
2. Discuss the morphology and physiology of protozoa. What is the importance of the habit of encystment?
3. Describe how protozoa reproduce. What is meant by: *simple fission, longitudinal division, multiple division, schizogony, merozoites, sporogony, sporozoites, conjugation, copulation, life-cycles*?
4. Name the four classes of protozoa, and name an organism belonging to each class.
5. Describe an ameba. Define *pseudopod, ameboid motion.*
6. Name a species of free-living ameba and two harmless parasitic species. What species of ameba causes amebiasis?
7. Describe the general structure of flagellates.
8. Name two common varieties of free-living, plant-like flagellates, and two genera of parasitic flagellates.
9. Name two genera of pathogenic flagellates, and an important disease caused by one of them.
10. Describe the general structure of infusoria. Name five varieties common in infusions.
11. Name a species of ciliate that is pathogenic for man.
12. Describe the general properties of the *Sporozoa.* What important disease is caused by organisms of this class?
13. Why are all the microbes, except the protozoa, classed in the plant kingdom? What are the two great classes of plant-like microorganisms?
14. Show, by a diagram, how the bacteria and the true fungi fit into the plant kingdom.
15. Describe briefly the *Thallophyta, Algae, Lichens.* What kinds of organisms are included in the great group of plants called *Fungi*?
16. What are the *Eumycetes*? Define *mycosis, mycotic disease.*
17. Describe the general morphology of: (*a*) the common molds, (*b*) the yeasts. Define *hypha, mycelium, septate* and *nonseptate mycelium, mold spores, budding.*

18. Give the scientific name for *bacteria,* and its literal meaning.
19. What is the scientific name for the *true* bacteria? Name and describe briefly the three morphological subgroups. How do these organisms compare, in size and form, with other kinds of microbes?
20. Name and describe briefly the species of mold-like higher bacteria which causes actinomycosis.
21. Name two other important disease germs which are usually classified as higher bacteria.
22. Describe the spirochetes, and name three important diseases that they cause.
23. Outline the chief characteristics of the rickettsiae. What are the two most serious rickettsial diseases?
24. Describe the outstanding properties of filtrable viruses. Compare these properties with those of rickettsiae. What can be said of the nature of these disease-producing agents?
25. Why are viruses so important? Name several human diseases caused by different viruses.

CHAPTER VI

MICROSCOPES AND MICROSCOPICAL METHODS

MICROSCOPES

In the modern compound microscope we have a truly beautiful instrument of precision. It meets its greatest test in the study of microorganisms, for the most powerful lenses must be used, and the instrument must be manipulated with all possible skill.

Parts of the microscope and their functions. The *mechanical parts* of the microscope are concerned with the support and adjustment of the *optical parts,* whose function is to make the enlarged image of the object which we see. Fig. 20 shows the principal parts of the microscope and how they are named.

Base, pillar, inclination joint. The *base* and the *pillar* serve to support the entire instrument. They are made heavy in order to minimize vibration as much as possible. The *inclination joint* in the pillar permits the microscope to be tipped back to any degree desired.

Arm, body-tubes. The *arm* supports the *body-tube,* to which the principal lenses are attached. The newer microscopes are so made that the instrument may be carried by the arm, but this may be harmful to some older makes.

Draw-tube, revolving nose-piece; tube-length. Fitting inside the upper end of the body-tube is the *draw-tube,* which may be drawn upward. Within it fits the *ocular.* The purpose of the draw-tube is to adjust the *mechanical tube length,* that is, the distance between the top lens of the ocular above and the attachment of the *objective* into the *revolving nosepiece* below. The system of lenses in objectives and oculars is made to function best when ocular and objective are a *definite distance apart,* that is, at a definite tube-length.

In Bausch and Lomb, Spencer, and Zeiss microscopes the proper

tube length is 160 millimeters; in Leitz microscopes it is 170 millimeters. The draw-tube is usually marked with a millimeter scale. The thickness of the nosepiece (usually 15 millimeters) must be subtracted from the scale on the draw-tube, in order to adjust it to the right tube-length. That is, instead of pulling out the tube to the mark 160 (or 170) on the scale, it should be placed at the mark 145 (or 155). The proper tube length in many microscopes is indicated by a line running completely around the draw-tube. Some microscopes have no draw-tube, and the proper tube length is fixed when the instrument is made.

When the draw-tube is pulled out beyond the point which gives the best tube length, the image is larger, but not quite so distinct in outline.

Coarse and fine adjustment (for focusing). The entire body-tube with its attached lenses is moved up and down by means of the rack and pinion of the *coarse adjustment.* The tube is likewise raised and lowered, by *very slight degrees,* by means of the *fine adjustment.* The purpose of these adjustments is to bring the object into *focus,* so that its outlines are sharp and clear. Both the coarse and the fine adjustment should be manipulated carefully, especially the latter, for it is a very delicate mechanism. The range of the fine adjustment screw is limited. At one end of its range it comes to a stop, and at the other it goes beyond the limit of movement and has no effect. *It should always be kept near the medium point.*

Stage. This is the part of the microscope on which the object to be examined is placed. In most bacteriological work, the object is a transparent smear or other preparation on a glass slide.

Mirror. The *mirror* collects and reflects light up into the microscope. One side of the mirror is a plane mirror, the other is a concave mirror. Since, in most bacteriological work, a large amount of light is needed, the concave mirror is most useful, for it helps to concentrate the light. When a bright artificial light is used, the plane mirror may suffice.

Substage condenser and diaphragm. Before the light reaches the object on the stage, it is condensed and focused by passage through the large condensing lens, commonly called the *Abbe condenser,* in the *substage.* The result is that the maximum amount of light is directed upon the object, a necessity when the highest powers of the microscope are used. Often, however, an object is too brilliantly illuminated if all the light from the mirror passes into the

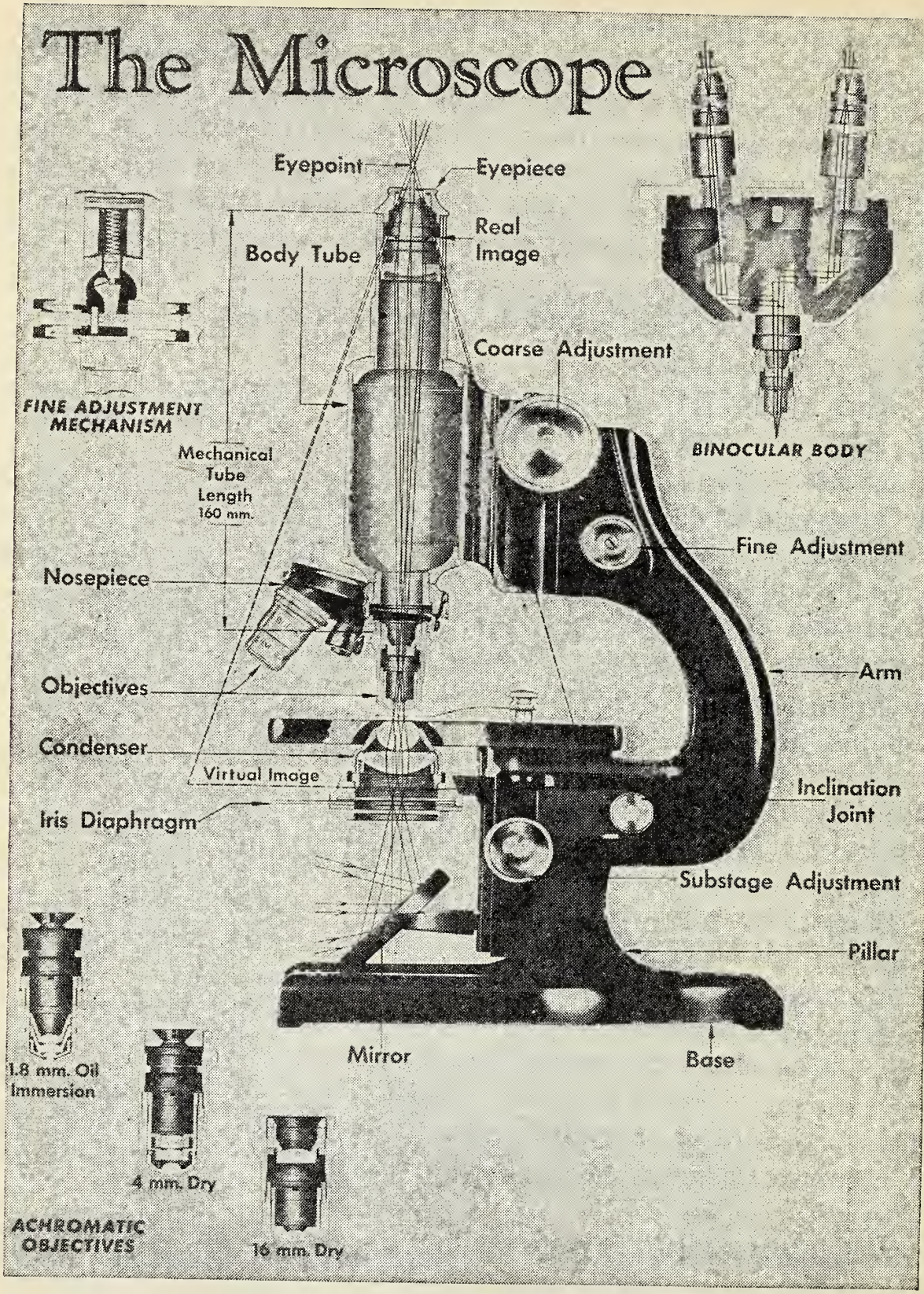


FIG. 20. The compound microscope, showing the parts, and the path of light through the instrument. (From O. W. Richards' *The Effective Use and Proper Care of the Microscope,* Scientific Instrument Division, American Optical Co., Buffalo, New York, 1941.)

condenser. For this reason, there is placed beneath the condenser an *iris diaphragm.* The size of the opening in the diaphragm may be reduced to that of a pinhead or any intermediate size by moving a hand lever; by this means the amount of light admitted to the condenser can be accurately controlled. If a diffuse light is required, as in the study of unstained, living microbes, the entire substage may be lowered. In microscopes having a divisible substage condenser, the top part may first be removed.

Objectives. The *objectives* are the most important of the optical parts. They limit the size of the image we see, and also they are largely responsible for the quality of this image. Most microscopes are equipped with three objectives, of different magnifying power—the *low power,* the *high power,* and the *oil-immersion objectives.*

Low-power objective. This objective is useful for the examination of protozoa and others of the larger microorganisms, and it may be used for study of colonies of growing organisms, but individual bacteria can scarcely be discerned with this lens. The low-power objective is usually much shorter than the other two, and it is certain to have a *much larger lens at its end* than either of the others. Different manufacturers use various systems for marking objectives. The low power is often marked "3" or "2/3," (meaning 2/3 inch), or "16 mm."

High-power objective. This objective is used in microbiology for the examination of living microorganisms suspended in drops of water or other fluids. In most microscopes, the high-power objective is longer and more slender than the low-power objective, and the visible lens at its end is *smaller than that of the low-power,* though still larger than that of the oil-immersion objective. It may be marked "6" or "1/6" (meaning 1/6 inch), or "4 mm."

Oil-immersion objective. This objective is indispensable to the bacteriologist. *It is always used for the examination of stained smears of bacteria.* The objective may be long or short, but it will always have a *very small lens visible at its end.* It is usually marked *"oil immer."* or *"homog. immer."* (meaning homogeneous immersion). Also, the figures "1/12" (inch), "1.9 mm," or "1.8 mm" often are engraved upon it.*

* Objectives are usually marked also with the magnifying power (e.g., 10x, 43x, 97x) and with the letters "N. A.," followed by a figure. "N. A." means *numerical aperture.* It is an index of the power of the objective to show, distinctly separated, two points close together in the object. The higher the N. A. figure, the greater the detail the objective will reveal.

The figures 16 mm, 4 mm, etc., on the objectives refer to what is called the equivalent focal length. They give an idea as to the distance there will be between the end of the objective and the object when in focus. Thus, the low-power objective will be in focus about 16 mm above the object on the microscopic slide, but the oil-immersion lens will be focused less than 2 mm above it.

Oculars. The *ocular,* or eyepiece, is a short tube with two lenses, which fits into the upper end of the draw-tube. The principal function of the ocular is to act with the eye itself to *magnify the image of the object formed by the objective.* Oculars are made with different magnifying powers. By one system, oculars are marked "5x," "10x," etc., indicating that these oculars magnify the image of the objective five times, ten times, etc. Other oculars are marked with the numbers 2, 4, etc. The higher the number, the greater the magnifying power. The most commonly used ocular is the 10x (or 4). This ocular used with the low-power objective gives a final magnification of about 100 times, with the high-power nearly 500 times, and with the oil-immersion about 1000 times.

Path of light through the microscope. Figure 20 shows how the microscopic image is made. It will be seen that the rays of light cross within the body-tube, with the result that as we look into the microscope the object appears upside down.

Principle of the oil-immersion objective. The oil-immersion objective differs from the low- and high-power objectives, in that the latter are "dry." No fluid is applied to the low- or high-power objectives in use, and there is a layer of air between the objectives and the object when in focus. The front lens of the oil-immersion objective, however, *is immersed in a special immersion oil* which fills the space between the front of the objective and the object slide. Immersion oil is a special preparation of cedar oil. It has the *same refractive index as glass,* that is, it bends rays of light passing through it to the same degree as glass. Thus, with the oil-immersion objective, light coming through the glass object-slide passes *straight* through the oil into the glass lens of the objective, without the deflection which a passage through air would cause (Fig. 21). This makes possible the use of lens combinations in these objectives which give a very high magnification.

Use and value of the oil-immersion lens; resolving power of the light microscope. Most of the time, microbiologists use the *oil-immersion objective.* This lens gives an initial magnification of 90 to

100 diameters (most commonly, 95x). When the usual 10x ocular is employed, we have a final magnification of 900 to 1000 diameters. A still greater enlargement is possible by using, for example, a 15x or 20x ocular, or by extending the body-tube, but in the ordinary student-type microscope this is likely to result in some fuzziness of the image, that is, in the loss of resolution. It is the *resolving power* of the microscope which determines the practical limits to which magnification can be carried.

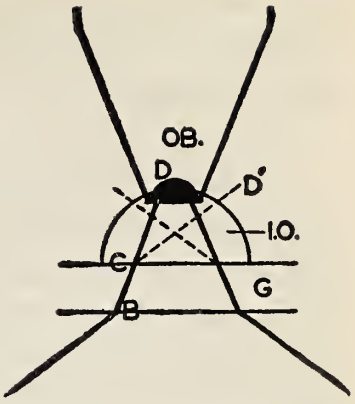


FIG. 21. Diagram illustrating the principle involved in the use of immersion oil. OB.: the oil immersion objective; BCD: path of a ray of light coming up from below into the glass slide (G), and through the drop of immersion oil (I.O.) into the glass lens of the objective. Since immersion oil is optically like so much glass (i.e., has the same refractive index as glass), the ray of light passes *straight* from the glass slide into the objective. If the oil were not there, this ray of light would be refracted by the air between the slide and the objective in the direction CD', and would not enter the microscope at all.

By resolving power is meant the capacity to form distinctly separate, sharp images of tiny points very close together in an object. Resolution is controlled in part by the numerical aperture of the particular microscope objective employed, and by the refractive index of the immersion oil used, but primarily it is limited by the *wave length of the light*. The eye cannot see clearly objects smaller than one half of the wave length of the light used. The light ordinarily used with the microscope has a wave length of about 0.5μ. This means that the smallest objects one can hope to see clearly under the oil-immersion objective of the ordinary microscope have a dimension of about 0.25μ to 0.3μ. The best research microscopes can do only slightly better than this.

Fortunately, many of the details of structure in bacteria and other microbes (except the viruses) are large enough to be revealed clearly by the usual type of microscope. Other details, however, such as flagella and possible nuclear structures, are seen with difficulty, and are only brought out by special illumination, or by special staining or chemical treatment of the microorganisms.

Use and care of the microscope. Skill in the use of the microscope must be acquired by practice, but if a few simple rules are followed, anyone can learn in a short time to use it without difficulty

and with enjoyment. If each of the following steps is carried out carefully, *in the order given,* the beginner will find most of the difficulties solved:

(1) Clean the microscope.
(2) Place the microscope so that it points toward a source of light, and take a comfortable position at the instrument.
(3) Place the object on the stage, swing into place the objective you wish to use, and, while watching from the side, lower it to a point just under the position it will have when in focus.
(4) Secure the proper amount of light by manipulation of the mirror, substage condenser, and diaphragm.
(5) Focus, first with the coarse, then with the fine adjustment; always focus up.
(6) Maintain the focus by continual manipulation of the fine adjustment.

The dark-field microscope. This is a highly useful adjunct to ordinary light-field microscopy. In the dark-field microscope a special condenser fits into the substage. The center of the top lens of this special condenser is opaque, so that none of the central rays of light can pass through it, and the object is illuminated only with very oblique rays. None of the light goes directly up the objective, as in the ordinary way, but instead the light-rays pass through the object almost at right angles to the objective, and nearly parallel to the stage. Through the microscope the field appears dark, but any microorganisms or other objects in the preparation stand out sharply as bright refractile bodies, just as the dust particles appear in a beam of light across a dark cellar.

The dark-field microscope is used for the examination of unstained microorganisms or other objects suspended in fluids. It is especially useful for the study of very small and delicate organisms, such as spirochetes, which are invisible or nearly invisible when viewed in the ordinary way.

Microscopy with ultraviolet light and with fluorescent light. It will be evident, from what has been said above with respect to the magnifying and resolving power of the ordinary microscope, that definite limits to microscopic vision are set by the wave length of the light used. When we employ dark-ground illumination, we increase to some extent both the visibility and the resolution. This is largely because it is easier to see a brightly illuminated object placed against a dark background than it is to discern a stained

particle of the same size viewed by direct light. Still, the limit of microscopic vision using ordinary light, even in a dark-field arrangement, remains substantially below that required for the direct observation of microbes as small as the viruses, and, indeed, many details of structure in the larger organisms remain obscure.

The use of light of shorter wave length, that is, *ultraviolet light,* offers a possibility of extending the limits of the size of particles which may be demonstrated with the microscope. Although not visible to the eye directly, the microscopic image may, of course, be caught upon a photographic plate. Little more has been learned, however, about microbes by ultraviolet microphotography than is revealed by careful studies with the ordinary dark-field microscope.

Ultraviolet light has a more promising use in connection with more recently developed fluorescence microscopy. The necessary apparatus for microscopy with *fluorescent light* includes an arc lamp, giving ultraviolet light, with filters to screen out most of the visible light, a microscope with special metallic mirror and condenser, and slides and cover glasses of quartz or ultraviolet glass.

Some tissue structures and virus particles have a natural fluorescence under irradiation by ultraviolet light. In other cases, fluorescence is induced by the addition of various activating substances (usually azo-dyes), known as fluorochromes.

The examination of tissue sections and of smears by fluorescent light has recently been recommended as a diagnostic procedure in tuberculosis and diphtheria.

The electron microscope. This newest device for revealing the intimate structure of minute objects is a remarkable instrument that has become an investigative tool of major importance, not only in microbiology but in many other fields of science and industry as well (Fig. 22). In this kind of microscope a stream of *electrons,* traveling at high speed in a vacuum, is substituted for light. The use of electron beams, instead of light-rays, is based upon two fundamental facts: (1) A beam of electrons exhibits wave-motion, just as light does, except that the moving electrons have an *extremely short wave length*—80,000 to 100,000 times shorter than that of visible light; (2) The electron beam can be deflected from its course, and focused to form an image, by passing the beam through suitably placed magnetic fields, just as light is refracted and focused in the ordinary microscope by glass lenses (Fig. 23).

Since, as we have learned above, resolving power is dependent

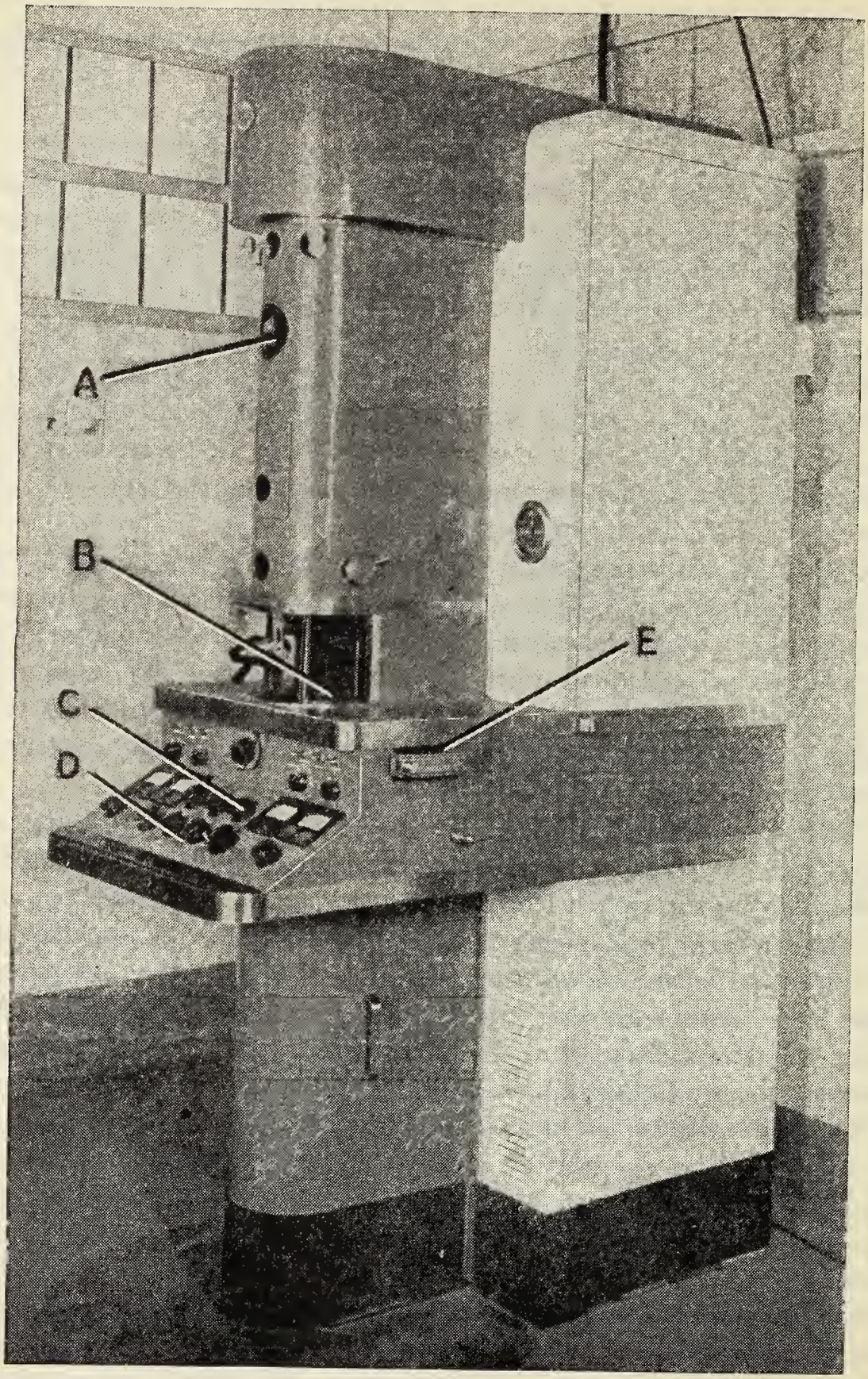


FIG. 22. Legend on opposite page.

primarily upon the wave length of the image-forming medium, and is greater the shorter the wave length, it is obvious that the electron microscope has a great advantage over the light microscope in this respect. Indeed, it is theoretically capable of resolving (showing clearly) particles separated by no more than 0.001μ. Actually, in the present-day instruments, which are usually operated at a voltage of 60,000 (60 KV), the smallest object that can be revealed clearly has a dimension of about 0.003μ. In practice, photographs of preparations under the electron microscope are commonly made at initial magnifications of about 12,000, or 16,000; then these original pictures are enlarged photographically to a final magnification of perhaps 60,000 or even 100,000. Photographic enlargement of the originals is possible without loss of detail, since the great resolving power of the instrument gives pictures that are remarkably sharp and clear.

It is well to realize that the electron microscope, too, has its limitations. Among them is the fact that observation of still-living microbes under it is not possible; the unstained preparation must be dried on a thin cellulose film, and the organisms are killed (and possibly somewhat altered) by the electrons. An electronograph is somewhat like an X-ray plate. It is a photograph of the shadows produced by the passage of electrons through the unstained, dried specimen, and the different parts of a bacterium (or other objects) are revealed only because they have different densities, i.e., different degrees of opacity to the electrons. It is necessary, then, to interpret with caution appearances in these pictures. We must have in mind

---

FIG. 22. The RCA electron microscope, type EMU, universal (research) model. The bacterial culture or other material to be examined is dried upon a very thin film of collodion and inserted into the specimen chamber at A. Vacuum pumps are now started and the entire system is quickly evacuated. The electron beam, which is formed at the top of the column, passes vertically downward. The operator sits in front of the sloping control board and observes the image of the object, as it appears on a fluorescent screen, by looking into the viewing chamber (B). The magnification desired (100x to 20,000x) is set by knob-controls (C). Other knobs move the stage so that different parts of the specimen may be examined, and still others adjust the focus and brilliance of the image (D).

Photographic plates are introduced at E. A picture in focus on the fluorescent screen is automatically in focus on the plate. Once an interesting field has been found and brought into focus, it is only necessary to close a sliding shield about the viewing chamber, press a cable control for the required exposure time, and the picture is made. (From Picard, R. G. and Smith, P. C.: "The Electron Microscope for Metals," *Metals and Alloys,* 1944, 20:637.)

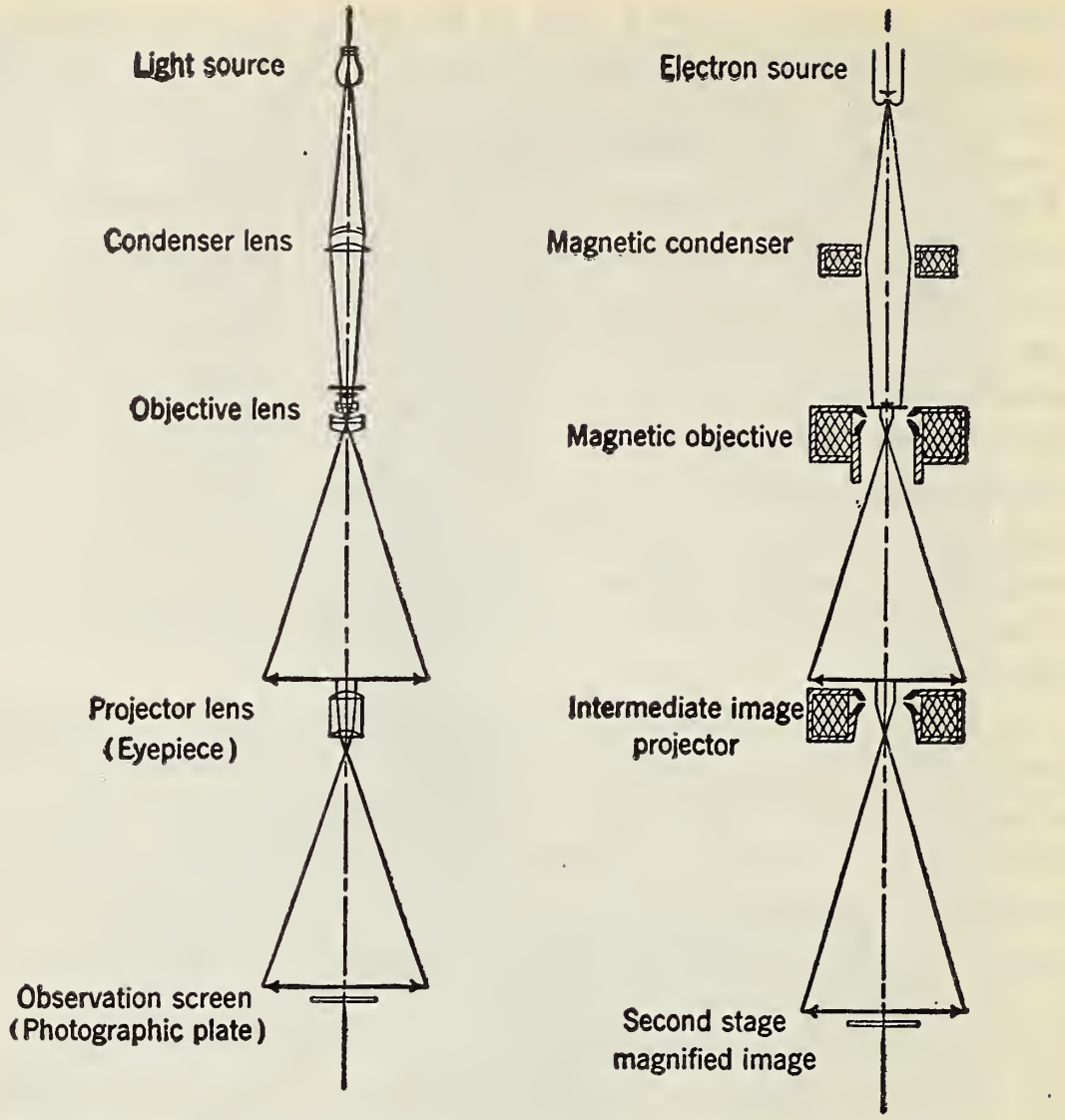


FIG. 23. Comparison of the optical system of the compound light microscope (left) with the RCA electron microscope (right). A series of magnetic fields in the electron microscope serves the same function as the series of glass lenses in the light microscope. The magnetic lenses, however, are not moved up and down as are the optical lenses. Instead, the focusing effect on the electron rays is produced by varying the current flowing in the coils, thus varying the intensity of the magnetic field acting on the electron beam. (Reprinted by permission from *Electron Optics and the Electron Microscope* by Zworykin, Morton and Ramberg, published by John Wiley & Sons, Inc., 1945.)

the possibility of mistaking mere thickenings in the object, or artifacts, for real structures.

Recent improvements and refinements in technique have greatly enhanced the value of electron micrographs, however, and revelations of immense practical and theoretical importance are being reported frequently. An important development is a method of coating the specimen with a thin metallic film so that objects cast shadows (Fig. 86).

MICROSCOPICAL METHODS

Examination of living microorganisms. The simplest way to examine living bacteria or other microorganisms is to suspend them in water or other fluid, place a drop of the suspension on an ordinary glass slide, and cover it with a cover glass. A much better method for most purposes, however, is to make a so-called *hanging-drop preparation,* as described below. In either case, such wet preparations are best studied by use of the *high-power objective;* only rarely is it necessary, or even advisable, to use the oil-immersion lens.

Hanging-drop preparations. A hanging drop is prepared as follows:

(1) Secure a perfectly clean cover glass, free from grease. (Cover glasses may be cleaned by rubbing them carefully between the fingers with soap and water, and then rinsing them in hot water. They may be dipped in alcohol and wiped dry with a clean, lint-free cloth, or they may be polished with cleaning powder. Gentle heating of the cover glass in the flame will remove the last trace of grease.)

(2) Place a thin ring of petroleum jelly about the edge of the concavity on a hollow ground slide.

(3) Place a drop of physiological salt solution or water in the center of the clean cover glass, and emulsify the material to be examined in this fluid, or use a drop of a broth culture. *Make a small mark close to the drop on the cover glass with a wax glass-marking pencil.*

FIG. 24. A hanging-drop preparation viewed from the side, and showing the drop hanging from the cover-slip in the depression of the hollow-ground slide.

(4) Invert the hollow ground slide so that the vaseline-ringed concavity is directly above the drop on the cover glass. Press the slide down lightly so as to seal the cover glass to the slide. Reinvert with a quick movement. The drop containing the material hangs suspended from the cover glass in the depression of the hollow ground slide (Fig. 24).

A hanging-drop preparation may be observed for a considerable period of time, so long as the fluid does not dry out. Motility of microbes, their natural groupings, their reactions in the presence of

certain chemical substances, and many other important facts may be learned from the study of hanging drops. It should be remembered that all small particles, including both motile and nonmotile bacteria, show *Brownian movement* in hanging drops.

The crayon mark on the cover glass facilitates the somewhat difficult task of focusing on a hanging drop. It is only necessary to locate this mark; then the edge of the drop will be easily found.

Examination of bacteria in stained smears. The shape and structure of bacteria are revealed most clearly when the organisms are dried upon a glass slide and colored with a stain, or viewed against a stained background.

Relief-staining (or negative-staining) methods. By these procedures, the bacteria themselves are not colored, but they stand out as in relief from a colored background. This technique is useful for the examination of ordinary bacteria, but is especially valuable for the study of spirochetes, or other microbes that do not stain well, and for the demonstration of special structures, such as capsules.

Nigrosin relief stain. An aqueous solution of nigrosin, a bluish-black dye, is especially useful as a background stain, and has largely replaced India Ink for this purpose (except for capsule demonstration, as described in Chapter VII). The staining solution is prepared by adding 5 (or 10) grams of nigrosin to 100 cc of distilled water and boiling the mixture for 30 minutes. Then 0.5 cc of formalin is added, as a preservative, and the solution is filtered through paper and stored in sterile tubes.

The material to be examined is emulsified in a drop of the nigrosin solution on a clear slide. The mixture may then be spread over the slide by one of two methods. The drop may be spread out slowly with a wire loop, by making several rotary movements through it, each time carrying the fluid further away, so that the smear will have the desirable thinness in some part of the outer portions. Or instead, the drop may be spread with the edge of another slide, as illustrated in Fig. 25. Another good way is to allow the drop to soak into the edge of a half-inch-wide strip of lens paper, then draw the paper slowly across the slide.

Let the film dry in the air, without heat. Examine the dry smear directly with the oil-immersion lens. Spirochetes and other organisms are not colored by the nigrosin, and in this kind of smear they stand out as white objects in a black field. The white marks made in the smear by particles of dust, scratches in the slide, etc., must be carefully distinguished from the microorganisms present. It is well to make a control smear of the nigrosin solution alone, for it may contain some extraneous bacteria.

Preparation of smears when the bacteria are to be stained. The most usual method of examining bacteria is to make first a thin film of the organisms on a clean slide, then apply stains to the dried smear. Smears are prepared in the following manner:

(1) *Secure a clean, grease-free slide.* Slides may be cleaned in hot water, covered with cleaning powder, and polished with a clean, lint-free cloth. They may be passed quickly through a Bunsen flame just before use, in order to remove the last trace of grease.

(2) When a *smear from a bacterial culture* is to be made, place a *very small* drop of water on the clean slide. Freshly distilled water should be used, unless the tap water is known to be free from microorganisms. Remove the organisms from the culture with an inoculating needle, with aseptic precautions, according to the technique for handling cultures described in a later chapter. Emulsify the organisms thoroughly in the water drop, then spread the drop to make a thin smear. To make a *smear from pus or other material* on cotton swabs, *roll* the swab along a clean slide to make a thin film. Usually no water is needed for a smear of this kind. Remember that smears must be *thin* enough to permit plenty of light to pass *through* them.

(3) Let the smear dry in the air. If the proper amount of water was used, the smear will dry almost as soon as it is made.

(4) After the smear is thoroughly dry, pass the slide, smear up, three times through the Bunsen flame. This process of heating is called *fixing* the smear. The bacteria in a fixed smear are not necessarily all dead, but they are affixed to the slide so that they will not come off in subsequent manipulations. After fixing, the smear is ready for staining. (Smears are sometimes fixed with methyl alcohol or other chemicals, instead of heat.)

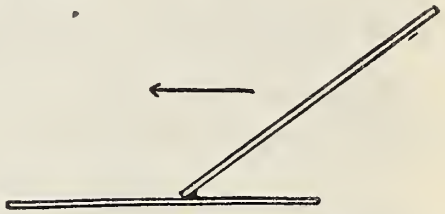


FIG. 25. Diagram illustrating the method of making blood smears, and relief-stain smears.

Impression smears. Colonies of bacteria or fungi upon agar or gelatin medium may be preserved and stained intact as a so-called *impression smear* (Fig. 26) (See also Fig. 56).

Stains. The stains now used are solutions of *anilin dyes.* These are artificial dyes made from coal-tar products. Anilin dyes were first introduced into bacteriological technique about 1880 by the early bacteriologists Koch, Weigert, and Ehrlich.

Only a few of the many kinds and colors of anilin dyes are used in bacteriology. There are three stains in most common use. These

are *carbol fuchsin* (a solution of the red-dye *basic fuchsin* in dilute carbolic acid), *methylene blue,* and *gentian violet* (or *crystal violet*). Any one of these stains may be used alone, or they may be used in combination with other stains and chemicals in one of the many special staining methods. Other stains in wide use are *safranin* (a faint red), *Bismarck brown,* and *toluidine blue.* Safranin and Bismarck brown are used as contrast stains (or *counterstains*), in combination with the blue or violet stains. Toluidine blue is frequently employed as a stain for diphtheria bacilli.

Carbol fuchsin. (Ziehl's formula)

Solution A

Basic fuchsin	0.3 gm
Ethyl alcohol (95%)	10.0 cc

Solution B

Phenol (melted crystals)	5.0 gm
Distilled water	95.0 cc

Mix Solutions A and B

Dilute carbol fuchsin. The solution given above, when diluted with 9 parts of distilled water, makes an excellent stain for general use.

Methylene blue. The most widely used preparation is called *Loeffler's alkaline methylene blue:*

Solution A

Methylene blue	0.3 gm
Ethyl alcohol (95%)	30.0 cc

Solution B

KOH (0.01% by weight)	100.0 cc

Mix Solutions A and B

Gentian violet. Various formulae have been devised for making up stains from this dye, and from the newer, related dyes called *crystal violet* and *methyl violet.* The following staining solution is widely used as a simple stain, and as the first step of the Gram stain (described below):

Kopeloff and Beerman's Alkaline Gentian Violet

Solution A

Crystal violet	1.0 gm
Distilled water	100.0 cc

Solution B

Sodium bicarbonate	1.0 gm
Distilled water	20.0 cc

Before use as a simple stain, mix 1.5 cc of Solution A with 0.4 cc of Solution B.

Safranin.

Safranin O	2.5 gm
Ethyl alcohol (95%)	100.0 cc

Mix 25 cc of this solution with 75 cc of distilled water.

Bismarck brown.

Bismarck brown	0.5 gm
Distilled water	100.0 cc

Dissolve by boiling; cool, and filter.

Toluidine blue.

Toluidine blue	0.25 gm
Glacial acetic acid	2.0 cc
Absolute alcohol	5.0 cc
Distilled water	100.0 cc

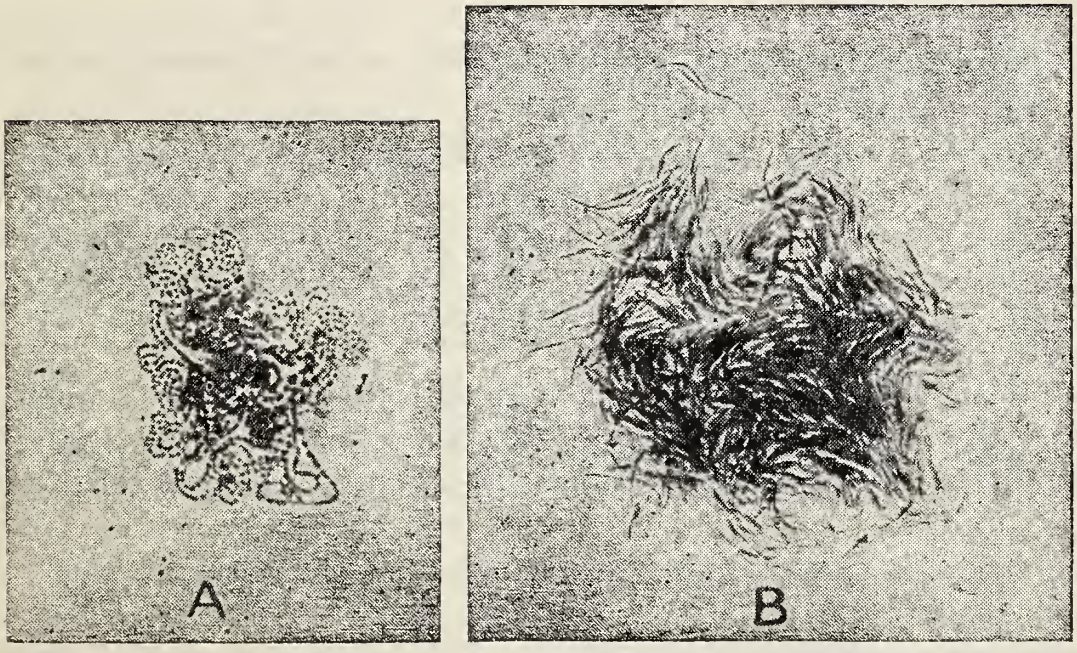


FIG. 26. A: an impression smear of a microcolony of streptococci about five hours old; B: an impression smear of a chain-forming bacillus. Note how these preparations reveal the internal structure of colonies, as well as the morphology of the individual organisms.

Use of simple stains. The actual process of staining with any single solution is as follows:

(1) Cover the *fixed* smear with the staining solution for a few seconds, or minutes, according to the particular stain in use, then wash off the stain with water. Plenty of the staining solution should be used, and it should never be allowed to dry upon the smear. The time needed for staining can be learned only through experience with the particular staining solution in use; even the weakest stains rarely need more than three minutes' application. Sometimes gentle warming of the slide over the flame is recommended.

(2) Dry the smear by pressing the slide between several layers of filter paper, or warm the slide slightly in the flame, then blow across it and drain off the water on a towel. The smear is then ready for examination.

The Gram stain. In 1884, a Danish physician named Gram described a special staining method which is of great practical value in bacteriology. Many modifications of the original Gram staining method have been described. At present one of the more widely used procedures is that suggested by Kopeloff and Beerman, in which the *gentian violet* solution described above is employed. Additional reagents needed include an *iodine solution,* a *decolorizing agent* (alcohol, acetone, or an alcohol-acetone mixture), and a *counterstain* (safranin, Bismarck brown, or dilute carbol fuchsin).

Color-blind persons will find it helpful to use Bismarck brown as the counterstain.

A recommended procedure is as follows:

(1) Cover the fixed smear with Kopeloff and Beerman's *alkaline gentian violet* (Solution A), and add a drop or two of *sodium bicarbonate* (Solution B). Let the stain act from one to two minutes.

(2) Wash off the stain with water.

(3) Rinse smear with *iodine solution,* then add more iodine solution and let it stand for two minutes. (This solution is made by dissolving 2 gm of iodine in 10 cc of normal NaOH solution, then adding water to make up to a volume of 100 cc.)

(4) Wash off iodine solution with water, and absorb excess water with filter paper.

(5) Apply an *alcohol-acetone solution* (3 parts of acetone to 7 parts of 95% ethyl alcohol), drop by drop, let it drain off, and *watch carefully.*

(6) When no more color is seen in the drippings, wash with water *immediately.* (Decolorization usually takes only five to ten seconds.)

(7) Cover smear with safranin solution (or Bismarck brown) for about one minute.

(8) Wash off counterstain with water and dry smear.

Results and interpretation of the Gram stain. The Gram staining method serves to divide bacteria into two classes: (1) so-called *Gram-positive* organisms, which retain the purple coloring of the gentian violet, and (2) so-called *Gram-negative* organisms, which lose the gentian violet when the smear is washed with alcohol or acetone, and which, therefore, are colored by the counterstain. When this staining process is successfully carried through, Gram-positive organisms have a purple stain, and Gram-negative organisms have the color of the counterstain (*i.e.*, pink when safranin is used, or brown when Bismarck brown is the counterstain).

In the case of Gram-positive organisms, the gentian violet plus the iodine forms with the bacteria a compound which is *relatively* insoluble in alcohol or acetone, so that the purple color does not come out after a few seconds' exposure to these fluids, while in the case of Gram-negative organisms the iodine solution has no effect and the color comes out very readily. *But if the alcohol or acetone is allowed to act too long, Gram-positive organisms will also be decolorized,* so that after the staining is completed they will appear to be Gram-negative. On the other hand, if the decolorization is not continued long enough, Gram-negative organisms may appear to be Gram-positive. Considerable practice is necessary before Gram staining can be carried out with consistent success. When studying an unknown organism, bacteriologists check the results of Gram stains by comparison with smears of *known* Gram-positive and Gram-negative organisms stained on the same slide, at the same time.

Under the same conditions, the normal individuals of any one species of bacteria react in the same way to the Gram stain. Most species can be classified quite definitely as either Gram-positive or Gram-negative; a few species show a borderline reaction. Occasionally, in old cultures, in which many of the cells are degenerate, and, in the case of some bacteria, in acid media, Gram-positive organisms may show a Gram-negative reaction.

The Gram stain is of great aid in the identification of unknown organisms, and much reliance is placed upon this staining method in connection with the diagnosis of gonorrhea, meningitis, and other diseases. Table V shows the reaction to the Gram stain of some of the principal pathogenic bacteria.

It is important to realize that the terms *Gram-positive* and *Gram-negative* have reference only to this staining method. Microorganisms of the most varied kinds, both pathogenic and nonpathogenic,

are found in each class. Nevertheless, fundamental differences between the Gram-positive and the Gram-negative organisms do appear to exist, as we explain in the following chapter.

Acid-fast staining method. There are a number of important bacteria which are distinguished from all other kinds by the fact that they are stained with considerable difficulty, but *when once colored with a powerful stain, they resist the decolorizing action of acids.* They are, therefore, said to take an "acid-fast" stain. They do not give up the stain when the slide is immersed in alcohol, as most bacteria do, nor even when it is placed in *acid alcohol,* i.e., alcohol to which has been added about 3 per cent of concentrated acid. Tuberculosis germs and other members of the genus *Mycobacterium* are acid-fast, and this is one of their most outstanding characteristics.

The method most widely used to demonstrate the acid-fast stain-

TABLE V. Classification of Important Pathogenic Bacteria According to Their Reaction to the Gram Stain

Gram-Positive (retain the purple stain)		Gram-Negative (lose the purple stain)	
ORGANISM	DISEASE WITH WHICH ASSOCIATED	ORGANISM	DISEASE WITH WHICH ASSOCIATED
Staphylococci (all pathogenic species)	—Furunculosis, etc.	Gonococcus	—Gonorrhea
Streptococci (all important pathogenic species)	—Erysipelas, tonsillitis, scarlet fever, etc., etc.	Meningococcus	—Epidemic meningitis
Pneumococci	—Lobar pneumonia, etc.	Eb. typhosa	—Typhoid fever
Cor. diphtheriae	—Diphtheria	Sal. paratyphi	—Paratyphoid fever, food poisoning
Myco. tuberculosis	—Tuberculosis	Shig. dysenteriae	—Dysentery
B. anthracis	—Anthrax	H. influenzae	—Influenza, etc.
Clos. tetani	—Tetanus	H. pertussis	—Whooping cough
Clos. perfringens (welchii)	—Wound infection, gas gangrene	M. mallei	—Glanders
Clos. botulinum	—Botulism	P. pestis	—Plague
Actinomyces	—Actinomycosis	Fusiform bacilli	—Vincent's Angina, etc.
		Brucella group	—Undulant fever
		V. cholerae	—Cholera
		Spirochetes	—Syphilis, Relapsing fever, etc., etc.

ing properties of an organism is the Ziehl-Neelsen technique. The steps are as follows:

(1) Place the slide on a ring stand or other metal support, and cover the fixed smear with carbol fuchsin (Ziehl's formula). Apply the Bunsen flame beneath the slide for a few seconds until the stain steams. *Do not boil* the stain, and do not allow it to dry on the smear. Steam from three to five minutes.

(2) Wash off carbol fuchsin with water.

(3) Dip the slide into acid-alcohol until the smear is decolorized. (A very faint pink color remains.) Acid-alcohol is 95% alcohol, containing 3% of concentrated hydrochloric acid. By its action the carbol fuchsin stain is removed from everything in the smear *except acid-fast staining organisms,* which retain the red color of the fuchsin.

(4) Wash off acid-alcohol with water.

(5) Cover smear with methylene blue for about thirty seconds. The methylene blue serves here as a contrast or counterstain, coloring everything in the smear except the acid-fast elements.

(6) Wash off methylene blue with water and dry smear.

Value of acid-fast staining method. This staining method is useful, of course, in the study of any of the acid-fast organisms, but it is especially valuable as an aid in the laboratory diagnosis of tuberculosis and leprosy (Figs. 108 and 110).

Fat stain.* A staining method has recently been developed which serves to demonstrate clearly the abundant fatty material contained within the cells of many bacteria and fungi. The lipoid matter is first stained a bluish color by a solution of the new dye called Sudan Black B, then the cytoplasm of the organisms is colored pink by a counterstain. The amount of intracellular lipid, and its position within the cells, are to a considerable degree characteristic for the bacteria of a particular genus, and even in some cases for a particular species (Fig. 30).

The procedure for the fat stain is as follows:

(1) Prepare smear, let it dry thoroughly in the air, and fix it by heat in the usual way. (Chemical fixation has no special advantages and may result in some loss of demonstrable lipid.)

(2) Flood the entire slide with Sudan Black B solution (0.3 gm of the powdered stain in 100 ml of 70% ethyl alcohol), and allow the slide to

* Burdon, K. L.: "Fatty Material in Bacteria and Fungi Revealed by Staining Dried, Fixed Slide Preparations," *J. Bact.,* Dec. 1946, 52.

remain undisturbed at room temperature from five to twenty minutes. (A staining period of less than 5 minutes will often suffice, but intracellular lipid is colored somewhat more intensely when the staining is continued for 5 minutes or longer. No further staining apparently occurs after the solution precipitates and turns a greenish or brownish color, but *no harm is done if the stain is allowed to dry completely over the smear.*)

(3) Drain off excess stain and blot the slide thoroughly dry.

(4) Clear the slide with C. P. xylol, by dipping it in and out of the solvent in a Coplin jar, or by adding the xylol from a dropping bottle. Blot the cleared slide dry.

(5) Counterstain with safranin (0.5% aqueous solution) for about five seconds (for ordinary bacteria or fungi), or with dilute carbol fuchsin (Ziehl's carbol fuchsin diluted 1:10 with distilled water) for one to three minutes (for acid-fast organisms). (Overstaining with the counterstain must be avoided.)

(6) Wash in water, blot, and dry slide.

REFERENCES

Bausch, E.: *The Use and Care of the Microscope.* Rochester, N. Y.: Bausch and Lomb Optical Co., 1932.

Conn, H. J.: *Biological Stains.* Geneva, N. Y.: Committee on Standardization of Biological Stains, 3rd Ed., 1940.

Crossman, G. and Loewenstein, E.: "Demonstration of Tubercle Bacilli in Tissue by Fluorescence Microscopy," *J. Lab. & Clin. Med.,* Aug. 1943, 28:1349.

Gage, S. H.: *The Microscope.* Ithaca, N. Y.: Comstock Pub. Co., 17th Ed., 1941.

Manual of Methods for the Pure Culture Study of Bacteria. Geneva, N. Y.: Society of American Bacteriologists, Biotech. Publications.

Richards, O. W.: *Effective Use and Proper Care of the Microscope.* Buffalo, N. Y.: Spencer Lens Co., 1941.

Zworykin, V. K. and Others: *Electron Optics and the Electron Microscope.* New York: John Wiley & Sons, Inc., 1945.

REVIEW QUESTIONS—CHAPTER VI

1. Locate on a microscope, and give the principal functions of, the following parts: base, pillar, inclination joint, arm, body-tube, draw-tube, nosepiece, coarse and fine adjustments, stage, mirror, substage condenser, and diaphragm. What is meant by *mechanical tube length*?
2. Name and describe the three objectives usually supplied on a modern compound microscope. What markings are to be found on these lenses?
3. Describe oculars. With a 10x ocular what is the final (approximate) magnification obtained: (1) by the low-power, (2) by the high-power, and (3) by the oil-immersion objective?
4. Trace on a diagram the path of light rays through a microscope, to show how the microscopic image is made.

5. Explain the principle of the oil-immersion objective. What special property has immersion oil?
6. Discuss the use and value of the oil-immersion lens. What is meant by *resolving power* of a lens system? Explain how resolving power is limited by the wave length of the light used. About what size is the smallest object that can be made out clearly under the oil-immersion objective of the ordinary microscope?
7. Outline six steps which should always be followed, in order, each time the microscope is used.
8. Explain the principle involved in, and the practical usefulness of, the dark-field microscope.
9. Discuss the value of using ultraviolet light and fluorescent light for the microscope.
10. Outline the construction and principle of the electron microscope, and explain why this instrument can give clear pictures at very high magnifications.
11. About what size are the smallest objects that can be revealed clearly in electronographs? What are some of the limitations of the present-day electron microscopes?
12. Describe the making of a hanging-drop preparation. How does one proceed to focus on a hanging drop? What uses have such preparations? What is Brownian movement?
13. What is meant by relief staining (or negative staining), and for what purposes is it used? Name one relief stain.
14. Outline the procedure for making and fixing a good smear.
15. Name six stains in common use, and describe the actual process of staining with a single stain.
16. Name the necessary reagents and outline the essential steps in performance of a Gram stain.
17. Define *Gram-positive, Gram-negative,* and discuss the results and interpretation of the Gram stain. Name several kinds of Gram-positive and of Gram-negative bacteria.
18. What is meant by an *acid-fast stain, acid-fast bacteria*? What important disease germ is acid-fast?
19. Outline the procedure of the Ziehl-Neelsen acid-fast staining method.
20. Outline a procedure for staining the fatty material in bacteria or fungi.

CHAPTER VII

MORPHOLOGY OF BACTERIA

In Chapter V we have characterized the several different kinds of microorganisms, and have shown how they are named and classified. We now turn to a more detailed consideration of the best known of them all—the *true bacteria* (*Eubacteriales*). Most of the descriptions given here and in the chapters that immediately follow apply also to that small group of the higher bacteria classed in the family *Mycobacteriaceae*.

FORMS OF BACTERIA

Shape of individual cells. Among the true bacteria there are three basic forms (Fig. 27). The individual cells of some bacteria are *spherical,* or nearly so; some are *cylindrical* or rod-shaped; and others have the form of a *curved rod* or *spiral.* The spherical forms are called *cocci* (singular, *coccus*), the cylindrical forms *bacilli* (singular, *bacillus*), and the spiral-shaped forms *spirilla* (singular, *spirillum*). Each one of the many kinds of the true bacteria is placed arbitrarily in one of these three morphological groups.

Cocci. The word *coccus* comes from a Greek word meaning "berry," and all the cocci have a spherical, or nearly spherical, form like that of a tiny berry. Many are not perfectly round, but are flattened on one side, or are more or less elongated (Fig. 27: A, B, C, D, E, F). There are many species of cocci, and they are widely distributed.

Bacilli. In Latin, *bacillus* means "a little stick or rod," and the bacilli (Fig. 27: G, H) have the shape of a little rod or cylinder. Individual kinds of bacilli show almost infinite variations of this basic shape. Some are so short and thick that they are nearly indistinguishable from cocci; others are long and slender. Their ends may be square-cut, rounded, or tapered to a blunt or a fine point. Most of the bacilli are straight, rigid rods, but some are slightly curved

and less rigid. The bacilli, like the cocci, are widely distributed, and many species are known.

Spirilla. In this group are placed bacteria having the shape of a spiral, like a corkscrew. Some of the spirilla are short comma-shaped organisms; others are longer, more delicately coiled threads (Fig. 27: I). The body of a spirillum is rigid, or nearly so; it moves

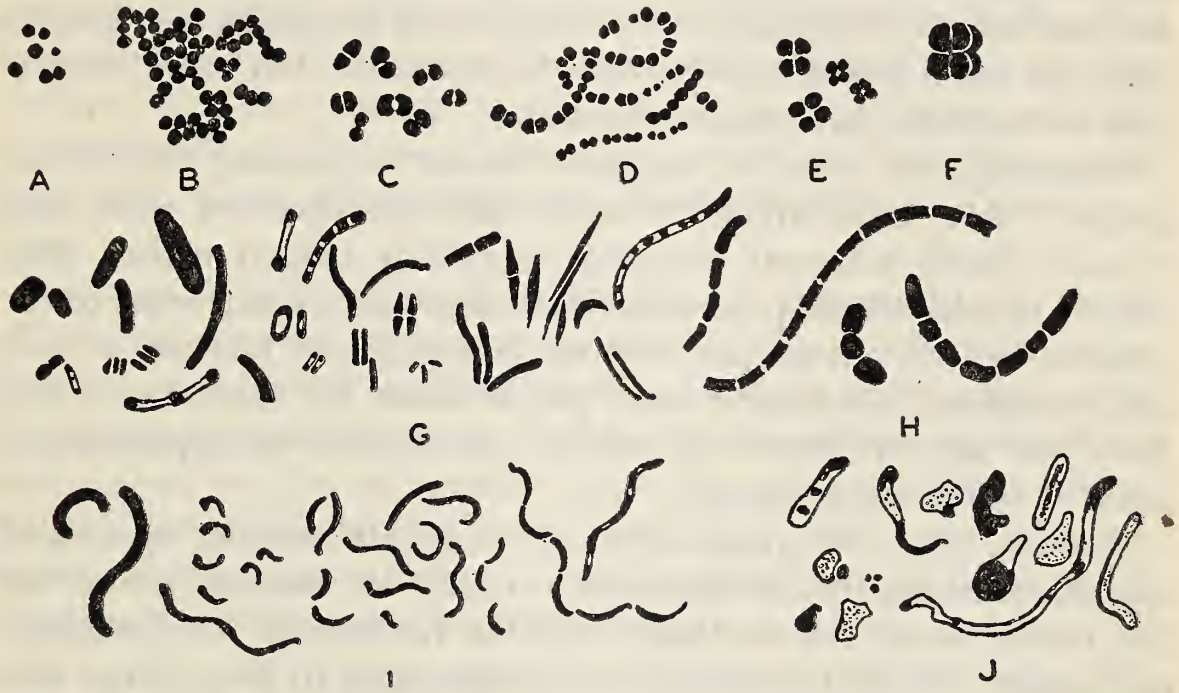


FIG. 27. Forms of the true bacteria. A: *single cocci;* B: *staphylococci;* C: *diplococci;* D: *streptococci;* E: *tetrads;* F: *sarcinae;* G: various forms of *bacilli;* H: *streptobacilli;* I: various forms of *spirilla;* J: involution forms.

through liquids by means of delicate, whip-like appendages, called *flagella.* There are fewer known varieties of spirilla than of cocci or bacilli.

Cell groupings. Among the cocci, and to a lesser extent among the bacilli and spirilla also, the actively growing cells tend to arrange themselves into characteristic *groups.* The character of the group is practically constant with any one kind of bacterium; therefore the grouping, as seen under the microscope, is of considerable help in the recognition of species.

(*a*) *Groupings of cocci.* Practically all the common cocci occur in groups of two or more cells, and cocci may be placed in one of the following classes, according to the number and arrangement of the organisms.

Diplococci; cocci in pairs. Usually the adjacent sides of the paired organisms are flattened. The germs of meningitis, gonorrhea, and

pneumonia, and several other familiar species of bacteria, have this form.

Streptococci; cocci in chains. When streptococci are multiplying, the cells always divide in the same plane of space and the new cells remain adherent, thus forming chains of the organisms resembling a string of beads. The length of the chain varies with the conditions under which the bacteria are growing, and also varies somewhat with the particular species of streptococcus. Streptococci are often found in connection with disease, but there are several varieties that are entirely harmless.

Staphylococci; cocci in irregular masses resembling clusters of grapes. These cocci divide in more than one plane of space and remain closely adherent, forming irregularly shaped masses. One can see in microscopic preparations of staphylococci that some of the cells lie behind others, just as some of the grapes forming a compact bunch will lie behind other grapes when the cluster is viewed from one side. There are a number of varieties of staphylococci, some of which cause disease.

Tetrads; cocci in groups of four. The only familiar species of bacteria showing this arrangement is called *Micrococcus tetragenus* (or, by the newer nomenclature, *Gaffkya tetragena*). This coccus is occasionally found in tuberculous sputum, and in some other disease conditions in man. In microscopic preparations the group of four cells is often seen to be surrounded by a capsule.

Sarcinae; cocci in cubical packets. The sarcinae are relatively large spherical cells which occur in compact masses, consisting of eight individual organisms arranged in the form of a cube. The *Sarcina lutea* is a harmless bacterium of this class. It occurs commonly in the dust, and can be cultivated readily from dusty air. The growth has a bright yellow color.

(*b*) *Groupings of bacilli and spirilla.* Some species of bacilli grow characteristically in pairs, and so may be called *diplobacilli.* Others may form long chains and are called *streptobacilli.* Some bacilli show a very striking *parallel* arrangement of the cells; others grow in a tangled, intertwined mass.

The spirilla do not group themselves in any characteristic way, but they frequently grow in long, wavy chains. Often the individual spirilla making up the chain cannot be made out, and the filament appears like a skein of twisted thread.

Variations from the typical form. In a laboratory culture of a

single kind of organism, the individual cells often vary considerably in size and shape, and some may have far from the typical form. This is natural, because all the individuals making up the population of the culture are not at the same stage of growth, and some may be degenerating, some may be dead. Very young cultures sometimes contain cells which look distinctly different from those in the same cultures a few hours later; usually the majority of the organisms in young cultures (before cell-division is occurring very actively) are distinctly larger than the predominant forms in older cultures. Not only the age of the culture, but the character of the culture medium, the temperature of incubation, and other conditions affect more or less profoundly the shape and size of the organisms.

Involution forms. The most conspicuous changes in the shapes of bacteria are seen when cultures become old, or when the organisms are growing in a medium which is for some reason unfavorable to their normal development. In these circumstances, the organisms may appear swollen or shrunken and may assume various bizarre shapes. They usually take stains irregularly, and some cells may appear as pale "ghost forms," which take almost no color at all. Sometimes, on the other hand, the organisms merely lose their distinct outline and become granular. These changes are signs of a simple degeneration or involution. Cells showing these abnormal appearances as a result of age or unfavorable environment are referred to as *involution forms* (Fig. 27: J).

Pleomorphism. There are a number of species of bacteria, however, which regularly show striking variations in the shapes of the individual cells in young and actively developing cultures, as well as in old and degenerate ones. Such species are described as *pleomorphic* (having-many-forms). Good examples are the bacilli of diphtheria (Fig. 82) and of tuberculosis (Fig. 108). The differences in size and shape shown by organisms of this kind are *not* due primarily to the influence of age or unfavorable environment. Apparently, these bacteria have less rigid cell walls than others, and they may exist as fragments of living protoplasm of irregular dimensions.

Changes accompanying dissociation. A single bacterial species may "dissociate" under certain conditions into so-called "smooth" and "rough" variants. These variants usually differ considerably in morphology. The *smooth* strain (smooth or S phase) of a bacterium is

made up of cells of relatively uniform size and shape, which, at maturity, divide by fission into two new cells that separate freely and slip past each other to form smooth masses of growth. On the other hand, the individual bacterial cells composing the *rough* variants (rough or R phase) of the same species are likely to be elongated forms which grow in filaments or chains and do not split into short elements of regular length but, instead, pile up into irregular, filamentous masses having a roughened surface (Fig. 56).

The important topic of *bacterial dissociation* or *variation* will be discussed more fully later in Chapter XIII.

Recognition of morphologic types under the microscope. Beginning students are often disappointed to find that, after studying the descriptions of staphylococci, sarcinae, and the other morphologic types listed above, they still cannot easily differentiate the various forms when they sit down at the microscope. The instructor, called to look at the same preparation, sometimes admits that he cannot make a positive diagnosis either, but often, for some reason puzzling to the student, he recognizes the correct morphologic group at once, without hesitation. This success of the instructor is doubtless due, in some instances, to keener and more careful observation as a result of which he sees more than a lackadaisical student does; but principally, of course, it is the result of experience—he knows *what to look for*. There is no real substitute for such experience, but the following remarks may be helpful in this connection.

The bacteriologist bases his decisions on the *relative size of the individual organisms* in a smear, as well as on the *character of the cell-groups which predominate,* and, of course, *the staining reactions.* In particular, he is not deceived into calling a pure staphylococcus culture a diplococcus just because he sees some of the cocci in pairs, or calling it a streptococcus just because he finds a few cocci apparently arranged in a short chain. He realizes that the particular groupings present are in part accidental, brought about by the mechanical breaking up of natural groups in the process of making the smear. He takes into account the character of the culture medium or other material from which the smear was made, as well as the age of the growth, and appreciates that a morphologically typical appearance would be unreasonable to expect in some circumstances—a streptococcus growing on a dry, solid medium, for instance, is not likely to have long chains.

Students should remember, too, that in the usual microbiology

course, especially when laboratory periods are spaced at intervals of forty-eight hours or more, it is often not practicable for them to make microscopic examinations of their cultures sufficiently early or often to catch the organisms when they have that textbook look.

SIZE OF BACTERIA

Unit of measure; the micron. As we have already learned, microorganisms are so minute that they cannot be measured accurately by any familiar scale. A special unit of measurement must be used. This is the *micron* (plural, *micra*). The Greek letter μ (pronounced "mu") is the symbol for a micron. 1μ is equal to 1/1000 of a millimeter. A millimeter is 1/1000 of a meter. The meter (about 39 inches) is the standard unit for measuring length in the metric system—the system of weights and measures used in all scientific work. In terms of inches, one millimeter is about 1/25 of an inch, and a micron is equivalent to about 1/25,000 of an inch.

Actual size of some common species. The actual size of some of the best known bacteria is given in the following table:

Table VI. Size of Common Bacteria

NAME OF ORGANISM	ACTUAL SIZE OF INDIVIDUAL CELLS
Staphylococcus aureus (boils, etc.)	0.8 to 1.0μ in diameter.
Streptococcus hemolyticus (sore throat, etc.)	0.4 to 0.75μ in diameter.
Pneumococcus (pneumonia)	0.8 x 1.2μ.
Gonococcus (gonorrhea)	0.8 x 0.6μ.
Meningococcus (meningitis)	0.8 x 0.6μ.
Corynebacterium diphtheriae (diphtheria)	Vary in length from 1.5μ to 6.5μ, in width 0.3 to 1μ.
Mycobacterium tuberculosis (tuberculosis)	2 to 4μ long, 0.3–0.5μ wide.
Eberthella typhosa (typhoid fever)	1 to 3μ long, 0.8–1μ wide.
Clostridium tetani (tetanus)	2 to 4μ long, 0.3–0.5μ wide.
Vibrio cholerae (cholera)	1 to 2μ long, about 0.4μ wide.
Treponema pallidum (syphilis)	8 to 14μ long, about 0.2μ wide.

Comparisons with familiar objects. It is difficult to conceive what these figures really mean and to visualize accurately how extraordinarily tiny these germs are. It may help to realize that as we look at them through the oil-immersion lens of the microscope, we see them magnified about a thousand times. Perhaps the most helpful object for comparison is a good rule marked off in millimeters. Most of the bacilli are about 1μ (1/1000 of a millimeter) wide. This means that 1000 *of them could be laid side by side very comfortably across a millimeter space.* The head of an ordinary pin has a diameter of about one millimeter.

STRUCTURE OF BACTERIAL CELLS

The fundamental elements of bacterial structure are: (1) the *cell wall,* (2) the cytoplasmic body or *protoplast,* and (3) the granules and other *cell inclusions.* In addition, *capsules, flagella,* and *spores* are found in some species.

Cell wall. Since the majority of bacteria are not spherical, but instead maintain a different and characteristic shape, such as that of a long rod, it is obvious that there must be an outer wall of considerable rigidity. Such a wall cannot ordinarily be seen, however, in either stained or unstained preparations of bacteria, because the living contents of the cell lie so closely pressed against it that its outline is obliterated. At times, however, the cytoplasm contracts and is pulled away from the wall, revealing the latter clearly as a very thin membrane which takes stains only faintly. Bacteriologists are accustomed to seeing in old cultures so-called "ghost forms" which have degenerated so that they contain little or no stainable protoplasm, and yet these still have a distinct wall, maintaining more or less exactly the shape of the original bacterial cell. The enveloping wall thus appears to be a firm, somewhat elastic structure, separate from the cell contents (Fig. 28).

The outer layer of Gram-positive bacteria. The separation of bacteria into what we call Gram-positive and Gram-negative varieties is accomplished by carrying through an arbitrary staining procedure, but there is good reason to believe that the differences revealed by Gram staining are associated with properties of a truly fundamental nature. Recent studies have substantiated the theory, originally proposed by Churchman, that the inner protoplasm of *all* bacteria cells is Gram-negative, and that the Gram-positive organisms possess an

additional outer layer containing Gram-positive material. The Gram-positive character appears to reside in a chemical complex, made up of protein and magnesium ribonucleate, which exists at the cell surface. The external cell cortex is apparently rather easily

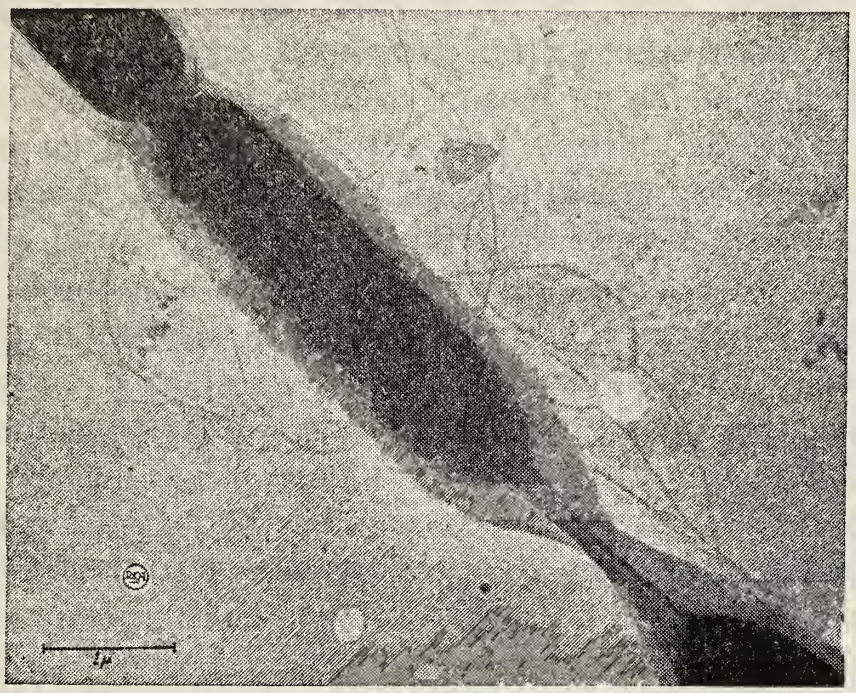


FIG. 28. A chain of cells from a young culture of *B. cereus* photographed with the electron microscope. The lighter external layer represents the cell wall. The fine, curved threads are flagella. Note the three different stages in cell division, and (at the lower right) the sharply defined connecting strand, enclosed by the drawn-out cell walls, between the almost completely divided cells. Compare with Fig. 35. (From Johnson, F. H.: "Observation on the Electron Microscopy of *B. cereus* and tyrothricin action," *J. Bact.*, 47:553, 1944.)

stripped away or destroyed by injurious chemical or physical agents. Hence old or degenerate Gram-positive bacteria may stain Gram-negative.

Cell contents: the cytoplasm or protoplast. When young, actively growing bacteria are examined in the living state, under the microscope, they appear as tiny, transparent, colorless masses in the shape of spheres, cylinders, or spirals. They are only slightly denser than the water in which they are suspended, and therefore they are hard to see. The interior of the cells—the cytoplasmic body or the *protoplast*—is in most cases entirely homogeneous, and appears to be made up of perfectly clear protoplasm. There is no head and no tail, and there are no special organs within the cells.

The protoplast, like the cytoplasm of other plant cells, is not

attached to the cell wall, but lies free within it. It has its own limiting wall—a semipermeable membrane called the *cytoplasmic membrane.* This, however, cannot be discerned in the fully turgid cell, because it is in such close contact with the other cell wall.

When dried upon a glass slide and colored with a stain, *young* bacteria usually show the same clear appearance as when unstained. In most species, the entire cell takes on a solid, deep color, like that of the nucleus of an animal cell. When lightly colored, or partly decolorized, however, a certain lack of uniformity in staining may be seen in the case of a number of species, even in young organisms. For example, the plague bacillus and some others take a stain only at the ends of the rods (polar staining). Electron micrographs have revealed unsuspected irregularities in the density of the protoplast within a number of common bacteria. But in general it is true that young, actively multiplying bacteria are usually homogeneous in internal structure.

Cell inclusions. In older bacterial cells, on the other hand, homogeneity of the cell contents is usually lost, granules and vacuoles appear, and the organisms show more or less conspicuous inequalities in staining. These new appearances are thought to be due to the formation, and separation from the cytoplasm, of granules or droplets of various nonliving substances, which thus may be regarded as *cell inclusions.* The more important of these inclusions are: (1) the so-called *metachromatic granules,* composed of *volutin,* (2) granules of *glycogen,* and (3) *fat* droplets.

Metachromatic granules. These appear as globules of deeply staining material within the cytoplasm. They are seen in several of the important pathogenic bacteria, but most conspicuously in the bacilli of diphtheria. In the latter species they are so prominent and so characteristic that they are of positive aid in the recognition of this germ under the microscope. The granules are sometimes referred to as Babes-Ernst granules, but are more commonly described as *metachromatic,* since when stained they take a color different from that of the stain itself. Thus, after staining with methylene blue, the granules in diphtheria bacilli appear mahogany brown. In some species they occupy characteristic positions within the cells (Fig. 29).

The nature, origin and significance of these structures have been variously explained by different investigators. Often the granules have been regarded as nuclei or as reproductive bodies. Most bac-

teriologists believe, however, that the granules in all cases represent nothing more than nonprotoplasmic cell inclusions, composed of what is called *volutin.*

Volutin is a viscous substance, which may occur in the form of droplets or irregular threads, not only in many bacteria but in yeasts, molds, and algae as well. The granules are not soluble in alcohol or in fat solvents, but the volutin may be dissolved out of bacterial cells (so that the granules disappear) by heating a bacterial suspension in water to 80° C for a few minutes. (Protoplasmic structures would be *coagulated, rather than dissolved,* by this hot-water treatment.) Thus, the volutin granules are regarded not as living matter, and not as necessary or permanent organs in bacterial cells, but rather as grains of reserve food material which may be stored temporarily in the mature cells of certain species.

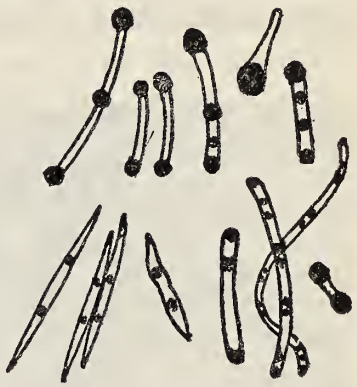


FIG. 29. Bacilli showing metachromatic granules.

Since volutin stains with basic dyes more intensely than the surrounding cytoplasm, metachromatic granules may be brought out prominently by various special procedures. For diphtheria bacilli, Albert's stain is widely used.

Albert's stain (Laybourn's modification). Stain for 5 minutes with the following solution:

Toluidine blue	0.15 gm
Malachite green	0.2 gm
Glacial acetic acid	1.0 cc
95% alcohol	2.0 cc
Distilled water	100.0 cc

Wash in water. Apply Lugol's iodine solution (I, 1 gm, and KI, 3 gm, dissolved in 300 cc distilled water) for 1 minute. Wash and dry smear.

Glycogen granules. The carbohydrate glycogen is commonly present in the cells of animal tissues, and occurs also in yeasts and molds and in some bacteria. Grains of glycogen in bacterial cells are not noticeable in ordinary microscopic preparations, but may be demonstrated as tiny, reddish-brown particles by treating the living organisms with a strong iodine solution. The glycogen apparently functions as a reserve foodstuff.

Fat droplets. The accumulation of globules of lipid within the cell is characteristic of various species of bacteria. The fat is especially conspicuous in the nitrogen-fixing soil organisms (genus *Azotobacter* and *Rhizobium*), in large, free-living spirilla (genus *Spirillum*), in various acid-fast bacilli (genus *Mycobacterium*), and in the common aerobic sporeforming bacilli of the dust and soil (genus *Bacillus*) (Fig. 30). Stainable intracellular lipid is present also

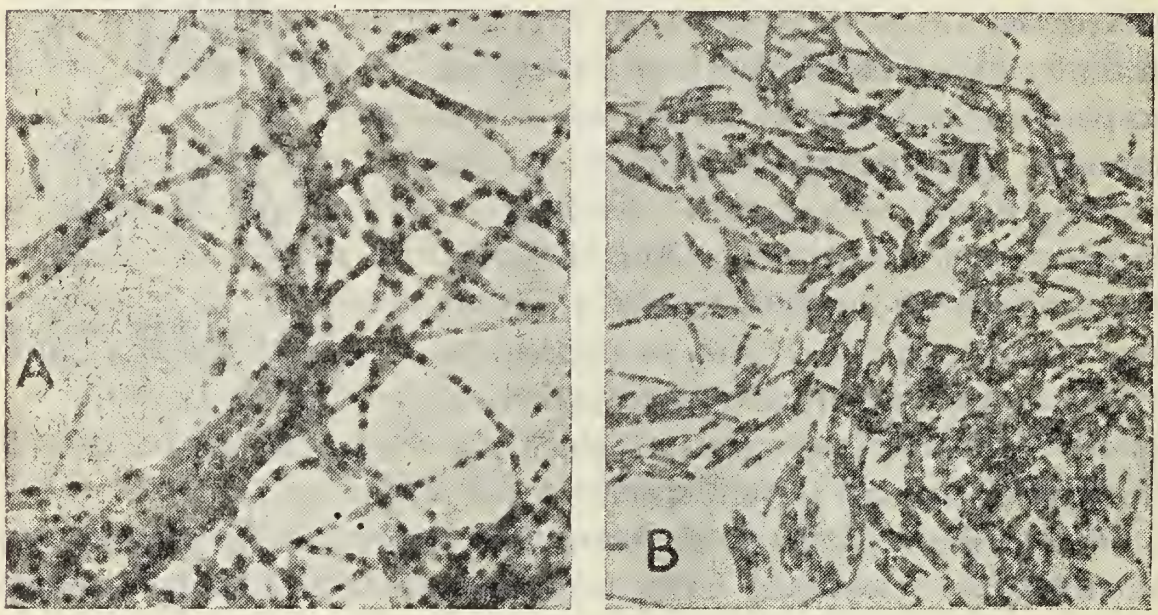


FIG. 30. Smears of aerobic sporebearing bacilli stained by Burdon's Sudan Black B fat-staining method. All the dark granules in these photographs are droplets of fat. A: *Bacillus anthracis* from a 12-hour-old culture; B: *Bacillus subtilis* (Ford) from a 24-hour-old culture.

in the diphtheria bacilli, the anaerobic bacilli of wound infections, and in both Gram-positive and Gram-negative cocci. Only a few species of the Gram-negative bacilli, however, seem to contain fatty material regularly. A certain number of the cells in cultures apparently undergo a kind of "fatty degeneration." Further studies with fat stains are needed before the origin and function of the intracellular lipid can be fully understood. A routine method for fat-staining of ordinary smears is outlined in the preceding chapter.

It is important to realize that, when a smear of a fat-storing bacterium is stained with one of the common simple stains, such as methylene blue, the larger fat droplets in the organism may appear as *colorless* spaces or vacuoles.

Nuclear material. A question long debated by bacteriologists is whether true nuclear material exists in bacterial cells and, if so,

whether it is organized into a separate structure comparable to a typical nucleus. As noted above, volutin granules may look like nuclei, but proof is lacking that these or other similar morophologically distinct chromatin particles sometimes seen in bacteria actually function as nuclei. There is little doubt, however, that matter chemically equivalent to a nucleus is present. Young bacteria stain heavily and evenly with basic dyes like the nuclei of animal cells. The most acceptable view, at the present time, is that bacteria do contain chromatin (material that takes nuclear stains), and that this chromatin is ordinarily uniformly distributed in the cytoplasm in the form of minute, invisible particles, although it is capable of being aggregated at times into larger, visible bodies.

Capsules. Surrounding many bacterial cells, there is a kind of gelatinous envelope. Knaysi refers to this as the *slime layer.* This enveloping material is ordinarily too thin to be seen in most species, but in some kinds it is regularly developed into a clearly visible *capsule,* external to the cell wall, with a sharply defined outer edge following the contour of the cell body. Often the capsule is much wider than the bacterial cell itself. Chained or paired organisms are enclosed within a single, continuous capsule (Fig. 31).

The origin of the capsular material is uncertain, and its relation to the cells is not clearly understood. It may arise from a modification of the cell wall; or it may be a product, secreted by the living bacteria, that remains attached in the form of a more or less firm, mucilaginous structure. In any case, capsule formation is markedly influenced by environmental conditions; it can be induced, in special circumstances, in strains of bacteria that ordinarily show no evidence of any appreciable slime layer. The organisms may then form *mucoid colonies,* and they are said to be in the M (mucoid) growth phase. Chemical studies have shown that the capsular material from different bacteria is of varying composition; most commonly it is made up of complex carbohydrates, but in some cases it consists of nitrogen-containing, mucin-like substances.

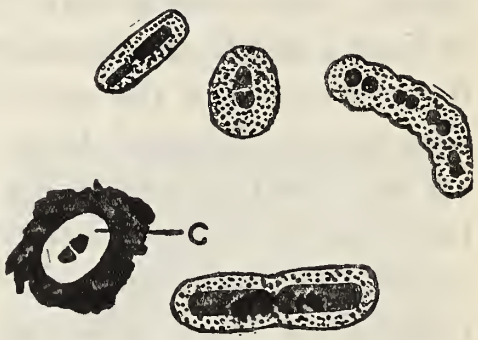


FIG. 31. Capsulated bacteria. C: appearance of the capsule in smears prepared by Gin's method (India ink smears).

The capsular matter does not take stains readily, and when a

comparatively weak dye, such as methylene blue, is used, the capsule remains uncolored, and appears as a clear zone about the cell. In smears, uncolored spaces about the bacteria *not* due to capsules are seen rather commonly, however; hence the presence of a true capsule is best demonstrated by a special capsule stain. The method of Hiss is widely used.

Hiss capsule stain. The smear should be made from a serum or ascitic fluid medium or from a body fluid, such as the peritoneal fluid, and preferably without use of water. Allow the smear to dry in the air, and fix by gentle heat. Cover smear with gentian violet solution (made by adding 5 cc of a saturated [15%] alcoholic solution of crystal violet to 95 cc water), and heat gently until steam is just visible. Wash off stain at once with an excess of 20% solution of copper sulphate (use no water); drain slide and blot dry immediately. The capsule appears as a faint purplish halo about the more deeply stained bacteria.

Among pathogenic bacteria, the phenomenon of capsule formation is of prime importance. Species which develop especially large capsules include the germs of lobar pneumonia (*Diplococcus pneumoniae*), the Friedländer bacilli (*Klebsiella pneumoniae*) and related species (found in some cases of pneumonia and other inflammations), and *Clostridium perfringens* (germ causing gas gangrene following wound infection). Probably all pathogenic bacteria develop at least a small amount of capsular substance when growing in the body tissues, and the most conspicuous capsules are seen on organisms freshly obtained from the infected host. The capsules seem to act as a kind of defense for the germ against bactericidal factors in body fluids. They contribute definitely, therefore, to the disease-producing power, or *virulence,* of the organism. They are lost by most species after cultivation for some time in the laboratory; and, accompanying the disappearance of the capsules, there is usually a marked loss in capacity to produce disease.

Encapsulation is significant, also, in another respect. It is the chemical structure of the capsular material that determines the specificity of the *antibodies* formed by the infected individual in response to the presence of the germ. The numerous "types" of pneumococci, for example, differ from one another in the chemical composition of the polysaccharides which make up their capsules, and consequently each type stimulates the formation of a different antibody. Immunity to a particular type of pneumococcus requires

an antibody that will react *with the capsulated organisms of that type.*

Flagella; motility of bacteria. All the known spirilla and about half of the more familiar species of bacilli, but none of the ordinary cocci, possess the power of locomotion through liquids—that is, they are able to move independently from place to place. Bacteria that possess this capacity are said to be *motile;* those kinds that cannot move about independently are *nonmotile.*

The movement of motile bacteria can be plainly seen when the living organisms are examined under the microscope. Some kinds travel sluggishly; others with really amazing rapidity. Sometimes active organisms pass across a microscopic field more rapidly than the eye can follow. It should be remembered, however, when examining motile organisms, that their apparent speed is magnified by the microscope in the same proportion as their size.

The organs of locomotion are extremely delicate, hair-like processes which extend out from one or more parts of the cell-bodies of the motile bacteria. These are called *flagella* ("little whips"). Their rhythmic contraction propels the organisms along.

Whether the flagella, the organs of locomotion, originate as extensions of the bacterial cell wall, or arise from the inner cytoplasm, has not yet been decided, although evidence recently obtained with the electron microscope strongly favors the latter view. The flagella are usually somewhat coiled, and are often much longer than the bacterium from which they arise. They are remarkably thin, having a width of considerably less than 0.1μ (estimated 0.03μ), and are easily broken off. Apparently, when the bacteria are in motion, the flagella often entwine to form a tail-like locomotor organ.

FIG. 32. The typhoid bacillus stained to show the flagella.

In unstained preparations examined by the ordinary microscope, flagella are quite invisible (although the movement they bring about is apparent). *They are also invisible in ordinary stained preparations;* therefore, one cannot tell by examination of a bacterium colored in the usual way whether it possesses flagella or

not. It is possible to stain the flagella, however, by special methods (Fig. 32).

True motility and Brownian movement. The independent movement of motile bacteria from place to place by means of their flagella is spoken of as *true motility*. When bacteria are being watched for motility, this true locomotion must be distinguished from the movement produced by currents in the liquid which cause the organisms to drift about. It is especially important that true motility be carefully distinguished from the mere vibration of the organisms in the fluid—a kind of motion known as *Brownian movement*. This is a physical phenomenon due to the rapid vibration of the molecules of the liquid itself which imparts an irregular bobbing or vibratory motion to any very small particles suspended in it. Nonmotile as well as motile bacteria, and in fact a particle of matter of any kind sufficiently small, will show Brownian movement when suspended in water. This motion is not progressive. A nonmotile organism will be impelled a little forward, then a little back, now a little this way, and now that, but it will not change its position in relation to neighboring organisms which are likewise bobbing around like buoys in a heavy sea. On the other hand, a motile organism propelled by its flagella will be continually changing its position with respect to its neighbors.

Different strains of bacilli or spirilla belonging to the same species may show marked differences in the degree of motility that they manifest in laboratory cultures. The age of the culture at the time of examination, the character of the culture medium, and other factors influence the activity of any given strain. Thus, motility is not a sufficiently constant character for use in the exact differentiation of species or varieties of bacteria although, in certain special instances, tests for motility do have practical utility.

SPORE FORMATION BY BACTERIA

Certain kinds of *bacilli* are capable of changing into resistant bodies called *spores* (or *endospores*). *Each individual bacillus becomes converted into a single spore.* The spore is able to withstand comparatively high temperatures and other unfavorable influences, and so serves to keep the organism alive when it would otherwise perish. When suitable conditions are supplied, the spore germinates and returns to the original bacillus form. This remarkable property of spore formation is confined to a few species of bacilli only, but it

has great practical importance. The aerobic sporeforming bacteria make up the genus *Bacillus,* while the anaerobic species are classified in the genus *Clostridium.*

Conditions under which spores form. Some of the sporebearing bacilli change to the spore form more readily than others, and certain types of culture media, or other special environmental conditions, tend to favor sporulation, while other circumstances suppress it. In any case, the principal stimulus to spore formation seems to be an accumulation of waste products resulting from the growth of the organisms themselves. Sporulation is always preceded by a period of active multiplication, and nearly all the organisms in the same culture begin to form spores at about the same time. The effect of the conversion of the bacilli into spores is the preservation of the life of the individual organisms when subjected to an unfavorable environment, thus perpetuating the species under circumstances which would destroy non-sporebearing organisms. It is important to note, however, that sporulation is clearly a *regular habit* of the sporeforming bacteria, and *part of their normal cycle of development,* irrespective of any special need for protection from injurious influences.

In some species, spore formation may be temporarily or permanently suppressed by cultivation under unusual conditions—for example, at abnormally high temperatures (as Pasteur showed in his work with anthrax bacillus vaccines), or on deficient media, or in circumstances where a sufficient access to oxygen is denied. In general, endospore formation is favored by the same conditions that favor active growth.

Only one spore is formed from a single bacterium. Therefore, *spore formation among the bacteria is not a method of multiplication.* The molds, on the contrary, multiply by forming many similar bodies, also called spores. (The student should distinguish carefully between *mold* spores and *bacterial* spores.)

Process of spore formation. The process of spore formation consists in a kind of condensation of the cell contents into a round or oval body with a thick wall. The actual manner of spore formation varies in minor details in different species of the sporebearing bacilli, but the process is essentially the same in all cases. There appears first a spot, near the center or the end of the rod, which stains more intensely than the rest of the cell. This spot enlarges until, in many cases, it is of greater diameter than the cell from which it is forming.

Very soon the center of this spot, instead of staining more intensely, ceases to stain at all when colored by ordinary methods. This is because the developing spore has now elaborated its dense outer wall, through which the stain does not penetrate. The wall itself may take a pale color, but the center of the spore does not stain; it

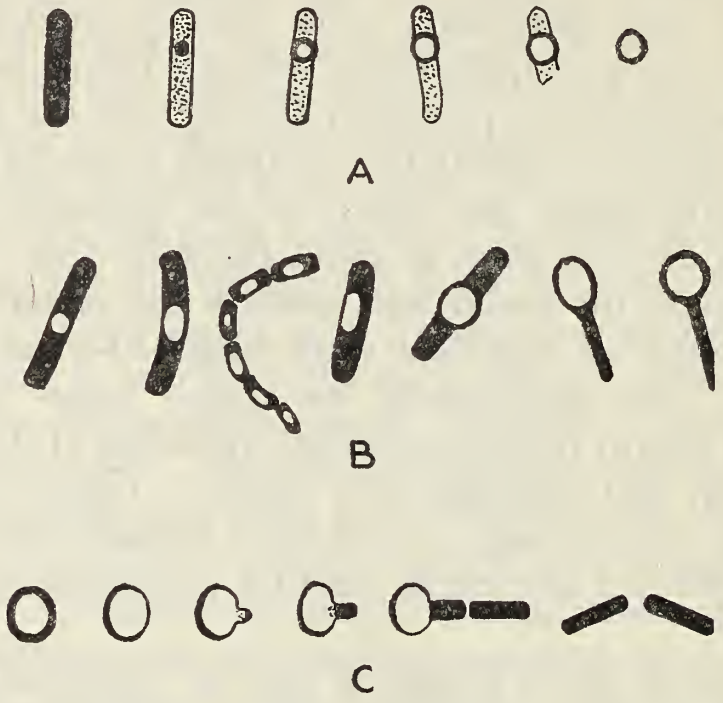


FIG. 33. Bacterial spores. A: stages in the formation of a spore. On the left is the vegetative form of the bacillus, on the right the free spore formed from it. B: the sporulating forms of different kinds of sporebearing bacteria, showing characteristic differences in the size, shape and position of the developing spore. C: one method of germination of a spore back to the vegetative form. The newly germinated rod is shown dividing at once into two actively growing bacilli.

appears as a bright, colorless spot. Meanwhile, the remainder of the original cell decreases in size, stains very faintly, and gives every evidence of having given up its vital matter to the spore. Finally, the remnant of the original cell disintegrates and the spore becomes free (Fig. 33:A). Often small tags of stainable cell substance stick to the spore for some time before it becomes *completely* free.

A bacillus in its original active, growing state is referred to as the *vegetative form* of the organism. A bacillus in the process of developing its spore is said to be in the *sporulating form;* it is sometimes called a *sporangium.* A completely formed spore detached from the bacterium in which it developed is called a *free spore.*

Each of the different species of sporeforming bacilli regularly makes a spore which is of a certain size and shape, and which occu-

pies a characteristic position in the sporulating form of the organism. Consequently, much reliance is placed upon the morphological features of sporulation in differentiating varieties of sporebearing bacilli (Fig. 33: B).

Properties of free spores. A free bacterial spore has two principal properties, in both of which it resembles a seed: (1) *It is highly resistant* to physical and chemical changes which would injure or destroy the original organism. (2) *It is capable of germination,* that is, it is able to return to the original growing vegetative form, *with all original properties intact,* when placed once more in a favorable environment.

Resistance of spores. The vegetative forms of sporebearing bacteria are no more resistant than non-sporeforming organisms, but the spores resist drying, heat, and injurious chemicals to a remarkable degree. Spores of the anthrax bacillus, for example, dried upon a piece of thread, have been found capable of germination after a lapse of as much as thirty-five years. Furthermore, the bacilli germinated from these very old spores were still capable of causing anthrax in laboratory animals. The vegetative forms of most bacteria die in a few minutes when exposed to a temperature of 65°–70° C (150°–160° F), but some spores resist the action of live steam at 100° C (212° F) for a half-hour or more. Dilute solutions of carbolic acid and other chemicals kill vegetative forms easily, but spores may withstand prolonged exposure to relatively concentrated solutions of these substances. There is often noted, however, a marked difference in the resistance of the spores from different strains, and also among the spores present in the very same pure culture. The majority of the spores of common aerobic species are actually more susceptible to heat than might be expected, and are likely to be killed by an exposure of no more than 10 minutes to moist heat at 75°–80° C. Only the minority that survive such an exposure will be possessed of the marked resistance to heat mentioned above.

Germination of spores. Spore germination is a reversal of the process of spore formation. The method of germination varies with different species, and seems to be as constant an attribute of a particular kind of sporulating bacillus as the character of the spores themselves. In the larger varieties of the common aerobic sporebearing bacilli (for example, *Bacillus cereus*), there is merely an *absorption of the dense outer wall* of the spore and a relatively slow return, requiring several hours, to the shape and size of the original vegeta-

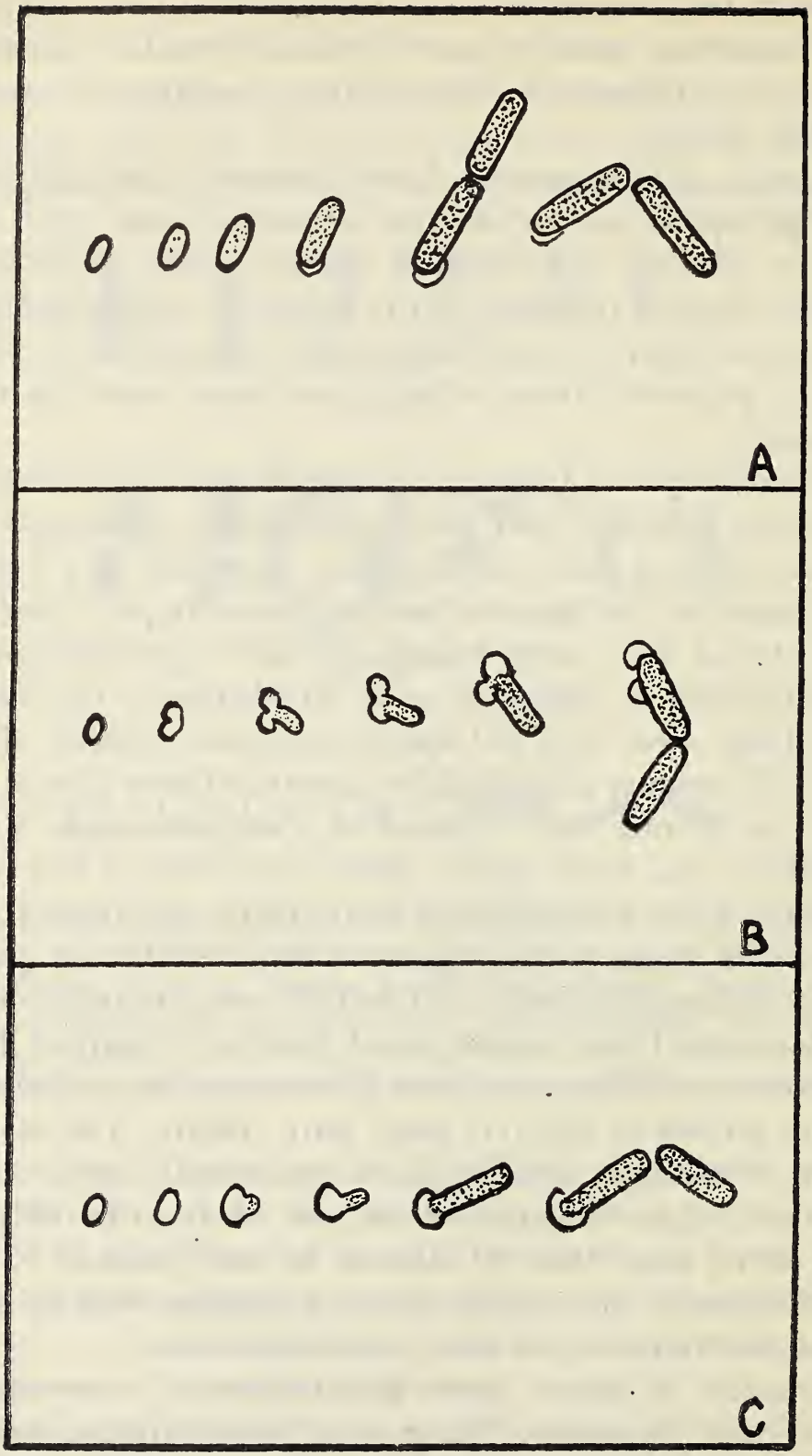


FIG. 34. The sketches show, from left to right, stages in the process of *spore germination* as observed with different varieties of bacilli of the genus *Bacillus*. A: germination by absorption of the spore wall and gradual return to rod form; B: equatorial germination with splitting of the spore-case into two parts; C: equatorial germination without splitting of the spore-case.

tive form. In the case of the smaller species, however, there is a *shedding of the outer wall,* or coat, of the spore. For example, in *Bacillus subtilis* (Ford) the wall of the spore thins at one place, and through this area the germinating rod quickly emerges from the now empty spore case. Since the bacillus comes out from the middle, or from near the middle, of the spore this is called *equatorial germination.* In the case of this species, the spore case remains intact, but in others, such as *Bacillus subtilis* (Marburg), equatorial germination is accompanied by a *splitting of the spore case* into two parts (Fig. 34).

Practical importance of spore formation. Spore formation is a property of a relatively small group of bacilli, the members of which have several characteristics in common. All the sporeforming bacilli can be found *in the soil,* where they exist in the spore state. Many of these organisms are native to the soil, and others are normal inhabitants of the intestinal tract of human beings and animals, and reach the soil through the intestinal discharges. Some kinds are always present in dust, and thus become widely distributed.

Fortunately, only a few of these sporebearing organisms are able to cause disease. Most of them are perfectly harmless saprophytes. The most important sporeforming bacilli which may produce disease are *Clostridium tetani,* the germ of tetanus; *Clostridium perfringens,* the principal bacillus of gas gangrene; and *Bacillus anthracis,* the cause of anthrax.

The existence of sporebearing bacteria must be taken into account in connection with practical methods used to destroy germs. When it is desired to render an object *sterile,* i.e., entirely free of living organisms, the great resistance of the spores that may be present must be considered. The result is that routine methods of sterilization are much more drastic than would be necessary if there were no spores to be destroyed.

REFERENCES

Bartholomew, J. W. and Umbreit, W. W.: "Ribonucleic Acid and the Gram Stain," *J. Bact.,* 1944, 47:415.

Burdon, K. L.: "Fatty Material in Bacteria and Fungi Revealed by a New Fat-Staining Procedure," *J. Bact.,* 1944, 47:414.

Dubos, R. J.: *The Bacterial Cell.* Cambridge, Mass.: Harvard Univ. Press, 1945.

Henrici, A. T.: *Morphologic Variation and the Rate of Growth of Bacteria.* Springfield, Ill.: Charles C Thomas, 1928.

Knaysi, G.: "Cytology of Bacteria," *Botan. Rev.,* 1938, 4:83. Also, *Elements of Bacterial Cytology.* Ithaca, N. Y.: Comstock Pub. Co., 1944.

LAMANNA, C.: "The Taxonomy of the Genus Bacillus—I. Modes of Spore Germination," *J. Bact.*, 1940, 40:347; "A Non-Life-Cycle Explanation of the Diphtheroid Streptococcus from Endocarditis," *J. Bact.*, 1944, 47:327.

LEWIS, I. M.: "Cytology of Bacteria," *Bact. Reviews,* Sept. 1941, 5:181.

MUDD, S. and ANDERSON, T. F.: "Pathogenic Bacteria, Rickettsiae, and Viruses as Shown by the Electron Microscope—I. Morphology," *J. A. M. A.,* Oct. 28, 1944, 126:561.

REVIEW QUESTIONS—CHAPTER VII

1. Name (singular and plural) the three morphological groups of the bacteria. What is the basic shape of the organisms in each group?
2. Define *coccus, diplococci, streptococci, staphylococci, tetrads, sarcinae, bacillus, diplobacilli, streptobacilli.* Describe and sketch characteristic shapes and groupings of cocci and bacilli. How do spirilla differ from spirochetes?
3. Define *involution forms, pleomorphic bacteria.* Give an example of a pleomorphic organism.
4. What morphological differences are usually found between "smooth" and "rough" strains of the same species?
5. What are some of the points to be remembered in attempting to differentiate the morphological types of bacteria under the microscope?
6. Define *micron, millimeter, meter.* What is the symbol for a micron? What is the equivalent of a micron in inches?
7. Give the sizes in micra of some important disease germs.
8. Describe the character of the cell walls of a bacterium. What is the significance of the outer layer of Gram-positive bacteria?
9. What is meant by "cell inclusions"? Name three kinds.
10. Describe and discuss the practical significance of the: (1) metachromatic granules, (2) glycogen granules, and (3) fat droplets that may be found in bacterial cells.
11. Summarize current opinion regarding nuclear material in bacteria.
12. Define *capsule.* How does the capsule appear in ordinary stained preparations? Name three species of capsulated bacteria. What is the relation between capsule formation and the disease-producing power of capsulated organisms?
13. Define *motile bacteria, nonmotile bacteria, flagella, true motility, Brownian movement.*
14. Describe flagella.
15. What distinguishes true motility from Brownian movement? Is motility constantly found in a given species at all times?
16. Describe spore formation in bacteria and its significance. Define *vegetative form, sporulating form, free spore.*

17. What are two principal properties of free spores? Describe spore germination.
18. Where are sporeforming bacteria to be found? Name three important pathogenic species. How does the existence of bacterial spores affect our methods of sterilization?

CHAPTER VIII

GENERAL PHYSIOLOGY OF BACTERIA

The physiology of bacteria is a subject of great practical, as well as theoretical, interest. Through it we may learn how we may, as we will, either cultivate the organisms and put them deliberately to work in the service of man, or prevent them from multiplying; and we may gain an insight into the immense capacity of these microbes to act as master chemists, transforming the substance of things about us with a seemingly magic power.

Reproduction of bacteria. Among the bacteria, multiplication is entirely an asexual process, that is, there are no male and female cells, and no fertilization is needed. After the parent cell has attained its maximum size, it splits across the short axis to form two new cells. This is the process of *simple transverse fission,* or *binary fission.* The new individuals grow until they are of maximum size and, if conditions are still favorable, each splits into two new cells as the parent did. The bacteria are potentially immortal. The daughter cells resemble the parent cells, because they are literally parts of them.

The actual process of cell division is illustrated in Fig. 35. A slight cleft or fissure develops across the short axis; this deepens and soon becomes a complete separation. New cell walls are formed at the point of division, and the two new cells, of nearly the same size, are now independent individuals. According to the species, they may remain close together, as happens often with the cocci, or they may separate entirely. There is often a marked *post-fission movement,* the new cells snapping apart, or swinging through an arc until they lie almost parallel to one another.

Rate of multiplication. The rate at which cell division occurs varies with the species and with the conditions in which the organism is growing. There are a few slowly dividing kinds, but most species are able to multiply with great rapidity. When conditions are the best, less than half an hour may represent the span of a

whole generation. In other words, the time necessary for many species to reach maturity and divide into two new cells may be about thirty minutes. This means that, theoretically, starting with just *one* organism of this kind at 9 o'clock, by noon there would be only 64, but by 9 o'clock the same night there would be very nearly

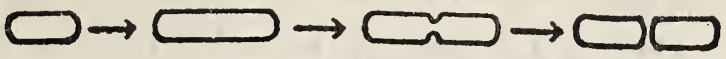


FIG. 35. Diagram illustrating the process of reproduction in the true bacteria. This method of multiplication is called simple transverse fission, and the bacteria are named *Schizomycetes* (the fission-fungi).

seventeen million (17,000,000), and in twenty-four hours the theoretical progeny would number about two hundred and eighty trillion (280,000,000,000,000)!

This enormous total resulting from the multiplication of a single original organism is possible only in theory. It could happen only if the half-hour division rate continued throughout the twenty-four hours, and this never occurs under any circumstances. After a short period of reproduction at the maximum rate, many factors combine to check multiplication, especially the decreasing availability of food and the accumulation of waste products. The bacteria cannot remain active in the presence of an excess of their own waste products, any more than other creatures can; and as these substances accumulate, growth and cell division become slower until, finally, reproduction ceases altogether. Despite these natural checks, the capacity of most bacteria to increase in number so rapidly, during the limited time in which conditions remain favorable, constitutes one of their most significant properties, and in large measure explains their great power.

Cell growth and cell division. It is interesting to note that mildly injurious circumstances cause, first of all, a cessation or reduction in the rate of cell division, without apparently affecting the capacity of bacterial cells to continue growing. Penicillin, for example, in certain low concentrations, has the effect of causing bacilli to grow as long, tangled threads. We often note in old cultures of bacilli, and especially in rough (R) strains, a tendency to produce unusually long rods or filaments, because of the failure of the cells to divide promptly, as they do in actively multiplying, young, smooth (S) cultures. Thus, cell growth and cell division (reproduction of new, independent cells) are separate phenomena, and they do not always

run parallel. In cultures at the most active period of multiplication, cell division commonly outstrips growth, so that the individual cells formed at that time are relatively small. (Bacilli, for example, appear as very short, coccoid forms.) On the other hand, when a culture gets old, or when the organisms are subjected to some mild injury, cell division tends to slow up or stop, while growth continues, so that relatively elongated, thread-like forms develop.

Chemical make-up of bacterial cells. The living matter of bacteria is essentially the same as the protoplasm of all other living organisms. It contains, first of all, a high proportion of *water*. On the average, as much as 80 to 85% of the bodies of growing bacteria may consist of water. *Proteins, carbohydrates,* and *lipids* are present, and also we may count among the constituents of bacteria, as in the case of other living cells, those all-important substances called *enzymes*.

Proteins. These complex, organic, nitrogen-containing compounds occur in bacteria as simple proteins (albumins or globulins), as partly degraded proteins (polypeptides, peptones, etc.), and as nucleoproteins, or as other conjugated proteins in combinations with carbohydrates or lipoids. It is usually true that the principal protein in a particular species of bacteria is characteristic of that species alone, but sometimes traces of the same protein exist in related organisms also.

Carbohydrates. Starches and glycogen, as well as the simpler sugars, may be present in bacteria. Most important are the *polysaccharides,* such as those in the capsules of organisms like the pneumococci. Characteristic group-specific or type-specific carbohydrate-protein complexes occur in many different bacteria. As we have already noted, the polysaccharides in the capsules of the different *serological types* of pneumococci differ slightly in chemical make-up, and these differences in capsular structure are responsible for the fact that each type stimulates in the animal body a different antibody.

Lipids. Fatty materials are present chiefly in the form of fats, waxes and phospholipids. Droplets of stainable lipid matter are found as conspicuous cell inclusions in many organisms, especially in Gram-positive bacteria, as previously described.

Lipoids, extractable from the dried bacteria by ether and similar solvents, are especially abundant in the bacilli of tuberculosis and related acid-fast organisms, the proportion of total lipoids in the

tubercle bacillus reaching as high as 41% of the dry weight. It has been shown that it is possible to extract, from waxes peculiar to these microbes, a compound called "mycolic acid," which, in combination with a polysaccharide, is probably responsible for their characteristic acid-fast staining properties.

Enzymes. These remarkable substances are complex proteins, or protein-like compounds, formed by the living organisms themselves. They act like catalytic agents, permitting complicated chemical reactions to go on in their presence that would otherwise occur with extreme slowness, if at all. Some enzymes remain within the bacterial cells, while others are given off into the surrounding medium, where they catalyze chemical changes *outside* the organisms.

One enzyme brings about just one kind of chemical reaction, and no other. Some enzymes cause decomposition of carbohydrates, others hydrolyze proteins, and still others attack fats. Moreover, a single enzyme usually can act upon a single kind of carbohydrate only, or a particular kind of protein or fatty substance only. Other enzymes, equally specific in action, bring about particular synthetic processes, cause oxidation and reduction reactions, and so forth.

Since the chemical nature of enzymes is uncertain, there has developed the custom of naming them according to their action. A name for an enzyme is obtained by adding the suffix *ase:* (1) to the name of the substrate acted upon, for example, *lactase, maltase, protease, lipase;* or (2) to a term indicating the type of reaction produced, such as *oxidase, reductase, hydrolase, catalase.*

Importance of enzymes. It can be truly said that there is no life without enzymes. It is through enzymatic action that living tissues are able to carry out, at ordinary temperatures and with relatively weak reagents, the extraordinarily complicated biochemical reactions characteristic of living matter—reactions that are quite impossible to reproduce with enzyme-free, lifeless materials in the laboratory.

In bacteria, numerous enzymes control all the complex chemical phenomena which accompany their growth. Enzymatic reactions are required in order that the organisms may: (1) secure food in a form that can be assimilated (this usually involves enzymatic decomposition of organic materials in the surrounding medium); (2) utilize these food elements for the synthesis of body protoplasm; and (3) have a supply of the necessary *energy* for these activities, and for reproduction, motility, and other life processes.

A good part of the enzymatic reactions which keep bacteria growing and multiplying takes place at the *surfaces* of the organisms. The external surfaces of bacterial cells supply places upon which various substances are *adsorbed* and concentrated, so that interreactions are facilitated. The smaller a cell is, the greater its surface area in proportion to its bulk. We can now appreciate the importance of the very small size of bacteria. The *total surface area* of the millions of organisms in a mass of bacteria is, of course, very great indeed. In fact, individual bacteria are so tiny that they are *almost all surface.* As they multiply in intimate contact with the substances on which they are growing, they act like so many drops of a concentrated reagent, producing rapid and profound chemical changes.

The complicated biochemical reactions accompanying bacterial growth are not brought about by single enzymes acting alone. Rather, a particular decomposition or synthetic process is usually catalyzed by a single enzyme only at the beginning, while the final reaction is the result of a chain of chemical changes involving a series of different enzymes. Thus, it is proper to speak of the *enzyme systems* of bacteria. These will be explained more fully below.

Nutrition of bacteria. Of course, the utilization of a particular food material is possible only when a bacterium possesses the necessary enzyme systems which permit it to decompose the substance, at least to some degree, so that food elements pass into solution about the growing organisms. Then nutritious elements, which are absorbed through the cell walls, must be assimilated by further enzyme action.

Autotrophic and heterotrophic bacteria. With respect to the kind of food materials utilized, bacteria may be divided into two classes: (1) *autotrophic* and (2) *heterotrophic.* The autotrophic bacteria utilize simple *inorganic* substances as the sole or principal source of nourishment, and are generally unable to use more complex organic materials. The heterotrophic bacteria, on the other hand, cannot utilize inorganic compounds, except to a limited extent, and are dependent for nourishment upon a supply of *complex organic substances.*

Nutrition of autotrophic bacteria. Only a small minority of bacteria belong to this interesting group. These organisms obtain carbon from carbon dioxide and carbonates, and nitrogen from am-

monia, nitrates, or nitrites. Organic compounds are not required; in fact, the strict autotrophs will not grow on ordinary media containing organic carbon and nitrogen compounds. A majority of the autotrophic bacteria, however, are not limited entirely to inorganic materials, but can utilize *some* organic substances. Consequently they are properly called *facultative autotrophs.*

It is worth noting that, since the protoplasm of the autotrophs is not essentially different from that of other kinds of bacteria, these organisms must possess truly remarkable powers of synthesis. Their ability to build up the complex organic constituents of living matter from simple inorganic chemicals makes them independent of preformed organic substances. It is customary to regard them as truly primitive bacteria, like those that must have been present on the earth before the more complex organic foodstuffs became available. Perhaps they represent the ancestral bacteria from which the common species of the present age have evolved. Their life-processes are fully as complicated, however, as those of heterotrophs, and their supposed primitiveness may be a false assumption.

Examples of typical autotrophic bacteria are the *nitrifying bacteria* of the soil (family *Nitrobacteriaceae*), which can build up nitrates from ammonia and nitrites (Chapter X).

Nutrition of heterotrophic bacteria; saprophytes and parasites. No sharp line divides the autotrophs from the heterotrophs. Instead, there is a gradual transition among bacteria from those forms that are strictly autotrophic and free-living, to those that are heterotrophic and parasitic. It is clear that, by a process of natural evolution, the free-living bacteria with wide enzymatic powers have been succeeded by species less and less able to synthesize their own food, and more and more dependent upon a close association with higher forms of life.

Among the great numbers of heterotrophs, some derive their food habitually from *lifeless matter* only. These are the *saprophytic bacteria.* Most of these are hardy species, able to grow well in almost any place where they are supplied with nonliving organic matter as food. Very few, however, are able to survive within the living body. The *natural habitat* of most saprophytes—i.e., the environment in which they are naturally and constantly found—is some part of the outdoor world, such as the soil or water. We find some species adapted to a particular kind of soil, or to the water of a

certain type of spring, or to some other particular place. Many saprophytic organisms grow naturally in the intestinal contents of healthy animals and human beings.

In contrast with the saprophytic types, there is a large group of *parasitic bacteria.* These are accustomed to living on, or in, the bodies of living things, from which they secure the necessary elements for their nourishment. Most of them die quickly when cast off from the body. The natural habitat of these parasitic varieties, then, is some part of the surfaces or tissues of living plants, animals, or human beings. Certain harmless species (commensals) exist habitually only in limited areas of the body—such as on the gum margins, or on the mucous membranes of the pharynx, or the external genitalia. The disease-producing parasitic bacteria are often still more closely adapted to growth in particular situations within the body tissues.

Laboratory cultivation of heterotrophs. When we attempt to cultivate bacteria in the laboratory, we find, as would be expected from the foregoing, a marked difference in the nutritional requirements of the saprophytic species, on the one hand, and the highly parasitic species, on the other. Although there are exceptions, it is generally true that the saprophytic bacteria may be cultivated on relatively simple media ("plain media"), such as broths containing only meat extract, peptone, and salt, or on milk medium, whereas the more fastidious parasites, and especially the pathogenic species, require media to which sterile whole blood, or blood serum, or some other enriching material has been added ("enriched media"). In the case of a number of common species, small amounts of essential *growth accessory substances* must also be supplied.

The requirements of the different kinds of *disease-producing bacteria* vary greatly, and in each case the type of media needed is a reflection of the general nutritional habits of the related nonpathogenic bacteria belonging to the same natural group or genus. Almost always, the pathogenic members of any natural group of bacteria are more fastidious, but their needs are only relatively greater than the nutritional demands of the whole group. The anthrax bacillus, for example, although decidedly pathogenic, grows on plain media, like the related nonpathogenic aerobic sporebearing bacilli common in the dust and soil. On the other hand, even the nonpathogenic members of the genus *Neisseria* are of parasitic habit, and generally demand enriched media, at least for primary isolation. It is not

surprising, then, that the pathogenic *Neisseria* (the germs of gonorrhea and meningitis) require especially enriched media, and are among the most difficult of bacteria to cultivate in the laboratory.

The formulae of the many different kinds of culture media actually used at present were worked out empirically, for the most part, and without any real understanding of the exact requirements of the organisms to be cultivated. By the force of tradition, bacteriologists cling to the use of these familiar media, and doubtless will continue to utilize them for an indefinite period. However, knowledge of bacterial nutrition is now growing by leaps and bounds, and it will eventually be possible to understand the real requirements in terms of chemical compounds of known structure. It will then be possible to cultivate the various bacterial species in *synthetic media* of known composition.

Growth-accessory substances. The so-called growth-accessory substances consist of organic compounds which are necessary to growing organisms only in very minute amounts. Presumably, these are substances which cannot be synthesized by the cell, and which thus have the same relation to the nutrition of bacteria as do the established vitamins to the nutrition of the human body. For example, it has been shown that the diphtheria bacillus requires nicotinic acid and beta-alanine in very low concentrations, and that the *Staphylococcus aureus* also requires nicotinic acid and thiamin (Vitamin B_1). It is interesting to note that these same substances are growth-promoting factors of great importance in higher forms of life, as well. A classical example of an organism requiring special growth factors is the "influenza bacillus" (*Hemophilus influenzae*). This organism must be supplied two different factors which have been known as the V and X factors. Both are present in blood. The V factor is now identified with cozymase (coenzyme I), a vitamin originally found in yeast extracts, while the X factor is supplied by hematin, a thermostable constituent of blood.

Other growth factors known to be required by one or more species of parasitic bacteria include most of the amino acids, purines, pyrimidines, and fatty substances, such as oleic acid.

At the present time, study of growth-accessory substances is being actively pursued by biochemists and bacteriologists, working in collaboration, and results are being obtained which are important not only in microbiology, but also in connection with problems of human nutrition.

Bacterial metabolism and respiration; bacterial enzymes. The chemical processes by means of which bacteria are nourished are carried out, as we have explained, through the action of enzymes. Each of the various species possesses a complicated *enzyme system* to supply it with food and energy. All the bacteria taken together are able to bring about an extremely wide variety of biochemical changes. Indeed, there is probably no natural organic substance which cannot be oxidized by at least one kind of organism.

Two sorts of bacterial enzymes are recognized: (1) *endo-enzymes* (or *desmolases*), and (2) *exo-enzymes*. The endo-enzymes function *inside* the living organisms (*desmo* = bound up with), whereas the exo-enzymes are secreted into the surrounding medium and act *outside* the cells.

Exo-enzymes. These are concerned chiefly with catabolic (decomposition) processes, that is, with the breakdown of the complex materials in the surrounding medium into simpler substances. The nutritive elements thus made available are then absorbed into the bacterial cells. Most of the exo-enzymes are properly called *hydrolases;* that is, they are enzymes that bring about the decomposition of large organic molecules, always with the introduction of a molecule of *water*. It is during these extracellular, hydrolytic reactions, brought about by bacterial exo-enzymes, that many chemical changes and products of great usefulness in human affairs are produced (Chapter X).

Endo-enzymes. The endo-enzymes, or desmolases, acting within or at the very surface of the cells, are concerned principally with the reactions that result in the synthesis of bacterial protoplasm, and in the furnishing of energy. The synthetic processes are in part the reverse of the hydrolytic changes mentioned above; that is, they are brought about by the extraction of water molecules. Others are due to more complicated condensations and molecular rearrangements, always carried out through the catalytic action of intracellular enzymes.

Energy-yielding enzyme reactions; respiration of aerobic and anaerobic bacteria. A certain amount of the *energy* necessary for life and multiplication is derived by bacteria from some of the molecular rearrangements just mentioned, but the principal source of energy is a complex set of chemical changes which may be grouped under the heading *oxidation-reduction*. This brings us at once to a curious fact. The bacteria may be divided into two groups,

according to the way in which they carry out these necessary energy-yielding oxidative processes; they are either (1) *aerobes* or (2) *anaerobes.*

The first group is made up of organisms that respire in the same manner as animals do; that is, they take in oxygen from the air, and give off carbon dioxide. They are said to be *aerobic.* The other, somewhat smaller group, however, contains bacteria which can live in the *absence* of free (molecular) oxygen, and thus must obtain energy from utilizing the oxygen which is *combined* chemically in available foodstuffs, or by other kinds of oxidation-reduction reactions. Many of these organisms refuse to grow at all in the presence of the least trace of free oxygen, and are killed by a few minutes' exposure to the air. They are called the *anaerobic* bacteria. Special methods designed to exclude atmospheric oxygen must be used to cultivate anaerobes in the laboratory (Chapter XI).

As might be expected, a gradation occurs in their relation to molecular oxygen between the strictly aerobic species, on the one hand, and the strictly anaerobic species, on the other hand, and it is more accurate to recognize three classes as follows:

(1) *Obligate aerobes:* Require free access to the air, since they are unable to reduce substances other than molecular oxygen. (Examples: the autotrophic, nitrifying bacteria [genus *Nitrobacter*]; the aerobic sporeforming bacilli [genus *Bacillus*]; the diphtheria bacillus; the cholera spirillum, etc.)

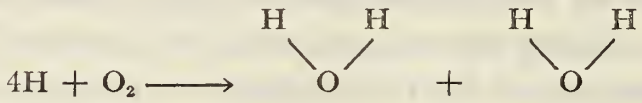
(2) *Facultative anaerobes:* Grow either in the presence or in the absence of air, but the majority show a preference for a low oxygen tension, that is, for an atmosphere containing only traces of free oxygen. These are often referred to as *microaerophilic* organisms. (Examples: many disease-producing bacteria, such as streptococci, meningococci, typhoid bacilli.)

(3) *Obligate anaerobes:* Grow only when atmospheric oxygen is totally excluded. (Examples: bacillus of tetanus and related sporeforming bacilli [*Clostridium*]; fusiform bacilli; the spirochete of syphilis.)

Oxidation and reduction. The chemical reactions through which these different groups of bacteria derive energy are too complicated for detailed explanation here. A good idea of what actually happens can be gained, however, from consideration of a few simple facts.

The word *oxidation* is used, in the present day, to include not only the simple combination of atmospheric oxygen with some sub-

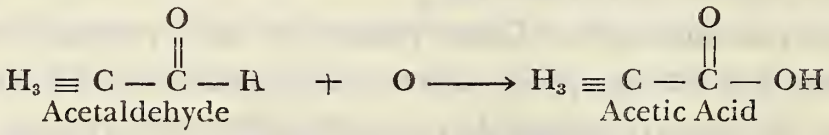
strate, but for any energy-yielding reaction, whether oxygen itself is actually involved or not. In all such reactions, there is a *transfer of electrons* from one atom to another. When hydrogen (H) is oxidized to water (H_2O), the single electron which each H atom carried is given over to the oxygen atom. Oxidation of hydrogen, then, means *loss of electrons* to oxygen. For its part, the oxygen has been reduced, that is, it has *gained in electrons:*



Now, in more complicated reactions, whether oxygen is concerned or not, a substance is said to be oxidized when it loses electrons; it is said to be reduced when it gains electrons. Obviously, every oxidation (removal of electrons) must be accompanied by an equivalent reduction of the partner in the reaction, that is, of the substance that is the recipient of the transferred electrons. If one compound is oxidized, another must be reduced, and vice versa. Hence oxidation and reduction reactions must be studied together.

In bacterial cultures, oxidation-reduction is brought about by two general classes of enzymes: *oxidases* and *dehydrogenases.*

Oxidases are active in the aerobic bacteria. They catalyze the direct combination of oxygen with various substrates, for example, the oxidation of acetaldehyde to acetic acid:

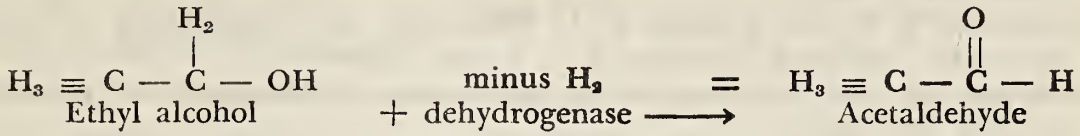


Also, they participate in the transfer of activated (nascent) oxygen by oxygen carriers. This "oxidase-reaction" is of practical use in the diagnosis of gonorrhea in man (Chapter XXXI).

Dehydrogenases are enzymes that activate hydrogen and are concerned in its transfer from one substrate (the hydrogen donator) to another (the hydrogen acceptor). A compound is reduced (gains electrons) by having H added, whether O has been removed from it or not. Hence, substances that are active hydrogen-acceptors may take the place of oxygen when dehydrogenases are present, and the transfer of electrons in this hydrogen-transport is an important source of energy for bacteria.

Dehydrogenases are present in both aerobic and anaerobic bacteria, but it is with the latter that they play an essential rôle in

bringing about indirect oxidations in the absence of molecular oxygen. An example would be the conversion of ethyl alcohol to acetaldehyde by the removal of hydrogen:



In this reaction the H_2 subtracted under the influence of dehydrogenase combines with O to make H_2O, or with some other H-acceptor, or passes off as gas.

Energy supply for the growth of anaerobes. From the foregoing we have learned that anaerobic bacteria are supplied with energy: (1) by utilizing the oxygen that is combined in such substances as sugars, Na_2NO_3, $(NH_3)_2\ SO_4$, and other O-containing compounds in the medium; (2) by reactions involving molecular condensations and rearrangements; and (3) by oxidation-reduction reactions, principally activated by dehydrogenases. It would be a mistake to say anaerobes do not need to utilize oxygen; they must have *some* O_2 for building into protoplasm. The point is, they cannot use free, molecular, atmospheric oxygen.

The reason why strict anaerobes are so sensitive to free oxygen is not yet completely understood. According to one theory, they are killed, or prevented from growing, in the air because they form hydrogen peroxide (H_2O_2), a harmful substance (often used as a disinfectant), when free O_2 is present. Aerobes produce H_2O_2 also, but these organisms are protected by an enzyme, called *catalase,* which decomposes the hydrogen peroxide as quickly as it is formed. Not all the known facts fit this theory, however; for example, it has been found difficult to prove that strict anaerobes actually produce inhibiting amounts of H_2O_2.

Probably more important for anaerobic growth than protection from H_2O_2 is the presence of a sufficient concentration of reduced substances in the medium. Many anaerobes can be grown in contact with the air if a strongly reducing substance, like fresh sterile animal tissue, or thioglycollate, is added to the medium. Thus, the *oxidation-reduction potential* of a medium may determine its suitability for cultivation of anaerobes. It has been suggested that the energy-yielding enzymes of anaerobic bacteria are inactivated in the presence of molecular oxygen, and can function only in a environment with a high proportion of reduced substances.

REFERENCES

ANDERSON, C. G.: *Introduction to Bacteriological Chemistry*. Baltimore: William Wood & Co., 1938.

BUCHANAN, R. E. and FULMER, E. I.: *Physiology and Biochemistry of Bacteria*. Baltimore: Williams & Wilkins Co., 3 vols., 1928–30.

FROBISHER, M., JR.: *Fundamentals of Bacteriology*. Philadelphia: W. B. Saunders Co., 3rd Ed., 1944.

GALE, E. F.: "Factors Influencing the Enzymic Activities of Bacteria," *Bact. Rev.*, Sept. 1943, 7:139.

PETERSON, W. H. and PETERSON, M. S.: "Relation of Bacteria to Vitamins and Other Growth Factors," *Bact. Rev.*, 1945, 9:49.

RAHN, O.: *Physiology of Bacteria*. Philadelphia: P. Blakiston's Son & Co., Inc., 1932.

SALLE, A. J.: *Fundamental Principles of Bacteriology*. New York: McGraw-Hill Book Co., 2nd Ed., 1943.

STEPHENSON, M.: *Bacterial Metabolism*. New York: Longmans, Green and Co., 2nd Ed., 1939.

VAN NEIL, C. B.: "Recent Advances in Our Knowledge of the Physiology of Microorganisms," *Bact. Rev.*, 1944, 8:225.

WAKSMAN, S. A.: *Principles of Soil Microbiology*. Baltimore: Williams & Wilkins Co., 2nd Ed., 1932.

WERKMAN, C. H.: "Bacterial Dissimilation of Carbohydrates," *Bact. Rev.*, 1939, 3:187.

REVIEW QUESTIONS—CHAPTER VIII

1. Explain the process of reproduction in the true bacteria. Define *simple transverse fission, Schizomycetes, post-fission movement.*
2. What is the rate of cell division of some common species under the most favorable circumstances? What is the significance of this rapid rate? What factors operate to check growth and multiplication? Discuss cell growth vs. cell division.
3. What classes of chemical substances are to be found in bacterial cells? Are the principal proteins usually characteristic of the species?
4. Mention some matters of practical importance in connection with: (1) the carbohydrate content and (2) the lipid content.
5. What are enzymes, and how do they act? How are they named?
6. Explain the importance of enzymes. Why are they required by growing bacteria? What is the importance of the large surface area of bacteria? What is meant by the *enzyme systems* of bacteria?
7. Define: *autotrophic, heterotrophic, saprophytic,* and *parasitic* bacteria. How do saprophytic and parasitic organisms differ in food requirements?
8. Give examples of autotrophs, and explain their nutritional habits. What is meant by *facultative autotrophs*?

9. Discuss briefly the nutritional habits of heterotrophic bacteria. What clue do we have to the usual requirements of disease-producing species? What sort of culture medium is likely to have more frequent use in the future?
10. Define: *growth-accessory substance.* Give examples of vitamin-like factors needed by bacteria.
11. Define: *endo-enzymes, desmolases, exo-enzymes, hydrolases.* What is the general function of: (1) exo-enzymes (hydrolases) and (2) endo-enzymes in the life of bacteria?
12. Into what two classes may bacteria be divided according to the way they carry out necessary energy-yielding oxidation-reduction processes? Define and give examples of: (1) *obligate aerobes,* (2) *facultative anaerobes* and (3) *obligate anaerobes.*
13. Explain the present use and meaning of *oxidation.* Why is an oxidation always coupled with a reduction? How do *oxidases* act in aerobic bacteria?
14. What are *dehydrogenases*? Describe how they make possible oxidation-reductions in anaerobic bacteria.
15. What are the energy sources for anaerobes? Is it correct to say that anaerobes do not need or use oxygen?
16. What are some possible explanations for the sensitiveness of anaerobes to atmospheric oxygen?

CHAPTER IX

INFLUENCE OF ENVIRONMENT ON BACTERIA AND EFFECTS OF BACTERIAL GROWTH

Bacteria are literally at the mercy of their environment, and may be affected favorably or unfavorably by slight changes in the physical or chemical state of their surroundings. Life is possible for them only within rather narrow limits set by the nature of the environment, and they are most active within still narrower limits. Anything that accelerates, or interferes with, the action of their essential enzymes will have its inevitable effect, of course, upon the development of the organisms. Their susceptibility is fortunate, for by controlling environmental conditions we can stimulate bacteria to grow, or stop their growth, or we can destroy them, as we wish.

Following are the principal factors of the environment that most directly affect the growth of bacteria: (1) the temperature, (2) the light, (3) the available moisture, (4) the reaction (hydrogen ion concentration, or pH), (5) the relation of the growth to atmospheric oxygen, (6) the osmotic pressure conditions, and (7) the character of neighboring microbes. Many other, but less important, environmental influences also play a part.

Temperature. *Optimum-growth temperatures.* For each species of bacteria there is a temperature at which the organism is in every way most active, and at which the most rapid growth and multiplication take place. This is called the *optimum-growth temperature.* It always corresponds to the usual temperature of the natural habitat of the species.

For the great majority of the saprophytic organisms inhabiting the soil and other places outside of living bodies, the optimum-growth temperature is 25° to 30° C, that is, just a little higher than the usual room temperature. Bacteria growing best at such temperatures are known as *mesophilic* (moderation-loving). There are some species found in the soil, in hot springs, and in intestinal contents of

animals, whose optimum temperature may be as high as 60° to 80° C (140° F) or higher. These are called *thermophilic* (heat-loving) bacteria. At the other extreme, there are a few exceptional species which grow best at temperatures only slightly above the freezing point (about 10° C). These are called *psychrophilic* (cold-loving) bacteria (Table VII).

The optimum temperature for the bacteria parasitic on the body of human beings or other warm-blooded creatures is the temperature normal to the body, i.e., 37° C (98.6° F). Laboratory incubators in which cultures of disease germs from the human body are grown are adjusted to keep a constant temperature of 37° C. Bacteria which are parasitic on the bodies of *birds* grow best at a higher temperature, 41°–45° C, because this is the normal temperature of birds.

Minimum- and maximum-growth temperatures. At temperatures above or below the optimum, organisms are less active, and grow and multiply more slowly. For each species there is a definite limit in each direction, beyond which growth will not occur. The lowest temperature at which growth is possible is called the *minimum-growth temperature,* and the highest, the *maximum-growth temperature.* The minimum temperature for most of the organisms living on the human body is about 20° C, and the maximum is about 42°–45° C. Some of the pathogenic germs are so closely adjusted to the body temperature (37° C) that they multiply scarcely at all at any other; most of them, however, will grow at temperatures between 36° C and 42° C.

Table VII. Classes of Bacteria According to Temperature Relations

Class	Approximate growth temperatures		
	Minimum	Optimum	Maximum
Psychrophilic	0° C	10°–15° C	30° C
Mesophilic	15°–25° C	25°–37° C	40°–55° C
Thermophilic	25°–45° C	50°–60° C	60°–90° C

Effect of low temperatures. At temperatures below the minimum-growth temperature, bacteria cease to develop, but most species are not killed by cold. Some organisms will withstand a short exposure to a temperature far below that of the freezing point of water, and

almost all species will remain alive for weeks at temperatures just above the freezing point. But *growth* of the ordinary bacteria does not occur in the cold, and the organisms will not multiply until returned to a higher temperature, approximating that of their natural habitat. Sudden changes of temperature are more harmful than abnormally low or moderately high temperatures.

Effect of high temperature; thermal death point. High temperatures are much more injurious than low temperatures. Sometimes an organism will continue to grow at an abnormally high temperature, but in doing so it will lose some of its characteristic properties. The anthrax bacillus, for instance, when cultivated for some weeks at 42° C, instead of 37° C, loses both its capacity to form spores and some of its disease-producing power.

If any bacterium is heated above the maximum-growth temperature to a sufficient degree, it will be killed. The lowest temperature at which an organism is killed by heat within a specified time (usually 10 minutes) has been called the *thermal death point.* This is an inexact expression, however, since, of course, not all the bacteria die simultaneously after the passage of so many minutes of time.

Thermal death time. A somewhat more exact term is *thermal death time,* which is the length of time required to kill all the bacteria in a given substance at a stated temperature. This is a useful conception; for example, it has been shown experimentally that, when market milk is held at 62° C for 30 minutes, all disease germs which might be carried in it are destroyed. That is to say, a 30-minute period at 62° C is well above the thermal death time at that temperature for all the dangerous microbes likely to be present in the raw milk. It is generally true that *all non-sporeforming disease-producing bacteria, and almost all other non-sporeforming organisms, when exposed in watery liquids to a temperature of 60°–65° C (140°–150° F), are killed within one-half hour.* This fact is the basis for the process called *pasteurization,* by which milk is rendered free of disease germs.

Light. Bacteria differ sharply from green plants in their reaction to light; as everyone knows, the development of green plants is in direct proportion to the hours of sunlight they enjoy. The bacteria, however, have no chlorophyll or other photosynthetic pigment, and their growth is not aided by sunlight. On the contrary, most species are injured by even diffuse daylight, and are killed in a few hours by direct exposure to the sun. It is the ultraviolet rays of the sunlight

which have this destructive effect. Some ways in which use is made of the destructive action of *ultraviolet light* on bacteria, viruses, and other organisms will be mentioned in later chapters.

Moisture; effect of desiccation. An abundance of water is just as essential for the growth of bacteria as an adequate supply of foodstuffs. In fact, bacteria cannot be nourished without water, because food elements must be in solution before they can be absorbed through the cell walls of the organisms. All kinds of bacteria grow best in a watery medium, and in an atmosphere saturated with moisture. A total lack of moisture prevents growth; therefore bacteria do not develop on thoroughly dry objects, and such objects do not decompose. Preservation of food by desiccation, a process much developed during World War II, is a well-known application of these facts.

However, the mere removal of moisture from the immediate surroundings of a growing bacterium does not necessarily result in its quick death; in fact, many species are remarkably tenacious of life in a desiccated state, especially if protected at the same time from light. Even when dried on a glass slide in the preparation of ordinary smears they may remain viable for several hours. Moreover, even the most sensitive species, such as the cocci of gonorrhea and meningitis, can be kept alive indefinitely in the dried state if cultures are first quickly *frozen, then dried rapidly in a vacuum.*

The method of preserving stock cultures of bacteria, filtrable viruses, enzymes, toxins, blood plasma, etc. in the form of dry powders by freezing and rapid vacuum evaporation is called the *lyophile process.* (The final product, the powder, is extremely *lyophilic;* i.e., water-loving.)

Reaction; pH. By reaction is meant the degree of acidity and alkalinity. This is now universally expressed in terms of *hydrogen ion concentration,* or pH, since it is the concentration of dissociated hydrogen $[H]^+$ (or hydroxyl $[OH]^-$) *ions,* that really determines the degree of acidity (or alkalinity) of a solution.

Most bacteria are highly sensitive to the pH of the medium in which they are growing. Each species prefers the particular reaction characteristic of its natural habitat. We find it necessary to adjust the hydrogen ion concentration of culture media with great care, in order to meet the exacting requirements of the various species.

A few bacteria, such as the so-called *aciduric* organisms found in the secretions about the human teeth, are adapted to an acid reac-

tion (pH about 5.5), but the majority are unfavorably affected by acid and grow best in a medium which is either neutral (pH 7.0) or slightly alkaline (pH 7.2–7.4). When bacteria decompose sugars, the acids produced often accumulate to such a degree that they stop the growth, or cause the death, of the organisms. Often chemical "buffers" are added to culture media to absorb acid as it is formed. There are some organisms that multiply best in a strong alkaline medium. The cholera spirillum, for example, prefers a pH of about 8.0.

Relation of the growth to atmospheric oxygen. Every living thing must have oxygen in some form but, as we have learned in the chapter immediately preceding, bacteria differ in their capacity to utilize the oxygen of the air. The majority do use atmospheric oxygen directly, just like human beings and animals. These are the *aerobes*. For an important group of bacteria, however, the free oxygen in the air is actually a poison. These remarkable organisms cannot use atmospheric oxygen or survive in its presence. These are the *anaerobes*. They must derive oxygen from the decomposition and rearrangement of oxygen-containing compounds in the medium. The complicated oxidation-reduction, energy-yielding reactions, principally catalyzed by dehydrogenases, previously described, serve for their respiration.

The strict anaerobes will grow only where air is totally excluded, except in the presence of strong reducing substances, like thioglycollate, or unless accompanied by aerobic organisms that use up the oxygen. Among important anaerobic pathogenic bacteria are the germs of syphilis, tetanus, and gas gangrene.

Intermediate between the strict aerobes and the strict anaerobes are the many species able to use either free or combined oxygen (facultative anaerobes). Each species of this large group shows a decided preference for growing either in the presence or absence of air. The relationship of microorganisms to oxygen is subtle, and the processes of bacterial respiration are complicated. It need only be realized that different varieties of bacteria are delicately adjusted to a particular form of oxygen supply, and develop best under a particular oxygen tension.

It is especially noteworthy that many pathogenic bacteria, even though they eventually grow well under ordinary aerobic conditions, develop much better *in the first generation or two* on artificial culture media in *anaerobic* or at least partially anaerobic cultures. In

other words, most pathogens are decidedly *microaerophilic* (liking a *little* air only). This is undoubtedly a reflection of the fact that within the body tissues the organisms have been adjusted to growing virtually without free oxygen.

The growth of a number of pathogens is aided by supplying them with an atmosphere in which the content of *carbon dioxide* has been increased to about 10 per cent.

Osmotic pressure conditions. Water passes into or out of bacterial cells, by the process called *osmosis,* according to whether the organisms are in a more dilute or a more concentrated solution than exists within the bodies of the bacteria themselves. Their protoplasm always contains more dissolved substances than pure water, so that if bacteria are suspended in distilled water, this water will diffuse *into* the bacterial cells, causing them to swell and perhaps to burst. When a cell is disrupted in this manner, the process is spoken of as *plasmoptysis.* On the other hand, if bacteria are placed in a very concentrated solution, such as a strong brine, water will pass *out* of the bacterial cells into this solution, so that the organisms may shrink and die. This is called *plasmolysis.*

Bacteria are not very sensitive to moderate changes in osmotic conditions, but the effect of highly concentrated solutions in destroying bacteria is of practical importance. Everyone is familiar with the practice of preserving meat by placing it in concentrated brine (salt solution). The meat is preserved because bacteria which would spoil it cannot multiply in the brine. Similarly, fruits or other perishable foods may be preserved in strong sugar solutions. We can see why sweetened condensed milk and thick sugary syrups keep so well.

In order that most bacteria may develop, their food must be supplied in diluted form. They grow best in watery solutions containing only a small percentage of sugar or other food substances, and particularly well if the proportion of dissolved substances is about the same in the food solution as within the bacterial cells.

Character of neighboring microbes. The life of any single organism in its natural habitat is profoundly affected by the presence of other microbes. In Nature, the various species are never found quite by themselves as we have them in our pure cultures in the laboratory. Instead, every particular place has its characteristic bacterial population, comprising a number of species, often of the most diverse types, living in intimate relationships with one another. For example, on the gum margins in the human mouth there is

always to be found a certain group of organisms of several different species. These bacteria constitute the *normal flora* of the gum margin. They grow there constantly, because they have become adapted to the natural conditions on the gums, and also because they have become adjusted to living together. There is, similarly, a characteristic normal flora on the skin, in the throat, at the various levels of the intestinal tract, etc., consisting in each case of several distinct species living, as it were, in a state of balance with environmental conditions and with one another.

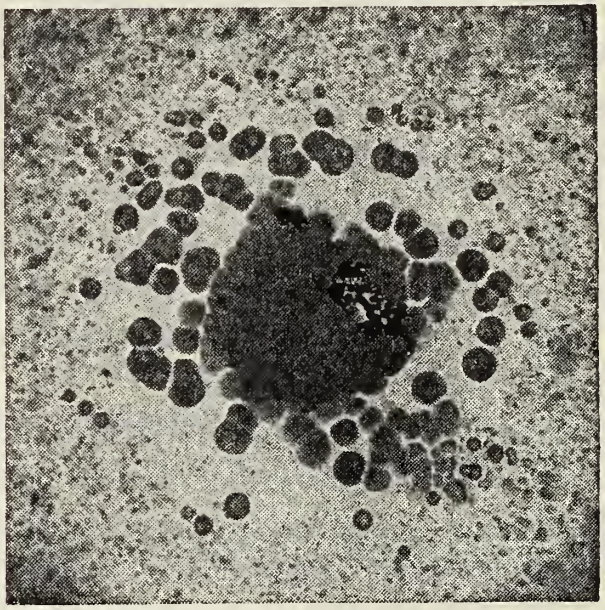


FIG. 36. The satellite phenomenon. The central dark mass is a colony of a staphylococcus. The smaller, translucent colonies clustered about it (like satellite moons around a planet) are colonies of a bacillus, *Hemophilus influenzae*. Substances diffusing outward from the staphylococcus colony have aided the growth of these bacilli. Note that the *Hemophilus* colonies farthest away from the staphylococcus colony are the smallest. (Courtesy of Dr. J. Ralph Wells, Kansas State Teachers College, Pittsburg, Kans.)

Symbiosis and commensalism among microbes. Often two organisms are so adjusted to each other that neither will grow well without its usual companion. When organisms live together in such a way as to be *mutually* helpful, we say that they are growing in *symbiosis*. When only one of the partners is benefited by the association, while the other is neither benefited nor harmed, we have an example of *commensalism* among microbes. This latter type of association is quite commonly observed. For example, an anaerobic organism may grow in an ordinary culture tube open to the air when an aerobic organism is also present. The aerobe, though not harmed

or helped by the anaerobe, does benefit the latter by using up the atmospheric oxygen. Usually the commensal relationship is far more subtle and complicated than in this example.

Sometimes one bacterium produces, and secretes into the medium, an essential growth-accessory substance needed by another. *Staphylococcus aureus,* for example, forms the so-called V factor (= zymase, or coenzyme I) required by *H. influenzae.* When the two organisms are growing together on a medium which otherwise lacks this growth factor, the separate small colonies of *H. influenzae* arrange themselves characteristically in a zone around the larger staphylococcus colony, like moons around a planet. This association of the two organisms is referred to as the *satellite phenomenon* (Fig. 36).

Mixtures of organisms sometimes accomplish what individual species are unable to do. The decomposition of complex organic matter—the bodies of dead plants and animals, for instance—is not carried through completely by any one kind of bacterium, but by successive groups of organisms living more or less in symbiosis. Even in connection with disease, we see many examples of the combined action of two or more kinds of microbes. Inflammations of the appendix, ulcerations of the mouth and throat, and wound infections are usually caused by a mixture of bacteria. Often the disease-producing power of a particular species (e.g., the diphtheria bacillus) is enhanced by the presence of certain other microbes (e.g., hemolytic streptococci). A striking illustration is the marked effect of a virus infection (e.g., a common cold, or influenza) in increasing the susceptibility of the individual to the accompanying invasion of common bacteria (such as streptococci or pneumococci).

Bacterial antagonism. On the other hand, the life of some organisms is antagonistic to the existence of others. The waste products or the by-products of one organism may injure or kill a neighboring organism.

It is the usual experience to find that in an artificially made mixed culture, prepared for class study, one bacterium tends to outgrow and suppress the others when the mixture is incubated. Sometimes the inhibiting effect of one microbe on another is explained by an obvious difference in tolerance to acid or other familiar growth-product. In the natural souring of milk, for example, a number of different species contribute to the production of the acid that clots the fluid, but they are killed off one by one as the process continues, until in many instances the only species that survives, when souring

is complete, is a certain streptococcus that is not injured by the high acid content of the fully curdled milk. The bacteria that vigorously decompose sugars, and thereby produce considerable amounts of acid, are, as a rule, antagonistic to those kinds most active in the decomposition of proteins, for the latter species are easily injured by acid. In many other cases of microbic antagonism, however, more subtle factors, not fully understood, account for the phenomenon. Unexplained is the fact that when certain disease-producing germs invade a particular spot, such as the tonsil, the eye, or the small intestine, they often cause the temporary disappearance of almost all the bacteria normally there.

Antibiotics. In recent years, the natural antagonistic action of one microbe on another has been studied intensively, with resulting practical applications of immense importance. From pure cultures of various microbes, bacteriologists have succeeded in isolating substances which have powerful inhibiting action on the growth of disease germs, or which sometimes will actually kill them. A substance produced by one kind of living organism which is injurious to another is called an *antibiotic substance,* or simply an *antibiotic.*

Products have been separated from bacteria, molds, and actinomyces that will dissolve the capsules, stop the growth, and sometimes promptly destroy the life, of many different organisms. Several of these products are now under trial as drugs for the treatment of germ diseases in man and animals. To make obvious the supreme importance of recent discoveries in this field, it need only be mentioned that *penicillin* is an antibiotic. The outstanding property of penicillin that makes it so valuable is its almost complete lack of poisonous effect upon the human body. It can therefore be administered with safety in sufficient doses to treat infections successfully. The various antibiotics will be considered further in Chapter XVI.

SOME IMPORTANT CHEMICAL CHANGES AND PRODUCTS RESULTING FROM BACTERIAL GROWTH

From a practical point of view, the real importance of bacteria lies in the chemical changes that accompany their growth. As we have already learned, bacteria are very active chemically. They are, in fact, master chemists, causing many remarkable transformations impossible to duplicate in a laboratory. These biochemical changes are brought about, for the most part, by the extracellular hydrolytic

enzymes (exo-enzymes) that diffuse out into the surrounding medium from the growing cells.

The most conspicuous biochemical activities are those concerned with the decomposition of proteins, carbohydrates, and fats. Other topics of interest are the production of light and pigments. An exceedingly important property of certain pathogenic bacteria is their ability to elaborate specific toxins.

Decomposition of proteins. The breakdown of protein substances by microorganisms is called *proteolysis;* the organisms are said to be *proteolytic* (protein-dissolving or protein-splitting). When native proteins, or proteoses, are decomposed under anaerobic conditions, yielding hydrogen sulfide and other foul-smelling compounds, the process is called *putrefaction.*

Proteolytic bacteria. Of course, all bacteria can attack the simpler nitrogenous compounds (peptones) to the degree necessary to obtain nitrogen for growth, but the species commonly called proteolytic are those which can *decompose complex native protein materials, such as meat, or egg, or blood-serum.* These are the organisms that bring about the foul-smelling rotting processes with which everyone is familiar—the decomposition of foods, of dead bodies, of feces, and of other lifeless matter. No one organism or group of organisms carries out the entire decomposition of the complex organic matter, but rather, successive groups take part, one group commencing where another leaves off. *Anaerobic* saprophytic bacteria play a dominant part in the earlier stages of decomposition, when foul odors are produced, but in the final stages aerobic bacteria predominate.

Products of protein decomposition. The protein molecule is first split into proteoses, then into simpler and simpler fractions—peptones, polypetids, and amino acids, which may finally be split to yield ammonia or free nitrogen. The decomposition does not necessarily occur in any regular order, and at the same time that more complex substances are being broken down, simpler compounds, such as carbon dioxide (CO_2), hydrogen (H), and water (H_2O), are continually being formed. The foul odors are due to the liberation of indol and skatol (the substances chiefly responsible for the characteristic odor of human feces), hydrogen sulphide (H_2S), and other malodorous compounds.

Examples of strongly proteolytic bacteria are saprophytic members of the *anaerobic* sporeforming bacilli, such as *Clostridium*

sporogenes and *Clostridium histolyticum,* and the almost equally active species of *aerobic* sporebearers, such as *Bacillus subtilis* (Marburg) and *Bacillus mesentericus.*

Fermentation of carbohydrates. The decomposition of sugars, starch, and other carbohydrates with production of acid, or acid and gas, is called *fermentation.* The power of microorganisms, especially the yeasts, to bring about fermentation has much practical importance.

Fermentation by bacteria. The various species of bacteria differ sharply in their capacity to ferment particular carbohydrates. Some few species can decompose cellulose, the most complicated substance of this kind. Others can decompose the relatively complex polysaccharides, such as *inulin, dextrin,* and *starch.* A larger number of species can attack disaccharides, like *saccharose* (ordinary table sugar, cane sugar); *maltose* (malt sugar); or *lactose* (milk sugar). Finally, the majority of bacteria can ferment the monosaccharide *dextrose* (glucose, or grape sugar). In virtually every genus of bacteria, however, there is at least one species that fails to ferment any carbohydrate at all, even dextrose.

Fermentation reactions furnish one of the readiest means of distinguishing one kind of bacterium from another. *Escherichia coli,* a normal inhabitant of the human intestine, can be distinguished from *Eberthella typhosa* (germ of typhoid fever), which it otherwise closely resembles, by the fact that it ferments lactose, while the typhoid bacillus does not. Similarly, the individual members of other groups of closely related bacteria can be identified by sugar-fermentation tests.

These reactions are remarkably specific; although the sugars differ only in the arrangement of OH groups and H atoms around the carbon atoms, some organisms ferment one and some another. Indeed, the specificity is so definite that we can use bacteria of known fermentative powers to *identify unknown sugars.* Thus, the reducing sugar in the urine of a nursing woman can be proved to be *lactose* by showing that fermentation occurs when the urine is inoculated with an organism that ferments lactose, but *not* when inoculated with a bacterium that cannot ferment lactose.

Products of fermentation. When carbohydrates are fermented by bacteria, various organic *acids* are formed, and often *gases* are produced also. Sometimes only acid forms, in other cases both acid and gas form.

One of the commonest acids produced is *lactic acid.* The so-called "lactic acid bacteria" cause the souring of milk and play an indispensable part in many commercial processes. *Acetic acid* is also commonly produced and *butyric acid* frequently results from anaerobic fermentation. A small amount of *alcohol* is produced, during fermentation, by some bacteria.

The gases most commonly formed from sugars by the few gas-forming bacteria studied in medical bacteriology are *carbon dioxide* (CO_2) and *hydrogen* (H). *Methane,* or marsh gas (CH_4), is produced from starch and cellulose by saprophytic organisms and can be observed arising as bubbles to the surface of the water of sluggish streams, ponds, or swamps containing decomposing vegetable matter.

Most of the bacteria which are active fermenters, and which produce large amounts of acid, can withstand an exposure to a higher concentration of acid than other species, i.e., they are *aciduric.*

Production of pigments. A number of bacteria are able to manufacture colored substances. These organisms are called *chromogenic* (pigment-producing) bacteria. The pigments may have any of the familiar colors of the spectrum; each species makes its own characteristic color. Some of the pigments are soluble in water, and therefore become diffused throughout the culture medium. Others are soluble only in chloroform or similar solvents.

Except for the chlorophyll-like pigment found in the purple and green sulphur bacteria, pigment production has no clear function and no relation to any other of the significant properties of the organisms. The pigments usually become noticeable when a culture has ceased to multiply most actively, that is, when the majority of the organisms are senescent and the culture is relatively old.

Both pathogenic and nonpathogenic organisms, of all morphological types, are found among the chromogens. A well-known species is the *Serratia marcescens* (old name, *Bacillus prodigiosus*) which produces a brilliant blood-red color. Colonies of this harmless organism, developing accidentally upon bread and sacrimental wafers, gave rise to the legend of the "bloody-bread," and sometimes this species is referred to as the "miracle bacillus." Other chromogenic bacteria include *Pseudomonas aeruginosa* (old name, *Bacillus pyocyaneous*), a mildly pathogenic organism that develops bluish-green pigments; the appropriately named *Bacterium violaceum;* and *Spirillum rubrum.* The common *Staphylococci* include orange and yellow pigmented varieties; and colonies of *Sarcinae* are a brilliant

yellow. Many of the saprophytic *acid-fast bacilli,* and also the germs of avian and human tuberculosis, develop yellowish or brownish pigments.

Production of light. There is a curious group of bacteria endowed with the property of emitting light. These organisms are most abundant in sea water. Some of them live as parasites upon the bodies of fish and other marine life. In the fish market there usually is to be found a barrel into which the heads and other discarded parts of fish are thrown. If one looks into this barrel after dark, one may see there a faint phosphorescent glow, produced by these bacteria, which are growing on the partly decomposed fish. The same ghostly glow may be seen in the waters of the sea at night. The phosphorescent appearance of jellyfish and other marine animals is due, in many instances, to luminescent bacteria growing on them. These remarkable organisms have no part in the production of disease in human beings.

Production of toxins. Almost all the bacteria which cause disease are in some degree poisonous. In many diseases the poison, or *toxin,* of the germ does the chief damage. The nature of these toxins is various, and to explain them a rather extended discussion is necessary. For the present, it will be sufficient to realize that bacterial toxins are of two chief kinds: (1) *exotoxins* and (2) *endotoxins.*

An exotoxin is a poisonous substance which diffuses into the surrounding medium from the body of a living bacterium. On the other hand, an endotoxin is a poisonous substance contained within the body of a bacterium, which is released only after the organism dies and the cell is disintegrated.

Further description of bacterial toxins will be deferred until we discuss the means by which microorganisms injure the body and cause disease (Chapter XX).

REFERENCES

See References for Chapter VIII.

REVIEW QUESTIONS—CHAPTER IX

1. Name seven environmental factors that directly affect the growth of bacteria.
2. Define: *minimum-, optimum-, maximum-growth temperature; thermophilic, mesophilic, psychrophilic bacteria.*

3. Give the approximate optimum-, minimum-, and maximum-growth temperatures for each of the three groups of bacteria classified according to temperature relations.
4. Discuss the effect of low temperature on bacteria.
5. Discuss the effect of high temperature. Define *thermal death point; thermal death time.* What is the temperature of pasteurization?
6. How do bacteria differ from green plants in their reaction to light? What is the effect of direct sunlight (ultraviolet light) on bacteria?
7. Explain how bacterial growth is affected by the presence or absence of moisture. Are growing bacteria necessarily killed by rapid drying? How can cultures be kept alive over long periods in the dried state?
8. What is meant by *reaction, hydrogen ion concentration*? Give examples of the pH requirements of different bacteria.
9. Define: *aerobic, anaerobic, facultative anaerobic,* and *microaerophilic* bacteria, and give examples.
10. Explain the influence of osmotic pressure conditions on the life of bacteria. What is the practical importance of the effect of concentrated solutions?
11. Explain how bacteria live under natural conditions in relationship with other microbes. What is meant by *symbiosis, commensalism, bacterial antagonism*? Give examples.
12. What is an *antibiotic*? Name one important substance of this kind.
13. Define: *putrefaction, proteolysis, proteolytic bacteria.* Mention some products of protein decomposition. Give examples of strongly proteolytic species.
14. Define *fermentation.* What are the usual products of fermentation?
15. How do bacteria differ in their capacity to ferment particular carbohydrates? Of what practical value are these differences?
16. Define *chromogenic bacteria,* and give examples.
17. Describe the light-producing bacteria.
18. Define: *bacterial toxin, exotoxin, endotoxin.*

CHAPTER X

ACTIVITIES OF NONPATHOGENIC, SAPROPHYTIC BACTERIA

According to the popular notion, all bacteria are harmful and only await an opportunity to injure us. But, as we have now learned, this is a totally false idea. On the contrary, of all the great invisible host of microbes, *only a small number* are ever pathogenic. The great majority of bacteria and other microbes belong to the class of harmless saprophytes. Many of them are not only harmless, but very useful in human affairs, and some play an essential rôle in Nature's scheme and really make possible the life of higher plants and animals.

Even if there were no disease-producing species at all, and no such thing as "medical bacteriology," the study of the activities of the harmless bacteria would still constitute an important and interesting science of great practical significance. Of course, it is the capacity of the free-living, saprophytic bacteria to bring about the biochemical changes, just mentioned in the preceding chapter, that gives these organisms so much importance. It is their power to hydrolyze proteins and related nitrogenous compounds, to ferment carbohydrates, and generally to decompose the great variety of natural organic substances found everywhere in nature, that makes their effects significant. In this chapter there is space to describe only a few outstanding examples of these activities.

Decomposition of lifeless organic matter. First, in importance, of the useful activities of saprophytic bacteria is the decomposition of the bodies of dead animals and plants, and nonliving organic matter of all sorts. The final result of this activity of the "rotting" bacteria is the conversion of the entire complex organic material into simple *inorganic* substances.

The process of decomposition itself is not pleasant to contemplate. A piece of putrefying meat or a rotten egg, with its disagreeable appearance and foul odor, is a disgusting object. But it

takes little imagination to realize the vital importance of these rotting processes. Suppose the dead bodies of plants and animals, and fecal matter and other waste materials excreted from man and animals, were to accumulate where they fell upon the soil, and not decompose! There would soon be no room on the earth's surface for living things. In bringing about the decomposition of lifeless organic remains, bacteria make the earth a fit place for the living.

Sewage purification. A clear example of the useful activities of saprophytic bacteria is their action, together with protozoa and other kinds of microbes, in bringing about the purification of sewage. The safe disposal of sewage from many communities involves its collection in septic tanks or in central sewage-disposal plants, and then the decomposition of the raw sewage there before the liquid remaining is allowed to run off. It is the enzymatic activity of aerobic and anaerobic proteolytic bacteria, and other saprophytic microbes, that converts the foul and dangerous raw sewage to harmless and inoffensive decomposition products, and so solves one of the major problems of the sanitary engineer.

Influence of bacteria on the fertility of the soil. The decomposition of nonliving materials by bacteria not only rids the earth of useless waste, but accomplishes a still more important result. It releases from dead or lifeless matter the elements needed for the

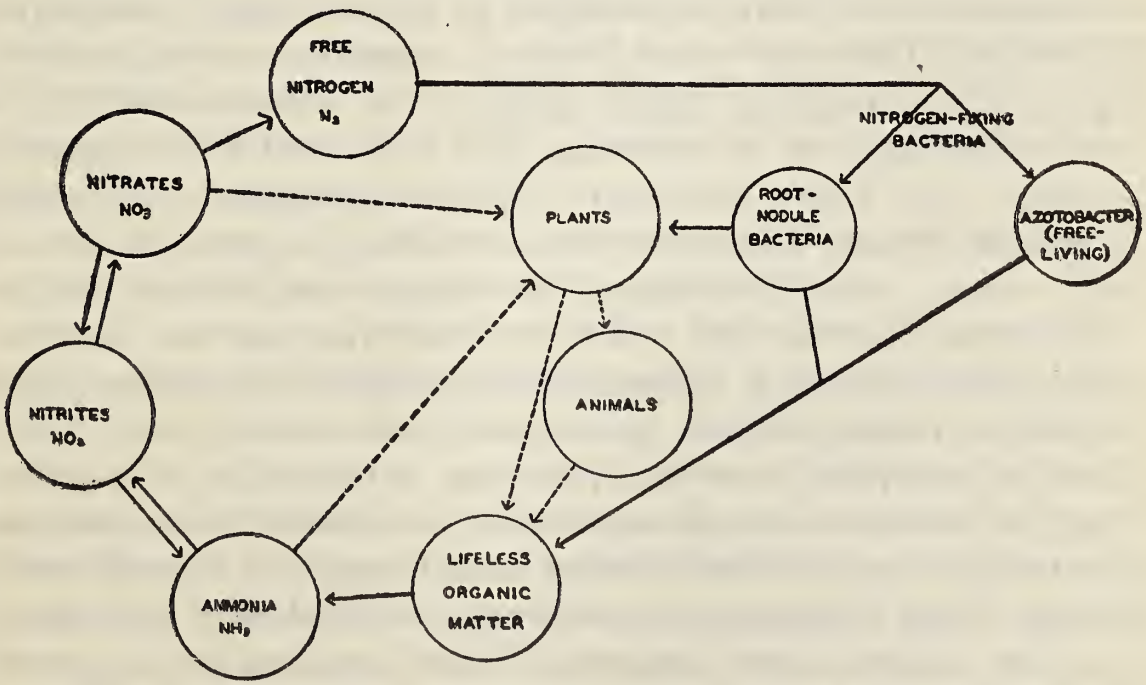


FIG. 37. The nitrogen cycle. The changes indicated by arrows drawn with a *solid* line are brought about entirely or chiefly through the activities of bacteria.

growth of plants, and returns these essential elements to the soil. The activities of saprophytic bacteria furnish the link between the dead and the living.

The all-important element, *nitrogen,* locked up in the bodies of dead plants and animals in the form of organic compounds, is released from its complex combinations by the action of putrefactive bacteria, and passes into the soil in the form of *nitrates*—simple inorganic compounds—*and plants use the nitrates for food.* Thus nitrogen, which is really the master element of living substance, passes through a perfect cycle—the *nitrogen cycle* (Fig. 37)—from the soil into the growing plant, then to the animal body when the plant is eaten, and back again to the soil through the agency of bacteria when the plant or animal dies. Other chemical elements necessary for living things, such as carbon, phosphorus and sulphur, pass through a similar cycle in which bacteria play an important part. The supply of these chemical elements on the earth is limited, and soon would be exhausted were it not for this necessary work of the bacteria. The fertility of the soil is, therefore, largely a result of bacterial activity.

Certain kinds of bacteria have an especially marked influence upon soil fertility. These are the denitrifying and nitrifying bacteria, and the nitrogen-fixing bacteria.

Denitrifying bacteria. Since plants are nourished by nitrates, the amount of this form of nitrogen in the soil largely determines its fertility. There are some kinds of organisms, called *denitrifying bacteria,* which can reduce *nitrates* (for instance $NaNO_3$) to *nitrites* ($NaNO_2$), or to ammonia (NH_3), or even to free gaseous nitrogen (N_2). These elementary nitrogen compounds are useless to growing plants. Therefore, the denitrifying bacteria in the soil really cause a waste of nitrogen, the all-important element for life.

Nitrifying bacteria. The action of denitrifying bacteria, however, in destroying nitrates is balanced by the activities of another group abundant in moist soil and natural waters all over the earth. These are called *nitrifying bacteria.* In contrast to the action of most bacteria, they do not cause decomposition, but instead they synthesize nitrates from ammonia and nitrites. In this way they directly enrich the soil. These organisms are autotrophs, or facultative autotrophs; some are aerobic, some anaerobic. Those classified in the genera *Nitrosococcus* and *Nitrosomonas* oxidize ammonia (NH_3) to nitrites ($-NO_2$), while those in the genus *Nitrobacter* oxidize nitrites ($-NO_2$)

to nitrates ($-NO_3$). The *Nitrosomonas* and *Nitrobacter* are nonsporeforming, pleomorphic, aerobic bacilli.

Nitrogen-fixing bacteria. The most important contribution to soil fertility is made by still another kind of bacteria—the *nitrogen-fixing bacteria.* These organisms are able to *fix nitrogen from the air,* i.e., they can capture atmospheric nitrogen and cause it to combine with other chemical elements in the soil, so that it becomes available in a form useful for the nourishment of plants. This remarkable property is possessed by two groups of soil bacteria.

The members of one group live free in the soil. An example is the organism named *Azotobacter chroococcum.* The *Azotobacter* are strictly aerobic, nonsporebearing, large rods, often occurring in swollen, oval, or yeast-like forms. The oval shape is due principally to the accumulation, inside the mature cell, of large globules of fat (Fig. 38). These free-living organisms utilize carbohydrates available in the soil as a source of energy, and obtain nitrogen directly from the air. The nitrogen is combined into their cell protoplasm, and later released in the form of nitrates, or other usable compounds, when the organisms die and are themselves decomposed by other soil microbes. The growth of *Azotobacter* also enriches the soil with another needed element—phosphorus—which, like nitrogen, is built into the growing cells.

FIG. 38. Fat in the cells of (A) *Azotobacter chroococcum,* and (B) *Rhizobium leguminosarum,* as shown by staining fixed smears with Sudan Black B-safranin. All the portions shown in black contain fatty material, and are colored blue by the Sudan Black B.

The second group of nitrogen-fixing organisms is composed of those bacteria that develop nodules on the roots of certain plants and are called *root-nodule bacteria* (Fig. 39). The plants which bear the nodules on their roots are called *legumes.* They include clover, peas, beans, and alfalfa. The organisms in the root nodules live in symbiosis with the plant, using carbohydrates from the plant and at the same time contributing to it nitrogen needed for its growth. The end result is to add large amounts of nitrogen to the soil.

It has been known for centuries that plants of this kind enrich the soil. It has long been a practice of farmers to carry out a rotation

of crops on a particular field, and to sow the field in clover or soybeans, or some other leguminous plant every so often. When the clover is plowed under and the field is planted again, the land is found to be more fertile. In order to be sure that an active development of the root-nodule, nitrogen-fixing bacteria will occur, it is a common practice to *inoculate* the soil or the seeds with these organisms before planting.

The name *Rhizobium leguminosarum* is given to one of the principal species of root-nodule bacteria. Members of the genus *Rhizobium* are similar to the *Azotobacter*. They are aerobic bacilli, highly pleomorphic, especially when seen in direct smears from root nodules.

Importance of agricultural bacteriology. Without going further into detail, or into other aspects of the subject, the foregoing should serve to give some notion of the fundamental significance of bacterial action in the soil. Also, it should make clear the value of good practices on the farm. When we recall how susceptible bacteria are to environmental influences, it becomes easy to comprehend the importance of the physical state and chemical composition of any particular piece of land which is to be used for planting. Scientific farming must take into account the fact that ground which is too wet or too dry, too acid or too alkaline, or plowed, harrowed, or fertilized improperly, or at the wrong time, is not going to support the luxuriant microbial population needed to insure a maximum crop yield.

Bacteria in industrial processes. Practical use is made of the capacity of bacteria to bring about putrefaction and fermentation in connection with numerous industrial processes. Among these are the manufacture of vinegar, and of various chemical products of fermentation; the curing of tobacco, cocoa, and coffee; the tanning of leather; and the retting of flax and hemp.

Vinegar manufacture. Vinegar is a solution containing acetic acid, and other substances which give it the characteristic color, aroma, and flavor. It is formed when wine, hard cider, or other liquor containing alcohol is soured (i.e., fermented) by bacteria which oxidize the alcohol to acetic acid. Organisms that do this belong to the genus *Acetobacter*. Wine itself is a product of *alcoholic fermentation* of a sugary solution (such as grape juice or molasses mash) brought about by *yeasts*.

The vinegar bacteria grow on the surface of the alcoholic liquor,

forming a thick scum which usually becomes slimy after a while (the M [mucoid] variation) and falls to the bottom. In the vinegar crock, on the dining-room table in many a home in years past, a more or less disintegrated mass of these bacteria was always to be seen. It was known as the "mother of vinegar."

Members of the genus *Acetobacter* are strict aerobes, and the chemical conversion of alcohol to acetic acid is an aerobic, oxidative process. To facilitate the reaction, vinegar is commonly made com-

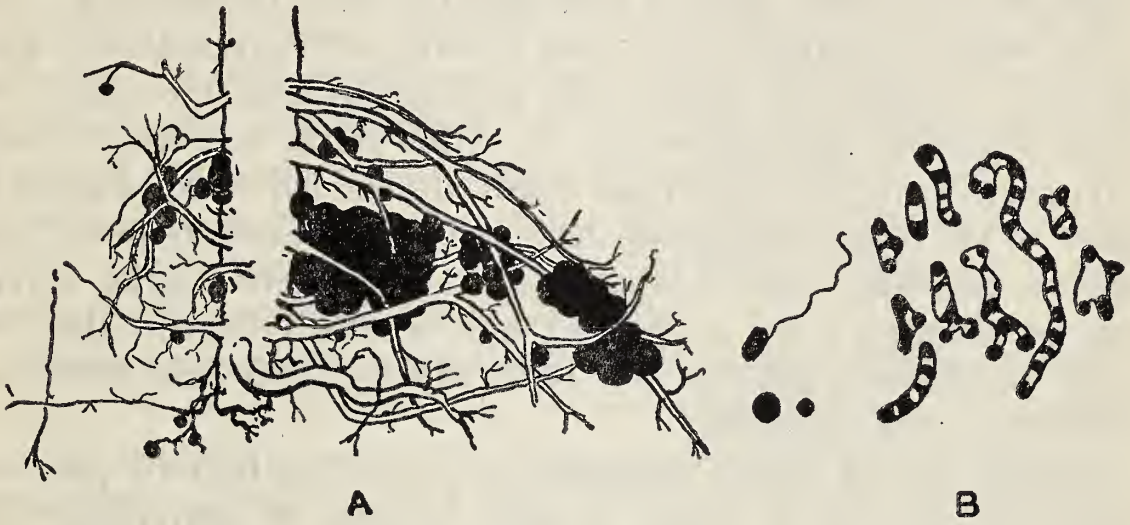


FIG. 39. A: nodules on the roots of a soy bean plant; B: forms of the bacteria that live in the root nodules. These organisms are capable of "fixing," i.e., combining with, nitrogen from the air. As a result of their activities, the soil in which plants with these root nodules grow is made more fertile. (B, based on Topley & Wilson's *Principles of Bacteriology and Immunity,* William Wood and Company, Baltimore, 2nd Ed., 1936.)

mercially by allowing the alcoholic fluid to trickle from top to bottom of a tall wooden cylinder filled with beechwood shavings previously inoculated with *Acetobacter aceti* or other species of the vinegar bacteria. Air circulates freely through the cylinder and the oxidation of the alcohol, first to acetaldehyde, then to acetic acid, occurs rapidly, so that the fluid drawn off at the bottom has already been changed to vinegar.

Manufacture of chemicals. Various chemicals of industrial importance may be prepared through the action of microorganisms. Examples are *lactic acid* (formed through fermentation of lactose, or other carbohydrate materials, by common saprophytic organisms like *Lactobacillus bulgaricus* or *Streptococcus lactis*); *glycerin* (a product of the fermentation of molasses and similar substances);

and *butyl alcohol* and *acetone* (derived from the anaerobic fermentation of corn mash).

Curing of tobacco, cocoa, and coffee. Some of the enjoyable tastes and aromas of certain brands of smoking *tobacco* are due to special fermentative processes (often patented, secret methods) to which the leaves are subjected. Bacteria of numerous kinds take part in these fermentations.

In the case of both cocoa and coffee, the original "beans" are fruits (of the small cocoa tree and of coffee bushes, respectively), which have an outer shell or covering that must be removed. The *cocoa* beans are spread out in vats, covered with green leaves, and allowed to ferment for several days. During this time, the outer coverings are slowly decomposed by the action of the saprophytic bacteria present. At the same time, fluids are exuded which impart a flavor to the beans. Afterward, the cured cocoa beans are dried and roasted. Similarly, *coffee* beans are freed from their exterior coverings by allowing them to soak in vats in which fermentation by yeasts and bacteria goes on. The coffee thus acquires some desirable qualities of taste or flavor.

Manufacture of leather. Saprophytic, proteolytic bacteria play an important part in the rather strange business of converting raw animal skins to fine leathers. The rôle of these bacteria can be summarized by saying that, at several stages of the process, they are allowed to act upon the hides to remove the last traces of hair and flesh, and to soften the skins and make them permeable by the tanning solutions. Since this involves the soaking of the hides in water containing dog or bird feces, or in other actively putrefying or fermenting mixtures, it is no wonder that the smell of the tannery yard is often alluded to by writers on this subject.

Retting of flax and hemp. The long, tough cellulose fibers in the stems of flax plants have been used for centuries in the making of linen. Similarly, the strong fibers in hemp are used for making rope. When these plants are harvested, the fibers are held fast to the woody part of the stems by a kind of resin, called *pectin*. It is necessary to soften this binding so as to release the fibers. The process of freeing the fibers is called "retting." It is really a "rotting," for it is the fermentation, and consequent decomposition, of the binding substances by the growth of bacteria that loosens the fibers. The *retting of flax and hemp* is, therefore, a bacteriological process of fermentation. In order to permit this fermentation to occur, the flax straw is some-

times spread on the ground in the fall and allowed to remain over the winter, or, more commonly, the flax is submerged in river water for several days.

RELATION OF BACTERIA TO THE PREPARATION AND PRESERVATION OF FOOD

Many foods furnish excellent nourishment for the growth of bacteria; and if the food is warm and moist, the organisms may multiply with great rapidity. In so doing, they produce in the food the characteristic changes associated with the putrefaction and fermentation of organic matter. Sometimes these changes are desirable from our point of view; sometimes they are not. The agreeable quality and flavor of a number of our common foods are due to the previous growth of bacteria in them. On the other hand, when foods of any kind "spoil," bacteria are again responsible, in this case causing undersirable decomposition of the foods.

Foods prepared by bacteria. *Fermented milks.* The most familiar kinds of foods prepared for us by microorganisms are those which result from the growth of bacteria in milk and cream. Soured (or fermented) milk is used the world over, and in many circumstances is preferred to sweet milk. Milk sours when the bacteria in it have multiplied and have fermented the milk sugar—lactose—changing it to lactic acid. In the presence of this acid the protein part of the milk, called the casein, becomes coagulated and separates as an insoluble mass forming the characteristic curd.

Sour milk may contain as many as 500,000,000 living bacteria in every cubic centimeter. It is still a wholesome and nutritious food, however, provided no disease germs are present in it. When milk sours in the usual way, the only types of organisms remaining in the fully curdled milk are the harmless aciduric bacteria, *Streptococcus lactis.* Soured milk keeps well, because putrefactive bacteria which might otherwise spoil it are prevented from growing in the presence of so much acid.

Buttermilk. The soured milk left in the butter churn after butter has been made, and many others forms of fermented milk, are very widely used throughout the world, especially in warmer climes where it is difficult to prevent spontaneous souring. The Armenians, Turks, Bulgars, and other peoples of the semiarid lands of southern Europe learned long ago that in such warm countries milk is kept

best when it is soured. They learned that milk could be soured more quickly if a bit of the curd from soured milk were added to new lots of freshly drawn milk. They deliberately inoculated fresh milk with carefully preserved pieces of clotted casein. The character of the fermented milk thus made by various peoples depended upon the kind of bacteria introduced. Thus, the fermented milk used for generations by the Bulgars was fermented by a particular bacterium, called *Lactobacillus bulgaricus.* The term *Bulgarian milk,* in a strict sense, refers only to milk fermented by this organism, but is popularly used to mean almost any type of fermented milk.

In America, fermented milks are sometimes used for the feeding of infants. Many infants find acid milk readily digestible, and the continuous feeding of such milk saturates the intestine with the harmless aciduric bacteria, and greatly restrains the development of putrefactive organisms which might form poisonous products. Milk soured by inoculating it with *Lactobacillus acidophilus,* called "acidophilus milk," is sometimes given to adults who suffer from excessive intestinal putrefaction.

Butter. Buttermaking is essentially a bacteriological process. It depends upon a "ripening" or fermentation of cream, followed by a mechanical churning of the soured cream. The first process, the bacteriological fermentation of the cream, is the important one, and to it are due the taste and aroma of the butter. The churning is purely a mechanical process by which the tiny globules of fat are made to coalesce into a solid yellow mass of butter.

When cream is separated from whole milk, it carries with it most of the microbes in the milk, so that cream always contains many bacteria. The farmer, making small batches of butter, depends upon these organisms naturally present to ferment the cream, and he permits the ripening to go on spontaneously to a point which he recognizes by experience as the proper stage for churning. He does not always get a batch of good flavor, because he does not always have the right combination of organisms in his cream.

In large creameries, constant quality and flavor of the butter are assured by first heating (pasteurizing) the cream to kill the bacteria naturally present, and then inoculating the cream with a little of a previous batch (called a starter) or with a pure culture of a particular species of the lactic acid bacteria, usually *Streptococcus lactis* or a related species, *Streptococcus cremoris.* When the cream has fermented and is ready for churning, it may contain as many as a

billion bacteria in every cubic centimeter; but after churning, the number of living bacteria rapidly decreases. Butter which has been kept in the cold for a few days usually contains not more than 50,000 living organisms per gram.

Cheese. All the many different varieties of cheese are products of the growth of microorganisms. In general, the method of cheese-making is as follows. The curd from soured milk is salted, then allowed to ripen. The ripening is brought about by the growth of bacteria or molds in the curd. The characteristic flavor and aroma of each of the different kinds of cheese are due to the character of the changes in the protein caused by the particular microbes concerned in the ripening. The special character of Roquefort and Camembert cheeses is due to the growth in them of certain molds, *Penicillium roqueforti,* and *Penicillium camemberti,* respectively. Limburger cheese is an example of the kind of cheese in which the putrefaction of the curd by bacteria (which occurs to some degree in all kinds of cheese) is permitted to go to an advanced stage.

Sauerkraut. This is another example of a food prepared by fermentation. Cabbage is cut into fine pieces, then layers of this chopped cabbage, sprinkled with salt, are packed tightly into casks. The salt draws much of the juice from the cabbage and this juice, rich in sugar, is fermented by the lactic acid bacteria always present. When the process of fermentation is complete, the cabbage has been converted into what we call sauerkraut. In this form it does not readily spoil, unless molds are permitted to get into it from the air.

Silage. In many parts of the United States, conspicuous landmarks throughout the farming country are the tall, cylindrical tank-like buildings called silos, which are placed out by the cow barns on nearly every farm. The farmer packs into his silo finely chopped corn (stalks, ears, and all), or alfalfa, straight from the field. (A machine chops the plants into pieces and conveys the material to the top of the silo on a conveyor belt, or by way of a blower). When the silo is filled, fermentation of the mass of plant tissue soon begins, brought about by the various species of aerobic and anaerobic bacteria always present. The material becomes strongly acid in reaction, and eventually the aciduric, lactic-acid species predominate. As the end result of this natural fermentation, the original raw plant material is converted, in the course of about one month, into what is called *silage,* a food for cattle which is apparently delicious, as well as evidently nourishing and economical.

Food spoilage and food preservation. Spoilage of food is due to the growth in it of the putrefactive type of bacteria. If food is to be preserved, it must be kept under conditions in which bacteria cannot multiply, or it must be freed of living organisms, and then it must be sealed away in such a manner that no microbes can gain access to it. In general, the more microbes in a food, the more quickly it will spoil. Therefore, *cleanliness* in the preparation and handling of any food will have a marked influence upon the length of time the food will keep.

There are five common ways of preserving food. These are: (1) *drying* the food, (2) *placing it in a preservative liquid,* such as vinegar, strong salt solution, or sugar solution, (3) *smoking* it, (4) *freezing or storing it in a cold place,* and (5) *canning* it.

Drying. Moisture is necessary for bacterial growth; therefore, food may be preserved if thoroughly dehydrated. Many kinds of dried fruits and vegetables, as well as dehydrated meat and milk, are on the market.

Salting and pickling. One of the most widely used methods of preserving meats is to rub them thoroughly with salt. It is a common practice to "pickle" meats in a very strong brine solution. Fruits are commonly preserved in the form of jellies and jams, which have a high concentration of sugar. The success of these methods depends upon the fact that bacteria will not develop in concentrated solutions, because in such solutions they are robbed of water through osmosis.

Smoking. Many farms have a "smoke house" where meats are hung to be preserved by smoking, and the method is also used commercially. A slow fire, usually made of green hickory wood, is maintained to furnish a dense smoke. The smoke deposits upon the food some chemical substances which serve to preserve it for some time.

Freezing and cold storage. If a food is kept continuously at a sufficiently low temperature, it will not spoil, because the ordinary bacteria do not multiply in the cold. Fish, meats, poultry, and a variety of other foods are commonly preserved by actual freezing. Eggs, fruit, and vegetables are kept at a temperature a few degrees above freezing, 4°–10° C (40°–50° F). Refrigeration is particularly important as a means of keeping *milk* sweet. In order to keep down the number of bacteria and to prevent early souring, milk must be kept continuously cold.

Canning. Every thrifty housewife is familiar with home canning. By the most common method, the food is first heated until thoroughly cooked, then placed while still hot in sterilized containers and sealed. By the so-called "cold pack" method the food is packed into the clean jar while cold, and then the jar and contents are steamed the necessary length of time. In either case, the purpose of the process is to destroy all the bacteria in the food by the heating, and to keep it sealed away in a container likewise freed from living bacteria.

Commercially, all sorts and varieties of foods are preserved in the familiar tin cans. After the cans are filled and sealed, they are placed in special steam chambers and subjected to a high temperature. They are then rapidly cooled and are ready for sale. Very rarely, foods spoil within the can. When this happens, it means that the bacteria in such a can were not all killed in the heating process, and that they have slowly multiplied. If a can shows a bulging end, or the contents look unusually mushy or otherwise appear abnormal, or have a bad odor, the can should be rejected without tasting the food. A safe rule would be to bring to a boil all canned foods before tasting them. Boiling will destroy not only harmful bacteria which might be present, but also the poisons they may have formed. Even the very dangerous toxins of *Clostridium botulinum* are destroyed by heating.

REFERENCES

Fabian, F. W.: *Home Food Preservation.* New York: Avi Publishing Co., 1943.

Fred, E. B.: *The Root-Nodule Bacteria of Leguminous Plants; Newer Knowledge of Bacteriology and Immunology* (Page 332). Chicago: Univ. of Chicago Press, 1928.

Frobisher, M. J.: *Fundamentals of Bacteriology.* Philadelphia: W. B. Saunders Co., 1944.

Haines, R. B. and Elliot, E. M. L.: "Some Bacteriological Aspects of Dehydrated Foods," *J. Hyg.,* 1944, 43:370.

Hammer, B. W.: *Dairy Bacteriology.* New York: J. Wiley & Sons, 2nd Ed., 1938.

Jensen, L. B.: "Microbiological Problems in the Preservation of Meats," *Bact. Rev.,* 1944, 8:161.

Prescott, S. C. and Dunn, C. G.: *Industrial Microbiology.* New York: McGraw-Hill Book Co., 1940.

Tanner, F. W.: *The Microbiology of Foods.* Champaign, Ill.: Garrard Press, 2nd Ed., 1944.

Tressler, D. K. and Evers, C. E.: *The Freezing Preservation of Foods.* New York: Avi Publishing Co., 1943.

Waksman, S. A.: *Principles of Soil Microbiology.* Baltimore: Williams and Wilkins Co., 1932.

REVIEW QUESTIONS—CHAPTER X

1. In general, why are the nonpathogenic bacteria important?
2. What causes putrefaction and decay of lifeless organic matter? Explain the importance of the decomposition of useless organic remains by bacteria.
3. How do bacteria contribute to the problem of safe disposal of sewage?
4. Explain how the decomposition of organic waste by bacteria: (1) contributes to the fertility of the soil and (2) prevents exhaustion of the supply of necessary chemical elements for living plants and animals.
5. Explain the "nitrogen-cycle" and its importance.
6. Define: *denitrifying bacteria; nitrifying bacteria.* Name and characterize three genera of nitrifying organisms. Explain the effect of denitrifying and of nitrifying bacteria on soil fertility.
7. What is meant by *nitrogen-fixing bacteria, root-nodule bacteria*? Name and describe the free-living, and the root-nodule organisms. What is a leguminous plant? Name some common legumes. Explain how these plants enrich the soil.
8. Explain the part that bacteria play in the preparation of vinegar. Name the vinegar bacteria.
9. Explain the part played by bacteria in: (1) the making of certain chemicals; (2) the curing of tobacco, cocoa, and coffee; (3) the manufacture of leather; and (4) the retting of flax and hemp.
10. Explain the process by which milk sours. Name four species of aciduric bacteria. Why does fermented milk keep well?
11. What is buttermilk, Bulgarian milk, acidophilus milk? What determines the quality of fermented milk? Discuss the use and value of soured milks.
12. Explain the process of buttermaking and the part played by bacteria. Explain the rôle of bacteria in the making of cheese.
13. How is sauerkraut made? Silage?
14. Why do foods spoil? Name five common ways of preserving foods.
15. Explain the principles involved, and discuss the practical use and value of drying, salting and pickling, and smoking as food preservation methods.
16. Explain why freezing and cold storage preserves food. What should be the temperature of refrigerators?
17. What are the essential steps in the preservation of food by canning? What is the purpose of the process?
18. Are canned foods generally safe? What would cause the rejection of a canned food? Give a general rule which would assure safety in the use of any canned food.

CHAPTER XI

CULTIVATION OF BACTERIA: CULTURE MEDIA AND METHODS

A peculiar technique, requiring the most meticulous attention to numerous important details, is employed in every step of the preparation and examination of cultures and in all other procedures in which microbes are handled. This technique is necessary in order that dangerous germs may be studied safely, and also in order to avoid contamination of the cultures, or other material being examined, with the microorganisms naturally occurring in the dust, on the fingers, and elsewhere. Microbiological technique is essentially an *aseptic technique,* that is, a procedure by which the worker excludes from his cultures microbes he does not wish to study, prevents infection of his own person, and avoids contamination of his surroundings. He creates for himself an aseptic environment in which to work. The glassware, the culture media, everything used for the study of germs in laboratory cultures is made *sterile* to start with. And once a culture is prepared, it is always handled in such a way that no microbes can gain entrance to it from without, and so that none of the bacteria in the culture can escape.

Sterilization of laboratory glassware. In the bacteriological laboratory the usual chemical glassware, such as beakers, test tubes and Erlenmeyer flasks, are supplemented with the *Petri dishes,* used for agar "plate" cultures, with *fermentation tubes* (Fig. 40), and *graduated pipettes,* and with a few other special kinds of glassware. More peculiar than the glassware itself are the special limitations on its use that apply only in microbiological work. Provided a beaker or other piece of glassware is chemically clean, the chemist is ordinarily free to use it as it comes off the shelf, but not so the bacteriologist. His glassware must not only be chemically clean and dry; it must be sterile as well. Hence, most of it is useless until it has been *sterilized.*

This is accomplished by subjecting the glassware to heat in some way. Every bacteriological laboratory has at least two types of sterilizers: (1) a *dry-heat oven* (hot-air sterilizer) and (2) an *autoclave* (steam-pressure sterilizer) (Figs. 57 and 58). It is the dry-heat oven that is used principally for sterilization of glassware, although some laboratories commonly employ the autoclave for this purpose. Test tubes, flasks, and bottles (clean and dry), are first plugged with cotton, and pipettes, syringes, and Petri dishes are wrapped in paper, then they are placed in the hot-air oven for 1½ to 2 hours at 160°–170° C, or in the autoclave for about 30 minutes at 121°–125° C (15 to 20 pounds' steam pressure). In use, these pieces of glassware are contaminated on the outside, of course, by handling, but the inside—the only part that actually comes in contact with the material under study—remains sterile. Moreover, these containers are always handled in such a way as to prevent the entrance of microbes from the laboratory dust or from any other outside source.

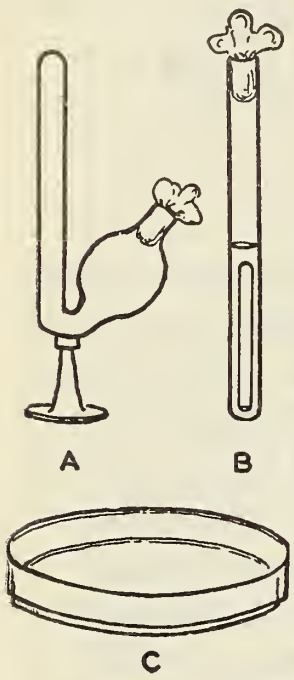


FIG. 40. Bacteriological glassware. A, B: two types of fermentation tubes. In B, gas, which may form as a result of fermentation, collects in the small tube inverted in the broth medium. C: a Petri dish.

CULTURE MEDIA

The most important factor in securing and maintaining a growth of bacteria in artificial laboratory cultures is the character of the food substances offered them. The various mixtures of nutritive substances used for the laboratory cultivation of microbes are spoken of collectively as *culture media*. The culture medium serves as the soil in which bacteria are planted for purposes of laboratory study.

Kinds of culture media. Although prepared primarily to support growth, special kinds of media serve other purposes as well. Many are designed to test or demonstrate particular biochemical activities or physiological properties of the growing organisms, such as the power to ferment a sugar or utilize citrate, while others are planned to differentiate one kind of bacterium from another, or to

encourage the growth of a particular kind, and so to aid in the separation and identification of species.

Plain nutrient broth and agar. The basic medium, from which many other important media are prepared, is a broth made either with an infusion or an extract of meat.

Plain infusion broth has the following ingredients:

Meat infusion	1,000 cc
Peptone	10 gm
Common salt	5 gm

Plain extract broth has the same composition, except that a beef extract is used instead of the meat infusion. The infusion or extract contains soluble extractives, salts, and some muscle sugar (dextrose), but little protein. The peptone supplies protein in a form which is readily assimilated by growing microbes. The salt is added to give the broth approximately the same salt content as that of blood, so that sterile whole blood may later be added to enrich the medium, if desired, without causing hemolysis of the added red blood cells.

After the ingredients have been dissolved and the reaction has been adjusted, the mixture is filtered through cotton, and the clear, yellowish solution is distributed into test tubes and flasks. The tubes and flasks are plugged with cotton, and the medium is sterilized by autoclaving.

Plain agar. Broth is converted into a solid medium by dissolving in it 15 or 20 grams of *agar-agar* (commonly called agar) per liter. Agar is made from the stems of a seaweed; it is chiefly of carbohydrate nature. It comes to the laboratory in the form of dry shreds or as a coarse powder. This substance has practically no value as food, but it has physical properties which make it ideal as the basis of a solid culture medium. It dissolves in water slowly, and only after boiling for some time, but once in solution it remains liquid until the temperature is reduced to about 40° C, when it solidifies. Agar medium is easily handled in the liquid state, and with simple precautions may be poured without contamination from one sterile vessel to another. It may be also inoculated in the liquid state just before it solidifies, that is, when cooled to about 45° C. It remains solid at 37° C, the temperature at which most cultures are incubated.

Sterilized agar medium may be allowed to solidify in test tubes

held in a nearly horizontal position, thus forming a long slanting surface for inoculation, called an *agar slant,* (or a *butt-slant,* when only the upper half of the agar is slanted), or the tube may be filled two-thirds full with agar, forming an *agar deep.* Agar media are used universally for *plate* cultures in Petri dishes.

Special and enriched media. A distinction may be made between the *plain media* just described (in which category are customarily placed also such media as those made with gelatin-agar mixtures, plain milk, or potato) and *special* or *enriched media.* Examples of the latter are the media made with coagulated blood serum (e.g., Loeffler's serum medium) or coagulated egg-glycerin mixtures (e.g., Dorsett's medium and Petragnini's medium), or with cooked meat or brain substance, (e.g., glucose brain broth), and those prepared by enriching plain broth or agar by the addition of carbohydrates, blood, blood serum, casein-digest, yeast extract, or other special nutritive substances. *Anaerobic* bacteria are often cultivated in a liquid or semisolid medium containing sodium *thioglycollate,* a chemical substance having a marked affinity for oxygen. In thioglycollate broth anaerobic organisms will grow, even though no special measures are taken to exclude air from the tube.

It must be understood that the number of different varieties of culture media is limited only by the imagination and the industry of bacteriologists, for each worker is free to concoct his own for his own purposes. Many special media will be mentioned in later chapters in connection with the work for which they were designed.

Differential and selective media. For the primary culture, bacteriologists sometimes use a medium so designed that it will reveal clear differences, when growth appears, between those organisms it is desired to study and other organisms likely to be present. An example of such a differential medium is *litmus lactose agar.* When this medium is inoculated with the feces of a patient, it serves to differentiate any colonies of typhoid bacilli that may appear from those of the nonpathogenic bacilli of the normal colon. The latter ferment the lactose (with acid formation), thus changing the indicator color, whereas the typhoid organisms do *not* ferment this sugar and hence do not affect the indicator (Fig. 41).

Bismuth-sulfite agar, desoxycholate agar, and *S.S. agar* are examples of media that are *both differential and selective.* They contain lactose and an indicator, like the medium described above,

but certain other chemicals or dyes are added which have an inhibitory effect upon the growth of the ordinary colon bacilli and Gram-positive organisms always present in feces. The development in fecal cultures of the typhoid bacillus, or of related types of bacilli causing intestinal infections, is therefore favored, while these undesired bacteria are suppressed (Table XXII, Chapter XXXIV).

Media that are selective for streptococci, cholera spirilla, fungi, etc., are used in various other phases of microbiological work.

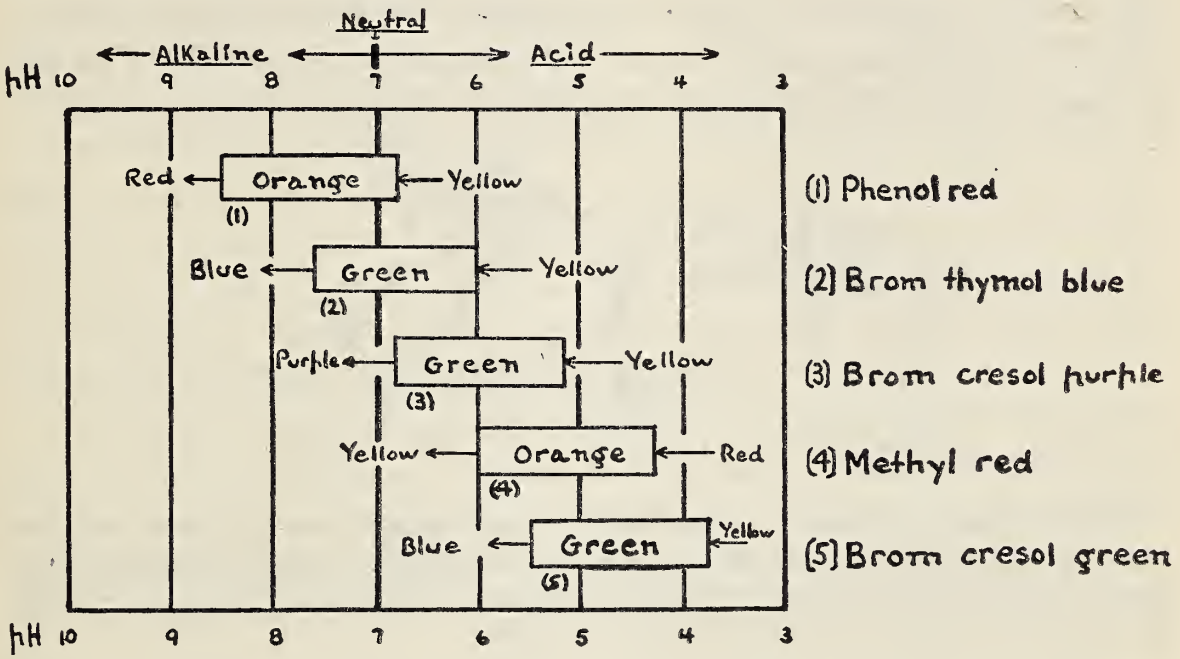


FIG. 41. Chart showing the range and color changes of five hydrogen ion indicators commonly used in the bacteriological laboratory. (Redrawn and modified from Mudge and Smith's *Fundamental Approach to Bacteriology*, J. W. Stacey, Inc., San Francisco, 1939.)

Synthetic media. Exactly reproducible nutrient solutions concocted from a definitely planned mixture of chemicals of known composition are being worked out at the present time for an increasing number of bacteria, including pathogenic species, and such media are widely employed for critical studies of bacterial nutrition and metabolism. Another important use for synthetic media is in the preparation of certain biologic products, notably diphtheria toxin and tuberculin.

Dehydrated media. Most of the commonly used kinds of media are available commercially in the form of dried powders. These dehydrated products are easily made into media ready for use merely by the addition of water, and sterilization. Successive lots of media

made in this way, at different times and in different laboratories, are likely to be uniform—a decided advantage—and in the case of the more complicated media, at least, it is economical of both time and effort to make use of a dehydrated product.

AEROBIC CULTURE METHODS

Handling test-tube cultures. When a smear is to be made from a test-tube culture, or when organisms are to be transferred from one tube to another, a definite procedure for handling the culture and the inoculating needle must be carried out (Fig. 42). The following is one commonly used method for a right-handed person.

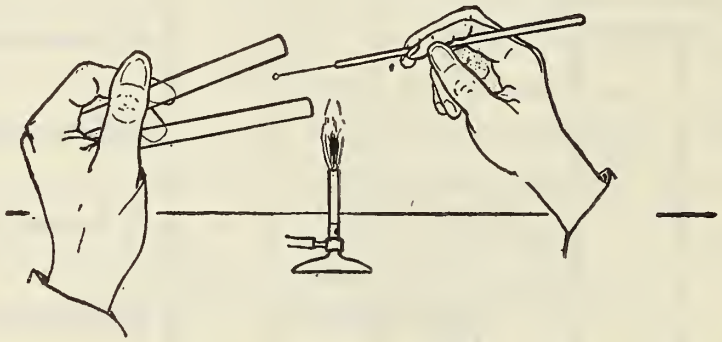


FIG. 42. Handling test-tube cultures. The cotton plugs have just been removed from the tubes, preparatory to making a transfer. The plugs are held by the fingers of the hand that holds the needle and can not be seen. Note that the tubes are held nearly horizontal, and close to the flame.

With the culture tube in the left hand, and the inoculating needle in the right hand, sterilize the needle by holding it *perpendicular* in the flame of a Bunsen burner until it is red hot. Remove the cotton plug from the tube with a twisting motion, by grasping it between the little finger and palm of the right hand. The portion of the plug extending outward from the hand should not touch the fingers or any other object. Pass the lip of the tube through the flame, then hold the tube *nearly horizontal* and close to the flame. Insert the sterilized needle into the tube and allow it to cool, then remove a minute portion of the growth with the tip of the needle. If the culture is on an agar slant, touch the growth very lightly; *do not plunge the needle into the agar.* Flame the lip of the tube again and replace the plug. If a smear is to be made, emulsify the organisms in a drop of water on a slide as previously described. If a tube of new medium is to be inoculated, take up the second tube with the left hand, remove the plug, flame the lip, inoculate,

flame the lip again, and replace the plug as before. Finally, sterilize the needle by holding it again in the flame before putting it down.

A quicker method of making a transfer from one tube to another is to hold both tubes parallel in the palm of the left hand. Remove both plugs, holding one between the little finger and palm of the right hand and the other between the third and fourth fingers. Flame the lips of both tubes, and with the sterilized needle quickly make the transfer. Then flame the tubes again, replace the plugs, and sterilize the needle.

Inoculation of test-tube cultures. *Broth,* milk, and other liquid media are inoculated by shaking the needle or swab bearing the organisms in the liquid a few times. Transfers from liquid cultures to new media are often made with sterile glass pipettes. *Slants* of agar or other media are inoculated by drawing the needle or swab *lightly* from bottom to top across the slanting surface. The surface of the agar must not be broken. Agar *butt-slants* are inoculated by making first a straight stab into the center of the butt, then withdrawing the needle and streaking the slant.

Two other kinds of test-tube cultures are often made. These are *stab cultures* and *shake cultures.* A *stab culture* is made by plunging a long, straight inoculating needle into the center of a deep column of semisolid agar or gelatin in a test tube; the needle is withdrawn by the same route. Most of the growth will occur along the line of the stab (Fig. 51: D). To make a *shake culture,* a deep column of agar is thoroughly melted, then cooled to about 45° C. At this temperature it is still liquid, and yet cool enough so that the organisms to be inoculated will not be killed by its heat. The inoculating needle is shaken vigorously through the melted agar; the medium is then rapidly cooled, so that the agar solidifies quickly. In these cultures, growth occurs in scattered colonies throughout the medium (Fig. 51: C).

Plate cultures. Cultures in agar or other solid media in Petri dishes are called plate cultures, and the Petri dish itself is commonly referred to as a *plate.* This term originated with the work of Robert Koch, who was the first to use gelatin and agar media. He had no Petri dishes and he poured his media on sterilized plates of glass.

Pouring a plate. The process of pouring melted agar from a test tube into a Petri dish is spoken of as *"pouring a plate."* The cotton plug in the tube of medium is removed and the lip of the tube is

heated in the flame. The cover of the sterile Petri dish is then lifted just enough to permit the neck of the tube to enter and to allow the agar to be poured in. Then the Petri dish cover is immediately dropped into place again. The dish may be gently revolved to distribute the medium evenly through the dish, but it must not be moved about or picked up until the agar has thoroughly hardened.

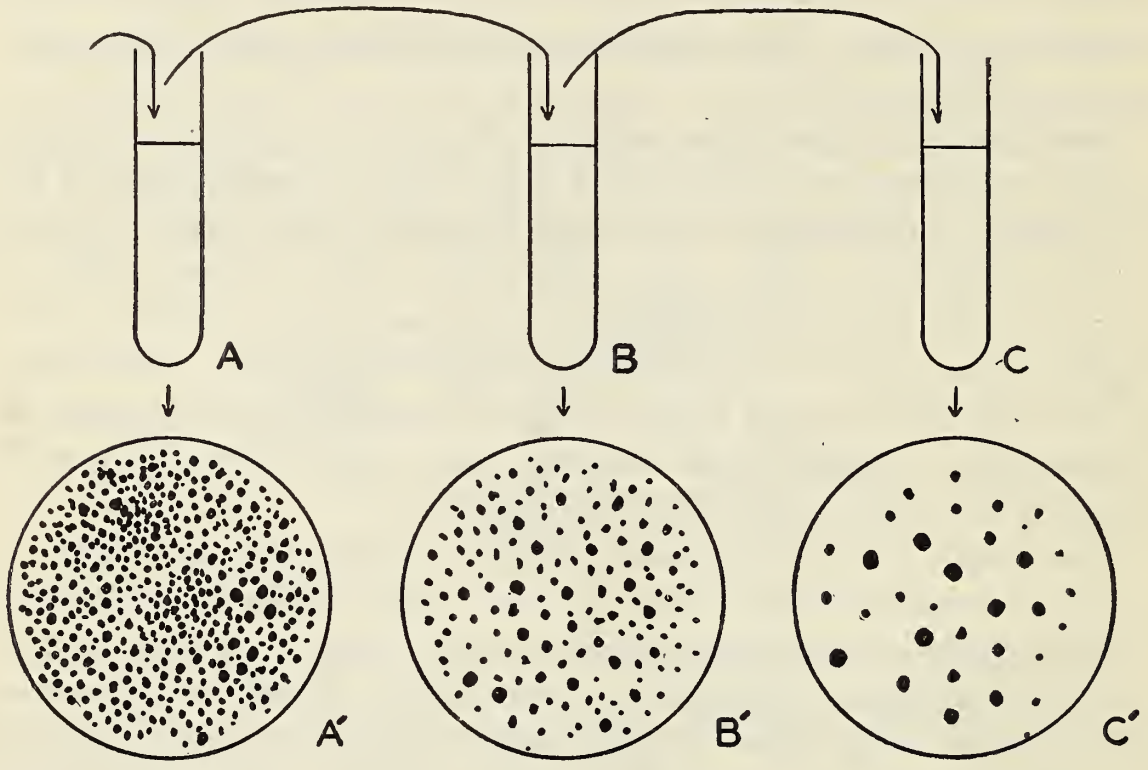


FIG. 43. Pour plate cultures, illustrating the effect of dilution of the inoculated material. A, B, C: tubes of melted agar, inoculated in series as indicated by the arrows. A′, B′, C′: pour plate cultures made from A, B, C, respectively, showing the growth resulting after incubation. Compare with streak plate cultures (Fig. 44) as a means of securing isolated colonies of bacteria.

Even this simple operation requires attention to a number of details. When agar plates are to be poured, the first essential is that the agar be *completely melted.* Otherwise there will be lumps in the plate. Several minutes' heating in boiling water is necessary to melt tubes of agar, and large amounts of agar will require prolonged heating. Once entirely melted, the second necessary step is to *cool the agar uniformly to about 45° C.* With a little experience, it is possible to tell when a tube of agar is at about this temperature, by the feel of the tube against the skin. When it is at the proper temperature, it has cooled just enough so that it can be held against the cheek or wrist for a few seconds without discomfort. The plate must then be poured *immediately,* before the agar has a chance to cool so far that it solidifies. Careful attention to the cooling of the agar, and quick action when it has reached the proper temperature, are neces-

sary. In case the agar solidifies before it can be poured into the dish, it must again be melted completely in boiling water, and cooled once more, before another try can be made. It is necessary to cool the agar to 45° C whenever it is to be inoculated while liquid, because a higher temperature might kill the bacteria introduced, and in any case it is desirable, in order that the medium may solidify promptly and not cause an excessive formation of water vapor. As agar cools in a Petri dish, the steam given off forms water drops on the under side of the cover. In order to prevent this water from dropping onto the surface of the culture, Petri dishes are *turned upside down* as soon as the medium has hardened, and they are incubated in this position.

There are two methods of inoculating agar Petri dish cultures. The resulting preparations are referred to as: (1) *pour plates,* and (2) *streak plates.*

Pour plates. To make this kind of culture the melted agar medium in a test tube, cooled to about 45° C, is inoculated by shaking the needle bearing the organisms through it just before it is poured into a sterile Petri dish. The growth will develop throughout the hardened medium, both on the surface and in the depths of the agar (Fig. 43).

Streak plates. The sterile, melted, and cooled medium is first poured into a sterile Petri dish and allowed to harden thoroughly; then the *surface* of the hardened agar is inoculated by streaking the needle or swab across it (Fig. 44). Here the growth occurs on the surface only.

Sometimes an agar plate is inoculated by streaking, and then a small amount of sterile melted agar is poured into the plate to make a thin layer over the inoculated surface. Such a *streak-pour-plate* culture is excellent for the cultivation of certain parasitic streptococci and other microaerophilic bacteria.

ANAEROBIC CULTURE METHODS

In the earlier days of bacteriology, it was thought necessary to employ elaborate and cumbersome methods to exclude air in order to cultivate the anaerobic bacteria. Most of the methods were so difficult, time-consuming, and "messy" that they had little actual use. Consequently, information about the anaerobes accumulated slowly, and it has been only in recent years, when easier and better techniques have been made available, that a real appreciation of

the importance of the anaerobic bacteria has become manifest. Even today anaerobic cultures are not made as frequently as they should be, in view of their proven value, especially in connection with the practical diagnosis of various infections.

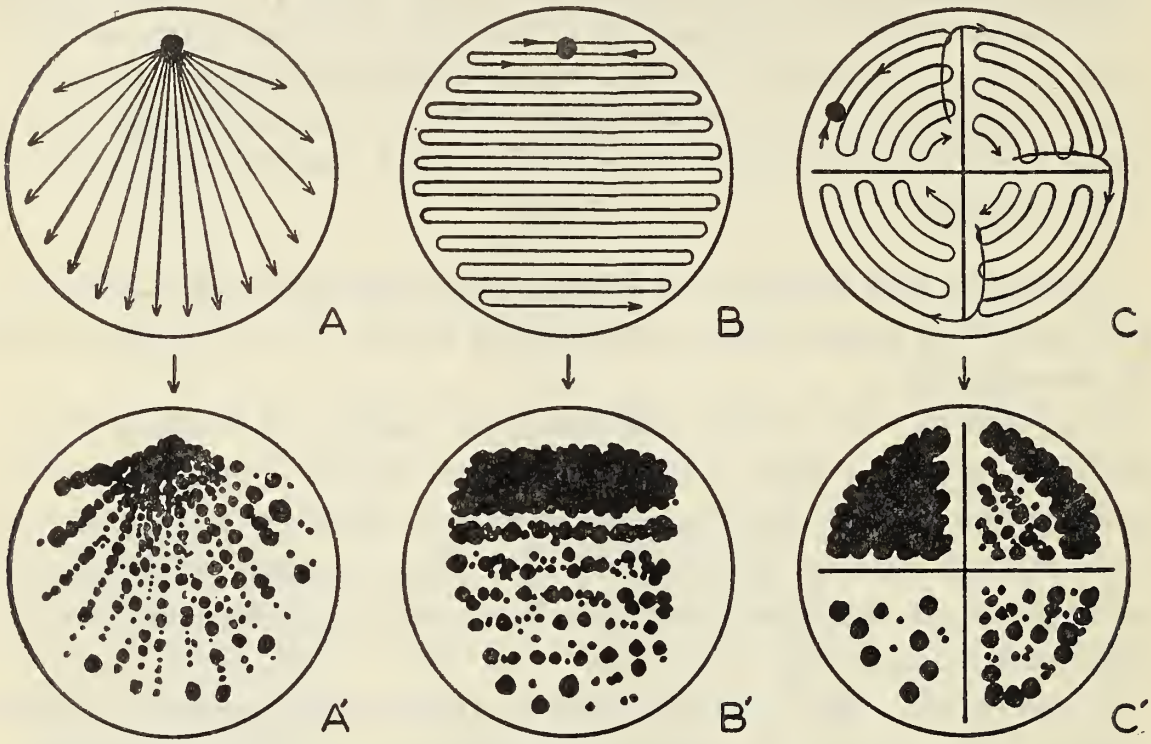


FIG. 44. Streak plate cultures. A, B, C: methods of inoculating streak plates. In method C the needle is sterilized after streaking a quarter of the plate, then recharged with the organisms by passing it across the section just previously streaked. A′, B′, C′: the growth resulting after incubation, when inoculated as in A, B, C, respectively. Compare with pour plate cultures (Fig. 43) as a means of securing single colonies of bacteria.

Cultivation of anaerobes in ordinary test tubes open to the air. Certain kinds of culture media are favorable for the growth of anaerobes and, once the contained oxygen is driven off by heating, these media will allow anaerobic growth without the use of artificial mechanical methods to exclude the air. Among these media are: glucose thioglycollate broth and agar; cooked meat medium; glucose brain broth; whole milk; deep columns of glucose infusion broth containing a small piece of fresh sterile tissue (such as rabbit kidney); and deep columns of glucose infusion semisolid agar, with or without the presence of fresh tissue, thioglycollate, or other reducing substance. The routine procedures to be followed before inoculating anaerobic organisms into such media are the following:

(1) Heat the medium in boiling water for about 20 minutes to drive off the oxygen contained in it.

(2) Cool the medium quickly under cold running water. Enriching substances, such as blood, ascitic fluid, or blood serum may be added to the partly cooled medium.

(3) Inoculate the depths of the medium.

Special anaerobic culture methods for test tubes. *Exclusion of air with a seal of petroleum jelly.* While the growth of most anaerobes in ordinary unsealed test tubes, in the media mentioned above, is entirely adequate for many purposes, a still better development is obtained if the air is totally excluded from the tube. One of the simplest ways to do this is to cover the broth or agar column with a half-inch layer of *sterile petroleum jelly.*

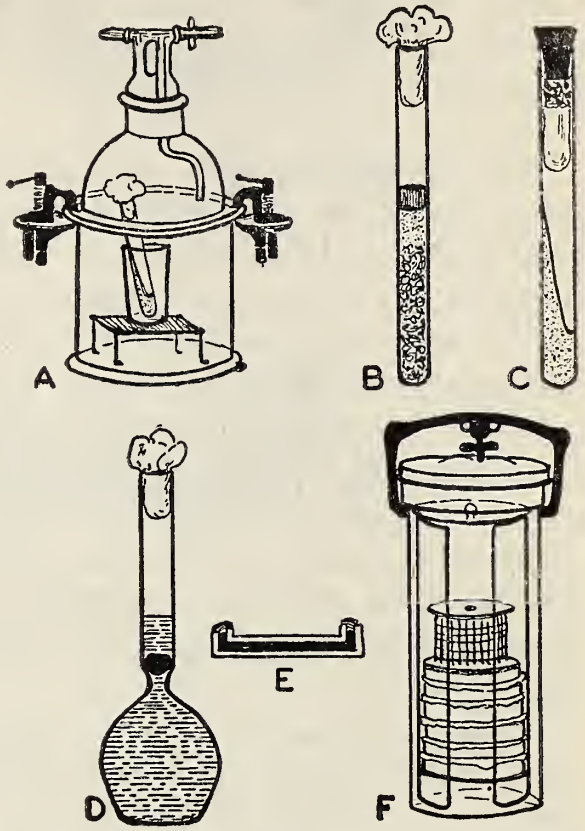


FIG. 45. Anaerobic culture methods. A: a Novy jar. The air in this jar can be exhausted by a vacuum pump, and replaced by some inert gas, such as nitrogen. B: a tube of meat medium sealed by a layer of petroleum jelly on its surface. C: a slant culture made anaerobic by the pyrogallic acid method. D: a flask with constricted neck and marble seal. The marble may be pushed aside for purposes of inoculation. E: the inverted Petri dish method. The inoculated agar is poured into the larger part (ordinarily the cover) of the Petri dish, then while the agar is still melted, the smaller part is placed upon it and the edges are closed with paraffin, thus sealing the agar between two layers of glass. F: the Varney phosphorus anaerobic jar. (See also Fig. 48.)

The sterile jelly may be kept at hand in an Erlenmeyer flask in which it has been autoclaved. After it is melted over a flame, it is added to the inoculated medium with a sterile pipette. When it cools, it hardens to make an air-tight seal. Examination of the growth in such cultures is best made by use of a fine sterile capillary pipette, the tip of which is sealed in the flame before plunging it through the jelly. Once beyond the jelly layer, the pipette is opened by breaking the tip against the side or the bottom of the tube.

Pyrogallic acid oxygen-absorption method for slant cultures. Cultures upon slants of blood agar or other common media are convenient, and consequently are widely used. The following is a satisfactory anaerobic method for slant cultures. It depends upon the use of an alkaline solution of pyrogallic acid to absorb the oxygen from the tube (Fig. 45: C).

Test tubes (preferably the large-sized, so-called "Board of Health" tubes) containing the sterile slanted medium should be plugged with a good grade of *absorbent* cotton. If the original plug is not of absorbent cotton, a roll of absorbent cotton may be made with the fingers. It need not be sterile. Cut or burn off the upper portion of the absorbent plug and push it into the tube to about 3/8 inch below the rim. The plug should be about 1½ inches long. Tap dry pyrogallic acid lightly into the space above the plug. Then add with a pipette about 1.0 cc of *warm* 10% sodium hydroxide solution—enough to dissolve the pyrogallic acid, but *no more*—and close the tube quickly with a solid rubber stopper. Twist the stopper into the tube as tightly as possible, then dip the top of the tube into melted paraffin. Incubate these tubes in an upright position like any other culture.

When examination of these cultures is desired, scrape off the excess paraffin and remove the stopper. Using forceps, remove the cotton plug with a slow twisting motion and close the tube with a new sterile plug. If the proper amount of hydroxide solution was used, removal of the original plug will leave the walls of the tube practically dry. After examination, if it is desired to incubate the culture further, it is merely necessary to roll another plug of absorbent cotton with the fingers, insert it, and repeat the process outlined above.

Anaerobic methods for plates. The following are among the useful procedures for growing anaerobes in plate cultures.

Specially made Petri dishes. Special Petri dishes have been devised to permit the production of anaerobic conditions within the plate by mixtures of pyrogallic acid powder and NaOH, without interfering with the inoculated agar surface (Fig. 46).

Anaerobic jars. Several types of jars have been designed to furnish anaerobic conditions by replacing the oxygen in the jar with hydrogen or nitrogen, or through chemical absorption of the oxygen.

One of the best methods depends upon the removal of the oxygen in the air by forcing it to combine with hydrogen in the presence

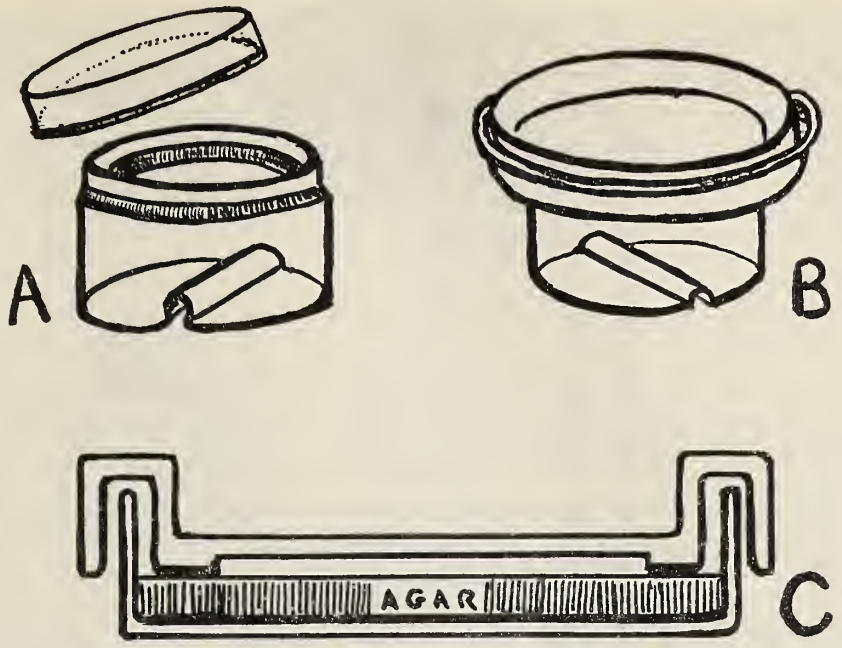


FIG. 46. Special types of Petri dishes used for cultivation of anaerobic bacteria. A: dish devised by Spray, B: a modification suggested by Bray. On one side of the center ridge in the deeper dish is placed a 10% NaOH solution, and on the other side a quantity of pyrogallic acid powder. The bottom of an ordinary Petri dish, containing the inoculated agar, is inverted, fitted over the other dish, and sealed by use of a vaseline-paraffin mixture, plasticine, or other sealing material. Then by gently tilting the assembled container, the reagents on the bottom are caused to mix together. The reaction thus produced establishes anaerobic conditions, and the culture is now ready for incubation. C: cross section of the Brewer anaerobic Petri dish. The cover is made so that its rim rests upon the agar, sealing in the central part of the plate surface, except for a thin layer of air above it, only about 1 mm in thickness. To absorb the oxygen from this, sodium thioglycollate (0.2%) is incorporated into the agar.

of finely divided platinum as a catalyst. A recently developed apparatus to carry out this principle is the Brewer anaerobic jar (Fig. 47).

In another efficient anaerobic jar, devised by Varney, the oxygen is removed by allowing a small piece of *phosphorus* to burn within the jar (Fig. 48).

CULTIVATION OF BACTERIA UNDER REDUCED OXYGEN TENSION

Many pathogenic organisms are *microaerophilic,* at least when they are first isolated from body tissues, and their growth is likely to fail altogether if they cannot find in the medium the somewhat reduced oxygen tension to which they are accustomed. One simple

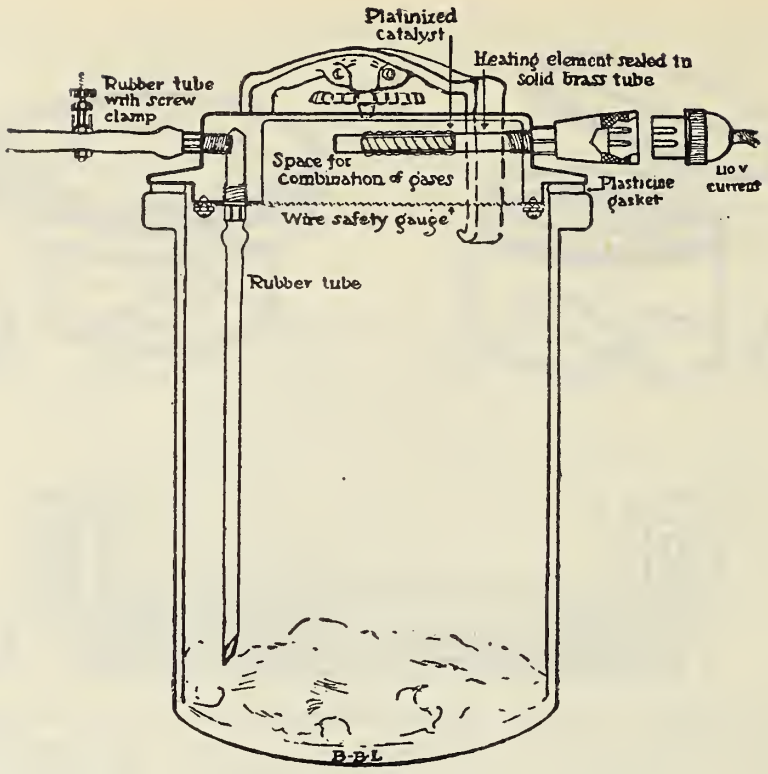


FIG. 47. The Brewer jar for cultivation of anaerobic bacteria. Hydrogen gas or illuminating gas is admitted through the rubber tube at the left. The hydrogen combines with the oxygen in the jar under the catalytic influence of platinized asbestos, which is warmed by an electric current. The combination of hydrogen and oxygen occurs in the upper part of the jar above the wire safety gauze, a feature designed to prevent development of an explosive mixture. (From Brewer, J. H., *J. Lab. and Clin. Med.*, 24:1190, 1939.)

way to improve the growth of such organisms on a *slant* culture is to heat the upper portion of the culture tube briefly in the flame (thus driving out much of the air), then to seal it at once by forcing in a solid rubber stopper. Another method to assure development of the microaerophiles is to make the primary inoculations into deep columns of semisolid enriched agar (shake cultures or stab cultures), or into deep tubes of glucose brain or meat medium, in which the organisms will find their own preferred level for active multiplication. The *streak-pour plate* technique described above is excellent for plate cultures.

CULTIVATION OF BACTERIA UNDER INCREASED CARBON DIOXIDE TENSION

The primary cultivation of several of the pathogenic bacteria is greatly aided if the cultures are incubated in an atmosphere containing from 5 to 10 per cent carbon dioxide. For some species,

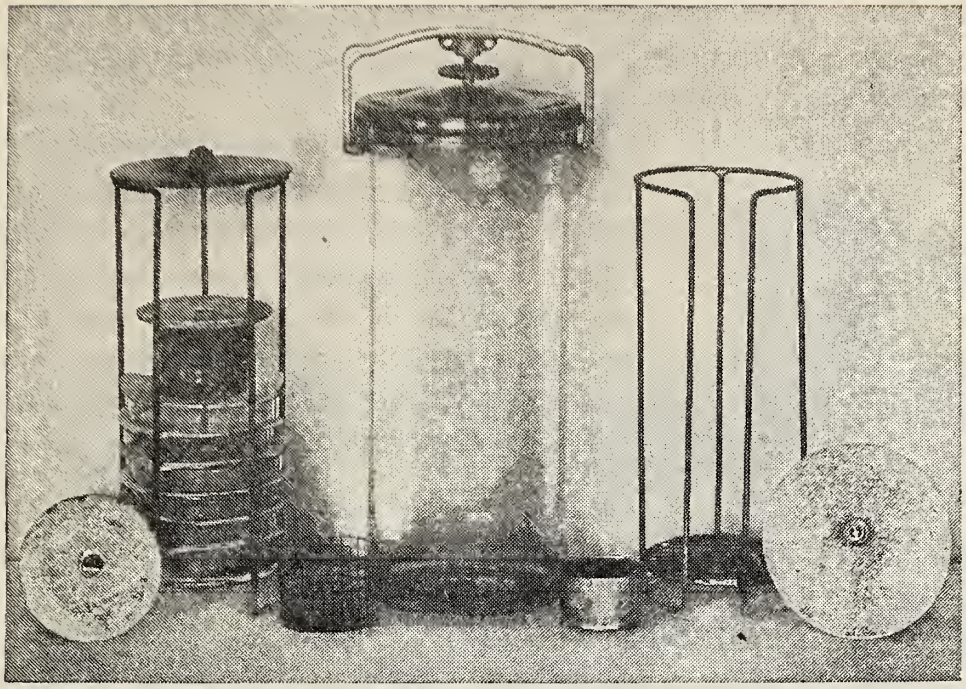


FIG. 48. The Varney phosphorus anaerobic jar. To the right and in the foreground are the separate parts. To the left is shown the entire assembly, ready to be placed in the jar. About 100 cc of water are first put into the jar; then the groundglass rim, rubber gasket, and cover are thoroughly greased with a mixture of paraffin and vaseline, or other sealing mixture. The metal cup is partly filled with powdered chalk, and now, with everything in readiness, a disc of yellow phosphorus (about 1/8 inch thick) is transferred with tongs from a bottle, where it has been kept under water, to the cup. The small asbestos circle with a central hole is then dropped onto the wire guard around the cup, the larger asbestos piece is put in place, and as quickly as possible the cover is clamped on. The phosphorus should begin to burn at once, and will continue to burn as long as free oxygen is available inside the jar.

After incubation, when the jar is opened, the remaining phosphorus will ignite again, and the worker must be prepared to remove the cup immediately with long tongs, and place it under water, or in a chemical hood where the phosphorus may burn off harmlessly. (Courtesy of Dr. P. L. Varney, Washington University School of Medicine, St. Louis, Mo.)

notably *Brucella abortus* and *Neisseria gonorrheae,* an increased amount of CO_2 in the atmosphere of the culture seems to be necessary for the first growth from the infected body tissues.

The following are among the practical methods that may be used to supply an increased CO_2 tension:

Single test tube method. Place the inoculated slant tube inside a larger (8″ x 1″) tube, which is plugged with absorbent cotton and has a small wad of cotton at the bottom (Fig. 49). Push in the plug of the large tube until it touches the plug of the smaller tube. Place in a small vial (10 x 35 mm) 1 cc of dilute sulfuric acid (1 cc of conc. acid in 29 cc distilled water).

then insert into this vial a #5 gelatin capsule containing 25 mgm of $NaHCO_3$. Now place the vial in the large tube *immediately,* allowing it to rest on the cotton plug; then quickly close the tube with a rubber stopper. Incubate in the upright position.

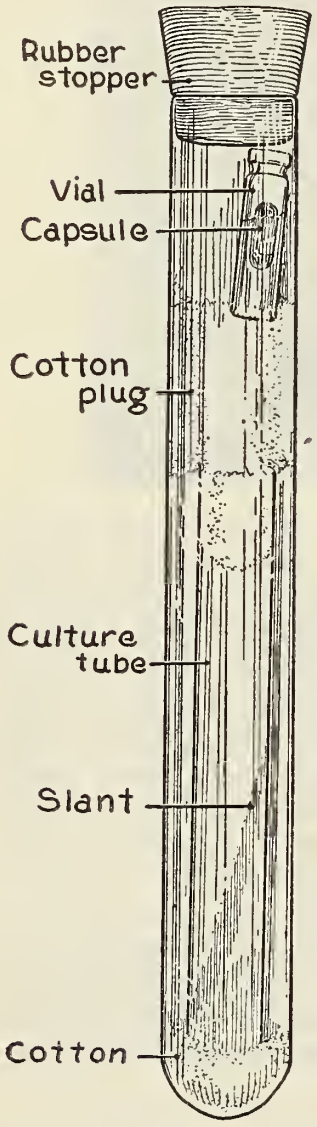


FIG. 49. A method for incubating a culture (e.g. *Brucella abortus*) in an atmosphere containing increased carbon dioxide. The procedure is described in the accompanying text. (Drawn by E. M. Shackelford.)

CO_2 jars. Tube or plate cultures may be incubated in a closed jar of some sort into which CO_2 gas is introduced or liberated. One simple method is to place a lighted, smokeless candle in a Mason jar, or similar container, together with the cultures, and then seal the jar. The candle burns until the CO_2 content of the atmosphere in the jar is about 10%.

COUNTING BACTERIA

In the examination of water, sewage, milk, and other materials, a count of the number of bacteria present is often made. The method of counting is simple, but the cultures must be made carefully in order to assure consistent results. The *standard methods* for counting the bacteria in water and milk, published by the American Public Health Association, are used almost universally. It is necessary: (1) to secure a representative sample of the material to be examined in a sterile container, (2) to dilute the sample aseptically in a definite quantitative manner, (3) to make agar pour plates with a constant amount of each of the dilutions; (4) to count the colonies which develop and, assuming that each colony represents a single living organism in the original sample, (5) to calculate the number of bacteria per cubic centimeter of the sample (Fig. 50).

Technique. The method of counting the bacteria in water may be taken as an example. The technique is as follows:

1. Shake the water sample thoroughly, and transfer, with a sterile pipette, 1 cc of the sample to a bottle containing 99 cc of *sterile* water. (This is called a *water blank.*) The sample is thus diluted 1:100. If 1 cc of this thoroughly mixed dilution is added to a 9 cc water blank, this will give a dilution of 1:1,000. A further transfer of 1 cc to another 9 cc water blank gives a dilution of 1:10,000. As many dilutions as are necessary (depending upon the number of living organisms suspected to be in the original sample) are made in this way.

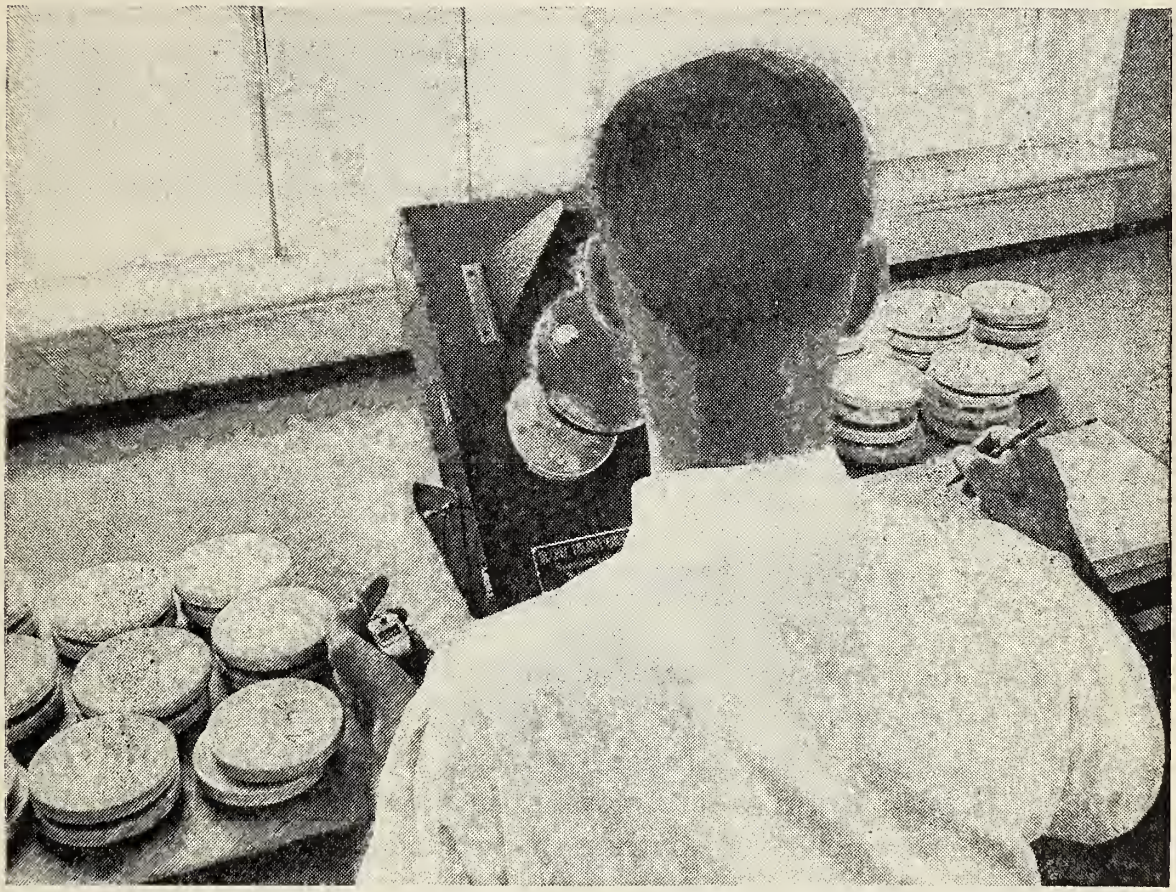


FIG. 50. Counting bacteria in milk samples. (Photographed at the Texas State Board of Health Laboratory, Austin, Texas. Reproduced through the courtesy of Dr. George W. Cox, State Health Officer, and of Dr. S. W. Bohls, Director of Laboratories.)

2. Place 1 cc of each dilution in the bottom of a separate sterile Petri dish. Mark the dishes accordingly. Then pour melted agar, cooled to about 45° C, into each dish, and mix it thoroughly with the water by gently tilting and rotating the dish. It is best to make these plates in duplicate or triplicate.

3. Allow the agar to harden. Then invert the dishes and incubate them at 37° C for 24 hours.

4. Discard those plates which contain less than 30 or more than 300 colonies, because counts based upon them may not be accurate. Count the

colonies in each of the remaining plates. Use a lens magnifying 2½ times. Each colony is assumed to represent a single organism in the original water sample. Multiply this individual plate count, in each case, by the dilution of the sample in that plate, so as to get the number in 1 cc of the original sample. Take the average of the individual plate counts as the final figure.

REFERENCES

BRAY, W. E.: *Synopsis of Clinical Laboratory Methods.* St. Louis: C. V. Mosby Co., 3rd Ed., 1946.

Diagnostic Procedures and Reagents. New York: Am. Pub. Health Assn., 2nd Ed., 1945.

HALL, J. C.: "Review of Development and Application of Physical and Chemical Principles in Cultivation of Obligately Anaerobic Bacteria," *J. Bact.,* 1929, 17:255.

KOLMER, J. A. and BOERNER, F.: *Approved Laboratory Technic.* New York: D. Appleton-Century Co., 3rd Ed., 1941.

LEVINE, M. and SCHOENLEIN, H. W.: *A Compilation of Culture Media.* Baltimore: Williams and Wilkins Co., 1930.

Manual of Methods for the Pure Culture Study of Bacteria. Geneva, N. Y.: Society of American Bacteriologists, Biotech Publications.

MUDGE, C. S. and SMITH, F. R.: *A Fundamental Approach to Bacteriology.* San Francisco: J. W. Stacey, Inc., 1939.

WADSWORTH, A. B.: *Standard Methods.* Baltimore: Williams and Wilkins Co., 2nd Ed., 1939.

REVIEW QUESTIONS—CHAPTER XI

1. Explain the peculiar features of microbiological technique. What is meant by the statement that it is essentially an aseptic technique?
2. Name some of the special glassware used by the bacteriologist. State precisely how this glassware may be sterilized.
3. Give the ingredients, and outline the method of preparation, of plain nutrient broth and agar. What is the difference between extract broth and infusion broth?
4. What is an agar *slant,* a *butt-slant,* an agar *deep,* an agar *plate.*
5. Compare *plain media* with *special and enriched media,* and give examples.
6. What is meant by *differential media, selective media*? Give examples. Define and explain the importance and value of *synthetic media, dehydrated media.*
7. Describe the proper way to handle test-tube cultures. Explain how broth, slant, butt-slant, stab, and shake cultures are inoculated.
8. Describe the proper technique for pouring a plate. Describe the procedure for making *pour plates, streak plates, streak-pour plates.*
9. Name at least six kinds of culture media that may support the growth of anaerobic bacteria without use of mechanical methods to exclude

the air. What steps should be taken routinely before inoculating such media with anaerobes?

10. Give one simple method of excluding the air from broth or agar-deep cultures in test tubes.
11. Outline a practical procedure for making single *slant* cultures anaerobic with pyrogallic acid and alkali.
12. Describe two anaerobic methods for plate cultures, and two types of anaerobic jars.
13. Describe some simple methods to promote the growth of microaerophilic bacteria.
14. Outline a practical method for supplying increased CO_2 tension to single slant cultures, and to tube or plate cultures in a CO_2 jar.
15. Outline the essential steps in making a count of the number of bacteria in a sample of water.

CHAPTER XII

ISOLATION AND IDENTIFICATION OF BACTERIA

Mixed and pure cultures. In nature, almost all kinds of bacteria or fungi, including the disease-producing species, live naturally in more or less intimate association with other kinds. Therefore, the primary culture from almost any source will be a *mixed culture* containing organisms of several different kinds. But in the laboratory the various species may be separated from one another and cultivated by themselves. A culture which contains just one species is called a *pure culture.* An organism must be studied in pure culture before the properties peculiar to itself may be learned.

The process of obtaining a pure culture by separating one kind of bacterium or fungus from a mixture with other kinds is spoken of as *isolation* of the organism.

Principle of isolation methods. The earliest workers to study bacteria in the laboratory used only liquid culture media. The task of securing pure cultures using liquids only was naturally very difficult, and only men of the highest mental attainment and possessed of the patience of genius, like Pasteur and Lister, could make much headway in the study of microorganisms. We have already explained how Robert Koch, in 1881, by the use of solid culture media and the plate method, simplified the entire procedure of securing pure cultures. The methods for isolating bacteria used today are essentially the same as those of Koch. These methods depend upon: (1) *dilution of the original material in solid media cultures in order to secure separated, pure colonies,* and (2) *transfer of a pure colony to a fresh sterile medium,* without contamination from the neighboring growth. The process of picking out a single colony for a smear or transfer is called *fishing.*

Dilution methods with agar media. In planting a vegetable garden it is necessary to scatter the seed over the ground so that the plants which later develop will not be too close together. In a

similar way, the bacteria which are to be planted upon culture media must be thinned out so that separate, typical, pure colonies will appear. Any method of diluting the original material will serve, so long as extraneous bacteria are not introduced.

Dilution methods for pour plates. One commonly used method of securing pure colonies is to make a series of pour plates, inoculating each plate in the series with decreasing amounts of the original material.

Dilutions may be made in a series of tubes of any sterile liquid medium, such as broth, or water, or physiological salt solution. The first tube of sterile broth, for example, may be inoculated with two loopfuls of the original material. After shaking this tube well to distribute the bacteria, two loopfuls from this tube may be transferred (with the usual precautions against contamination) to a second tube. This process may then be repeated until a series of four, five, or more tubes have been inoculated. Then 1 cc, or a loopful, of the contents of each tube may be added to separate tubes of melted agar and the mixtures poured into sterile Petri dishes.

By another common method, the dilutions are made directly in the liquid agar (Fig. 43). A series of tubes of agar is heated until the agar melts, then cooled to about 45° C. A needle bearing a loopful of the material from which the bacteria are to be isolated is shaken vigorously through the melted agar in one tube, then passed, without being sterilized, to a second melted-agar tube, then to a third, and so on until four or five or more tubes have been inoculated. Then separate plates are quickly poured with the contents of each tube before the agar solidifies (p. 164).

Dilution by means of streak plates. Streak plates, instead of pour plates, may be used for isolation. It is merely necessary to spread out the original material over the *surface* of agar plates to such a degree that separate pure colonies will develop.

A very minute amount of the material (which may or may not have been previously diluted with broth or other sterile liquid) may be placed on the surface of one plate. With a looped or a straight needle, or a sterile, bent, glass rod, this material may be streaked over the entire surface of the plate; then, without sterilizing the needle or rod, a second plate, and a third, or more, may be streaked in the same way. Some of these plates should develop well separated pure colonies.

The making of good streak plates is not as easy as it may appear, and much patience and skill are required. In order to avoid contamination, the cover of the Petri dish must not be lifted more than is necessary. The

agar must be thoroughly hardened before inoculating, and great care must be taken not to break the surface of the medium in streaking. The needle should be held so lightly that practically the only pressure upon the agar surface comes from the weight of the needle itself. *The streaking must be done patiently according to some definite plan.* As much as possible of the surface of the medium should be utilized. Some of the common methods of streaking are illustrated in Fig. 44, p. 166.

Fishing. The process of picking up bacteria from a single colony with a sterile needle, without touching neighboring colonies, is often a delicate task requiring care and skill. This is particularly true when pathogenic organisms are being isolated, because their colonies are usually small.

A straight needle is always used for fishing. Often it is an advantage to have the end of the needle bent at a right angle and filed to a fine point. Colonies to be fished from plate cultures may be marked by drawing a ring about them, with a wax pencil, on the bottom of the dish. The colonies may be given numbers, and smears or cultures made from them may be correspondingly numbered. In fishing, as in any other procedure, the culture must be protected from contamination, and the complete removal of the cover of a Petri dish is avoided whenever possible. Fishing from plate cultures is much easier if performed with the aid of a low-power dissecting microscope.

STUDY AND IDENTIFICATION OF PURE CULTURES

Once a pure culture is obtained, a long study is usually required before the organism can be fully identified and named. It is necessary to determine: (1) its morphological properties and staining reactions, (2) its cultural characteristics, and (3) its physiological requirements and biochemical activities. It is often necessary also (4) to test its possible pathogenicity (disease-producing power), and (5) to perform serological tests to see whether the organism will agglutinate, or otherwise react specifically, with an immune serum known to contain antibodies for the species to which the organism appears to belong. This latter procedure will identify the unknown organism positively in most cases.

(1) Morphological study; staining reactions. Microscopic studies and staining will reveal the form (coccus, bacillus, or spirillum), and the characteristic arrangement and grouping of the cells, their size, and the presence or absence of intracellular fat, spores,

capsules, granules, or other peculiarities of structure. Examination of hanging-drop preparations will show whether the organisms are motile or not. The reaction to the Gram stain is of great help in determining the class to which the unknown bacterium belongs. In case the organism is acid-fast, this will be revealed by the acid-fast staining method.

These studies supply a great deal of information about an organism, and may give a strong hint as to its identity, but *a species of bacteria can never be identified with certainty on the basis of morphology and staining alone.* Nearly every type of disease germ is duplicated, so far as morphology and staining are concerned, by other species which are harmless.

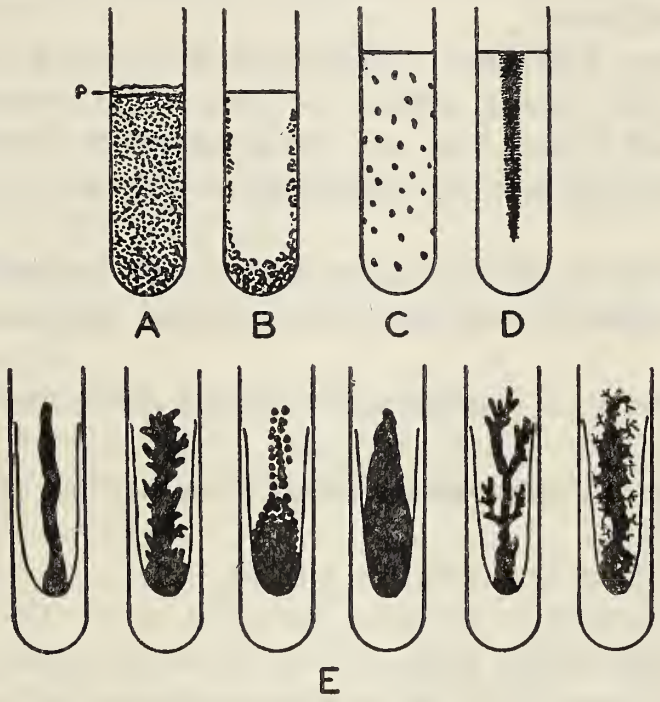


FIG. 51. Types of bacterial growth. A, B: appearances shown by cultures of bacteria in broth. Most species produce a uniform cloudiness throughout the broth, as in A; others grow only as flocculent masses along the sides and at the bottom of the tube, as in B. Some organisms form a membrane-like film over the top of the broth. This is called a pellicle, (P). C: an agar shake culture, showing colonies distributed throughout the medium. D: an agar stab culture. E: forms of growth shown by different species of bacteria on the surface of agar slants.

(2) **Cultural characteristics.** Further information about an organism is obtained by observing the way it grows in various kinds of culture media. Certain species have a characteristic manner of growth in broth or milk, in agar, or other media. Figures 51 and 52 illustrate some of the forms of growth commonly seen.

In noting cultural characteristics, certain descriptive terms

suggested by committees of the Society of American Bacteriologists, and incorporated in their Descriptive Chart for the pure culture study of bacteria * are widely used. Most of these will be mentioned here, and for those terms whose meaning is not obvious a brief definition will be given. The student is free, of course, to employ other terms and any familiar adjectives which seem appropriate.

Growth in broth. The following points are usually noted:

Surface growth—ring, pellicle (film over the fluid), flocculent, none.

Clouding—slight, moderate, strong, transient, persistent, none; granular growth.

Odor—absent, decided resembling ————————————.

Sediment—absent, scanty, abundant, compact, flocculent, granular, flaky, viscid on agitation.

Growth on slants. If a slant is inoculated by drawing the needle from bottom to top with a single stroke, the growth which develops may take any one of several forms which may be characteristic of the species. The principal forms of growth are illustrated in Fig. 51:E; these are, from left to right:

Filiform—uniform growth along the line of inoculation.

Echinulate—growth along line of inoculation, with toothed or pointed margins.

Beaded—discrete or semiconfluent colonies along the line of inoculation.

Spreading—growth extending considerably beyond the line of inoculation.

Arborescent—branched, tree-like growth.

Rhizoid—growth of an irregular branched or root-like character.

Other characters of the slant growth to be noted include the *luster* (whether glistening or dull), the *surface characteristics* (whether smooth, rough, or wrinkled), the *consistency* when touched by the needle (whether butyrous, viscid, like a leathery membrane, or brittle), and *pigment formation* with color in the growth itself and perhaps changes in the color of the medium.

Agar colonies. Single colonies developing on agar plates are most conveniently used for the study of colony characteristics. The colonies may be observed with a hand lens, or they may be studied under a dissection microscope or under the low-power objective of the ordinary microscope. A fundamental point to be noted is whether the colonies appear to be Smooth (S), Rough (R), or Mucoid (M), and whether some are in one and some in another one of these growth phases.

---

* Charts may be ordered from the Biotech Publications, Geneva, N. Y.

In description of the colonies, the following points are usually considered: *form, size, color, optical characteristics, surface, elevation, edge, internal structure,* and *consistency* when touched with a needle. Thus, a colony of staphylococcus might be described as follows: a circular, orange-colored, opaque mass, about 2 mm in diameter, with a smooth, glistening surface and regular edge, slightly convex, showing a homogeneous granular internal structure, and having a soft consistency.

The following are some of the more precise terms which have been suggested to describe colony characteristics. Several of these are illustrated in Fig. 52.

Form (Fig. 52: A).

Punctiform (pinhead colonies barely visible to the naked eye, under 1 mm in diameter), *circular* (round colonies over 1 mm in diameter), *filamentous, irregular, rhizoid* (root-like).

Elevation (Fig. 52: B, left to right).

Flat, raised, convex, pulvinate (like a cushion), *umbonate* (with a raised button-like center).

Surface (Fig. 52: C, left to right).

Smooth, rough, concentrically ringed, radiately ridged.

Edge (Fig. 52: D, left to right).

Entire (with even, regular margin), *undulate* (wavy border), *lobate* (having margins deeply lobate, producing lobes), *erose* (border irregularly toothed), *curled.*

Internal structure (Fig. 52: E, left to right).

Amorphous (homogeneous), *coarsely granular, finely granular* with coarser center, *concentric, curled* (with a parallel series of wavy threads).

Optical characteristics

Opaque, translucent, transparent, iridescent (exhibiting changing rainbow colors).

Growth in blood agar. The colonies in blood agar media would be described as above, but, in addition, the effect of the growth on the blood may be noted.

Hemolysis (definite clear zones about colonies)—slight, marked, none. (Called beta type hemolysis in reference to streptococci.)

Greenish-brown coloration—slight, marked, none. (Called alpha type hemolysis in reference to streptococci.)

Growth in stab cultures. In soft agar or gelatin medium a stab growth may be described with attention to the following points:

Position of best growth—at top, at bottom, in a zone —— mm below the surface, uniform throughout.

Form of growth along line of puncture—filiform, beaded, aborescent, irregularly branched.

Surface growth—present, absent.

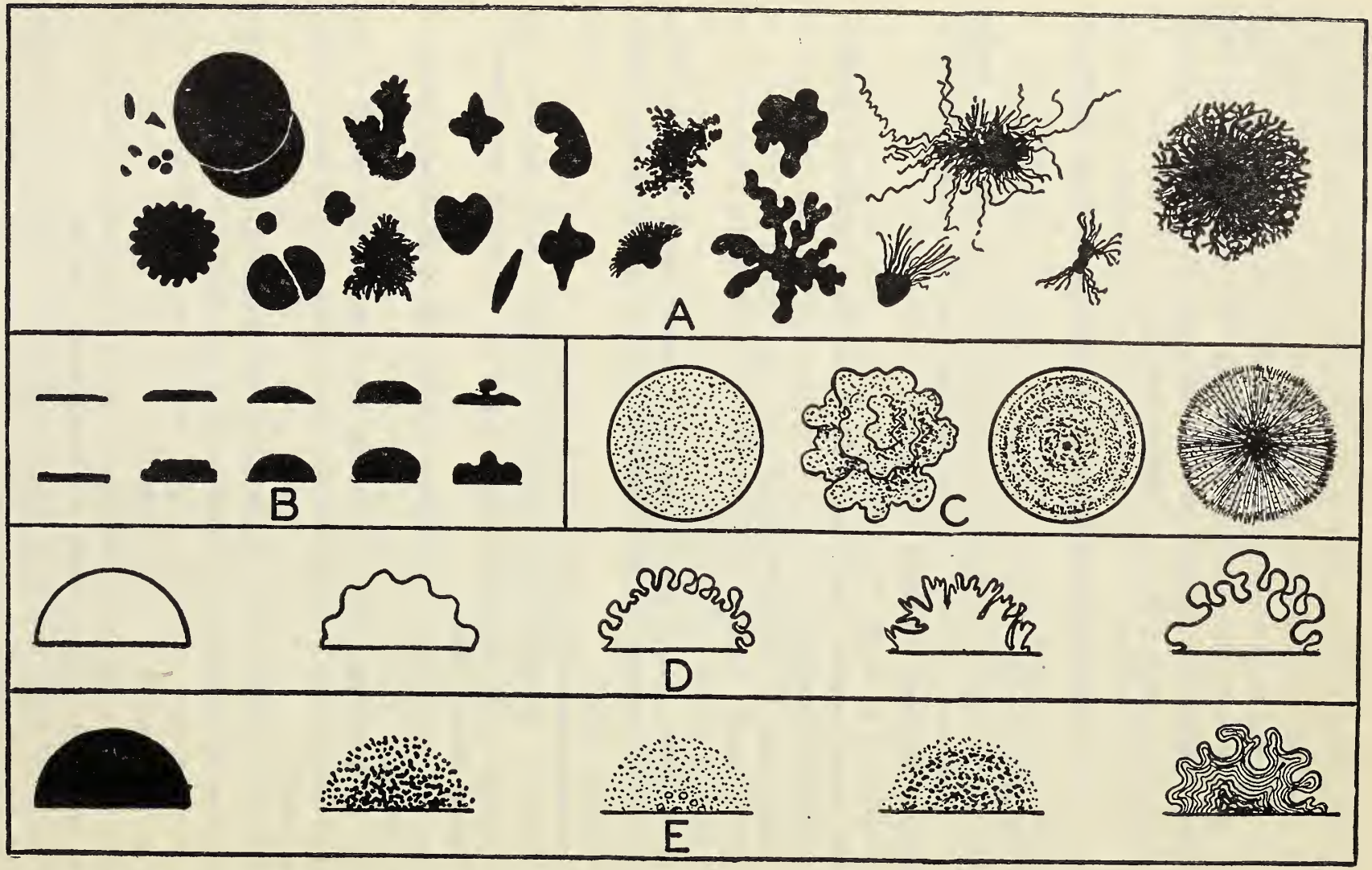


FIG. 52. Colonies of bacteria in agar cultures. A: various forms of colonies; B: cross sections; C: surface appearances; D: character of edges; E: internal structure.

Color, optical qualities—as for slants.

Medium—colored, digested, unchanged.

Liquefaction (in gelatin stabs)—*crateriform* (saucer-shaped), *napiform* (liquefied area in shape of a turnip), *infundibuliform* (liquefied area in shape of a funnel or inverted cone), *saccate* (liquefaction in an elongated, sac-like area), *stratiform* (liquefying to the walls of the tube at the top of the stab and then proceeding slowly straight downward).

Milk cultures. Bacteria may be roughly classified into several groups according to the type of changes they cause in milk medium. Tanner recognizes the following groups:

Inert bacteria bring about no visible change in the medium.

Acid-forming, non-coagulating bacteria ferment the lactose, or the very small amount (about 0.1) of dextrose in the milk, causing any indicator present to change toward the acid side, but they do not produce enough acid to coagulate the milk. If only the dextrose is fermented (as, for example, by the typhoid bacillus), there is produced only a transient acidity, the medium returning to a neutral or alkaline reaction within a few hours after the acidity first appears. In any case, the degree of acidity (pH) in the culture at any stage of growth may be determined by testing drops of the medium with different indicators.

Acid-coagulating bacteria form sufficient acid to clot the milk, the casein precipitating as an insoluble curd. Gas also may be formed, as evidenced by bubbles in the curd. An extreme form of this is the "stormy fermentation" brought about by *Clostridium perfringens*.

Peptonizing bacteria cause the proteolysis, i.e., digestion, of the proteins of the milk. Usually, but not always, clotting of the milk occurs first, then digestion of the curd occurs. The medium becomes darker and clearer. This process may go on until the medium consists only of a clear, yellowish fluid with only a slight undigested residue.

Rennin-curd-producing bacteria are those that cause coagulation of the milk while the medium is neutral or slightly alkaline. They possess special enzymes. This type of curd may be the first step in peptonization.

Alkali-producing bacteria form considerable amounts of alkaline substances, with the result that indicators are changed to their alkaline colors.

Some organisms cause *reduction* of litmus or other indicators in milk, that is, loss of the color of the indicator as the result of its reduction to a colorless base by bacterial enzymes. This usually occurs first in the bottom portion of the tubes. It must not be confused with other changes in the indicator color.

(3) Physiological requirements and biochemical activities. The most significant properties of an organism are learned through

a study of its physiology and its biochemical reactions. The points usually investigated are mentioned in the following paragraphs:

(1) *Food requirements.* Does the organism grow upon the simpler agar and broth media, or does it require media enriched with blood or other body fluids?

(2) *Temperature relations.* Does it grow best at body temperature? If not, what is its optimum growth temperature? Minimum and maximum growth temperature? Thermal death time?

(3) *Relation to free oxygen and* CO_2. Does it grow best under aerobic, or under anaerobic conditions, or under partially reduced oxygen tension? Is its growth improved by cultivation in an atmosphere containing an increased proportion of carbon dioxide?

(4) *Relation to the reaction (pH) of the medium.* What is the optimum pH for growth? Growth occurs within what limits of pH?

(5) *Pigment production.* Does it develop a colored growth after prolonged incubation on agar slants, Loeffler's serum medium, potato, or other media?

(6) *Proteolytic action.* Does it decompose gelatin? Cause digestion of coagulated blood serum, meat, and similar substances?

(7) *Fermentation of carbohydrates.* Does the organism produce acid, or acid and gas, from glucose or other carbohydrates?

(8) *Indol production.* Does it form indol?

(9) *Production of hydrogen sulphide.* Is H_2S formed?

(10) *Reduction of nitrates.* Are nitrates converted to nitrites?

(11) *Reduction of litmus and other indicators.* Is litmus decolorized in litmus milk cultures? Are other indicators similarly reduced to a colorless compound?

(12) *Hydrolysis of starch.* Is starch broken down to glucose?

(13) *Production of acetyl-methyl-carbinol (the Voges-Proskauer Test).* Positive or negative?

(14) *Final pH in glucose broth (the Methyl-Red Reaction).* Positive or negative?

(15) *Special Tests.* A variety of other tests are used in connection with the study of certain groups of bacteria. Several of these are described in the appropriate places in later chapters.

When all the facts derived from these tests are assembled, it often becomes possible to recognize the species, or at least the group of closely related species, to which the unknown organism belongs.

(4) **Pathogenicity.** Will the organism produce disease in laboratory animals? The animals most commonly used in laboratories are guinea pigs, rabbits, and white mice. For certain special work,

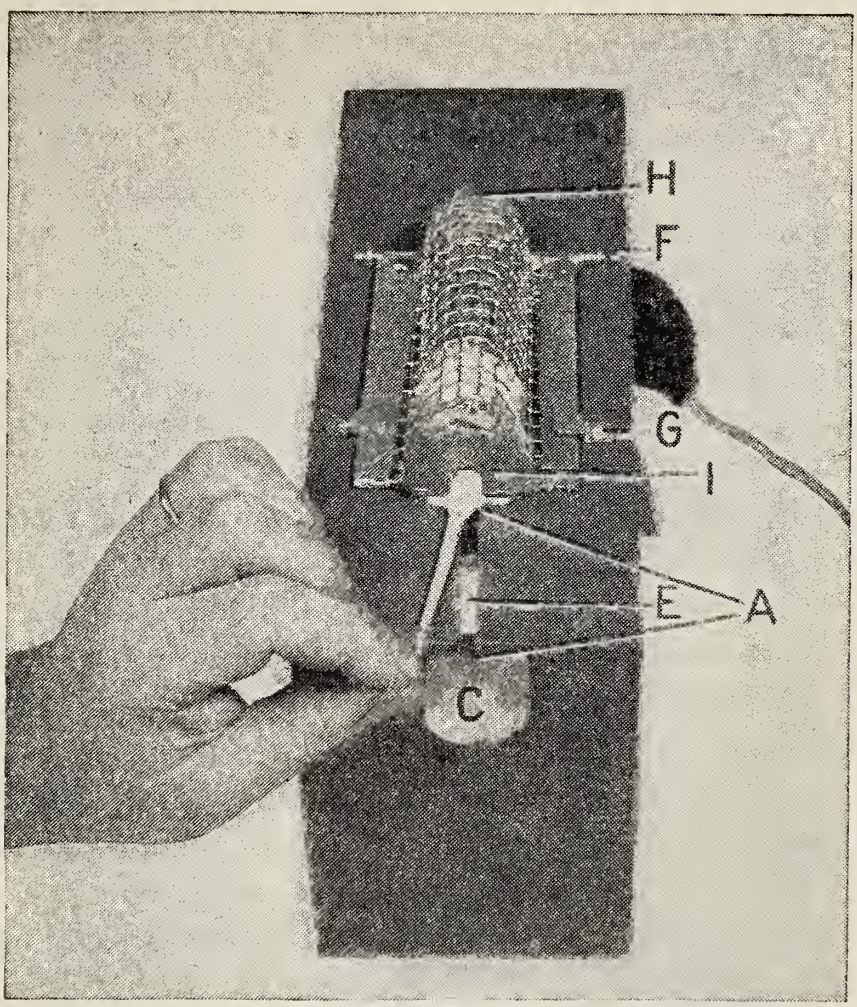


FIG. 53. A holder facilitating the intravenous inoculation of mice. The animal is held in a wire cage of roughly semicylindrical shape, which is raised to admit the mouse, then lowered over the animal and secured by the pivoting buttons (G). One hand holds the protruding tail. The mouse is prevented from moving forward by placing in the open end of the cage a soft, cotton-stuffed pillow (H). In making intravenous injections, the tail is held near its tip by the fingers of the left hand, and directly over the slot (E) which is illuminated by light from below. The tail veins are clearly seen through the almost transparent tissues, and material may be injected into one of them from a syringe held in the right hand. A 26- or 27-gauge needle must be used. (From Burdon, K. L., *J. Lab. and Clin. Med.*, 23:1293, Sept. 1938.)

monkeys, dogs, cats, or other animals may be needed. Pathogenicity tests are usually made by injecting the organisms under investigation into these animals *subcutaneously* (just under the skin), *intraperitoneally* (into the peritoneal cavity), or *intravenously* (into a vein) (Fig. 53). Other possible routes of inoculation are *intradermal* (into the skin just under the epidermis), *intramuscular* (into the muscles, as of the thigh), *intracerebral* (directly into the brain through the skull), and *intranasal* (dropping the material into the nostrils) (Fig. 54).

Such animal inoculations are often of great value in identifying a particular organism. Also they help in securing pure cultures of disease germs. If a material contains acid-fast bacilli, for example, and there is doubt as to whether they are tubercle bacilli or not, the matter can be settled by inoculating a guinea pig. If the material injected contains tubercle bacilli, the guinea pigs will develop tuberculosis in a few weeks. It is difficult to cultivate the tubercle germs upon culture media directly from sputum or other matter containing them, but if this material be inoculated into a guinea pig, the germs can easily be obtained in pure culture from the lesions in the animal.

(5) Identification by agglutination reactions and other serological tests. One final method of identifying a species of bacteria is by use of serological tests. These tests depend upon the fact that when a given bacterium is injected into a suitable animal it acts as an *antigen,* stimulating the formation of specific *antibodies,* which become abundant in the blood serum of the injected (vaccinated) animal. The serum of this animal then, by virtue of its contained antibodies, will react specifically with the particular bacterium injected, and not, as a rule, with any other organism.

One of the simplest of the several kinds of reactions that may be demonstrated when bacteria and a specific antibody-containing serum are mixed is the *agglutination reaction,* the end result of which is that the organisms are clumped together and fall out of suspension in visible aggregates. With an unknown bacterium to be identified, and sera of known antibody content at hand, it is only necessary to mix the organisms and each serum in succession and discover in which mixture agglutination occurs. There may be certain "group reactions," or cross-reactions with closely related species (which usually disappear, however, in high dilutions of the serum), and it must be remembered, too, that complications in all serological tests may arise because of the differences in antigenic structure of types or variants within a species, but when all these matters are properly controlled, identification can be made with confidence by these tests. If, for example, we have isolated a Gram-negative bacillus which has the characteristics of the typhoid germ, a final test of its identity may be made by mixing a suspension of the organisms with a blood serum known to contain antibodies for that species. If agglutination occurs under properly controlled conditions, the organism must be a typhoid bacillus.

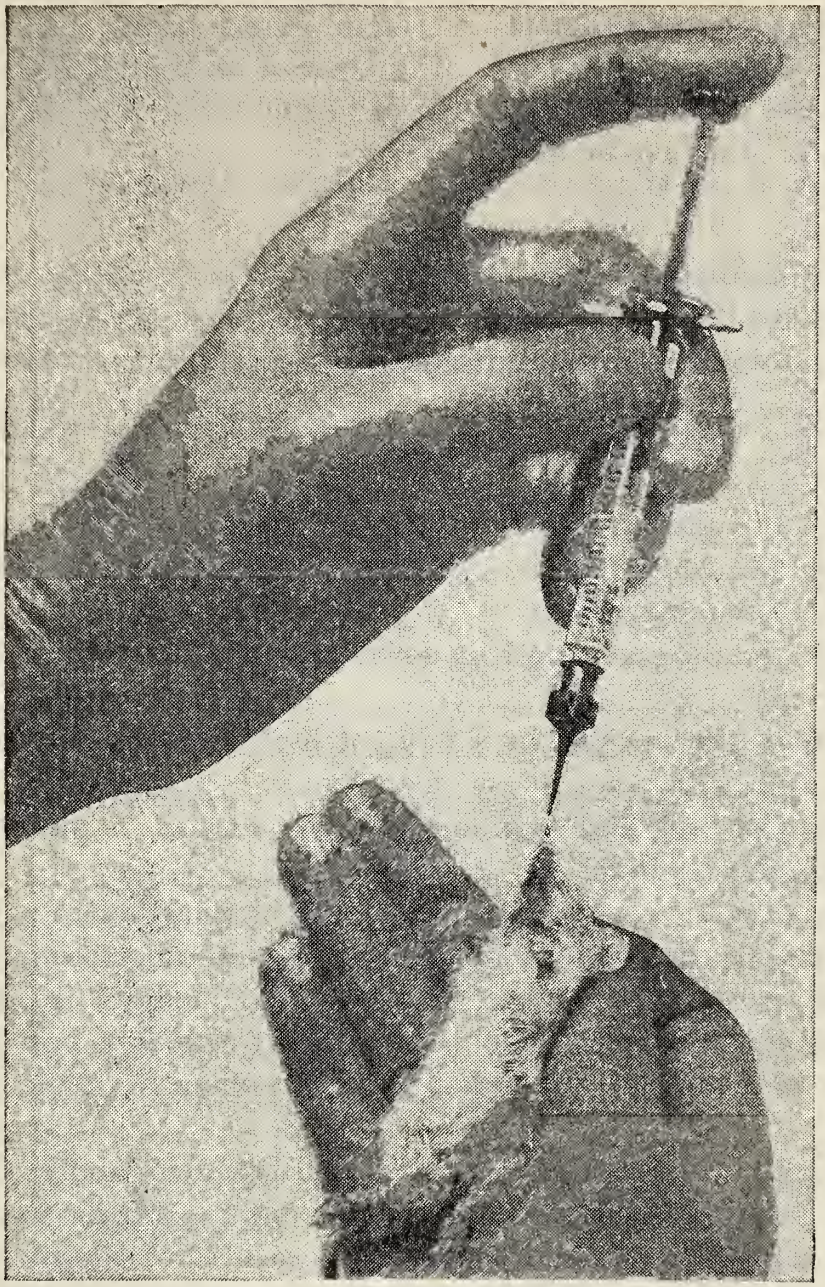


FIG. 54. The intranasal inoculation of a white mouse. This technique is used in studying influenza virus. (Photographed at the Texas State Board of Health Laboratory, Austin, Texas. Reproduced through the courtesy of Dr. George W. Cox, State Health Officer, and of Dr. S. W. Bohls, Director of Laboratories.)

The technique of agglutination, and other serological tests, is further explained in Chapter XXII.

REAGENTS AND TECHNIQUE USED IN SOME COMMON BIOCHEMICAL TESTS

Tests for indol formation. In order to test for indol formation, an organism must be grown from 48 to 72 hours in a medium made with a peptone

containing the amino acid tryptophane, since indol is a product of the decomposition of tryptophane. A casein digest broth, called tryptone broth, is especially favorable. One of the best of several methods to demonstrate the indol is Gore's modification of Ehrlich's test. The following solutions and procedure are used:

Solution I:

Para-dimethylamidobenzaldehyde	1 gm
Ethyl alcohol (95%)	95 cc
Hydrochloric acid (concentrated)	20 cc

Solution II: Saturated aqueous solution of potassium persulfate ($K_2S_2O_8$)

Procedure:

1. Remove the cotton plug from the culture tube.
2. Moisten the under surface with 4–6 drops of Solution II, then an equal amount of Solution I.
3. Replace the plug and push it down to within an inch of the surface of the medium.
4. Place the tube upright in a bath of boiling water and heat for 15 minutes.
5. Remove the plug and examine its under surface. A pick (rose) color on the plug indicates indol.

Production of hydrogen sulphide. One of the best methods for testing the capacity of bacteria to produce H_2S is to make stab inoculations into a soft agar medium devised by Treece. Another good medium is Kliger's iron agar. Development of black color about the growth is a positive sign of production of hydrogen sulphide. Treece medium is made up as follows:

Prepare separately a 2% solution of ferrous sulphate and a 2% solution of sodium thiosulphate. Sterilize these solutions by autoclaving. Add 1 cc of each of them (with aseptic precautions) to each 90 cc of a melted, sterile, semisolid, nutrient agar (pH 7.4), and tube the medium aseptically.

Nitrate reduction test. Cultures are made in nitrate broth, i.e., in broth or peptone solution to which has been added about 0.1% of nitrite-free potassium *nitrate* (KNO_3). After incubation for two or three days, or at later intervals, the culture is tested for the presence of *nitrites* (KNO_2). To the whole broth, or to a portion removed aseptically from the bottom of the culture tube, a few drops of the following test reagents are added, in the order named:

Solution I. Sulphanilic acid:

Water	250.0 cc
Sulphanilic acid	2.8 gm
Glacial acetic acid	100.0 cc

Dissolve the sulphanilic acid in the water, and add the acetic acid.

Solution II. Alpha-naphthylamine:

Water	250.0 cc
Alpha-naphthylamine	1.7 gm
Glacial acetic acid	100.0 cc

Dissolve the alpha-naphylamine in the water by heating, then add the acetic acid.

A rose-red color indicates the presence of nitrites. This positive test should be controlled by a check on a tube of uninoculated medium. In case the test for nitrite is negative (no red color), it may be assumed that the nitrate was not attacked by the organism. However, a negative test may possibly be due to the decomposition of the newly formed nitrite itself (to free nitrogen or ammonia) by the further action of the organism. This may be checked by testing the culture to see if there is any nitrate still present in it. This is done by adding a little powdered zinc, to reduce any residual nitrate to nitrite. Repeat, now, the test for nitrite. If positive, this indicates that the original nitrate in the medium is still there, and confirms the inability of the organism to reduce it. Of course, if this test is negative, as well as the original test for nitrite, the organism must have utilized and decomposed *both* the original nitrate and the nitrate formed from it.

Hydrolysis of starch. Cultures in broth containing 0.2% of soluble starch are used, and, at intervals during incubation for about ten days tests are made for evidence of breakdown of the starch. To test, remove about 0.2 cc of the culture, aseptically, to a depression in a porcelain test plate, and add a few drops of dilute iodine solution (Gram's iodine diluted 1:3 with distilled water). Note the color that appears *immediately* after adding the iodine. Blue means no hydrolysis (a negative test); reddish-brown means hydrolysis is occurring, but is only partial (a weakly positive test); whereas no color means that hydrolysis is complete (a strongly positive test). The presence of reducing sugar in those cultures showing complete hydrolysis may be established by testing them with Fehling's or Benedict's solution.

Voges-Proskauer (V.P.) Test. This is a test for the presence in a culture of *acetyl-methyl-carbinol,* a product formed by some organisms from glucose. The V.P. test is usually conducted on cultures in a 1% glucose, phosphate broth after incubation for two or three days. It is only necessary to add about 2 cc of a strong (10%) KOH solution. The acetyl-methyl-carbinol is thereupon slowly oxidized to a pink compound, and after the culture tube has stood for a variable time (but usually within 30 minutes) the pink color appears, indicating a positive test. Failure to develop this color means a negative test.

Methyl-red (M.R.) reaction. Methyl-red is an indicator; a solution is prepared by dissolving 0.1 gm in 300 cc of ethyl alcohol and then diluting with 200 cc of water. The M.R. test consists merely of adding a few drops

of this indicator to a portion of a glucose broth or peptone-water culture after incubation for about four days. (Commonly, the same culture used for the V.P. test above is employed.) A distinct red color is recorded as *methyl-red positive;* this means that the organisms have produced sufficient acid to lower the pH to at least pH 4.5. On the other hand, a definite yellow color is read *methyl-red negative;* this means that the final hydrogen-ion concentration is only about pH 5.4. *Escherichia coli* cultures are usually methyl red +, while those of *Aerobacter aerogenes* are usually methyl red —.

REFERENCES

See References for Chapter XI.

REVIEW QUESTIONS—CHAPTER XII

1. What is meant by *mixed culture, pure culture, isolation* of an organism, *fishing* a colony?
2. Explain the principle of isolation methods. Who invented plate cultures?
3. Describe dilution methods that may be used with: (1) pour plates and (2) streak plates. What is the technique of fishing?
4. Name five phases of study usually necessary before an unknown pure culture can be fully identified and named.
5. Discuss the value and limitations of a study of the morphology and staining reactions.
6. Describe some of the characteristic forms of growth that may be seen in cultures in broth, on slants, on blood agar plates, in stab cultures, in milk cultures. What features are usually considered in describing colonies of bacteria?
7. At least fourteen specific physiological or biochemical properties are usually investigated in attempting to identify an unknown pure culture. How many can you recall?
8. What animals and what routes of inoculation are commonly used in testing the pathogenicity of a culture? How may such animal experiments help in identifying or isolating an organism?
9. What are the reagents used in an agglutination test? What happens in a positive test? What is the practical value of such tests?
10. What is the origin of indol in bacterial cultures? Outline one procedure for testing for indol.
11. How may the power of a bacterium to form H_2S be tested?
12. Outline one procedure for determining capacity to reduce nitrates to nitrites.
13. Outline a test for hydrolysis of starch.
14. What is the Voges-Proskauer test? How may it be performed?
15. Describe the methyl-red reaction and its significance.

CHAPTER XIII

GROWTH CHARACTERISTICS OF BACTERIA IN LABORATORY CULTURES BACTERIAL VARIATION

In the laboratory, the majority of common bacteria grow rapidly and luxuriantly on a great variety of culture media, and the student will soon learn to recognize different types by their appearance and behavior in these cultures. The forms of growth which may be seen in liquid and solid media, and the terms used in describing cultural characteristics have been briefly considered in Chapter XII and illustrated in Figs. 51 and 52. Here it will be helpful to mention certain fundamental aspects of bacterial growth, knowledge of which adds greatly to the interest and the understanding with which any culture may be studied.

GROWTH CHARACTERISTICS OF BACTERIA

Colony forms. The character of bacterial colonies is influenced to some extent by the nature of the medium and by other environmental factors, but is related primarily to fundamental properties of the organisms themselves, especially to their vigor of growth, their manner of cell division, and the nature of post-fission movements. In consequence, any one variety of bacterium tends to form constantly the same general type of colony, and identification of species may be based *in part* upon colony characteristics.

The student will become acquainted early in the Microbiology course with the relatively large, heavy, and often irregularly shaped colonies of the saprophytic sporebearing bacilli of the dust and soil, and with the dense, frequently pigmented colonies of cocci from the same sources, so that when these colonies appear unbidden in Petri-dish cultures that have been opened to the air, they will be recognized at once as *contaminants*. He cannot fail to note the marked contrast between the large, coarse colonies of these saprophytes and the diminutive, delicate colonies

formed, even upon enriched media, by most parasitic bacteria, and especially by pathogenic species.

He will observe also that colonies of actively *motile* organisms tend to have irregularly spreading edges, and there may be pencils of growth extending out of the main mass of the colony where little rivulets of moisture allowed the bacteria to migrate. Sometimes motile organisms will make a continuous film of growth over the whole surface of a moist medium, covering all other kinds of colonies that may be present. Such a "spreader" is the proteus bacillus (*Proteus vulgaris*), one of the common intestinal bacteria. Nonmotile variants of the same species (lacking flagella) form raised, discrete colonies. Bacteria with large capsules usually make sticky, mucoidal colonies.

The more common types of colonies, with a smooth appearance, regular outline and soft consistency, but not mucoidal, usually contain organisms, without large capsules, that separate readily after division, slipping past each other to form compact, evenly contoured masses. On the other hand, the rougher, flatter, more granular colonies, the root-like or tree-like forms, and the ridged and convoluted colonies with sharp and irregular angles are formed by bacterial cells that adhere more or less firmly to each other after division, forming sheets of closely packed organisms, or long chains, packets of bacilli in parallel rows, or tangled masses of rods. The student will find himself well repaid if he will observe colonies closely, and compare their appearance and structure with the morphological features of the organisms present in them.

Types of colonies formed by the same species. Apart from differences in colony structure, due to species differences, the *bacteria of a single species* may form several distinct types of colonies, as follows: (1) so-called *Smooth* (S) *colonies* of regular outline and form; (2) *Rough* (R) *colonies,* of more granular and irregular character; (3) a *Mucoid* (M) *type,* of a soft and often sticky nature; (4) tiny colonies, called *Dwarf* (D) *colonies;* or (5) colonies of *mixed type,* such as SR (predominantly smooth), RS (predominantly rough), SM, etc. (Fig. 56). These differences in colony form are important, for they are the outward expression of significant morphological and physiological differences in the particular organisms concerned. They are evidence of the *variation* within a species which we discuss later in this chapter.

Growth and death of a bacterial population. Whether growing in a liquid or a solid medium, any bacterial culture must be thought of as a *population,* made up of millions of individual organisms. It is instructive to analyze the development and decline of such a

population in a single culture tube. If a given bacterium is inoculated into a tube of broth and the medium is then incubated, the growth of the organism will follow a definite course (Fig. 55).

Growth curve. It is customary to divide the growth curve into several parts, but these may be combined into four chief phases:

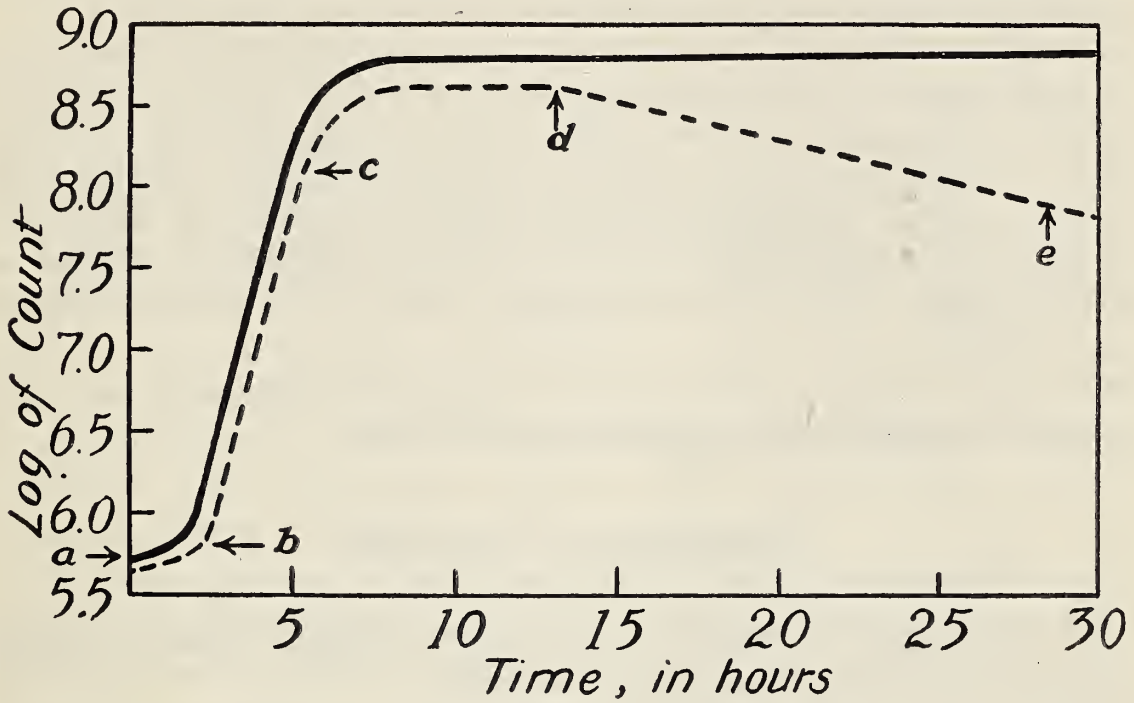


FIG. 55. The growth curve of a bacterial culture. Solid line: total number of bacteria, alive or dead; dotted line: number of living or viable bacteria. (Based on a figure in Topley & Wilson's *Principles of Bacteriology and Immunity*, William Wood & Company, Baltimore, 2nd Ed., 1936.)

(1) the *lag phase* (a to b), (2) the *logarithmic phase* (b to c), (3) the *stationary phase* (c to d), and (4) the *phase of decline* (d to e).

The *lag period* usually lasts not more than a few hours. During the first part of this period there is no apparent increase, and in fact there may be a reduction in the number of living organisms, for some of the bacteria in the inoculum die, while others are as yet unprepared for active multiplication. The lag period ends when the organisms finally begin to divide rapidly.

During the *logarithmic phase* the organisms are multiplying regularly and at a maximum rate. The bacteria increase by geometrical progression (2 divide to make 4, 4 to make 8, 8 to make 16, etc.), and if the logarithms are plotted in relation to time we get a straight, steep-rising line as in Fig. 55: b to c. We have previously mentioned that in the case of a number of bacteria, for example the colon bacilli and the cholera spirilla, the generation time, i.e., the time between the formation of a new

bacterium and its division to form two new cells may be as short as 20 minutes. Organisms such as the staphylococci and streptococci are said to multiply almost as rapidly, while other species, such as diphtheria bacilli, increase at about half of this rate. A few bacteria always multiply, even during this logarithmic phase, at a markedly slow rate; the generation time of the tubercle bacilli, for example, is said to be about 5 hours. Whatever the species of organism, the logarithmic period of growth is brief.

The reduction in available food substances, accumulation of waste products, and possibly a lack of sufficient oxygen, and other harmful influences not well understood, force the culture into the *stationary phase* during which the organisms are less active, divide less frequently, and the total number of living organisms remains practically constant, the death rate balancing the multiplication rate. The bacterial population reaches its greatest density, in the case of most bacteria, within 24 to 48 hours' incubation at 37° C.

The *phase of decline* then sets in and gradually the organisms cease entirely to multiply, and eventually they all die off.

VARIATION IN BACTERIA

No description of bacteria would be complete without special mention of the phenomenon of *variation.*

Among higher forms of life *nothing is more invariable than variation.* No two plants or animals of the same species are *exactly* alike; individuals varying widely in some particulars from the "normal" members of the species are common, and some of these variant types breed true, for a time at least, so that the variations are perpetuated through several generations. So it is also among bacterial species.

Bacteriologists were slow to recognize the existence of *variation in bacteria.* They failed to realize that a tendency to vary would cause differences in the appearance and behavior of different cultures of the same species. For many years, under the influence of the early teachings of Koch, the attributes of a particular kind of bacterium were thought to be constant under all circumstances, and the textbooks described for each species what was supposed to be its fixed and characteristic cell form, colony structure, cultural properties, biochemical activities and pathogenic powers. Also, it was generally believed for some time that the organisms belonging to any one species could be expected to behave always in the same way as antigens; that is, every culture of the same species was supposed to produce the same immunological response in in-

fected (and vaccinated) individuals, and to react always with exactly the same antibody.

Since about 1920, however, it has been realized that these antigenic qualities, and also practically all the other properties of bacteria are subject to considerable variation. A pure culture containing only bacteria of a single species, and *even a culture which has originated from the multiplication of a single bacterial cell,* may break up ("dissociate") into two or more strains, each with clearly different characteristics. The variant strains may breed true for generations, although, under suitable environmental influences, there is a tendency for them to revert to the parent type.

Kinds of variations. Aside from variations in shape and size, an ordinarily motile species may have variants that have lost their flagella and are therefore nonmotile; a sporulating organism may give rise to a nonsporebearing variant, and a capsulated bacterium to noncapsulated strains. Variations in physiological properties are common; there may appear within a single species strains with unusual resistance to acid, or to some disinfectant, or to high concentrations of salt, or to the injurious effects of sulfonamide drugs. Organisms like the whooping-cough bacillus, and the meningococcus, that are fastidious in their nutritional requirements when first isolated from the human body, give rise to variants that grow relatively well on ordinary media. This change toward a more saprophytic physiological state is usually accompanied by a reduction in the disease-producing power (*virulence*) of the organisms. Virtually all the properties of pathogenic bacteria that contribute to their virulence, including toxin-forming capacities, are subject to variation. Consequently, some strains of a particular germ may be highly dangerous, while other strains (having a different origin and history) are less virulent, and still others have lost the ability to cause disease, although they still retain other properties of the species.

By far the great majority of variations represent departures from the "normal" appearance or behavior of species which are not far on one side or the other of a common mean and, moreover, there is a constant tendency for the variants to revert to the "normal" form. Most of the variant strains which we study have developed rather slowly, through the cumulative effect of environmental factors. Occasionally, however, variant strains that breed true seem to arise suddenly, like the mutations, or "sports," which occur among

higher forms of life. It is doubtful, however, whether true mutation, in the sense used by biologists generally, actually occurs among bacteria.

Obviously, the occurrence of variant strains increases the difficulty of identifying and classifying bacterial species, and complicates the problems of infection and immunity, laboratory diagnosis, and many other phases of bacteriology.

Variations in biochemical reactions; constitutive and adaptive enzymes. Most obvious of all variations are changes in the ability to bring about some of the expected chemical reactions generally characteristic of the species.

In working with laboratory cultures, it is especially common to find strains which have *lost,* in greater or lesser degree, the capacity to form pigment, or to liquefy gelatin, or to ferment a particular carbohydrate, or to carry out some other biochemical activity ordinarily expected of that species. Variations of this sort cause real difficulties in practical work. The student should not be surprised to encounter, in some of the stock cultures issued to him for study, such variations from the expected set of biochemical reactions.

Fundamentally, all the physiological and biochemical variations are due, of course, to an alteration in the enzyme content, or at least in the degree of activity of certain enzymes, of the variant strain. Usually it is a matter of depressed activity, rather than a complete absence of the enzyme, that explains failure of a culture to bring about some expected biochemical change, for it often happens that the seemingly lost enzymatic powers are restored when the organism is repeatedly cultured in the presence of the particular substrate utilized by the enzyme. Thus, a stock strain of *Proteus* bacillus that seems to have lost all power to ferment sucrose (as fully active strains do), may again become an active fermenter of this sugar if transferred through several generations in sucrose broth.

Occasionally it has been noted that a culture, placed *for the first time* in a culture medium containing a particular substance never before utilized by the organism, decomposes this new substance immediately, thus apparently bringing into use a brand new enzyme. It is questionable, however, whether microbes suddenly elaborate an altogether new enzyme-complex. It is more likely that what happens is the rapid activation of an enzyme already present, though not before detected. It has been suggested that bacterial enzymes may be of two types: (1) *constitutive* or (2) *adaptive.* By definition,

constitutive enzymes are *always possessed* by a particular bacterium, irrespective of the medium on which it is grown, whereas adaptive enzymes are those *formed by a bacterium in response to the presence of a particular substance in the medium.* Actually, the true situation is probably more complex than this, and the difference between the so-called constitutive and adaptive enzymes is perhaps more academic than real.

The important point is that the enzymatic constitution of individual bacterial cells may vary over a wide range and be influenced by many different factors. For each clearly defined species of bacteria we can make a list of the biochemical changes which are exhibited by the most active known strains; these might be said to constitute the potentialities of the species. But the *actual* enzymatic make-up of the cells of any particular strain may differ considerably from that of preëxisting parent strains, and this seems to be largely determined by the physical and chemical conditions prevailing at the very moment these cells were dividing from their mother cells.

Correlated variations; Smooth and Rough strains. It would be a hopeless task to follow variations if it were not for the fact that the different morphological and physiological changes are to a certain degree correlated with one another, and the variations observed in many different species follow the same general pattern. Many of the most significant changes, moreover, are reflected in certain easily visible characters of the colonies formed by the bacteria on solid media.

Five different kinds of colonies that may be formed by different strains of the same species have been mentioned on page 192. The two principal types of colonies that represent different growth phases of organisms of the same species are the *Smooth* or *S type* and the *Rough* or *R type*. A culture consisting wholly or predominantly of S colonies is called an S strain, or if the opposite type of colony predominates, an R strain (or the organisms are said to be in the *smooth phase* or in the *rough phase,* respectively). With a few notable exceptions, such as the pathogenic streptococci and anthrax bacilli, smooth colonies are formed by the "normal" organisms possessing all the usual attributes of the species, while rough colonies contain the variants.

Smooth strains grow diffusely in liquid media, form stable and homogeneous emulsions in saline, and when agglutinated by

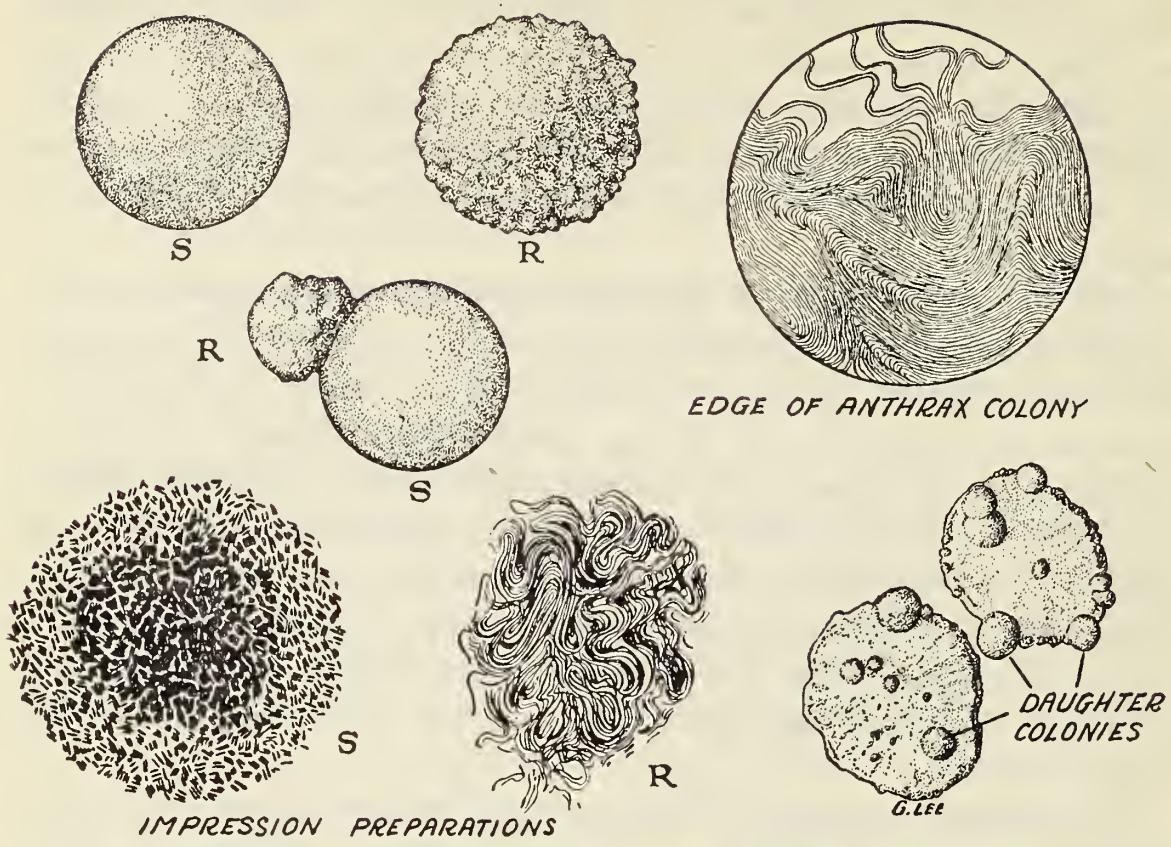


FIG. 56. Smooth and rough bacterial colonies. Upper left, a diagrammatic representation of the surface appearance of Smooth (S) and Rough (R) colonies. Lower left, actual arrangement of the bacilli in a Smooth and in a Rough colony of colon bacilli as revealed by impression preparations from young agar colonies. Drawn from photographs of the preparations made by Bisset (*J. Path. and Bact.*, 47:223, 1938). Upper right, a low power view of the edge of the characteristically rough colony of the anthrax bacillus. The long, parallel chains of bacilli are thrown into folds and look (under low magnification) like tresses of hair. (Drawn from a photograph in Kolle and Hetsch.) Lower right, "daughter colonies" which sometimes develop after a pure colony has reached its full growth. The organisms in the daughter colonies frequently have distinctly different properties from those in the parent colony. This is clear evidence of variation or "dissociation" within a species.

immune serum form large, coarse clumps. All the structures characteristic of the species, such as flagella and capsules, are well developed. In most pathogenic species smooth (S), or mucoid (M) strains are the *virulent* ones, and are generally isolated from patients in the early stages of acute infectious diseases. They possess all the surface structures and chemical make-up (e.g., the type-specific polysaccharides) of the fully active organism, and hence should be used in vaccines, for it is this form of the germ to which we wish to be immunized.

Rough strains commonly show a granular growth in broth, a

tendency to clump spontaneously in saline, and, when agglutinated by immune serum, they form small, fine clumps. They often have lost flagella or capsules, and also the antigenic elements that go with these structures. In the Pneumococci, for example, the change from S to R involves loss of capsule and of the specific capsular polysaccharide responsible for type specificity, leaving only the uncapsulated body (somatic portion) of the organism, which contains a nucleoprotein antigen common to all types. Rough strains may show changes in somatic antigens also. Often there are accompanying alterations in morphology, with unusual or bizarre forms. There is commonly an altered sensitivity to different bacteriophages. The R forms of many pathogenic species are reduced in virulence, or are actually nonvirulent. They are likely to be found in individuals who are resistant to the smooth, virulent form of the same species, such as in convalescents, healthy carriers, and chronically infected persons.

$S \rightarrow R$, $R \rightarrow S$ changes. Smooth to rough *phase variation* occurs spontaneously under laboratory conditions, especially in old stock cultures not often transferred, and also it may be induced deliberately by several means. In general, it is only necessary to expose the smooth strain to some mild injury. For example, a weak bacteriophage may induce the change, or the organisms may be grown in a medium made unfavorable by the addition of a dilute disinfectant, or by incorporating in it specific anti-S immune serum. Reversion of an R to an S form is more difficult, but it may be accomplished in some instances by very frequent transfers on especially favorable medium, by growing the organisms in a medium containing anti-R immune serum, or by passing them through a series of susceptible animals.

Practical importance and general significance of bacterial variation. The occurrence of variation obviously adds to the difficulties in classification of bacteria, making it hard to define the limits of a species. Variations also introduce practical difficulties in bacteriological diagnosis of infectious diseases, for the laboratory worker must have means of identifying not merely the "normal" members of the species of pathogenic organisms involved, but also the common variants, which often differ immunologically. Identification of a bacterium on the basis of fermentation and other biochemical activities must be made with caution, for not every strain of a species will give "typical" reactions. A most important point

is that strains to be used for the making of vaccines must be carefully selected to include those actually representing the virulent form of the organism against which we wish to be protected. It is probable, though not yet proved, that in many infectious diseases recovery occurs when the invading bacteria, under the influence of the antibodies formed by the infected host, become "dissociated" or degraded into relatively nonvirulent forms which may be phagocytized. Variations associated with changes in virulence of the causative germs probably account in large part for the development and decline of epidemics.

In judging the general significance of variation, it must always be remembered, as we have previously noted, that any bacterial culture is a *population*. When such a collection of bacterial cells is subjected to influences of a mildly injurious nature, those individual bacteria best suited to survive in the new circumstances do survive and multiply, and thus by *selection* the final cultures are made up predominantly of cells having characteristics varying more or less from those displayed by the original bacterial population. This does not explain the whole phenomenon of variation, but does account for the manifestations we commonly see.

The question of bacterial life cycles. The remarkable changes, especially in size and shape, shown at times by variant strains, have been given a much deeper significance by some observers. They have been regarded as expressions of cyclic changes. It has been suggested that bacteria may pass through a kind of *life cycle* as, for example, the malaria parasites do, and that at different stages of the cycle they may assume different forms, appearing perhaps as a bacillus at one time, and a coccus at another time, or again as a filtrable virus. But the evidence for this view is generally considered to be unconvincing. Despite the frequent occurrence of variant forms, all the common kinds of true bacteria may be safely regarded as permanent species which breed true to themselves, and which, *under favorable circumstances,* will always have, within narrow limits, the same morphological and physiological characteristics.

REFERENCES

Bisset, K. A.: "The Structure of 'Rough' and 'Smooth' Colonies," *J. Path. & Bact.,* 1938, 47:223.

Buchanan, R. E.: *Growth Curves of Bacteria. Newer Knowledge of Bacteriology and Immunology.* Chicago: Univ. of Chicago Press, 1928.

Bunting, M.: "Factors Affecting the Distribution of Color Variants in Aging Broth Cultures of *S. marcescens* 274," *J. Bact.,* 1942, 43:593.

Dubos, R. J.: "The Adaptive Production of Enzymes by Bacteria," *Bact. Rev.,* 1940, 4:1; *The Bacterial Cell.* Cambridge, Mass.: Harvard Univ. Press, 1945.

Gale, E. F.: "Factors Influencing the Enzyme Activities of Bacteria," *Bact. Rev.,* 1943, 7:139.

Morton, H. E. and Sommer, H. E.: "The Variation of Group C Hemolytic Streptococci," *J. Bact.,* 1944, 47:123.

Roepke, R. R., Libby, R. L. and Small, M. H.: "Mutation or Variation of Escherichia coli with Respect to Growth Requirements," *J. Bact.,* 1944, 48:401.

Severens, J. M. and Tanner, F. W.: "The Inheritance of Environmentally Induced Characters in Bacteria," *J. Bact.,* 1945, 49:383.

Winslow, C.-E. A. and Walker, H. H.: "The Earlier Phases of the Bacterial Culture Cycle," *Bact. Rev.,* 1939, 3:147.

Zinsser, H.: "On Postulates of Proof in Problems of the Bacterial Life Cycle," *Science,* Mar. 4, 1932, 75:256.

REVIEW QUESTIONS—CHAPTER XIII

1. What is a *colony*? Explain some of the factors that influence the appearance and structure of bacterial colonies.
2. Contrast the colonies formed in Petri-dish cultures by contaminants from the air with those of most parasitic bacteria. What is meant by a "spreader"?
3. What five types of colonies may be formed by bacteria of the same species?
4. Make a sketch to illustrate the growth curve of a bacterial culture, and name the four chief phases in the development and decline of a bacterial population. Describe briefly what happens during each of the four phases.
5. What is meant by *variation* of bacterial species? Mention some kinds of variation commonly encountered with bacteria.
6. Discuss the relation of alterations in enzyme activity to the general phenomenon of variation. Define the so-called *constitutive enzymes, adaptive enzymes.*
7. What is meant by a *Smooth* (S) strain, a *Rough* (R) strain, of a bacterial species? Outline the usual properties of S strains and contrast these with the qualities of R strains. What influences induce the S to R change, the R to S change?
8. Mention some definite examples of the practical importance of bacterial variation.
9. Explain how *selection* of particular variants within a bacterial population, together with the influence of adaptive enzymes, account for most of the observed variations.
10. What is the prevalent view concerning the question of life cycles in bacteria and the permanence of bacterial species?

CHAPTER XIV

CLASSIFICATION AND NAMING OF BACTERIA

Bacteriologists have always been more interested in what bacteria *do* than in the question of how they should be named and classified. Few of the older workers bothered to provide complete and accurate characterizations of the organisms they studied, and bacteriology has reached its present complexity without ever passing through a purely descriptive phase. The indifference of most bacteriologists toward these matters, and the inherent difficulty of the task, have combined to obstruct the development of a truly logical scheme of classification. Our present system of nomenclature and classification is therefore somewhat of a hodge-podge, with many illogical features and inconsistencies.

Nevertheless, it is not too difficult for the student to become well oriented in this phase of bacteriology, so that the names of the principal kinds of bacteria will become as familiar as those of his human friends and neighbors.

Orders of the Schizomycetes now recognized. The present-day system of bacterial classification, used in the United States and, with minor differences, throughout the world, is based upon a scheme originally proposed by a committee of the Society of American Bacteriologists, in 1920, which has been further modified by Bergey and his associates in successive volumes of the *Manual of Determinative Bacteriology* (published by Williams & Wilkins Company) the fifth and latest edition of which appeared in 1939. A sixth edition of this important book is expected in 1947. In the meantime, the arrangement of Orders and Suborders of the class *Schizomycetes* to be recognized in the forthcoming new edition has been published. It is given below in Table VIII:

Table VIII. Key to the Orders and Suborders of the Class Schizomycetes *

Order I. *Eubacteriales* (True bacteria. Rigid cells that are flagellate when they are motile.)
- Suborder I. *Eubacteriineae* (Unattached and do not possess photosynthetic pigments. Includes Family *Corynebacteriaceae*.)
- II. *Caulobacteriineae* (Sessile and stalked, attached bacteria.)
- III. *Rhodobacteriineae* (Sulfur purple and nonsulfur purple bacteria.)

Order II. *Actinomycetales* (Branching, nonmotile, mycelial threads.)
- III. *Chlamydobacteriales* (Filamentous, alga-like bacteria.)
- IV. *Myxobacteriales* (Slime bacteria, creeping motility.)
- V. *Spirochaetales* (Flexuous, spiral cells.)

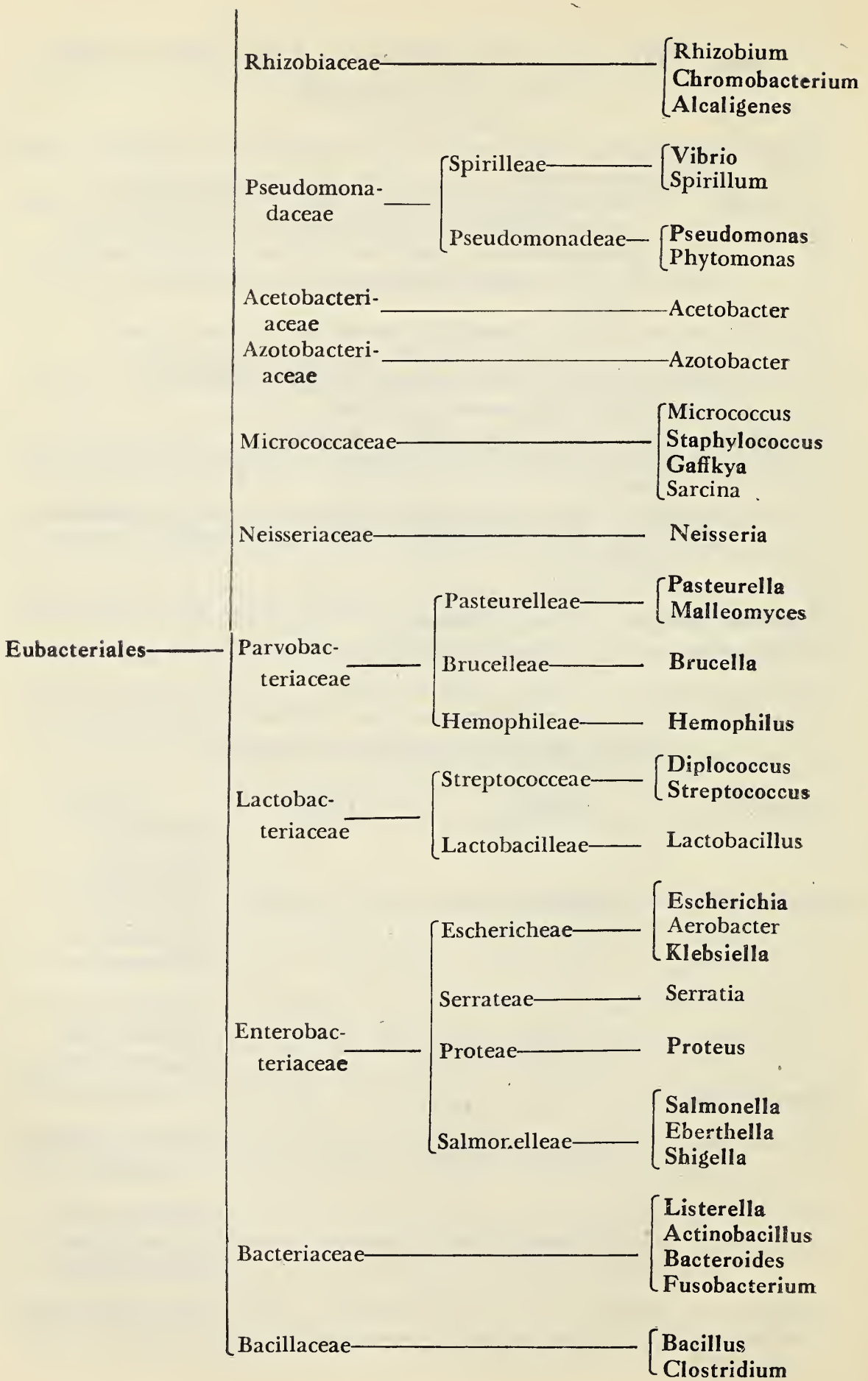
Supplement: Groups whose relationships are obscure
- Group I. Family *Rickettsiaceae* (Intracellular parasites carried by arthropods.)
- II. Order *Virales* (Filter-passers that grow in living protoplasm.)
- III. Family *Borrelomycetaceae* (Highly pleomorphic parasitic organisms.)

Principal genera. In the following chart (Table IX) is presented a classification of the principal genera of bacteria (true bacteria,

Table IX. Principal Genera of the Spirochetes, Mold-like Higher Bacteria, and True Bacteria

ORDER	FAMILY	TRIBE	GENUS
Spirochetales	Spirochetaceae		Spirocheta
			Saprospira
			Cristispira
			Borrelia
			Treponema
			Leptospira
Actinomycetales	Actinomycetaceae		**Actinomyces**
			Leptotrichia
			Erysipelothrix
			Proactinomyces
	Mycobacteriaceae		**Mycobacterium**
			Corynebacterium
	Nitrobacteriaceae		Nitrobacter
			Nitrosomonas
			Nitrosococcus

* Breed, R. S., Murray, E. G. D. and Hitchens, A. P.: "The Outline Classification Used in the Bergey Manual of Determinative Bacteriology," *Bact. Rev.*, 1944, 8:255.


Eubacteriales
Rhizobiaceae
Rhizobium
Chromobacterium
Alcaligenes
Pseudomonadaceae
Spirilleae
Vibrio
Spirillum
Pseudomonadeae
Pseudomonas
Phytomonas
Acetobacteriaceae
Acetobacter
Azotobacteriaceae
Azotobacter
Micrococcaceae
Micrococcus
Staphylococcus
Gaffkya
Sarcina
Neisseriaceae
Neisseria
Parvobacteriaceae
Pasteurelleae
Pasteurella
Malleomyces
Brucelleae
Brucella
Hemophileae
Hemophilus
Lactobacteriaceae
Streptococceae
Diplococcus
Streptococcus
Lactobacilleae
Lactobacillus
Enterobacteriaceae
Escherichieae
Escherichia
Aerobacter
Klebsiella
Serrateae
Serratia
Proteae
Proteus
Salmonelleae
Salmonella
Eberthella
Shigella
Bacteriaceae
Listerella
Actinobacillus
Bacteroides
Fusobacterium
Bacillaceae
Bacillus
Clostridium

mold-like higher bacteria, and spirochetes) which follows the arrangement in Bergey's Manual, 5th Edition, 1939. Doubtless there will be some changes in the forthcoming 6th Edition. The names given are those now most widely used in the American scientific literature. In order to simplify matters, a considerable number of the less important genera, families and tribes recognized by Bergey have been omitted. The genera containing frankly pathogenic species are indicated by use of darker type.

Common names for familiar groups among the bacteria. Some of the generic names (e.g., *Brucella, Salmonella*) are so generally known and accepted that they are familiarly employed in ordinary speech. It goes without saying, however, that bacteriologists do not habitually use the full scientific names of the microbes they talk about, nor do they ordinarily have in mind a formal taxonomic scheme, like that given above, when they think about relationships among the different kinds of bacteria. Rather, they make frequent use of common names and of a rough working classification based on practical experience, without much regard for the strict rules of taxonomy. The common species of bacteria are thought of as falling into certain natural groups, made up of organisms sharing a particular habitat, or showing some outstanding morphological, physiological, biochemical or pathological property. Some of the adjectives applied to these different groups have already been used in earlier chapters of this book; for example, aciduric, chromogenic, nitrifying, autotrophic. The following are some of the descriptive words or phrases most widely employed, together with notes on the identity and scientific nomenclature of the organisms concerned in each case. Note that the arrangement is alphabetical for easy reference.

Acid-fast bacilli—the bacilli of tuberculosis and leprosy, and related bacteria of the genus *Mycobacterium*.

Aciduric bacteria—active fermenters, forming large amounts of acid, especially lactic acid, and able to endure relatively high concentrations of acid; sometimes called *acidophilic*. Typical aciduric organisms are bacilli of the genus *Lactobacillus* and the "lactic group" of streptococci (*Streptococcus lactis*).

Aerobic sporeforming bacilli—the Gram-positive, *aerobic,* saprophytic spore-bearing rods, common in the dust and soil; also the causative organism of anthrax; genus *Bacillus*.

Anaerobic sporeforming bacilli—the group of Gram-positive, *anaerobic* spore-bearing rods common in the soil and in the intestinal tract of man

and animals; many are saprophytic, but group includes causative germs of tetanus, gas gangrene, and botulism; genus *Clostridium.*

Autotrophic (and facultative autotrophic) bacteria of the soil and water—organisms of the family *Nitrobacteriaceae,* sulfur bacteria of the genus *Thiobacillus* and Order *Thiobacteriales,* iron bacteria of the Orders *Chlamydobacteriales* and *Caulobacteriales,* nitrogen-fixing bacteria of genera *Azotobacter* and *Rhizobium.*

Bordet-Gengou bacillus—causative organism of whooping cough, *Hemophilus pertussis.*

Bulgarian bacillus—*Lactobacillus bulgaricus*—one of the lactobacilli found in the fermented milks commonly consumed as food in Central European countries, in Egypt, and elsewhere.

Butter bacillus—one of the saprophytic acid-fast bacilli found in butter, genus *Mycobacterium.*

Butyric acid bacteria—group of anaerobic sporebearing bacilli capable of attacking carbohydrates with the production of butyric acid and butyl alcohol; principal species is *Clostridium butyricum.*

Chromogenic bacteria—a term generally applied to organisms forming conspicuous amounts of pigments; typical examples are *Chromobacterium violaceum* (violet pigment), *Pseudomonas aeruginosa* (green and blue pigments), *Serratia marcescens* (red pigment), and *Sarcina lutea* (orange pigment).

Coliform bacilli—includes *Aerobacter aerogenes* and related Gram-negative bacilli, not of intestinal origin, as well as the colon bacilli (*Escherichia coli*) which are normal inhabitants of the intestine (colon).

Colon bacilli—the nonsporebearing, Gram-negative bacilli always present in intestines of human beings and animals; *Escherichia coli.*

Crown-gall bacillus—organism causing tumor-like growths on roots and stems of plants (*Phytomonas tumefaciens*).

Diphtheroids—bacilli morphologically similar to the diphtheria bacillus, but otherwise distinct; members of the genus *Corynebacterium* other than the diphtheria bacillus, e.g., *Corynebacterium xerose.*

Döderlein's bacillus—a large, aerobic, Gram-positive bacillus probably identical with *Lactobacillus acidophilus,* found often in the human vagina.

Ducrey's bacillus—the causative organism of chancroid; *Hemophilus ducreyi.*

Dysentery bacilli—the group of Gram-negative, nonsporebearing, nonmotile bacilli responsible for dysentery in man; members of the genus *Shigella.*

Enteric bacteria—usually refers to the large group of Gram-negative, nonsporebearing bacilli found in intestinal tract of man and animals both in health and in disease, including the causative organisms of typhoid fever and dysentery; often spoken of as the colon-typhoid-dysentery-group. Important genera include *Escherichia, Proteus, Eberthella, Salmonella,* and *Shigella.*

Food-poisoning organisms—usually refers to certain species of the "paratyphoid group," of the genus *Salmonella,* e.g., *Salmonella enteritidis,* but may properly be applied also to certain strains of *Staphylococcus* incriminated in cases of acute gastroenteritis following eating of certain foods. The anaerobic sporebearing bacillus, *Clostridium botulinum,* is the cause of a special kind of food poisoning known as *botulism.*

Friedländer's bacillus—*Klebsiella pneumoniae,* a Gram-negative bacillus with a conspicuous capsule, type species of the genus *Klebsiella.*

Fusiform bacilli—anaerobic nonsporebearing, spindle-shaped bacilli found in the normal mouth, and (mixed with spirochetes) in Vincent's angina, and other so-called fuso-spirochetal infections; genus *Fusobacterium.*

Gas bacillus—*Clostridium perfringens,* formerly known as *Clostridium welchii,* or the Welch bacillus. The principal anaerobic bacillus present in most cases of severe gangrenous wound infection (gas gangrene).

Gas gangrene bacilli—the group of anaerobic, Gram-positive sporebearing bacilli of the genus *Clostridium* causing gas gangrene of wounds. Includes *Clos. novyi, Clos. septicum, Clos. histolyticum,* etc.

Gram-negative diplococci—the germs of gonorrhea, meningitis, and related species of the genus *Neisseria.*

Gram-positive cocci—the large and varied group of cocci commonly encountered on the skin and mucous membranes in both health and disease, including dangerous germs of pneumonia, scarlet fever, septicemia, etc., as well as numerous harmless varieties. Members of the genera *Micrococcus, Staphylococcus, Gaffkya, Sarcina, Diplococcus* and *Streptococcus.*

Hansen's bacillus—the true causative organism of leprosy; an acid-fast bacillus, found in direct smears from leprous lesions, *Mycobacterium leprae.*

Hay bacillus—a harmless, aerobic, sporeforming bacillus often cultivated from hay infusions; *Bacillus subtilis* or a related species (not identified definitely with any one species).

Hemophilic bacteria—the group of small, Gram-negative, nonsporebearing bacilli requiring the presence of hemoglobin, or certain growth-accessory substances, in culture media; includes the so-called influenza bacillus, and the germs of whooping cough and chancroid; genus *Hemophilus.*

Higher bacteria—organisms having a more complex structure or mode of life than the ordinary, or true, bacteria. Members of the order *Actinomycetales,* particularly the *Actinomyces;* this term is often applied also to some of the iron and sulfur bacteria.

Hoffmann's bacillus—a common diphtheroid, *Corynebacterium pseudodiphthericum* (*hoffmanni*).

Hog-cholera bacillus—*Salmonella choleraesuis,* a member of the paratyphoid bacilli often associated with food infection in man; originally thought to be the cause of hog-cholera (now recognized as a virus disease).

Influenza bacillus—*Hemophilus influenzae,* the organism originally isolated by Pfeiffer from influenza patients, and for some time regarded as the cause of that disease; now recognized as a pathogenic organism often associated with, but not the primary cause of, epidemic influenza; same as Pfeiffer's bacillus.

Intestinal bacilli—same as enteric bacteria.

Iron bacteria—organisms that metabolize iron or store it within a sheath around the cells; most typical are members of the Order *Chlamydobacteriales;* others are classified in the Order *Caulobacteriales.*

Kleb's-Loeffler bacillus—the causative organism of diphtheria, *Corynebacterium diphtheriae.*

Koch-Week's bacillus—the causative organism of "pink-eye"; often called *Hemophilus conjunctivitidis,* but probably is a variety of *Hemophilus influenzae.*

Lactobacilli—the nonpathogenic, aciduric organisms of the genus *Lactobacillus.*

Mesophilic bacteria—organisms growing well in the medium range of temperature, that is, from about 25° C to 40° C; this term includes the vast majority of common bacteria.

Microaerophilic bacteria—organisms liking *small amounts* of free oxygen, preferring to grow in an atmosphere of low oxygen tension; e.g., *Neisseria meningitidis,* and many other pathogenic, parasitic species.

Mold-like bacteria—organisms showing branched growth like molds, or otherwise resembling fungi; e.g., the *Actinomyces;* same as higher bacteria.

Morgan's bacillus—one of the intestinal bacilli sometimes suspected of causing gastroenteritis, particularly in infants; has been variously classified, most recently as *Salmonella morgani* or *Proteus morgani.*

Morax-Axenfeld bacillus—a small bacillus found in cases of subacute conjunctivitis in man; *Hemophilus lacunatus* (or *duplex*).

Nitrifying bacteria—autotrophic soil organisms able to synthesize nitrites and nitrates; genera *Nitrosococcus, Nitrobacter* and *Nitrosomonas.*

Nitrogen-fixing bacteria—autotrophic soil organisms able to combine with (fix) atmospheric nitrogen; genera *Azotobacter* and *Rhizobium.*

Nonsporebearing anaerobes—a large group of parasitic bacteria whose properties are incompletely understood at present; some cause serious tissue-destroying lesions in man; included are the fusiform bacilli, classified by Bergey in the genus *Fusobacterium,* and other bacilli placed in the genus *Bacteroides;* e.g., *Bacteroides melaninogenicus.*

Paracolon bacteria—Gram-negative, nonsporebearing bacilli obviously related to typical colon bacilli (*Escherichia coli*) but characterized by a slow and irregular fermentation of lactose.

Photogenic bacteria—saprophytic bacteria capable of producing light; e.g.,

Pseudomonas phosporescens; occur on luminous fish and other marine life.

Pfeiffer's bacillus—the "influenza bacillus," *Hemophilus influenzae.*

Psychrophilic bacteria—cold-loving organisms having an optimum growth temperature of approximately 4°–10° C.

Sheathed bacteria—the iron bacteria of the order *Chlamydobacteriales.*

Slime bacteria—organisms of the Order *Myxobacteriales.*

Smegma bacillus—a saprophytic, acid-fast bacillus found often in human smegma; *Mycobacterium smegmatis.*

Spiral bacteria—the relatively rigid, corkscrew-shaped spirilla of the genera *Vibrio* and *Spirillum.*

Sulfur bacteria—saprophytic organisms that utilize sulfur in metabolism; e.g., members of the genus *Thiobacillus* (Order *Eubacteriales*).

Thermophilic bacteria—heat-loving organisms, growing best at temperatures as high as 60°–80° C.

Timothy grass bacillus—one of the saprophytic, acid-fast bacilli found in grass and grains; *Mycobacterium phlei.*

True bacteria—the organisms of the order *Eubacteriales.*

Tubercle bacillus—the causative organism of tuberculosis, *Mycobacterium tuberculosis.*

Vinegar bacteria—aerobic, saprophytic organisms capable of converting alcohol to acetic acid (*Acetobacter aceti*).

REFERENCES

Bergey, D. H., Breed, R. S., Murray, E. G. D. and Hitchens, A. P.: *Bergey's Manual of Determinative Bacteriology.* Baltimore: Williams and Wilkins Co., 5th Ed., 1939.

Breed, R. S., Murray, E. G. D. and Hitchens, A. P.: "The Outline Classification Used in the Bergey Manual of Determinative Bacteriology," *Bact. Rev.,* 1944, 8:255.

REVIEW QUESTIONS—CHAPTER XIV

1. What are some of the reasons for the present state of bacterial classification and nomenclature?
2. How many orders of *Schizomycetes* are now recognized? Name the orders to which belong the true bacteria, the mold-like higher bacteria, and the spirochetes?
3. The chart (Table IX) lists 31 genera of medical importance; how many of them can you recall?
4. Give examples of some of the common names applied to familiar groups of the bacteria, and give an appropriate definition of each.
5. How should the scientific name of a bacterial species be written?

PART TWO

METHODS OF DESTROYING MICRO-ORGANISMS AND OF CONTROLLING THE SPREAD OF COMMUNICABLE DISEASES— SOURCES AND MODES OF INFECTION

CHAPTER XV

STERILIZATION AND DISINFECTION: PHYSICAL METHODS

In previous chapters we have discussed the effect of environment upon the life of microbes and have pointed out their sensitiveness to sunlight, heat, and other physical and chemical conditions. We shall now consider in this and the following chapters the practical ways in which microorganisms can be destroyed by deliberately exposing them to these harmful influences, and how the spread of germ diseases may thus be prevented. But first it will be necessary to define the various terms which are used in connection with this subject.

DEFINITIONS

Sterilization. By *sterilization* is meant the *complete destruction of all microorganisms.* An object which is quite free of any living microbe is said to be *sterile.* The meaning of these terms, sterile and sterilization, is absolute; there is no such thing as a "practically sterile" or "nearly sterile" object. A thing is either sterile, or it is not sterile.

Disinfection. *Disinfection* means the *destruction of pathogenic microorganisms.* The term *disinfectant* is applied to an agent, usually a chemical, which is *used to destroy disease-producing organisms.* Of course, it may kill harmless bacteria, too, but it happens that disease germs are, in general, more easily destroyed than the harmless saprophytic types, so that disinfection may be accomplished in many instances with agents that do not truly sterilize. Thus, the feces of a typhoid patient may be successfully disinfected with a chemical which kills the typhoid germs, even though it does not destroy all the bacteria present.

Germicide. Any agent that *kills* microorganisms may be called a germicide.

Bacteriostasis. This refers to a condition in which bacteria are *prevented from multiplying,* though not killed. Low temperatures, weak concentrations of disinfectants, and certain dyes, for example, may keep bacteria in a state of suspended animation, and so are said to have a bacteriostatic effect.

Antisepsis. This term and the more widely employed derivative, *antiseptic,* cannot be defined very satisfactorily. Literally, an antiseptic is a substance that opposes *sepsis,* a word derived from the Greek meaning rotting, putrefaction, decay. Since it is the growth of microorganisms that causes sepsis, an antiseptic must have the property of *preventing the multiplication of microbes,* or, in other words, it must have a bacteriostatic effect. It may be a much weaker agent than a disinfectant, for the latter actually destroys germs.

Unfortunately most laymen and some doctors do not use *antiseptic* in this strict and literal sense, but give it the same meaning as disinfectant. In consequence, an "antiseptic" in current usage may refer to a truly germicidal agent in one case, or to a substance that has merely a bacteriostatic action in another case. It is best for professional people to employ the term only in its literal sense, to mean an agent that inhibits the growth of microbes, without destroying them.

Asepsis; aseptic technique. *Asepsis* means "without sepsis"—that is, it connotes the *absence* of the germs that cause infection. In the modern operating room, earnest efforts are made to create and maintain asepsis, and during an operation the surgeon and all his assistants follow certain careful *procedures designed to exclude and avoid germs.* Similar methods are used in the wards, especially in the handling of patients with communicable diseases; and indeed, to a limited extent, aseptic methods are called for in any sickroom, whether in the hospital or at home. Also, the microbiologist in his laboratory must be an expert in aseptic technique.

PHYSICAL METHODS OF STERILIZATION AND DISINFECTION

Sunlight and ultraviolet light. Direct sunlight has a powerful germicidal action. Exposure for a sufficient time will kill spores, as well as vegetative cells. Tubercle bacilli are destroyed in a few hours. It is often possible to make deliberate use of sunlight for the disinfection of clothing, bedding, mattresses, and other materials. The germicidal property of the sunlight is not due to the ordinary light

which we see, but to the very short, invisible, light rays beyond the violet end of the spectrum—the ultraviolet rays. It should be remembered that these rays are filtered out by ordinary glass, and that the sun must shine directly upon an object in order to exert its germ-killing effect.

Ultraviolet rays may be produced artificially by passing an electric current through vaporized mercury in quartz tubes. The rays pass through the tubes of quartz, and so can be applied to any object. A great variety of ultraviolet ray lamps have been developed for special purposes, and these devices are finding increasing use. One of the most important developments in this connection is the recent employment of ultraviolet rays for the treatment of viruses, so that they may be safely used as *vaccines*. By this treatment the viruses are not entirely destroyed, but only weakened in disease-producing power.

Disinfection of the air. The employment of ultraviolet rays for the destruction of bacteria, fungi, and viruses *in the air* is an important development of recent years. Remarkable success in preventing air-borne infection has been reported through the use of ultraviolet lamps in the wards of hospitals where patients with contagious diseases are housed, in public schoolrooms, and in operating rooms. It seems likely that this method of air-disinfection will some day become commonplace. The equipment installed in any particular place, however, will have to be most carefully planned and arranged, to avoid danger of injury to human eyes or other tissues, and at the same time to provide effective intensity of the ultraviolet rays.

Complete control of air-borne germs involves also methods for reducing room dust and for controlling humidity. Chemical mists (so-called *aerosols*) of propylene glycol or diethylene glycol also have a rôle in air-disinfection.

Cold. Cold prevents the multiplication of the ordinary bacteria, and refrigeration serves as an admirable method for the preservation of food and other substances which are easily decomposed by micro-organisms. Some of the common disease germs, such as those causing meningitis, gonorrhea, and syphilis, are actually killed by cold. But most bacteria are not destroyed, even at very low temperatures. The bacillus of typhoid fever, for example, may be frozen in a block of ice and still be able to grow when carefully thawed out and returned to a favorable temperature.

Heat. The application of heat in one form or another is the most widely used method of destroying microbes. It will be remembered that, although the vegetative cells of most of the ordinary bacteria are destroyed in a few minutes at about 65° C, spores and some unusual organisms are more resistant. For this reason, if we wish to sterilize an object truly, we must apply high temperatures and continue them for considerable periods.

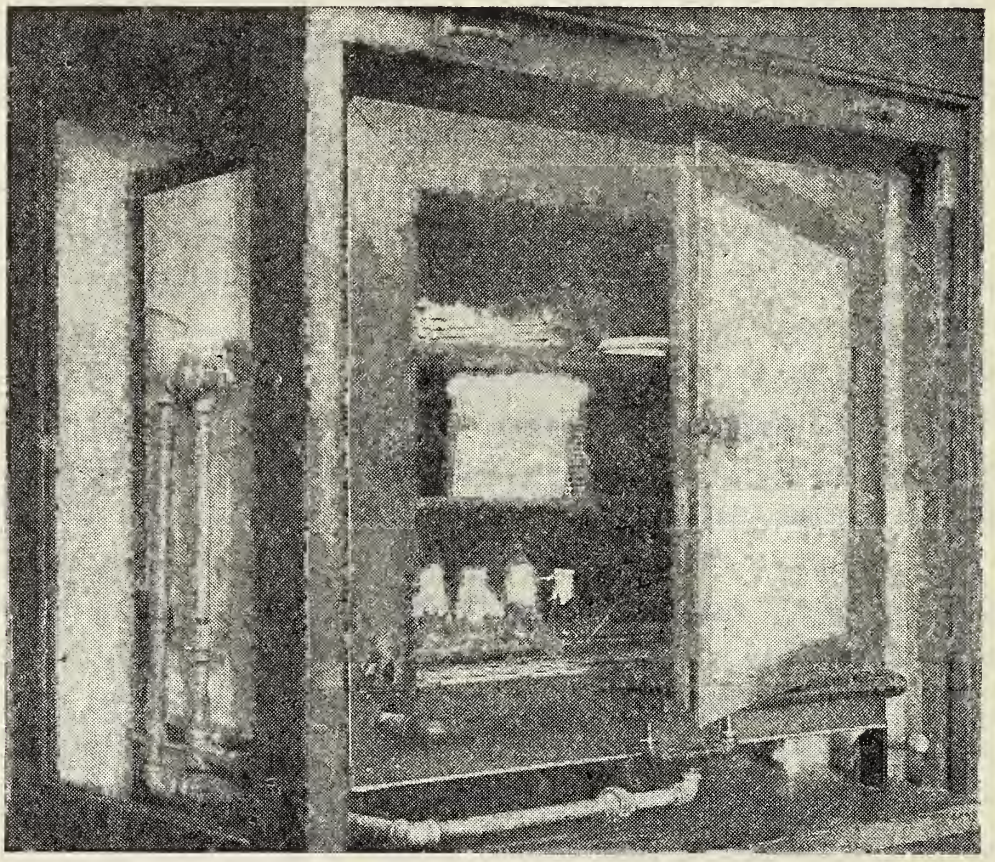


FIG. 57. A dry heat oven, showing types of laboratory glassware which are sterilized in this apparatus.

Moist heat versus dry heat. Heat destroys microbic life by causing coagulation of the protoplasm. This process is accelerated by the presence of abundant water, since proteins coagulate at lower temperatures when they are well hydrated. Consequently, moist heat—in the form of hot water, live steam, or steam under pressure—is a much better sterilizing agent than dry heat (hot air).

Heating in the open flame. Heating small glass or metal objects by holding them in the free flame, as in the Bunsen burner, is an effective method of sterilizing them. This is the way the bacteriologist sterilizes his inoculating needle and the glass capillary pipettes he sometimes uses. The method may be used in an emergency for

sterilizing a strip of adhesive tape or the tips of forceps or other instruments.

The inside of a medicine glass may be sterilized by first wiping it out with alcohol or ether, and then touching a flame to the adherent liquid; the fluid will burn and leave the glass sterile. A small instrument might be sterilized in a similar way.

Burning or incineration. Burning is a safe and cheap method of disposal of small objects of no value, such as used swabs, or dressings, or paper sputum cups. Whenever possible, incineration of articles contaminated with disease germs is to be recommended.

Exposure to hot air (dry-heat oven). In bacteriological laboratories, one of the most commonly used pieces of apparatus is a sterilizer called the *dry-heat,* or hot-air, *oven* (Fig. 57). This oven is similar to the ordinary baking oven used in the home. It is a double-walled chamber heated by gas or electricity, and so constructed that it will stand a high temperature. This apparatus is used in the laboratory principally *for the sterilization of test tubes, flasks, Petri dishes, and other kinds of glassware.* It may also be employed for sterilization of instruments, hypodermic needles, dressings, and the like, and for vaseline or other nonvolatile fluids. It cannot be used, of course, for culture media or other watery or alcoholic solutions, for these would simply evaporate and be ruined.

An exposure of at least one hour at a temperature of 160°–170° C is necessary to effect the sterilization of objects in the dry-heat oven. *As a routine method, glassware is held in the oven at 170° C (338° F) for one and one-half hours.* Cotton and paper are liable to be burned if the temperature runs over 180° C; this must be avoided, but a slight charring is desirable. After the period of heating, the oven must not be opened until the temperature has fallen to 100° C or less, because too sudden cooling of the glassware may crack it.

Boiling. Boiling is such a commonplace, everyday procedure that its use is sometimes neglected for a more complicated method. Boiling water has a temperature of 100° C (212° F). The vegetative forms of most bacteria are killed by a few minutes' exposure to this temperature, but it must be remembered that spores may resist boiling for as long as one hour, or more. The time required to sterilize by boiling will vary with the nature of the material. In any case, it must be remembered that, in order that an object may be sterilized, it *must be completely immersed in the boiling water,* and the boiling must be continued long enough for all parts of the object to reach

the sterilizing temperature. Boiling is especially useful for the disinfection of dishes and tableware, handkerchiefs, bed linen, bedclothes, and other things contaminated by patients, and for the sterilization of surgical instruments, hypodermic needles, and syringes.

Heating in flowing steam. Flowing steam, that is, steam which is not confined under pressure, has the same temperature as boiling water, 100° C, and never rises above this temperature. For this reason, flowing steam is not effective against bacterial spores unless it is applied for a long time. In practice, a method of intermittent exposure to flowing steam, called the *fractional or discontinuous method of steam sterilization,* is employed. The material to be sterilized is exposed to the steam for half-hour periods on each of three or four successive days. The apparatus used in the laboratory is called the *Arnold sterilizer.* An ordinary double-boiler or steamer might be used at home. At the first heating, most of the bacteria in the active vegetative stage are killed, but not the spores. Then, in the interval between the first and the second heating, the spores which survived the first heating germinate into vegetative cells. These should be killed the second day, but in order to sterilize the material surely, the process of intermittent heating is usually continued for another day or two. The method is somewhat uncertain, at best, and at the present time the Arnold sterilizer is not often used.

Heating in steam under pressure. When steam is confined under pressure in a closed chamber, it is a much more effective sterilizing agent than flowing steam, because it penetrates better, and particularly, *because it attains a higher temperature.* It is the temperature, not the pressure, which is the more important. The relation between the pressure of steam and the temperature is shown in the following table:

Table X. Relation of Steam Pressure to Temperature in the Autoclave

Pounds of Pressure of Steam (Above atmospheric pressure)	*Temperature*	
	Centigrade	Fahrenheit
0	100°	212°
5	107.7°	227°
10	115.5°	240°
15	121.6°	250°
20	126.6°	260°

The autoclave, or steam-pressure, sterilizer. Sterilization by steam under pressure is carried out in laboratories and hospitals in an apparatus called an *autoclave* (Fig. 58). The "dressing sterilizer" of the hospital is an autoclave. The pressure cookers used at home for cooking or canning of food are small autoclaves. So also are the large retorts used in canning factories for sterilization of tin cans of food. The autoclave is essentially a double-walled

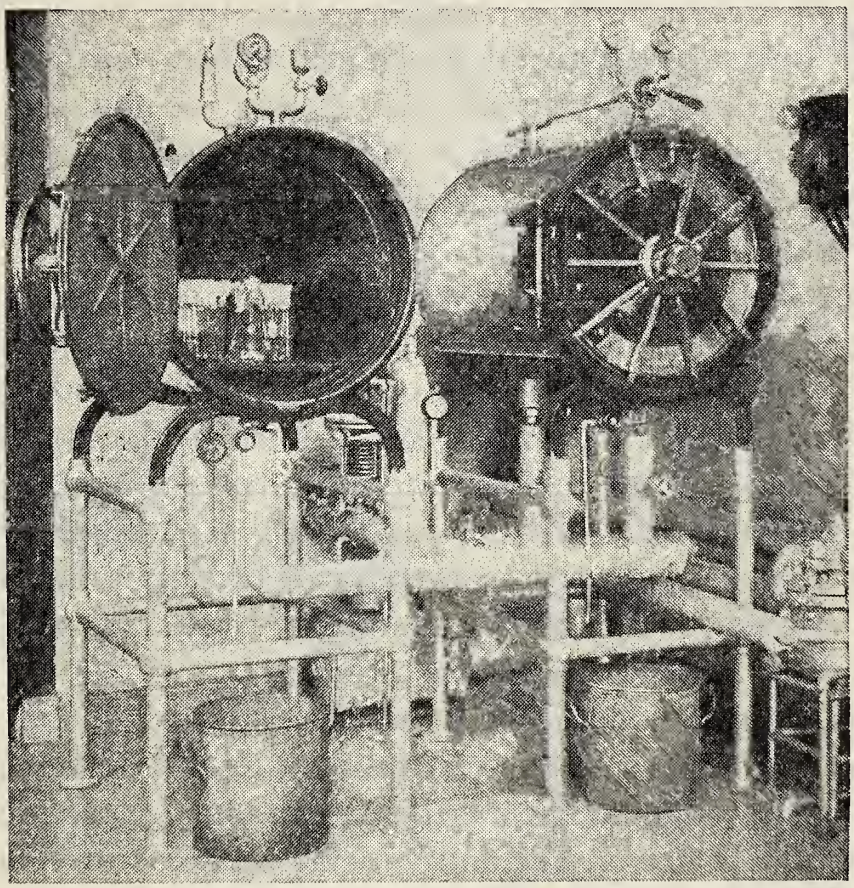


FIG. 58. Autoclaves (steam pressure sterilizers). The steam for the autoclaves shown here is supplied directly from the main steam line.

metal chamber, fitted with a door which can be closed very tight. There is a safety valve to permit the escape of the steam, should the pressure get too high. There are usually two gauges, one to show pressure of steam in the outer jacket, and another to show the pressure in the inner chamber. There are valves to hold the steam in the jacket and to send it into the sterilizing chamber. There is an exhaust valve to let the steam escape from the inner chamber. There should also be a thermometer (best attached just beyond the exhaust valve) to show the temperature of the steam within the chamber, but some autoclaves are made without thermometers. The steam is often generated by a gas-heated boiler arranged beneath the auto-

clave, although in some large laboratories and hospitals steam from the main boiler room is piped directly to the apparatus. It is important, for efficient operation, that the autoclave be one that can be brought to the desired steam pressure *quickly,* in order that prolonged heating of the contents at less than the sterilizing temperature may be avoided.

Articles to be sterilized in the autoclave are usually exposed to a pressure of *15 pounds of steam* (that is, to a temperature of about 121° C) *from twenty to thirty minutes.* The time required depends upon how bulky the material is—larger articles naturally require longer to heat through—and also how closely the material is packed into the chamber. In order to be sterilized, *every part of the material must be heated to the sterilizing temperature for the necessary length of time.*

Both the hospital and the laboratory find constant use for the autoclave; in the hospital for the sterilization of sponges and dressings and other materials for the operating rooms and wards, and in the laboratory for bacteriological culture media, solutions, discarded cultures, rubber articles, etc. The correct operation of the sterilizer is an important matter, particularly in hospitals where this apparatus is depended upon to prepare the dressings and other materials to be used in surgical and obstetrical work. Often nurses have the responsibility of operating the hospital autoclaves.

The following are three essential precautions: (1) keep the size of packages to be autoclaved relatively small, and do not pack articles too close together in the sterilizing chamber, (2) see that all air in the chamber is driven out and entirely replaced by steam before closing the exhaust valve, and (3) see that the necessary steam pressure and sterilizing temperature is maintained for a sufficient length of time.

Pasteurization. This is a special method of applying a mild degree of heat to milk and other liquids for destroying undesirable and harmful nonsporeforming microbes. It is not a process of sterilization, but rather of *disinfection.* It was originated by Louis Pasteur and used by him for killing microorganisms which cause undesirable fermentations in the process of making wine.

Pasteurization is now extensively used for freeing *milk* from disease germs (Fig. 67). *The process of pasteurization of milk consists of heating it to 61°–63° C (142°–145° F) for one-half hour, followed by a rapid cooling.* In most modern dairies the raw milk

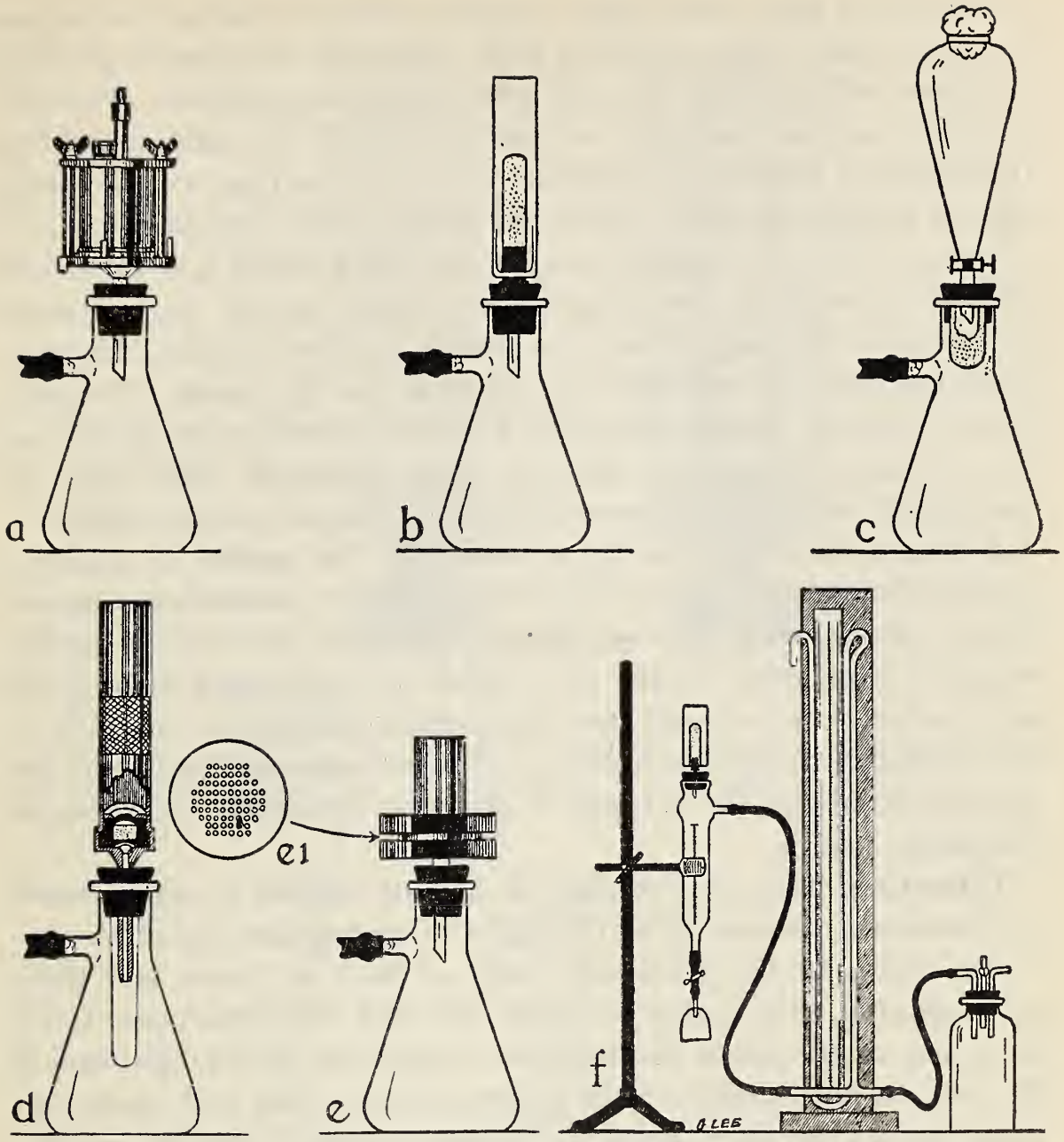


FIG. 59. Filters: a, one form of a Seitz filter; b, a Berkefeld filter; c, a Chamberland filter, with part of the candle cut away to show that the fluid filters from within outward; d, a Jenkins filter, with a portion cut away to show the square porcelain block which is the actual filtering material; e, one type of ultrafilter, and e1, the perforated plate over which is stretched the collodion membrane which forms the actual filtering surface. f, a Mudd filtration apparatus, illustrating the necessary arrangements for critical work with filters. There is (at the right) the usual trap bottle to catch the back flow when the suction is released and, in addition, a manometer, so that the actual negative pressure used may be regulated. The filtrate drops into a graduated burette, from which measured samples may be collected at any desired time intervals, and tested separately for sterility.

is carried in pipes from large receiving tanks to the pasteurization machines. Just before entering these machines, it is passed through hot pipes which warm it to the pasteurizing temperature. The pasteurizers consist essentially of tanks in which the milk is held at the necessary temperature (usually 143° F is used) and continuously stirred for half an hour. Then the milk passes through long, cold pipes, in which it is rapidly cooled, and from which it is carried to automatic bottling and capping machines. As we have already learned, this heating destroys the vegetative forms of most bacteria, including those of any disease germs that may be present. The milk is *not sterilized*—pasteurized milk will sour, though more slowly and in a manner somewhat different from unheated milk—but all pathogenic bacteria or viruses likely to be found in it are killed.

Pasteurization does not appreciably alter the quality or flavor of milk, although its property of preventing scurvy is somewhat diminished. Infants need the antiscorbutic principle, but this is readily supplied from other foods, and boiled or pasteurized milk is the only proper kind of milk for them. Pasteurization of *all* milk is encouraged by health authorities everywhere and is required by law in many localities, for it is one of the most important measures for the public health.

Filtration. This is a method of making liquids or gases sterile by permitting them to *filter* through the appropriate material.

Air filters. In the laboratory most of our test tubes and flasks are stoppered with a plug of cotton. Air and other gases pass freely in or out of the tubes through the cotton, but microorganisms in the dusty air are caught in the meshes of the cotton and cannot get through into the tube (unless the cotton is wet). Therefore, the cotton plugs serve as efficient filters.

Filtration of liquids. Filters for making *liquids* free of any visible or cultivable microbes have important uses in microbiology. There are several types of laboratory filters, and the same type of filter may be obtained in various sizes and in several different grades of porosity (Fig. 59).

In Fig. 60 is shown a Mandler filter assembled for use. The filter candle and a suction flask to receive the filtrate are sterilized, usually in the autoclave, and then carefully fitted together. The liquid to be filtered is poured into the glass "mantle" about the filter candle, suction is applied, and the liquid is drawn slowly through the porous filter and falls into the sterile flask below. As the fluid

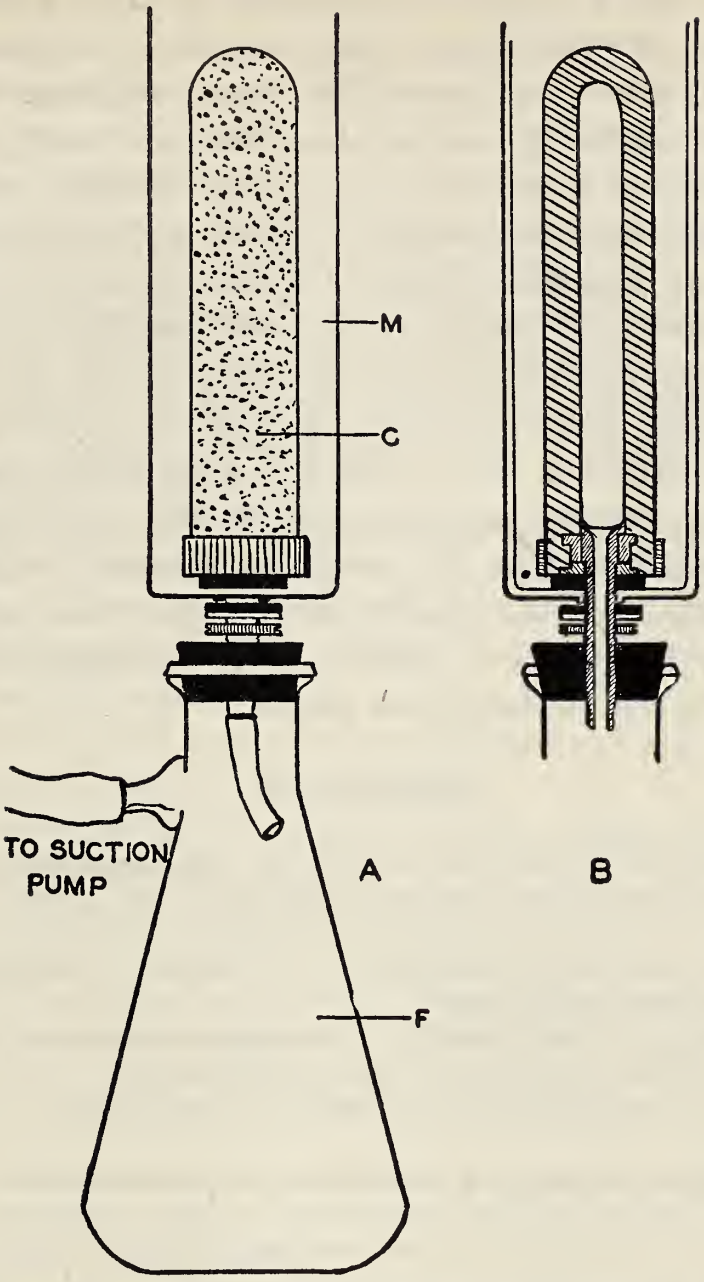


FIG. 60. A: a Mandler filter set up for use. A glass test tube of larger diameter than the filter candle (C) is inverted over the candle (this tube is not shown in the drawing), then the culture or other material to be filtered is poured into the glass mantle (M). The fluid rises inside the inverted tube so that it covers the entire candle. Then as suction is applied the fluid is drawn through the filter, while the ordinary bacteria are held back, and a clear filtrate, free of any visible microbes, flows down into the sterile flask (F) below. B: section through the filter, showing the hollow construction of the filter candle.

passes through the filter candle, all the microorganisms in it are held back, and the filtrate is bacteriologically sterile, that is, free of any microbe that can be cultivated on lifeless media.

Uses of laboratory filters. Filters are used in the laboratory to remove microbes from blood serum, ascitic fluid, sugar solutions, or

other liquids which cannot be sterilized by heat without altering them in some undesired way. Also *exotoxins,* enzymes, and other products of the growth of bacteria are separated from the organisms which produce them by use of these filters. Finally, the *filtrable viruses* are isolated from the body tissues or fluids which contain them, and separated from ordinary microbes, by filtration.

Filtration of drinking water. In most communities, filtration through sand and gravel is one of the most important measures used for the purification of the public water supply (Fig. 66). In this kind of filtration there is more than a mechanical straining. There develops on the surface of the sand filter a scum which consists largely of putrefactive microorganisms, and as the water passes slowly through the sand, most of the contained bacteria are held back in the surface scum, and there destroyed in competition with the hardy putrefactive forms. Properly filtered water is nearly free of all bacteria, and quite free of dangerous germs.

REFERENCES

Brigg, E., Jennings, M. E. and Olson, F. C. W.: "Epidemiological Observations on the Use of Glycol Vapors for Air Sterilization," *Am. J. Pub. Health,* Aug. 1945, 35:788.

Hollaender, A.: "Ultraviolet Irradiation as a Means of Disinfection of Air," *Am. J. Pub. Health,* 1943, 33:980.

Hoyt, A., Chaney, A. L. and Cavell, K.: "Studies on Steam Sterilization and the Effects of Air in the Autoclave," *J. Bact.,* 1938, 36:639.

McCulloch, E. C.: *Disinfection and Sterilization.* Philadelphia: Lea and Febiger, 2nd Ed., 1945.

Rahn, O.: "Physical Methods of Sterilization of Microorganisms," *Bact. Rev.,* 1945, 9:1.

Underwood, W. B.: *Textbook of Sterilization.* Erie, Pa.: Am. Sterilizer Co., 1934.

————: *Some Features Relating to Pressure Steam Sterilization of Media and Solutions of Particular Interest to the Laboratory Technician.* Erie, Pa.: Am. Sterilizer Co., 1937.

Walter, C. W.: "Sterilization of Dressings and Dry Goods," *Internat. Abstract of Surgery,* Nov. 1940, 71:414.

REVIEW QUESTIONS—CHAPTER XV

1. Define: *sterilization, sterile, disinfectant, disinfection, germicide, bacteriostasis, antiseptic, asepsis, aseptic technique.*
2. What is the effect of sunlight and ultraviolet light on microbes? Discuss the practical use of ultraviolet-ray lamps for air-disinfection.
3. What practical value has cold as a means of controlling bacterial life?

4. What is the approximate thermal death time for the vegetative cells of most bacteria? Why must higher temperatures be used in the practice of sterilization?
5. Give examples of the practical use of the open flame and burning for sterilization purposes.
6. Describe the dry-heat oven. For what is it used? Give the essential details for the successful operation of the oven.
7. Give practical examples of the use of boiling as a means of sterilization. What factors bear upon the success of the method?
8. Describe the Arnold sterilizer. What is the temperature of flowing steam? What is the discontinuous method of sterilization, and what is the principle underlying it?
9. What happens to the temperature when steam is confined under pressure? What is the temperature of steam under 15 pounds' pressure? Which is the more important in bringing about sterilization, the pressure or the temperature of the steam?
10. Describe an autoclave.
11. What are the usual time and the pressure of steam required for sterilization of articles in the autoclave?
12. Describe five steps in the operation of an autoclave, and discuss the precautions necessary for success.
13. Why is it important not to pack articles too closely into the autoclave? In general, what is the relation between the amount and bulk of material in the autoclave and the time required to sterilize it?
14. Why is it necessary to replace all the air in the chamber with steam?
15. Define *pasteurization*. What is the principal use of the method at present?
16. How do cotton plugs serve as filters to keep out microbes?
17. Name and describe briefly four types of laboratory filters. Describe the operation of a Mandler filter.
18. How is filtration used for the purification of water? What other factors besides simple mechanical filtration account for the result?

CHAPTER XVI

DESTRUCTION OF MICROORGANISMS BY CHEMICAL GERMICIDES: CHEMOTHERAPY

Uses of disinfectants and bacteriostatic (antiseptic) chemicals. The chemical agents employed as disinfectants or antiseptics may be said to fall into three categories: (1) compounds (like cresol) used freely to destroy microorganisms in bodily excretions and in our general physical surroundings; (2) germicides (like tincture of iodine) used for direct, local application to human tissues for the prevention or treatment of infection; and (3) bacteriostatic or bactericidal preparations (like the sulfonamide drugs and penicillin) given internally for the treatment of generalized infections.

It is in the field of *chemotherapy*—the treatment of infectious diseases with drugs—that such sensational advances have been made in recent years. The most remarkable property of these new therapeutic agents is their low toxicity for the human body. A few of the germicides used in earlier times (for example, acriflavine and other dyes) were safely used within the body for the treatment of infections of the urinary bladder, kidneys, or other internal organs. The great majority of strong disinfectants, however, can never be used in this way, for they are almost as poisonous to the human tissues as they are to bacterial cells. Even the local application of such preparations to infected areas is often unwise, since this would cause unnecessary irritation of the tissues, and delay healing. Consequently, most of the older disinfectants are of little value in treatment. They are most useful, however, in *preventing* infections, particularly for disinfection of the skin before a surgical incision, or prior to a hypodermic injection. The common disinfectants are of special value for the disinfection of the feces, sputum, and other germ-laden discharges from a patient with a communicable disease.

Many laymen share certain popular misconceptions about disinfectants. They tend to overrate the germ-killing power of well-

advertised preparations, and also often fail to realize that most successful germicides are poisonous in some degree to body tissues. Sometimes, with a too simple faith in their efficacy, and without regard for their irritating qualities, germicidal preparations are applied repeatedly and indiscriminately to the nose, throat, or skin. Overtreatment, with harsh chemicals, of skin infections such as "athlete's foot" is a common practice. This must be because people generally lack a proper appreciation of the truly remarkable *natural* defensive and recuperative powers of *normal, healthy tissues.*

Factors affecting the action of germicides. How the various chemical germicides act to kill microbes is not known exactly. The organisms are doubtless injured in different ways by different chemicals but, in any case, the end result is probably a precipitation of the cellular proteins and a coagulation of the protoplasm.

The germicidal power of a chemical naturally differs according to the strength or *concentration* in which it is applied. A disinfectant in a very weak solution may have no effect upon bacteria, or it may actually stimulate their growth. In somewhat greater concentration the same chemical may have only an antiseptic or bacteriostatic action, while a still stronger concentration may be an excellent germicide. *A disinfectant solution must be of proper concentration, and it must be used in sufficient amounts* to be effective. It is well to remember that a liquid disinfectant is always more or less diluted when mixed with the material to be disinfected. Thus, if one pint of a 5% lysol solution is mixed with a pint of feces, its strength is reduced to that of a 2.5% solution.

The commonest mistake made in the use of disinfectants is the failure to allow sufficient *time* for them to act. The effect of chlorine and a few other germicidal chemicals is almost instantaneous, but these are the exceptions to the general rule. The great majority of disinfectants do *not* act quickly, and in practice *it is important that sufficient time be allowed for complete disinfection of any material.* The germicidal agent must be given time to penetrate all parts of the material, because the active chemical must reach the germs themselves in order to kill them.

Finally, the action of a disinfectant varies with the *character of the material* acted upon. *Organic matter interferes* with the germicidal action of any chemical, and some disinfectants are of no value in the presence of abundant organic material. Bichloride of mercury, for example, cannot be relied upon to disinfect sputum

or feces, because it combines with the albuminous matter and may not reach the bacteria at all. *The proper disinfectant must be chosen according to the nature of the material to be disinfected.*

Measurement of the germicidal power of chemicals. *Phenol coefficients.* In 1903, Rideal and Walker suggested a test-tube method of evaluating disinfectants, known as the *phenol coefficient method.* Since that time, Reddish and others have introduced many improvements and modifications. In the United States, at the present time, the procedure proposed by Ruehle and Brewer (1931) is the standard test employed by the Food and Drug Administration, U. S. Department of Agriculture.

Whatever the particular technique employed, the principle of all phenol coefficient tests is the same. The action of pure phenol on cultures of *Eberthella typhosa, Staphylococcus,* or other bacteria is taken as the standard, and the relative germicidal value of other disinfectants is determined by comparing their germ-killing power with that of phenol under the same conditions. Definite dilutions of phenol and of the disinfectant to be standardized are used, so that the result of the test can be expressed by a number. This number is called the *phenol coefficient.* The coefficient of phenol itself is 1.0. If a disinfectant is less active than phenol in killing germs under the same conditions, its phenol coefficient is some figure less than 1.0; if it is more active than phenol, its phenol coefficient will be greater than 1.0.

Typical results of a test are shown in Table XI.

The phenol coefficient is determined by dividing the highest dilution of the disinfectant that killed the test organism in 10 minutes, but not in 5 minutes, by the greatest dilution of phenol giving the same results. Thus, for Disinfectant X (Table XI) the phenol coefficient is $\frac{350}{90} = 3.89$. (This would be recorded as the figure to the nearest 0.1, that is, as 3.9.) Strictly speaking, this is only the *E. typhosa phenol coefficient,* since this species was the test organism.

Limitations of phenol coefficients; other methods. Because of the limitations of the standard phenol coefficient tests a variety of other methods for evaluating disinfectants have been tried in recent years. These newer methods include tests of the toxic effect of the germicides upon living leukocytes, or upon other living tissues, as well as trials of their bactericidal power in test tubes or in experi-

mental skin infections. No one test has yet been found entirely satisfactory, however. In order to discover its full potentialities, a disinfectant would have to be tested repeatedly against many different organisms, by a variety of methods, and under a variety of conditions, especially in circumstances simulating as closely as possible those prevailing where it may actually be used. It would then

Table XI. Results of Phenol-Coefficient Test with Disinfectant X (Test Organism *E. typhosa*)*

Germicide	Dilution	Growth in subcultures after		
		5 min.	10 min.	15 min.
Phenol	1:90	+	—	—
	1:100	+	+	+
Disinfectant X	1:300	—	—	—
	1:325	+	—	—
	1:350	+	—	—
	1:375	+	+	—
	1:400	+	+	+

* Adapted from Ruehle, G. L. A. and Brewer, C. M.: *U.S. Food and Drug Administration Methods of Testing Antiseptics and Disinfectants,* U.S. Dept. of Agriculture, Circular 198, 1931.

be found that for each disinfectant there are certain zones of concentrations within which it is, in turn: (1) without effect, (2) stimulating to the growth of the test organisms, (3) bacteriostatic, (4) definitely germicidal in a useful way, and (5) too concentrated to be of practical value. Few disinfectants have been so thoroughly studied, and at present our information about their germicidal power is based almost entirely on rather crude trial-and-error tests.

COMMON DISINFECTANTS

Carbolic acid (phenol). This is the germicide used by Lister in his epoch-making work which introduced antiseptic methods into

surgery. It is employed, as described above, as a standard for determining the relative strength of other disinfectants.

A 5% solution of the chemical is an effective germicide for disinfection of feces, sputum, or other excrement.

Cresol and lysol. Cresol is a derivative of phenol. It has about three times the bactericidal activity of pure phenol and is no more poisonous. Many commercial disinfectants contain cresol in some form, combined with tar oil, soap, or some other substance. A 2% solution of this compound in water makes an excellent disinfectant for feces or sputum, and for general purposes.

Lysol is a mixture of cresol and linseed-oil soap. It is more soluble than pure cresol, and makes a milky emulsion in water. This product has many uses, both in the hospital and in the home. Its action is not seriously interfered with by organic matter. It can be used in 2–5% solutions for disinfection of feces, sputum, and similar materials; for contaminated bed linens; and for the hands of hospital attendants. A solution of about 1:500 is employed as a vaginal douche.

Formalin. This is a solution of formaldehyde gas (about 37%) in water. One part of this solution with 9 parts of water makes a good disinfectant which is an effective germicide, even in the presence of considerable albuminous matter, as in the case of sputum. The solution is unstable, however, and hence is reliable only when freshly prepared. In the hospital, formalin solutions are sometimes used to disinfect instruments or rubber gloves, as well as excreta.

Formalin has a hardening, as well as a preservative, effect upon tissues, and is universally used for the preservation of anatomical and pathological specimens.

In the preparation of bacterial vaccines, the organisms are often killed by adding about 0.2% of formalin. Formalin is also of great value as the agent used to convert deadly exotoxins (such as diphtheria and tetanus toxin) to harmless *toxoids*. This is done by adding about 0.4% of formalin to the toxin, and allowing the material to stand at an appropriate temperature for a few weeks.

Chlorine and its compounds. Chlorine as a gas, or as a liquid, or in combination with various other elements, is one of the most effective germicidal agents we possess. *Chlorine gas* and *liquid chlorine* are used extensively for the sterilization of drinking water, swimming-pool water and sewage. About 0.5 to 1.0 part of active

chlorine to a million parts of water is the usual amount needed.

A widely used chlorine compound is *chlorinated lime,* $Ca(ClO)_2$, also called calcium hypochlorite, chloride of lime, and bleaching powder. This substance is made by passing chlorine gas through freshly slaked lime. The chlorine is not firmly bound to the compound, so that the powder decomposes and becomes useless as a disinfectant rather rapidly when exposed to the air. A freshly made bleaching-powder solution is one of the cheapest and best chemicals we have for disinfection of such places as dairies, slaughter houses, cellars, outhouses, etc.

Iodine. Iodine is not as powerful a germicide as chlorine, since free I (the truly bactericidal element) is not as readily released as free Cl is from its compounds. Nevertheless, alcoholic or aqueous solutions of iodine are among the most useful and practically effective of common disinfectants.

Tincture of iodine. An alcoholic solution is the preparation most widely employed. The tincture is made in various strengths; the regular U. S. Pharmacopeia formula calls for 7% of I and 5% of KI dissolved in 83% alcohol. A milder tincture, however, containing 3–3.5% iodine and about 2.5% potassium iodide in 50 or 70% alcohol, is even more useful in many circumstances. As a disinfectant for cuts and minor wounds, and for small areas of the healthy skin (prior to injection or incision), tincture of iodine is probably superior to any other chemical agent. The mild tincture is especially to be recommended; it kills bacteria and fungi readily, and at the same time has relatively little toxic effect on body cells. It must be remembered, however, that if too strong a tincture is applied, it may burn the tissues. Care must be taken to prevent the solution from becoming too concentrated through evaporation of the alcohol. A freshly made tincture is always best; it will have the proper strength and, also, its germicidal power will be most active.

Aqueous iodine solutions, such as Gram's iodine, are also germicidal.

Alcohol. The stock solution of ethyl alcohol (C_2H_5OH), as purchased by laboratories and hospitals, consists usually of about 95% of pure (absolute) alcohol. In this form, alcohol is *not* a good disinfectant. When, however, 95% alcohol is diluted with water to a concentration of 60–70% it becomes a useful germicide, although *its action is slow and uncertain, and it is less efficient than other agents.*

Seventy per cent alcohol is universally used on the hands and arms of surgeons and nurses as the final step in "scrubbing up" before an operation. It is also widely employed to disinfect a small area of the skin, especially to prepare a site for a hypodermic injection. A safer procedure is to paint the area first with mild tincture of iodine. Then, when the iodine has dried, rub it off with a sponge wet with 70% alcohol.

Like formalin, alcohol is also employed as a dehydrating and preserving fluid for anatomical specimens.

Acids and alkalies. All the mineral acids, like hydrochloric acid, are germicidal, but have a limited practical use as disinfectants. *Fuming nitric acid* is commonly applied to wounds caused by the bite of a mad dog, in an effort to kill the virus of rabies (hydrophobia) that may be there. *Boric acid* has a weak antiseptic action, and is often used as an eyewash. Hot boric-acid solutions are employed in wet packs applied to boils or similar lesions. (Nurses should take care not to confuse boric-acid solution with plain water. The drinking of any considerable amount by an infant may produce toxic symptoms, and even death.) Vinegar, which contains about 6% *acetic acid,* is germicidal.

Lye and other strong alkalies are germicidal, but naturally they are seldom used in that capacity in practical disinfection. In general, watery solutions having an alkalinity greater than about pH 9.0 will inhibit the growth of most microbes, and will destroy many of them outright.

Mercury compounds. *Bichloride of mercury* ($HgCl_2$), also called "corrosive sublimate," is a powerful germicide, under favorable circumstances. Despite its marked germ-killing power in test-tube experiments, however, this substance has a number of undesirable properties which distinctly limit its practical value as a disinfectant. It is a highly poisonous substance if taken internally. Bichloride of mercury solutions should be carefully labeled, and colored with a little methylene blue so that they cannot be mistaken for harmless liquids. This chemical is unreliable as a disinfectant for feces, sputum, or other albuminous materials. It is irritating to the skin. It is corrosive, and cannot be used for instruments or other metal objects. In the hospital, the use of bichloride of mercury is restricted almost entirely to the disinfection of glass and rubber articles, such as catheters and clinical thermometers. Solu-

tions with a strength of 1:2000 or 1:1000 are most commonly employed.

Because of the obvious limitations of the inorganic bichloride, numerous organic mercury compounds have been prepared. Among these are *mercurochrome* and *merthiolate*. Mercurochrome, despite a considerable popularity, is only a moderately strong antiseptic. Merthiolate has a greater germicidal power than phenol, and may be used as a skin disinfectant. It has found its greatest usefulness, however, as a preservative and safeguard against outside contamination when added in small amount (about 1 part in 10,000) to vaccines, antisera, and similar materials.

Silver compounds. Various compounds of silver are germicidal, and several are employed in medical practice. These compounds are of two kinds; soluble silver salts, like *silver nitrate* ($AgNO_3$), and silver-protein preparations, like *argyrol*.

For clinical use, these substances are dissolved in water in strengths varying from 0.01% to 20% or more, depending upon the intended use. It is now the practice, everywhere, to introduce into the conjunctival sac of *every* new-born infant a few drops of dilute silver nitrate solution (1 or 2%). The purpose of this procedure (Credé's method) is to prevent the possible development of gonorrheal ophthalmia, or other form of ophthalmia neonatorum. The installation of the silver solution should be followed by washing out of the eyes with physiological salt solution. This stops the action of the silver nitrate at once, since the silver is precipitated as insoluble and inert silver chloride.

Oxidizing agents. *Hydrogen peroxide* (H_2O_2) is a popular, but not very reliable, disinfectant of limited clinical usefulness. It gives up nascent oxygen readily, and in the process will destroy bacteria, provided it is in intimate contact with them. But when applied to a wound or to the mucous membranes, it is rapidly decomposed by reacting with the blood and other organic matter present. Although the consequent appearance of many bubbles suggests great activity, its practical effect as a germicide is often only slight. It may, however, have a useful cleansing action.

Dyes. A number of the dyes used for the staining of smears, and as indicators in microbiology, are germicidal or bacteriostatic, and some are employed in clinical practice. Prontosil, the forerunner of sulfanilamide, was a red dye.

Gentian violet, methylene blue, and other common dyes, are sometimes used as treatment for infected wounds, and for acute inflammations caused by cocci, or fungi (*Monilia*) in the mouth, urethra, or vagina. The therapeutic results are variable.

Generally regarded as superior to other dyes for clinical use are the acridine dyes *acriflavine,* and *proflavine.* The latter is said to be less irritating to tissues and a more reliable antiseptic. Proflavine sulphate was used successfully in World War II, in powder form, for dusting into badly infected wounds.

Soaps and the newer detergents. In general, the soaps are not to be relied upon as disinfectants. Even the so-called germicidal soaps do not in themselves kill bacteria much more readily than ordinary soaps. It is true that soap and water are important agents in practical disinfection. However, this is principally because washing and scrubbing remove and destroy many bacteria mechanically. The more delicate pathogenic organisms, such as those of meningitis, gonorrhea, and syphilis, will not survive on the skin after a thorough scrubbing with warm water and any good cleansing soap. But staphylococci, the germs of typhoid and dysentery, and some other bacteria may not be killed by even a prolonged exposure to soap solutions. The clinical thermometer is not sterilized by the soap solution on the thermometer tray, but only after it has been first cleansed by the soap, wiped dry, and then immersed for a sufficient time in bichloride of mercury or other disinfectant.

Recently a group of cleansing compounds or *detergents* characterized by an especially *low surface tension* have been synthesized. Among these are some that have much promise as disinfectants. Substances of this class are often referred to as "wetting agents." They markedly reduce the surface tension of fluids to which they are added, and so tend to wet all surfaces closely. Those that wet the fatty materials in the skin and mucous membranes are likely to penetrate these tissues readily. It has been found that a number of the cationic detergents (those compounds that yield on dissociation a positively charged radical [cation] representing the larger portion of the molecule) are strongly germicidal, both in test-tube tests and *in vivo* trials, and are apparently nonirritating to living tissues. Examples are the compounds known by the trade-names Zephiran and Phemerol.

Lipoid solvents. *Ether, chloroform, toluol, acetone, benzene, carbon tetrachloride,* and related fat solvents, represent a class of

substances not generally recognized as disinfectants. Several of these compounds, however, actually possess considerable bactericidal power, and some deserve practical use in medicine as germicides. Chloroform and toluol have long been employed as bacteriostatic agents to control bacterial contamination in meat suspensions, or other protein preparations undergoing enzymatic digestion, or for the preservation of samples of urine, blood serum, and the like. Ether and benzene are sometimes used on the skin, prior to scrubbing up for surgery, with the purpose of removing grease, but they have some disinfecting action as well.

Acetone is an excellent agent for disinfecting a small area of the intact skin. It is definitely superior to 70% alcohol, so widely used for that purpose. It is especially to be recommended for preparing the skin for inoculation with smallpox vaccine. Acetone also serves well in the laboratory for the sterilization of knives or other small instruments, and for the emergency sterilization of hypodermic syringes and needles.

Fasting has called attention to the value of *carbon tetrachloride* for the local treatment of ringworm and other skin infections. Also he has found this noninflammable substance (the principal ingredient of commercial dry-cleaning fluids, and the liquid present in common types of fire extinguishers) to be useful as a first-aid treatment for small burns.

Aerosols. Recently, sprays which send into the air very fine mists of bactericidal substances have been successfully used for the disinfection of the air in confined spaces. Such mists, made up of finely dispersed droplets, only about 1–2 microns in diameter, are called *aerosols*. Of the several different chemical substances that have been tried as bactericidal aerosols, propylene glycol and triethylene glycol are among the best. When sprayed into the atmosphere by an efficient atomizing and blowing apparatus, and when the temperature and humidity of the air are favorable, these substances remain suspended for long periods and act to free the air of bacteria, viruses, and other microbes, without injuring persons or objects.

CHEMOTHERAPY

Chemotherapy may be defined as *the treatment or prevention of infectious diseases by specific chemical agents.* It is the aim of chemotherapy to accomplish the destruction, or to inhibit the

growth, of the infecting microorganisms without causing marked or serious toxic effects in the host. A good chemical remedy will have a high "chemotherapeutic index," i.e., it will be one showing a considerable difference between: (1) the largest dose that can be tolerated without harm, and (2) the smallest dose necessary for cure. The ratio between these two factors is the important point:

$$\frac{\text{largest tolerated dose}}{\text{smallest curative dose}} = \text{chemotherapeutic index}$$

This, obviously, is not the whole story, and many other considerations finally determine the practical effectiveness and usefulness of any chemotherapeutic agent.

Older chemical remedies. The search for curative drugs for the germ diseases is very old, but until recent times it has met with only limited success. For a few diseases, however, good chemical remedies were discovered early in medical history. The use of *mercury compounds* for syphilis, for example, was introduced in the sixteenth century. The value of *quinine* (from cinchona bark) in the treatment of malaria was discovered by the Indians of Peru, and the drug was first used in Europe in the early seventeenth century. About the same time, *ipecac,* and somewhat later *emetine* (both derived from certain plants native to Brazil), were introduced for the treatment of amebic dysentery.

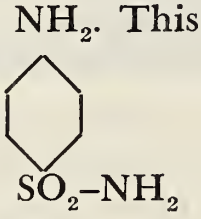
Modern chemotherapy may be said to have begun with the work of Paul Ehrlich (Fig 9). Ehrlich set out deliberately to synthesize, in the laboratory, chemical compounds which could be used safely and effectively in the treatment of trypanosomiasis, syphilis, and other diseases. An arsenical compound called *atoxyl* was shown by Thomas, in 1905, to be successful in controlling experimental trypanosome infection in mice. This work stimulated Ehrlich to undertake his epoch-making studies of the development of arsenical compounds for the treatment of syphilis, and led eventually (1909) to the preparation of *salvarsan* (arsphenamine). This was the famous compound "606," Ehrlich's "magic bullet." *Neosalvarsan* (neoarsphenamin) is a modified form of this important drug.

The sulfonamide drugs. For years after Ehrlich's day, there were few important advances in chemotherapy. New synthetic drugs for malaria—*plasmochin* and *atabrine*—were developed, and improvements were made in syphilis therapy, such as the use of bismuth compounds. But the prospect of finding chemotherapeutic agents for the common *bacterial* diseases seemed remote indeed as late as 1935.

In that year, a German pathologist and chemist, Domagk, published reports describing the therapeutic effect of *prontosil* and *neoprontosil* on experimental and clinical streptococcus infections. These observations changed the picture entirely, and opened up a new era in chemotherapy, promising great benefits to mankind. Prontosil was a red dye that had been synthesized and patented by chemists of the I. G. Farbenindustrie in 1932. Neoprontosil, a related soluble compound, was first described by Domagk in 1935. It was soon found by other workers that the prontosils break down within the body to para-amino-benzene-sulfonamide—the compound that came to be called *sulfanilamide*—and that this substance is as active by itself as the original prontosil.

Sulfanilamide, fortunately, was not patented, and, shortly after these discoveries, the manufacturing and testing of this material, as well as of numerous new derivatives, began on a large scale. In these early critical studies, which laid the foundation for the success now attained in the use of these products in treating many different diseases, English and American bacteriologists and pharmacologists took a leading part.

The formula of sulfanilamide is NH_2. This reveals the presence



of the characteristic SO_2 group. More widely used than sulfanilamide itself at the present time are several derivatives. Among the best known and most valuable are *sulfapyridine, sulfathiazole,* and *sulfadiazine.* These are spoken of collectively as the *sulfonamide drugs* or, less formally, as the *sulfa drugs.*

The sulfonamides have been found highly effective in the treatment of localized streptococcus infections, septicemias due to hemolytic streptococci or staphylococci, lobar pneumonia, and numerous other infectious processes. They are *not* effective in tuberculosis, or in spirochetal, virus, and rickettsial diseases. They are distinctly toxic for the human system, and absolutely should not be used by the public without professional guidance. But if the doses are kept within safe limits, carefully controlled by tests of the concentration of the sulfonamide reached in the blood stream, there is little danger.

An important complication that creates practical difficulties is

the tendency for various pathogenic bacteria to become resistant, i.e., to become *drug-fast* with respect to the sulfonamides. This tendency is particularly marked in gonorrhea, and has seriously interfered with effective treatment of that disease with sulfa drugs.

The action of all the sulfonamides is mainly bacteriostatic. This bacteriostatic power is virtually abolished in the presence of a sufficient amount of *para-amino-benzoic acid* (PABA). According to a widely accepted theory, based upon this effect of PABA, the sulfa drugs are thought to compete with PABA for combination with some bacterial enzyme that ordinarily uses the latter substance in some essential metabolic cell-reaction. The enzyme, not being able to distinguish between the needed PABA and the chemically similar, but harmful, sulfa drug reacts with the latter, unless an excess of PABA is present. When blood cultures are to be made from persons who are under intensive treatment with sulfa drugs it is now the practice to add a quantity of PABA to the medium, in order to counteract the bacteriostatic effect of the sulfonamides in the blood sample, and so to permit the organisms present to grow out.

Penicillin and other antibiotics. Antibiotics may be defined as *chemical substances produced by one microbe which are destructive or inhibitory to other microbes* (Fig. 61). Certain substances of this kind have been known from the earliest days of bacteriology. Little attention was given them, however, until recently. In 1939, Dubos isolated from a common species of sporeformer (*B. brevis*) an antibiotic agent which he called *tyrothricin*. This was later found to consist of two distinct substances, *gramicidin* and *tyrocidine*. The marked bacteriostatic activity of these products attracted much notice, and helped to revive interest in antibiotic agents in general.

But it was the still more recent demonstration of the extraordinary potentialities of *penicillin* in the chemotherapy of various infectious diseases that focused the attention of the medical world upon the antibiotics, and led to the intensive search for new substances of this kind which is still going on. The English bacteriologist Fleming, in 1929, first separated from a mold, *Penicillium notatum,* the substance he called penicillin, and made the original observations upon its antibacterial action. It was not until a decade later, however, that Florey and his associates at Oxford began a series of studies on penicillin that demonstrated its truly phenomenal

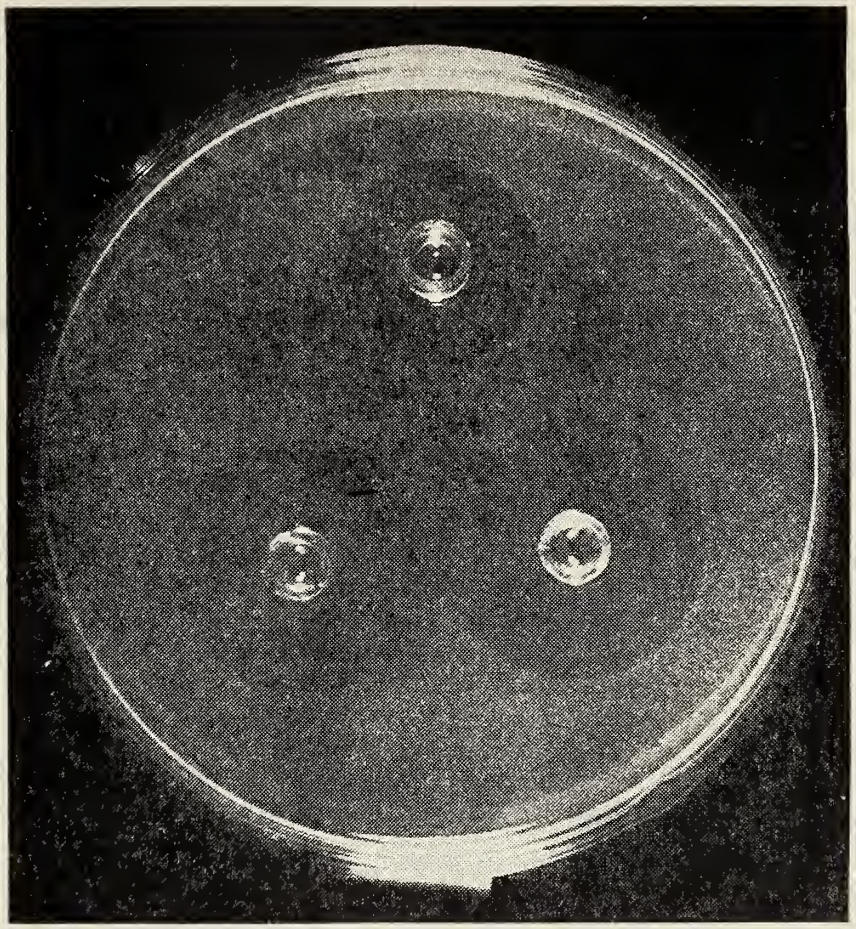


FIG. 61. The cup method for testing the antibacterial effect of streptomycin. The test organism (*Bacillus subtilis*) is mixed with the agar just before pouring into the Petri dish. After the agar has solidified, small glass cylinders are placed on the agar surface, as shown. Different dilutions of the solution of streptomycin to be tested are placed in the cylinders; then the plates are incubated for 12–16 hours. The bacilli grow throughout the agar except where inhibited by the antibiotic substance. The diameter of the zone of inhibition is measured, and compared with the zone produced by a streptomycin solution of standard potency. The activity of penicillin and other antibiotics may be measured in the same way. (From S. A. Waksman, *Microbial Antagonisms and Antibiotic Substances*, The Commonwealth Fund, New York, 1945.)

powers as a chemotherapeutic agent for human infectious diseases.

Since 1940, a score of other antibiotic substances have been isolated from microorganisms of various kinds. These differ greatly in chemical make-up, range and degree of antibacterial activity, and toxicity for animals. Their action is primarily bacteriostatic. The majority are effective against Gram-positive bacteria almost exclusively, although a few inhibit the growth of certain Gram-negative organisms. Some are active against fungi and acid-fast bacilli. Properties of the more important of these new antibacterial agents are summarized in Table XII.

It is safe to say that no one of the antibiotic agents will prove to be a panacea. Yet study of these substances is scarcely more than well started, and no one can say what triumphs over infection may be just around the corner. In any case, the prospects are good for an immense saving of human life and suffering through this new kind of chemotherapy.

Table XII. Properties of Some of the More Important Antibiotic Substances *

Aspergillic acid

Formed by: Aspergillus flavus.

Active against: Both Gram-positive and Gram-negative bacteria.

Comment: A nitrogenous compound soluble in water, alcohol, ether, and acetone. This substance is relatively toxic and of doubtful value *in vivo.*

Clavacin

Formed by: Actinomyces clavatus and several species of *Penicillium.*

Active against: Gram-negative and some Gram-positive bacteria, and fungi.

Comment: Highly bactericidal, but too toxic for clinical use.

Eumycin

Formed by: Certain strains of *Bacillus subtilis.*

Active against: Filamentous pathogenic fungi (e.g., *Trichophyton*) and higher bacteria, including *Mycobacterium tuberculosis* and *Corynebacterium diphtheriae.*

Comment: Soluble in neutral or alkaline water, alcohol, and acetone; precipitated by acid; heat stable; of low toxicity; potentially highly useful, but not sufficiently tested as yet.

Notatin (also called *penatin,* and *penicillin B*)

Formed by: The same molds that produce penicillin.

Active against: Gram-positive and Gram-negative bacteria, having a considerably wider range of activity than penicillin. The purer preparations have been found bacteriostatic to certain organisms in extremely high dilutions—for example, 1:400,000,000.

Comment: A water-soluble, oxidative enzyme, active in the presence of glucose; its activity is neutralized by catalase. Apparently no more toxic than penicillin.

Penicillin

Formed by: Penicillium notatum and *Penicillium chrysogenum.*

* Summarized principally from Waksman, S. A.: *Microbial Antagonisms and Antibiotic Substances.* New York: The Commonwealth Fund, 1945.

Active against: Streptococci, pneumococci, staphylococci, gas-gangrene bacilli, and various other Gram-positive organisms; also gonococci, meningococci, and some other Gram-negative bacteria, and the spirochetes of syphilis, relapsing fever, etc.

Comment: Very low toxicity for human and animal tissues, and highly effective in treatment of many common infections. Soluble in water and alcohol; thermolabile. Destroyed by gastric juices. Not of value in virus diseases, or in infections caused by Gram-negative bacilli.

Recent refinements in methods of production have resulted in preparation of several penicillins (called Penicillin G, X, etc.), which differ somewhat in effectiveness against different pathogenic organisms.

Certain resistant bacteria form an enzyme (*penicillinase*) which destroys penicillin. Penicillinase may be added to the culture medium when blood is to be cultured from a patient under treatment with penicillin, in order to destroy any active penicillin in the blood sample, and so to permit the growth of any organisms that may be present.

Pyocyanase

Formed by: Pseudomonas aeruginosa.

Active against: Many Gram-positive and Gram-negative bacteria.

Comment: The active principle is a thermostable lipoid present in culture filtrates; these filtrates were formerly used in local treatment of diphtheria, etc. In mixed cultures, *Pseudomonas* often overgrows the other bacteria present. This is largely explained by the presence of pyocyanase, together with the chloroform-soluble blue pigment *pyocyanin,* which is also bactericidal.

Streptomycin

Formed by: Actinomyces griseus.

Active against: Gram-negative bacteria especially.

Comment: Of value principally in treatment of tularemia, *H. influenzae* infections, and urinary tract infections due to Gram-negative bacteria, it may prove helpful in tuberculosis also. A promising antibiotic, but organisms develop resistance to it very rapidly.

Streptothricin

Formed by: Actinomyces lavendulae.

Active against: Various Gram-negative and some Gram-positive bacteria.

Comment: A water-soluble thermostable compound of low toxicity; active *in vivo.*

Subtilin

Formed by: Bacillus subtilis.

Active against: Gram-positive bacteria, including *Mycobacterium tuberculosis.*

Comment: Soluble in alcohol; heat stable; stable in acid but not in strong alkali; active *in vivo.*

Tyrothricin

Formed by: Bacillus brevis.

Active against: Gram-positive bacteria.

Comment: Relatively low toxicity; has been used to treat mastitis in cattle, and as a topical application in certain human infections. Contains two distinct antibiotic substances: (1) *Gramicidin,* a thermolabile polypeptide soluble in ether and alcohol, active only against Gram-positive organisms, and (2) *Tyrocidine,* a thermostable polypeptide soluble in alcohol, but not in ether, which causes lysis of both Gram-positive and Gram-negative bacteria.

REFERENCES

American Medical Association, Council on Pharmacy and Chemistry: "Criteria for Evaluation of Skin Disinfectants," *J.A.M.A.,* Feb. 20, 1943, 121:593.

Goodman, L. and Gilman, A.: *The Pharmacological Basis of Therapeutics.* New York: The Macmillan Company, 1941.

Green, T. W. and Birkeland, J. M.: "The Use of the Developing Chick Embryo as a Method of Testing the Antibacterial Effectiveness of Wound Disinfectants," *J. Infect. Dis.,* Jan.–Feb. 1944, 74:32.

Hoffmann, C. E. and Rahn, O.: "The Bactericidal and Bacteriostatic Action of Crystal Violet," *J. Bact.,* Feb. 1944, 47:177.

McCulloch, E. C.: *Disinfection and Sterilization.* Philadelphia: Lea and Febiger, 2nd Ed., 1945.

McIlwain, H.: "Theoretical Aspects of Bacterial Chemotherapy," *Biol. Rev.,* 1944, 19:135.

Mudd, S.: "Current Progress in Sterilization of Air," *Am. J. Pub. Health,* June 1944, 34:578.

Waksman, S. A.: *Microbial Antagonisms and Antibiotic Substances.* New York: The Commonwealth Fund, 1945.

REVIEW QUESTIONS–CHAPTER XVI

1. In what three general ways may disinfectants or antiseptics be employed? Discuss some popular misconceptions.
2. Name and discuss three factors which affect the germicidal action of chemical agents.
3. Define *phenol coefficient.* Outline the general procedure followed in performing a phenol-coefficient determination.
4. Mention some other kinds of disinfection tests. If a disinfectant were completely studied, what effects would it be likely to have at different concentrations?
5. Explain the practical use and value of: (1) phenol, (2) cresol and related compounds, and (3) formalin.
6. What practical uses have gaseous and liquid chlorine, chlorinated lime?

7. Explain the practical use and value of: (1) iodine, (2) alcohol, and (3) acids and alkalies.
8. How is bichloride of mercury used as a disinfectant? What undesirable properties limit its practical value? Name other mercury compounds used as antiseptics.
9. Name two compounds of silver that are widely used. Describe one important use of silver nitrate.
10. Name an oxidizing agent, and describe its use and value as a disinfectant.
11. Name four kinds of bacteriostatic dyes sometimes used in clinical practice.
12. Discuss the value of soaps and the newer detergents as disinfectants. What is a "wetting agent"?
13. Discuss the use and value of lipoid solvents, especially acetone and carbon tetrachloride, as germicides.
14. What are aerosols? Name two chemicals that have been used in the form of aerosols for the disinfection of the air in enclosed spaces.
15. Define *chemotherapy, chemotherapeutic index.* What is the aim of chemotherapy?
16. Name four chemical remedies known from early times. What famous synthetic drug was developed by Ehrlich?
17. What are the sulfonamide drugs? Discuss their practical usefulness. What theories are proposed to explain their mode of action?
18. Define *antibiotics.* Name, and characterize briefly, ten of the more important antibiotic agents.

CHAPTER XVII

PRACTICAL ASEPTIC TECHNIQUE IN SICKROOM AND OPERATING ROOM

While the practice of personal cleanliness to minimize chances of infection should be an everyday matter, the need for careful habits becomes acute in those who are called upon to care for a sick person, particularly if the patient suffers from an easily communicable infectious disease. The responsibility of avoiding infection is again especially great upon the doctors and nurses who take part in surgical operations.

Not every disease is caused primarily by germs, but there are few severe illnesses of any kind not complicated, at least, by an infection of some part of the body. In any case many phases of the routine care of all patients require the application of microbe-destroying methods.

Obviously, the use of these methods becomes especially important when a case of a disease caused primarily by microorganisms must be cared for, because *nearly all germ diseases are more or less readily communicable from one person to another.* The doctor and nurse who are called upon to attend such a case assume a grave responsibility. Their task is not merely to aid in the recovery of the patient, but also to *prevent the spread of the disease* to themselves or to others.

This means that they must carry out a rigorous *aseptic technique;* that is, they must practice those *methods by which germs are excluded and infection is prevented.* They must see to it (the nurse especially has this responsibility) that, so far as is humanly possible, all the dangerous microbes cast off from the body of the patient are destroyed before they have an opportunity to infect another susceptible person. They must also endeavor to prevent reinfection of the patient with the germs already injuring him, and try to avoid the entrance of other harmful microorganisms into his weakened body.

Principles of medical and surgical aseptic technique. The methods used for the care of patients with communicable diseases are comparable to those used to prevent infection in the operating room, but there are important practical differences.

In *surgical aseptic technique,* a distinction is made between objects that are *sterilized* (i.e., known to have been freed of all living microorganisms) and those that are *nonsterilized.* The latter may or may not be frankly contaminated. If an outsider enters an operating room, after it has been "set up" for an operation, and touches with the unscrubbed hands, or otherwise comes in contact with a sterilized object, that object then becomes no longer sterile. It must be resterilized if it is to be used in the operation.

In *medical aseptic technique,* on the other hand, distinction is made between objects that are *contaminated*—because they have been in contact with the patient, and thus may be conveyors of disease germs—and those that are *clean,* i.e., free from contamination, although not necessarily sterile. When any person enters the contaminated area in the immediate vicinity of a patient with a communicable disease, and touches anything in this zone, he himself becomes contaminated, and must disinfect his hands before leaving.

MEDICAL ASEPTIC TECHNIQUE IN THE SICKROOM

Isolation of cases of communicable diseases. *The contaminated unit.* To prevent the spread of germ diseases the first necessary step is the isolation of the patient. Around each patient a sharply defined zone of contamination, called the unit, is established. This may consist of a separate room, or, more commonly, the space enclosed within a glass-walled cubicle, partitioned off in the ward. It is not absolutely necessary that the bed be walled in but, in practice, separate rooms or cubicles are used whenever possible. It is more convenient to carry out isolation technique under those circumstances, and the presence of some kind of physical barrier serves as a reminder to the nurse and others that the patient is a source of danger. Thus the infected individual is cut off from direct contact with all other persons, except those who must necessarily attend him. Isolation means that the patient and his immediate surroundings become like the bacteriologist's culture tube containing powerful pathogenic bacteria. *The organisms must not*

be permitted to escape to cause harm elsewhere. Also, *germs from outside must not be permitted to enter.*

Control of air-borne infection. Some modern hospitals have installed ultraviolet-ray lamps over the doors of the cubicles or at other places marking the borders of contaminated zones, to serve as barriers against air-borne infection. These have been found effective if properly installed, and if the air is nearly dust-free and of suitable humidity.

General precautions in the care of the isolated patient. The medical aseptic technique used in the care of the isolated patient is essentially nothing more than the application of common sense to the problem of preventing the spread of the disease, and has its basis in definite knowledge of the ways of microbes. The actual methods of disinfection employed and the details of the isolation technique naturally differ in different hospitals, because more than one agent or method may be used to achieve the same result. Any of the methods must be modified, of course, to meet the actual conditions in a private home.

Cleanliness. Whether the patient is in the hospital or at home, cleanliness in the sickroom is a primary consideration. Truly clean things do not carry germs, and cleanliness really forms the very basis of disease prevention.

The sickroom should contain no unnecessary furnishings, so that everything in it can be truly cleaned. Dust must be removed, but it should not be stirred up into the air. Sweeping is best done with soft brushes, and after wet sawdust has been sprinkled on the floor. The dust rag should not be dry, but should have a little oil on it. There is little reason to fear room-dust as a carrier of disease germs. It is possible, however, that some pathogenic organisms, such as the tuberculosis germs or the spores of pathogenic molds or higher bacteria, might exist in the dust of a room in which a patient, who has been excreting these germs, has lived for a while. In some hospitals, treatment of floors with special oil preparations has been found valuable for reducing dust generally. At any rate, dust cannot be tolerated in the sickroom. The floor, the woodwork, and the bed frames may be scrubbed vigorously with hot water and soap. In addition, a disinfectant, such as cresol, or carbolic acid, may be applied to all surfaces.

The person of the patient himself must be kept as clean as possible. Prompt disposal of all soiled bedding, dressings, and dis-

charges of any sort will go a long way toward preventing reinfection of the patient and spread of the disease. In cases of intestinal disease, and in other cases where necessary, the *patient's hands* should be disinfected before eating. They may be scrubbed with soap and water, then held in a basin of 50% alcohol for two minutes, and dried on a sterile towel.

Food and drink for the patient must be protected from contamination. The nurse who is caring for a case of typhoid fever or dysentery should have nothing to do with the preparation of food for the patient or for his family. In the hospital, and in the home as well, cleanliness in the kitchen is of prime importance. Flies and vermin of all kinds must be kept out of food. Milk, vegetables, and other perishable foods must be protected from dirt and dust, handled with clean hands, and kept cold in a clean refrigerator.

Conduct of the doctor and nurse in the sickroom; gowns and masks. Obviously, the doctor and nurse in attendance upon an isolated patient must conduct themselves with care. Immediately upon entering the room, or before approaching the bed, the nurse and doctor put on long *gowns* which cover them from the neck to the feet. The gowns are always handled in such a manner that the outside surface (possibly contaminated) is not touched by the clean hands. The nurse may wear a special cap to cover her hair. If the case is one of influenza, pneumonia, or any one of the many other diseases of the respiratory organs in which the germs are present in the nose and mouth secretions, and are commonly transferred to others by coughing or sneezing, the nurse and doctor wear a gauze *mask* covering both mouth and nose (Fig. 62). Sometimes, when it is necessary to touch the patient, or to handle materials which might grossly contaminate the hands, rubber gloves are worn.

Visiting is usually forbidden, unless the patient is critically ill. In cubicled wards, visitors observe the patients through the closed glass partitions. If visitors are permitted to enter contaminated zones, they too must wear gowns and masks, and refrain from any direct contact with the patient.

When ready to leave the patient, the doctor or nurse first removes the rubber gloves, if these have been worn. The gloves are usually dropped into a basin containing a disinfectant, such as lysol solution or bichloride of mercury, or they may be boiled. Then the doctor removes his gown and mask, with the assistance of the nurse,

and proceeds to the nearest scrub stand to disinfect his hands. The nurse usually scrubs her hands before removing her gown. She then takes off the gown, with proper care to avoid touching the contaminated outer surface, returns it to its hanger beside the bed, then scrubs up a second time. Soiled gowns are commonly disinfected by soaking them for several hours in a solution of lysol or other disinfectant, or are sterilized by boiling or autoclaving. Gauze masks are usually disinfected with steam, laundered, then resterilized by autoclaving, for use again. The efficiency of these masks is *increased by repeated washing,* so long as the fabric remains intact.

Disinfection of the hands after contact with the patient. By far the most important of the steps in the technique just outlined is the disinfection of the hands. This step is indispensable, whether or not gloves have been worn, and whether or not the hands have knowingly been contaminated. The careful nurse will take pains not to contaminate her hands unnecessarily, but it will be practically impossible to avoid getting some germs on the fingers from the patient's body, or from some articles soiled by his excretions. The commonest method of germ transfer is by way of contaminated hands. It is therefore of the utmost importance that the nurse and doctor conscientiously disinfect their hands as soon as they leave the patient's bedside. Under no circumstances must they approach another patient until this has been done.

Scrubbing up. Methods and rules of hand disinfection after contact with an isolated patient vary in different hospitals. The safest method is to give the hands and forearms a vigorous washing and scrubbing with soap and water, for at least two minutes. Scrubbing should be carried out with a stiff brush, warm water (preferably running water), and soft soap, beginning with the wrists and backs of the hands, and giving special attention to a thorough cleaning about the nails. The scrubbing should not be so violent as to break the skin. No rings should be worn on the hands. After the washing, the hands may be placed for two or three minutes in lysol, 70% alcohol, or other disinfectant.

Where running water is not available, it is the usual practice to soak and rub the hands first in a disinfectant, then wash them in soapy water.

Sometimes the use of a disinfectant can safely be omitted, because the more delicate disease germs will be destroyed and removed by the scrubbing alone, but if the case is one of typhoid fever or other

intestinal disease, or one in which staphylococci or sporebearing bacilli are abundant, it is far better to follow the scrub-up with the use of a good chemical germicide. It must always be remembered that the careful washing cannot be omitted under any circumstances, and also that the mere dipping of the hands into a strong solution of disinfectant is of no value. The disinfectant solution should be diluted to the proper concentration, and the hands should be covered with it *for at least two minutes.*

When hand disinfection after every contact is carried out carefully, cases of measles, diphtheria, and other highly contagious diseases may be successfully cared for in the same ward, and the nurses may pass from bed to bed without carrying infection.

A certain amount of *cross-infection* (i.e., spread of infection from one patient to another, or from patient to doctor or nurse) *does* occur, however, even in the best-managed hospitals. It is now thought that this is due principally to transmission of infectious particles *through the air.* Where air-borne infection is effectively controlled, cross-infection is reduced practically to zero.

Concurrent disinfection of articles contaminated by the patient. Aside from hand disinfection, the most important feature of the isolation technique is the thorough disinfection of all articles used by the patient and all discharges from his body, continuously throughout the illness and as long after recovery as the patient is still carrying the disease germs. This is spoken of as *concurrent disinfection.*

Eating utensils. The isolated patient should have his own set of dishes and tableware, kept apart for his exclusive use. The dishes should be disinfected after use, by boiling in soapy water, and they should be rinsed finally in very hot water. Most hospitals will have a special dishwashing and sterilizing machine. The tray on which the food was served may be boiled or disinfected with a chemical solution. Remnants of the patient's food should be burned or thrown into a basin of disinfectant.

Bath water. The bath water should be disinfected before it is poured into the drain. An excess of chlorinated lime may be added and the mixture may be allowed to stand for an hour.

Bedclothes, bed linen, and other articles used by the patient. These must be disinfected as soon as possible after they become soiled. Every article that has been in contact with the isolated patient is considered contaminated, and must be disinfected before

it may be used for another purpose. The bedclothes (sheets, pillow cases, etc.), towels, napkins, and so on are gathered up by the nurse, wearing a gown, and taken directly to a waiting pan of water, which is then heated to boiling; or they are taken to a steam sterilizer; or they are put to soak in a dilute solution of carbolic acid, lysol, or other disinfectant. Only after they have thus been disinfected are these articles sent to the laundry, unless the laundry has special arrangements for the safe handling of contaminated materials. The disinfecting action of sunlight is often put to use by placing pillows, mattresses, and similar articles outdoors in the sunshine for several hours.

Rubber articles, such as hot-water bottles, ice caps, air-cushions, sheets, are usually soaked in bichloride of mercury or other disinfectant solution, then rinsed in clean water; or they may simply be washed thoroughly in soap and water. Rubber catheters are boiled after use. *Enameled basins,* pitchers, and pans are thoroughly cleaned in boiling soapy water, and may be autoclaved or allowed to stand in a disinfectant solution. *Mops* used to assist in cleaning bedpans and urinals are kept constantly, when not in use, in a pail of disinfectant. *Toilet articles* used by the patient are disinfected by boiling, if possible, or by immersion in a solution of a chemical germicide.

Clinical thermometers. The isolated patient has a separate clinical thermometer for his own use. In the wards, however, one thermometer may serve for several patients. In any case, the thermometer must be clean (uncontaminated) when placed in the patient's mouth, and after removal it must be disinfected before it is used again. In order to facilitate the handling of clinical thermometers, a "thermometer tray" is prepared in the hospital. On the tray there are glasses or tubes containing: (1) soap solution, (2) clean water, and (3) a disinfectant, such as 1:20 carbolic acid, 70% alcohol, or bichloride of mercury. A 1:2000 solution of bichloride of mercury is probably the best disinfectant for this purpose. On the tray, also, is a receptacle for clean cotton, another for soiled cotton. The thermometer is kept immersed in the disinfectant solution, and just before placing it in the mouth (or rectum, for rectal temperatures) it is wiped off with a bit of cotton wet with the clean (not necessarily sterile) water. After the thermometer has been removed from the mouth or rectum, and the temperature recorded, it is carefully and thoroughly wiped with a piece of cotton wet

with the soap solution, in order to remove all adherent mucus. *This step is essential, because the germicidal action of the disinfectant is interfered with unless the thermometer is properly wiped.* But it must be remembered, also, that *the thermometer is not disinfected merely by wiping with soapy water, nor even by allowing it to stand in the soap solution. It must be placed, after wiping, in the disinfectant, and must be permitted to remain there for a few minutes before it is used for another patient.* Three minutes in 1:2000 bichloride of mercury solution is sufficient.

Concurrent disinfection of discharges from the patient. All the things in contact with the isolated patient which have been mentioned above—the bed linen, dishes, and so forth—must be disinfected for just one reason, namely, *they are liable to be contaminated with some of the discharges from the mouth, nose, bladder, bowels, wounds, or open sores of the patient. These excreta —nasal discharge, sputum, urine, feces, pus—are the truly dangerous things.* Some one or more of them (according to the disease) contain the disease germs, usually in great numbers, and at the very height of their disease-producing power. The aim of concurrent disinfection is to destroy these germs at the bedside, before they can carry disease to other persons.

Discharges from the nose should be received on gauze or cloths, which may be collected in a paper bag, and the bag and contents burned. Handkerchiefs may be soaked in solutions of lysol, cresol, carbolic acid, or chlorinated lime, or thoroughly boiled.

Sputum must be collected and disinfected with the greatest care. Probably the best method is to collect the sputum in paper cups, which may be placed in paper bags, and then burned. Sometimes an enameled cup with a cover which can be raised with the thumb is used, or a wide-mouthed bottle. The cup or bottle should contain about 10 cc of 5% carbolic acid solution or a 10% formalin solution. This amount of chemical cannot be relied upon to disinfect the sputum completely, however, and the vessel and its contents must be disinfected by autoclaving, or by soaking for several hours in an excess of a disinfectant. The sputum of patients with advanced tuberculosis of the lungs is especially dangerous, because there is likely to be a great deal of it, and the patient, and often the nurse and doctor as well, become indifferent to its danger. Tuberculous sputum should never be carelessly handled, and every drop of it should be conscientiously disinfected.

Feces and urine from patients with typhoid fever and other intestinal dieases must always be disinfected. Great thoroughness and care are

required. The disinfection of *feces* is best carried out in the bedpan. Lysol, formalin, milk of lime, or carbolic acid, in an amount at least twice the volume of the feces, should be added and the fecal masses broken up and thoroughly mixed with the solution. The mixture should stand at least two hours before the pan is emptied. The empty pan may be disinfected by pouring in about a quart of disinfectant, allowing it to remain there for an hour or two, or by placing the entire pan in boiling water or in a steam sterilizer. *Urine* may be disinfected by allowing it to stand for a time with an excess of chlorinated lime. *Vomitus* may be disinfected with chemicals, or burning.

Pus from wounds and open sores is best collected on pieces of gauze, which may be dropped into paper bags, then burned.

Terminal disinfection. After his removal, disinfection of the bed and room occupied by the isolated patient (*terminal disinfection*) is not nearly as important as the concurrent disinfection just described, which was practiced while the patient was there. *Persons, not things, are the most important sources of infection,* and when the infected person is removed, most of the danger is gone. If cleanliness has been insisted upon, and concurrent disinfection properly carried out during the illness, the bed and the room can easily be made ready for use again.

Fumigation of rooms and contents with formaldehyde gas after use by a case of a contagious disease was formerly practiced widely everywhere. But now it is not often done, for it has been found that fumigation is troublesome, expensive, and often ineffective; and, furthermore, it is unnecessary.

The simplest and best procedure is give the room and contents a thorough scubbing with hot soapsuds, followed by wiping of the bed, furniture, doorknobs, and other surfaces with a dilute disinfectant. In a private home repainting and repapering the room may seem desirable, but it is not at all necessary. The room should be thoroughly *sunned and aired.* The rugs, books, pillows, blankets, and mattress should be exposed directly to the sun out-of-doors. If the mattress is badly soiled, it may be necessary to take it apart, wash and sterilize the filling, and put it together again, or the entire mattress may be sterilized in a large autoclave.

SURGICAL ASEPTIC TECHNIQUE IN THE OPERATING ROOM

The most conspicuous feature of modern surgical practice is the scrupulous care taken to avoid infection of the operative wound.

Every surgeon endeavors to carry out in minutest detail a strict *aseptic technique.* If he operates in a sterile field, he tries to keep it sterile. If he operates in a field already infected, as he is often obliged to, he tries to stop the infection and to prevent it from spreading beyond the wound. Similarly, the obstetrician takes the greatest care to avoid infection of the uterus at childbirth.

The vital importance of avoiding infection at operation and at childbirth can scarcely be overstated. Many operations, especially those in which the head or the abdomen is opened, are certain to lead to death if pathogenic organisms enter the operative wound. Hence, the perfection of the aseptic technique at operation is as important for the welfare of the patient as the skill of the surgeon.

Nurses must understand this aseptic technique thoroughly and learn to practice it conscientiously, because they are directly responsible for it, and everything depends upon how intelligently and faithfully they meet this responsibility.

Surgery before the use of germ-excluding methods. We of a more fortunate and enlightened generation can scarcely appreciate the horrors of the older time, when, to the hazards of an operation itself, no matter how trivial, was added grave danger from the infection that inevitably followed. In those days, before the introduction of antiseptic methods by Lister (in 1867), practically every wound, whether accidental or deliberately made by a surgeon, became a running sore, discharging quantities of pus. This was regarded by the best surgeons of the day as inevitable, and even necessary for the healing of the wound. The ill-smelling "matter" from wounds was so highly regarded that it was referred to as "laudable pus."

It seems a wonder that any patients recovered from an operation, or survived an extensive accidental wound. Some of the more fortunate did recover, but, as a rule, only after weeks of pain and fever. Many other patients did not fare so well, and the end was more commonly tragic than happy. The majority of wounds of all sorts became infected with powerful disease-producing germs, and the patients developed *erysipelas* (a streptococcus infection of the surrounding skin), or *tetanus* (lockjaw), or "blood poisoning" (a generalized infection of the entire body). Many wounds were invaded by the worst disease of all, a horrible gangrene which caused the flesh to blacken and putrefy. The latter infection was so common in hospitals seventy or eighty years ago, especially in the overcrowded, ill-ventilated unsanitary surgical wards of charity hospitals, that it was known in every country as *hospital gangrene.* The gangrene

became epidemic and spread from patient to patient. It is said that the wards reeked with the nauseating smell of rotting flesh, and frequently resounded with the piteous cries of the dying.

In those days, infection of the uterus and blood of mothers at childbirth was very common, and often fatal. In many hospitals, as many as one half or even two thirds of the mothers did not live to nurse their babies.

It must be remembered that this was before microbes were well known, and before their part in disease was understood. Surgeons stood helpless before this mysterious and malignant foe, which so often made useless their best efforts. They were able to perform only a limited variety of operations with any hope of the patient's survival. Amputations were common, especially after compound fractures, for it was felt that there was no other way to save the patient's life, although amputations themselves were frequently fatal.

Joseph Lister and his antiseptic technique. In a previous chapter, it has been recited how the great English surgeon, Joseph Lister, pointed the way out. Lister was the first surgeon to disinfect his hands, instruments, and dressings, and the first to demonstrate that infection of wounds can be prevented in this way.

The methods used by Lister himself to avoid infection at operation were very simple. He disinfected his hands, his instruments, ligatures, sponges, and dressings in carbolic-acid solutions. At one time, in his earlier experiments, he was so impressed with the danger of infection from bacteria falling into the wound from the air that he employed a spray of carbolic acid about the wound during the operation. Later, however, the spray was abandoned as unnecessary.

Not uncommonly, Lister performed operations in private homes. No special preparations were made beforehand, except the provision of trays and basins of carbolic-acid solution, 1:20 and 1:40, which Lister often brought to the house himself. On his arrival, the instruments, which had been cleaned but not sterilized since the last operation, were placed in a tray of 1:20 carbolic acid, where they remained for some twenty or thirty minutes before the operation began. The sponges used to wipe out the wound during operation (marine sponges were used exclusively at that time) were soaked in the 1:40 lotion. When everything was conveniently arranged, the patient was brought in. While the anesthetic was being administered, the site of the operation, which had not been especially

cleansed, was merely wiped with the 1:20 carbolic-acid solution. Lister then "took off his coat, turned up his shirt sleeves, pinned an ordinary unsterilized huckaback towel over his waistcoat (for his own protection, not for that of the patient) and washed his hands in 1:20 lotion, or even what he called 'the strong lotion' (1:20 carbolic acid in 1:500 sublimate—bichloride of mercury—lotion), an ordeal that few of his adherents could endure. . . . He wore no mask or gloves." * Towels wrung out in carbolic-acid solution were arranged about the field of operation. During the operation, the hands of every one concerned were frequently dipped into the carbolic lotions, and the sponges constantly conveyed small amounts of the disinfectant to the wound. Bleeding blood vessels were tied with catgut sterilized merely by soaking for a half hour in a 1:20 solution. The wound was dressed with a thick gauze pad, the part next the wound being saturated with carbolic acid.

Hospital operations were performed in the same way, under conditions of cleanliness no better than would be found in any decent home. Crude as this technique may seem to us, it must be remembered that it was a great advance over the habits of other surgeons of that day.

Surgical aseptic technique of today. What Lister accomplished with his crude method is now assured by a much more elaborate technique and a somewhat different plan. Now the use of chemical disinfectants is restricted as much as possible, and infection of the wound is avoided by the use of instruments, dressings, etc., which have been *sterilized by heat.*

In a modern operation, the surgeon is assisted usually by several other persons, other doctors and nurses, each of whom has a definite task to perform during the course of the operation (Fig. 62). The surgeon depends upon all his assistants, but *especially upon the nurses,* for the perfection of the aseptic technique. They must be constantly alert to avoid contamination of instruments and supplies which have been sterilized. There must be undivided attention to the task, in order that even the slightest mistake may be detected. The nurse should develop an acute "aseptic conscience" which will not permit her to overlook any break in technique, even though no one but herself is aware of it.

Preparations of the surgeons and nurses. Before an operation, the surgeon and all his assistants "scrub up"—that is, they *disinfect the*

* Godlee, Sir Rickman: *Lord Lister,* New York: The Macmillan Co., 1917, p. 460.

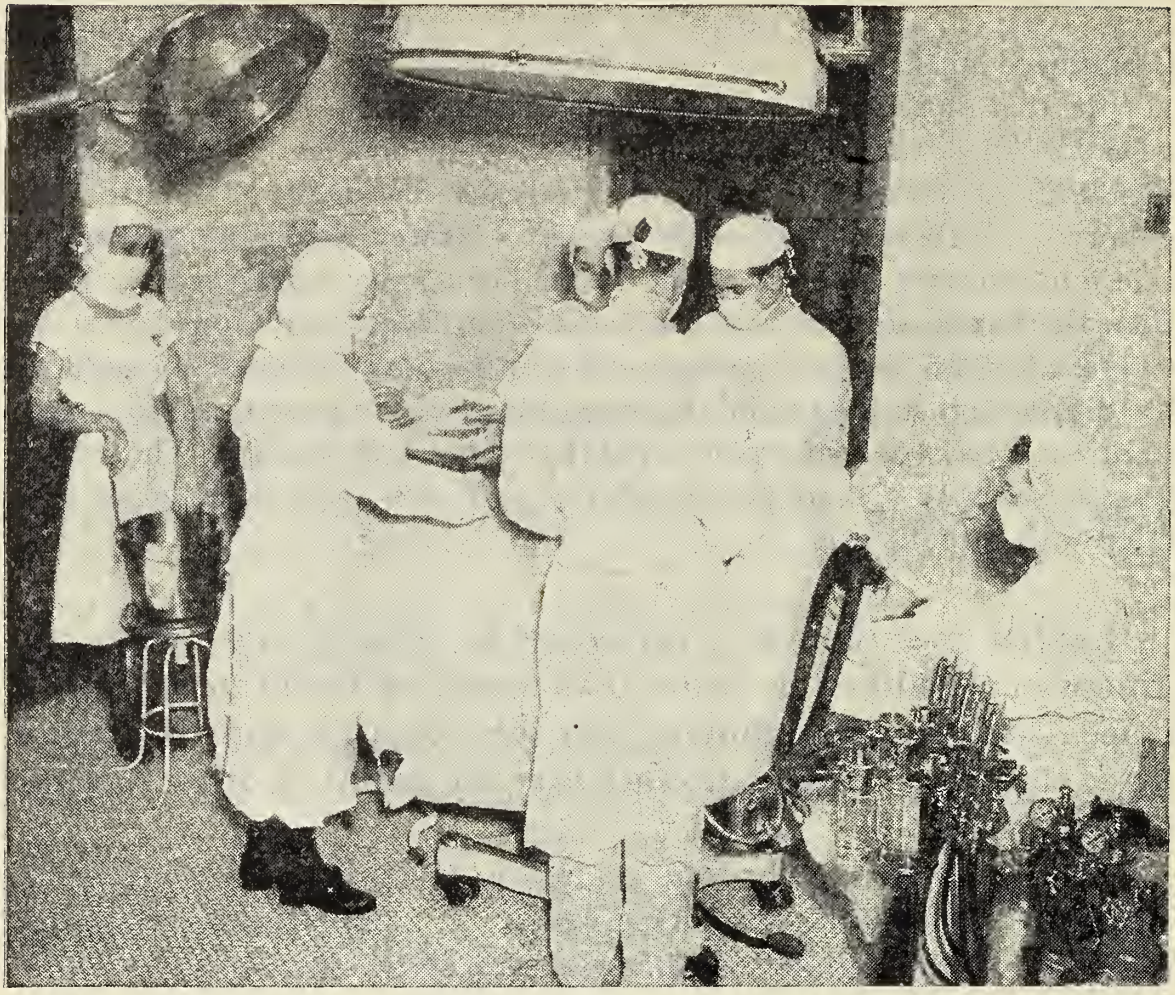


FIG. 62. Aseptic technique in the operating room. Note the gowns, gloves, caps and masks worn by the surgeon and his assistants, and the use of sterile drapes to isolate the operative field. (Photographed by Dr. J. G. Morris, Jr., at Memorial Hospital, Houston, Texas.)

hands and forearms by a vigorous scrubbing followed by the use of a chemical disinfectant. This must be carried out even though rubber gloves are to be worn. This hand disinfection is not expected to make the skin sterile, but it is intended to render the hands as free of microbes as possible. The method is essentially the same as that used after contact with an isolated patient, only more prolonged and even more careful.

In some hospitals, the surgeon first dips the tips of his fingers in half-strength (3.5%) tincture of iodine, and allows the iodine to dry. Then he scrubs his hands and forearms with a stiff sterile brush, using liquid soap and warm running water, for five minutes. He thoroughly cleans his nails with a sterile nail file. After the scrubbing, he rinses his hands and forearms in running water, allowing the water to drain off at the elbows. Then he immerses his hands

in 70% alcohol for two or three minutes. He raises his hands, to allow the alcohol to drip off at the elbows, then dries them on a sterile towel. There is no short-cut to this process of hand-disinfection; the prolonged and thorough scrubbing is essential and, likewise, the exposure to alcohol cannot safely be omitted.

A sterile *gown* is next put on with precautions not to touch the outside surface. Nurses put a *cap over the hair.* A sterile *gauze mask* is adjusted to cover the mouth and nostrils.

The hands are dusted with sterile powder, then thrust into *sterilized rubber gloves.* Each pair is prepared previously, by placing the gloves in a folded towel or special muslin bag and sterilizing the package in the autoclave.

Preparation of the patient. The *field of operation,* that is, the patient's skin at the place where the incision is to be made, is usually shaved the evening before the operation is scheduled. On the morning of the operation, this area is scrubbed with soap and water and rinsed with 70% alcohol. Immediately before operation, the area is disinfected in one of several ways, depending upon the particular site and the preference of the surgeon. Two applications of mild tincture of iodine (3.5%) followed, after the iodine has dried, with 70% alcohol constitute a common method. Another agent frequently used is picric acid (5% solution in 95% alcohol). This is applied in two or more coats, immediately before operating.

Sterile towels are placed about the operating field, and sterile sheets are draped about the patient and the operating table, so that the actual area of skin in which the operative wound will be made is isolated as much as possible, and protected from contamination.

Sterilization of instruments and other necessary materials. The *autoclave* is widely used for the sterilization of most instruments and supplies. Sometimes instruments are sterilized by *boiling* in water for ten minutes. A little bicarbonate of soda is usually added to the water. The instruments must be *completely immersed* in the boiling water; otherwise they will not be sterilized. Also, it is assumed that the instruments are *clean* to begin with, and thus carry no bacterial spores. (Soiled instruments, likely to be contaminated with spores, cannot be surely sterilized by boiling; they should be autoclaved.) Clean knives, scissors, and other sharp instruments are usually boiled for a short period—about three to five minutes. Very sharp, delicate knives are often sterilized by immersion for several minutes in a germicidal solution. Seventy per cent alcohol is widely

used for this purpose, but better disinfectants, such as formalin solution or acetone, are to be preferred. A reliable method, but one seldom employed, is to sterilize clean, dry, cutting instruments in the dry-heat oven.

Hypodermic syringes, when clean, are often sterilized by boiling for five minutes. The barrel and plunger should be separated during the boiling, and a little cotton should be placed in the pan to prevent breaking of the parts by bumping. Syringes may also be sterilized in the autoclave. The parts are first enclosed separately in gauze, then wrapped in paper or in a towel. In an emergency, hypodermic syringes may be sterilized by drawing acetone in and out several times. The barrel and plunger must then be warmed slightly over a flame (without touching any object) until the acetone is all evaporated and the glass is entirely dry.

Hypodermic needles demand great care. They should be washed and dried as soon as possible after use, so that they will not rust. They should be sharpened frequently. Like syringes, needles may be sterilized by boiling; or dry, clean needles may be placed, point down, in a small test tube with a bit of cotton at the bottom. The plugged tube may then be sterilized either in the autoclave or in the dry-heat oven.

Ligatures made of catgut or other material, and used for tying off blood vessels, and *sutures* of silk thread or other substances used for sewing up the wound, are usually purchased already sterilized from the manufacturer. Sometimes they come in vials of alcohol in which they have been boiled. Sometimes the threads come wrapped in paraffin paper, and have been sterilized in a dry-heat oven. Some kinds of suture materials are boiled just before use.

Since catgut is made from the intestines of sheep, it is unavoidably contaminated with intestinal organisms, including sporeforming bacteria, and it must therefore be sterilized in some manner before use. Too much heat will destroy the desirable physical qualities of catgut, however, and, in the past, unsterile catgut not infrequently appeared on the market. At present, catgut sold in the United States must pass sterility tests imposed by the Pure Food and Drugs Act.

Sponges, dressings, towels, and similar materials are all sterilized in the autoclave. The "sponges" now used are made of folds of absorbent gauze.

Dressing-room technique. As much care is taken in the dressing room as in the operating room to avoid infection. The doctor and

nurse usually wear gowns and sterilized rubber gloves. The soiled dressings are removed carefully, and promptly disposed of. New sterile dressings, drains, etc., are handled with sterile forceps, and are protected from contamination with the most meticulous care.

REFERENCES

CHRISTOPHER, F.: *Textbook of Surgery*. Philadelphia: W. B. Saunders Co., 4th Ed., 1945.

Control of Communicable Disease. New York: Am. Pub. Health Assn., 6th Ed., 1945.

CUTLER, E. C.: "Sterilization and Aseptic Operating-Room Technique," *Surg., Gyn., & Ob.*, Nov. 1940, 71:414.

HARRIES, E. H. R. and MITMAN, M.: *Clinical Practice in Infectious Diseases*. Baltimore: Williams & Wilkins, 1944.

HASENJAEGER, E.: *Asepsis in Communicable Disease Nursing*. Philadelphia: J. B. Lippincott Co., 2nd Ed., 1944.

MELENEY, F. L.: "Postoperative Infections," *Internat. Abstr. of Surg.*, Nov. 1940, 71:403.

TOP, F. H.: *Handbook of Communicable Diseases*. St. Louis: C. V. Mosby Co., 1941.

REVIEW QUESTIONS—CHAPTER XVII

1. Define *aseptic technique*. Explain why this technique is necessary in the care of the isolated patient.
2. Contrast *surgical aseptic technique* and *medical aseptic technique*.
3. What is meant by *isolation* of the patient? Must the patient necessarily be surrounded by a wall of some sort? What can be done about airborne infection?
4. Discuss the importance of cleanliness in the sickroom, and tell how it can be secured.
5. Describe the proper dress and conduct of attendants in the sickroom of an isolated patient.
6. Why is it necessary to disinfect the hands after contact with the isolated patient? Describe a practical method of hand-disinfection after such a contact. What is "cross-infection"?
7. What is meant by *concurrent disinfection*?
8. Discuss precautions necessary to protect food and drink from contamination.
9. Give a practical method, with all essential details, for the disinfection of: (1) eating utensils, (2) patient's hands, (3) bath water, (4) bedclothes, bed linen, etc., (5) rubber articles, (6) enameled ware, (7) patient's toilet articles.
10. Explain the necessary steps for the disinfection of clinical thermometers in routine use.

11. Explain why it is necessary to carry out concurrent disinfection of the articles mentioned in Question 9. Explain the importance of disinfecting discharges from the body of the patient.
12. Give a practical method for the safe collection and disinfection of the following: (1) discharges from the nose, (2) sputum, (3) feces and urine, (4) pus.
13. What is meant by *terminal disinfection*? What is its importance as compared with concurrent disinfection? What procedures are necessary to prepare a bed and room for use after occupancy by a case of communicable disease?
14. Describe conditions prior to the introduction of aseptic methods into surgery and obstetrics. Review the work of Joseph Lister, and its results.
15. In general, how has Lister's technique been modified in present-day practice? Discuss the importance of the part played by the nurse in modern aseptic surgery.
16. Describe a practical method for the disinfection of the hands in preparation for an operation. How are the surgeon and his assistants dressed? Why do they wear masks? Why rubber gloves? How are the gloves sterilized?
17. How is the patient's skin at the site of the operation disinfected? What measures are taken to isolate the field of operation from possible contamination?
18. Give practical methods, with all essential details, for the sterilization of: (1) surgical instruments, (2) hypodermic syringes and needles, (3) sponges, dressings, and similar materials.

CHAPTER XVIII

SOURCES OF INFECTION: THE SPREAD OF DISEASE THROUGH PERSONAL CONTACT

Older ideas versus modern knowledge. It is only in comparatively recent years that we have come to understand how we "catch" disease, and how disease germs are transferred from one individual to another. Widespread epidemics of cholera, typhoid fever, smallpox, and other serious diseases were familiar enough a hundred years ago, but their origin and manner of spread were not understood. Most of the theories advanced to explain these epidemics were based upon the belief that some evil influence was exerted by the surroundings in which people lived. Epidemics were said to be caused by some unusual configuration of the stars, by earthquake, or volcanic eruption, or by a peculiarity of the weather. The causes of disease were thought to reside in something *external* to the bodies of men.

Even after the work of the pioneer bacteriologists, during the last quarter of the nineteenth century, had demonstrated that most of our common diseases are caused by microscopic germs, there was still a great deal of misunderstanding as to the way these germs reach our bodies and pass from person to person. It was commonly believed, for example, that disease was spread through poisoned air. Indeed, there are many persons even today who fear night air and "sewer gas."

When it was shown, about sixty years ago, that cholera and other intestinal diseases may follow the drinking of water polluted with waste matter from the human intestines, the attention of all sanitarians and medical men was attracted to *filth* in general as a source of disease. It became a widespread notion that filth of any kind was the most important cause of illness. The efforts of all early public-health officers were directed toward cleaning up the environment. Much good was accomplished by this work, for at that time

people lived in a much less cleanly manner than they do now. A marked reduction in cases of typhoid fever, and other intestinal diseases, followed wherever general sanitary conditions were improved. But merely cleaning up the rubbish and filth did not prevent the spread of measles, or influenza, or many other communicable diseases. We now understand the reason for this. *The germs of these diseases do not live in filth.* The important fact brought out by modern studies is that *the germs which cause communicable diseases have no natural existence outside of the bodies of men, animals, or insect carriers. Nearly all disease germs die quickly when cast off from the bodies of their natural hosts.*

It is true that the germs of typhoid fever, cholera, and dysentery may exist in contaminated water or food for a few days, and the germs of tuberculosis, scarlet fever, and a few other diseases may live in milk, but these are exceptions to the general rule. Whenever disease does arise from the use of contaminated food or water, the germs have always been introduced *a short time before* from the discharges of some person or animal.

The true sources of infection. The important point to remember is that most of the disease germs are so closely adapted to life in the bodies of living men or animals that they can exist only for brief periods on any external object. They do not travel through the air, except to a limited extent within closed spaces. They do not remain alive and virulent for long periods on clothing, baggage, furniture, and similar articles. On the contrary, *germs remain closely associated with infected individuals.*

The real source of infection lies in the secretions and excretions from the persons or animals harboring the germs. The feces, urine, sputum, nasal discharge, saliva, or droplets expelled from the mouth and nose contain the organisms, and these are the truly dangerous things. It is through actual contact with these body discharges that germs pass from one individual to another. External objects used by infected persons act only incidentally as bearers of pathogenic organisms when freshly contaminated with germ-laden excretions.

Modern methods of preventing the spread of communicable diseases are based upon these conceptions and upon an intimate knowledge of the germs concerned. A pure water and milk supply, adequate disposal of sewage, and, in general, a clean environment are recognized as essential, but the greatest emphasis is now placed

upon the discovery, isolation, and treatment of the *persons* who are carrying germs, and upon the teaching of personal hygiene.

Persons who carry germs. Individuals harboring germs of specific diseases, eliminating them from their bodies, and therefore acting as sources of infection for others, include: (1) *persons who have a typical case of a disease,* (2) *persons who have a mild, atypical, unrecognized case,* and (3) *persons who harbor and disseminate germs without having symptoms of disease*—that is, the so-called *carriers.*

Typical cases. Persons who are suffering from a typical case of a communicable disease are obviously a source of infection to other individuals who come in contact with them. However, the typical case of any of the common diseases is usually promptly recognized and *isolated,* so that the infection is not likely to spread to any great extent.

Unrecognized cases. Persons whose illness is so mild or so unusual that its true nature is not recognized are much more likely to be responsible for the occurrence of new cases. Mild and atypical cases of many of the common communicable diseases occur frequently. In epidemics of dysentery, scarlet fever, poliomyelitis, and other communicable diseases, there are numerous individuals infected with virulent organisms whose symptoms, nevertheless, are so slight, or so unusual, that a proper diagnosis is never made, and their cases never come to the attention of physicians or public-health authorities. Contact with such persons accounts for many of the typical cases which develop in more susceptible individuals.

Carriers. The healthy carrier constitutes a still more important focus from which infection may spread. In many of the common infections—for example, scarlet fever and typhoid fever—there is a marked tendency for the causative organisms to persist in the body of the infected individual for some time after all the symptoms of the disease have disappeared. Many cases of scarlet fever may be traced to contact with such *convalescent carriers* who had recovered from their own attack of the disease more than a month before. Some investigations have indicated that about 2% of those who recover from typhoid fever still excrete typhoid bacilli occasionally in the feces or urine for many months after they are entirely well, and that in some instances they remain carriers for the rest of their lives.

Another class of carriers includes persons who harbor virulent

germs but have not themselves developed the illness at any time. Usually such a healthy carrier is totally ignorant of the fact that he is a source of infection for others. He mingles freely with his associates, and may be the innocent cause of many cases of disease. He is himself immune.

Importance of carriers as sources of infection. Obviously, the existence of numerous healthy carriers of disease germs greatly increases the opportunities for the spread of infection. The discovery of carriers, and their control, are important and difficult problems in connection with the prevention of diphtheria, scarlet fever, typhoid fever, epidemic meningitis, and other communicable diseases. New cases originate most commonly from contact with carriers, rather than with other cases. This is especially true in times of epidemic, when many individuals become temporary carriers.

Principal methods of germ transfer. The most common way in which communicable diseases are spread is through (1) *personal contact* with infected persons or carriers. This may be: (*a*) an *actual bodily contact,* or (*b*) an *indirect contact through contaminated hands,* or (*c*) *through the common use of articles soiled by discharges (fomites),* (*d*) *by droplet infection,* or (*e*) *by air-borne infection.* Other important methods of germ transfer are: (2) *infection through contaminated water,* (3) *infection through contaminated milk and other foods,* and (4) *insect-borne infection.* Of lesser importance are: (5) *prenatal or congenital infection,* and (6) *soil-borne infection.*

The last-mentioned modes of infection may be dismissed with a few words. The remainder will then be considered more extensively.

CONGENITAL INFECTION

By congenital infection is meant infection transmitted *from the mother to the fetus before birth,* so that the baby is born with the disease. Syphilis is often transmitted in this way, and rarely, other diseases. This is *not inherited* disease—that is, the causative organisms are not present in the human germ cells (which contain all we directly inherit)—but rather it is infection acquired during uterine life from the mother, by passage of the organisms through the placenta. In congenital syphilis the fetus may be killed by the infection, resulting in a stillbirth, or the baby may be born with more or less obvious stigmata of the disease.

SOIL-BORNE INFECTION

There is little reason to fear the dust and dirt as sources of infection, because we know that the germs of most communicable diseases do not long survive there. But there are a few instances in which the soil may be regarded as the origin of infectious disease. The germs of tetanus and gas gangrene are deposited on the earth in the intestinal discharges of men and animals, and their spores survive for long periods in the soil. When dirt containing these spores is introduced accidentally into a wound, tetanus or gas gangrene may result. In a similar manner, anthrax in animals is a soil-borne infection. When germ-laden excreta are deposited upon the soil, and drainage from this contaminated ground pollutes water supplies, causing typhoid fever, the soil might be regarded as a source of infection.

INFECTION THROUGH PERSONAL CONTACT

(*a*) **Actual bodily contact.** Obviously, a person may become *inoculated* with germs by coming into immediate contact with lesions in some other person or animal. Except for those diseases transmitted solely by the bite of insects, any of the communicable diseases might conceivably be contracted in this way. This very direct transfer of germs is the rule, however, in only a few diseases. The most important examples are the venereal diseases, *gonorrhea* and *syphilis*. The organisms of these infections are so delicate and so closely adapted to the living tissues that they have practically no existence outside of the body. Therefore, these diseases are rarely contracted except by actual contact with lesions containing the organisms.

The great majority of other infections pass from person to person less directly, and by some one of the methods of indirect contact described below.

(*b*) **Infection through contaminated hands.** First importance must be given to contaminated hands as the means by which disease germs are carried about. A vivid description of the part dirty hands may play in the spread of infection is given by Sedgwick in his report of an outbreak of typhoid fever in 1892, quoted by Chapin.*

* Chapin, C. V.: *Sources and Modes of Infection*. New York: John Wiley & Son, 2nd Ed., 1916, p. 169.

"The families are of that grade in which food always stands upon the table; meals are irregular except for those who must obey the factory bell. The children play awhile, then visit the privies, and with unwashed hands finger the food upon the table. Then they eat awhile and return to play. Or, changing the order of things, they play in the dirt and eat and run to the privy, then eat, play, and eat again, and this in various houses and various privies. For them, so long as they are friendly, all things are common—dirt, dinners and privies; and, to illustrate exactly how secondary infection may go on, I may describe in detail one case which I personally witnessed. A whole family (of six or more) was in one room. Four of them had the "fever." Two of these were children in the prodromal stage. A table stood by the window covered with food, prominent among which was a big piece of cake. . . . By and by one of the children having the fever withdrew to the privy, probably suffering with diarrhea, but soon returning, slouched over to the food, drove away some of the flies, and fingered the cake listlessly, finally breaking off a piece, but not eating it. Stirred by this example, another child slid from his seat in a half-stupid way, moved to the table, and, taking the same cake in both hands, bit off a piece and swallowed it. The first boy had not washed his hands, and if the second boy suffered from secondary infection I could not wonder at it."

Such ignorance and squalor are not found everywhere, of course, and such conditions are less common now than in 1892. Nevertheless, it must be true that the hands of the best of us are seldom really clean. Most individuals frequently bring their fingers to the nose or mouth. Then they deposit saliva and nasal discharges on the objects they handle. This must result in a continual swapping of bacteria with our associates, who finger the same objects, and who are likewise busily engaged in distributing saliva. *The hand-to-mouth, hand-to-nose transfer of germs is more common than is generally realized,* and in many instances no more mysterious method need be imagined to account for the spread of communicable diseases.

(*c*) **Infection through fomites (articles freshly soiled with discharges).** Next to fingers themselves as bearers of germs come inanimate objects, such as handkerchiefs, drinking cups, and the like, which have been *freshly* contaminated with secretions or excretions from an infected person or carrier. Such an object is called a *fomite.* Those articles in immediate contact with the patient or carrier and used by him, such as bed clothing, bed linen, tableware, handkerchiefs, towels, toilet articles, emesis basins, and other things used in the sickroom, and the candy and toys of children,

are most likely to be contaminated with pathogenic bacteria. We know that most of these germs can remain alive on such objects only for a short period, but it is easy to see how the common towel and drinking cup in public places, and the innumerable small objects used in common by members of the same household, might occasionally serve to transfer virulent organisms from one person to another.

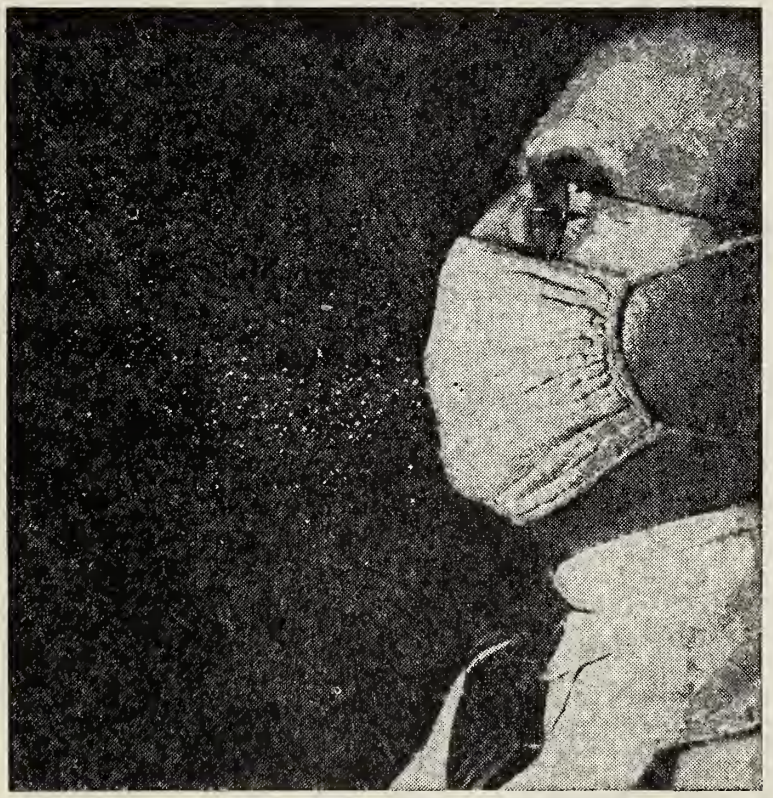


FIG. 63. A sneeze through a mask of a type commonly worn by surgeons and nurses. The picture was made by use of a special high-speed photographic technique, with an exposure of only 1/30,000 of a second. Droplets expelled from the mouth by the sneeze evidently passed through the mask, although most of them were held back, as can be seen by comparison with the heavy cloud of germ-laden particles sent into the air by an unstifled sneeze (Fig. 64). It is evident, however, that even a mask as well made as this one (which is thicker than most) may not prevent droplet infection altogether. (From Jennison, M. W.: "Atomizing of Mouth and Nose Secretions into the Air as Revealed by High-speed Photography," *Aerobiology,* American Association for the Advancement of Science, Washington, D. C., 1942.)

Among children, especially, freshly contaminated articles probably are important means of spreading germs. Children put all manner of things instinctively into their mouth, and they do not stop to inquire who was mouthing the thing last. In this way, children who play together are continually swapping saliva, and a cold or similar infection in one child is quickly passed to the other

children. Youngsters naturally have no regard for personal cleanliness; this is something that has to be taught. Very young children do not seem to be able truly to appreciate an object until they have gotten at least a corner of it into their mouths. The habit of carrying the fingers to the mouth and nose, and of putting pins, pencils, and all kinds of other small objects into the mouth, begins very early, and the practice of a stricter personal hygiene, which we know to be essential to prevent infection, begins, if at all, rather late in the lives of most individuals. This largely explains why communicable diseases transmitted by contact with nose and mouth secretions still occur so frequently, despite an abundance of knowledge about them.

(*d*) **Droplet infection.** When a person is infected by inhaling droplets of germ-laden moisture expelled from the mouth or nose of another person, the method of germ transfer is referred to as *droplet infection.* This is another common means of spreading germs. A sneeze or a cough sends a spray of moisture into the air, every droplet of which may be loaded with virulent organisms dislodged from the inflamed lungs, bronchi, throat, mouth, or nose (Figs. 63, 64). Even in ordinary talking, we occasionally expel a fine spray of droplets, especially in pronouncing forcefully words beginning with such consonants as *f* and *p*. A person standing a few feet away can scarcely avoid taking some of these droplets into his own nose or mouth with the breath. In this way the germs of diphtheria, influenza, measles, and all the other organisms which may be present in saliva or nasal discharges are readily passed from person to person, especially when individuals are brought into close contact in crowded living quarters, street cars, schools, and the like.

The wearing of a gauze mask over the nose and mouth by hospital attendants, while caring for a patient with influenza or other respiratory disease, is commonly practiced as a precaution against droplet infection. Masks are worn, also, in the children's wards and nursery, primarily for the protection of the patients. The surgeons and nurses in the operating room are masked to prevent droplets from contaminating the operative field.

(*e*) **Air-borne infection.** The germ-laden droplets just mentioned above are, of course, of varying sizes. The larger ones will tend to settle out rather quickly under the influence of gravity. The droplets of smaller size, however, may remain suspended in

the air for considerable periods and, as they evaporate, they leave a residue, or nucleus, of fine dust-like matter which may contain still viable pathogenic organisms. In recent years, Wells and others have brought forward strong evidence showing that these so-called *droplet nuclei* are important in the transmission of infection.

These particles are readily dispersed over wide areas by small currents of air, and persons at a distance may become infected by inhaling them. Of course, the ability of different kinds of disease germs to survive on the droplet nuclei will vary; also, survival will be markedly influenced by the physical state of the atmosphere, and such factors as the presence or absence of sunlight. Experimentally, it has been shown that various bacteria—for example, streptococci, pneumococci, and diphtheria bacilli—and also viruses, such as the influenza virus, may survive for twenty-four hours or more in the air of closed rooms, and that animals may become infected simply by breathing in such contaminated atmospheres.

Thus, it is now well established that *air-borne infection* in enclosed spaces, through infectious droplet nuclei, is to be reckoned with as a common means of germ transfer. It apparently explains most of the cross-infections that occur in the contagious wards of hospitals; seems to contribute to the origin of operating room infections; and must play a large part in the spread of disease among children in the schoolroom, and among the general population wherever people are crowded together. The need for effective disinfection of the air and for dust-control offers a new challenge to sanitary engineers.

Importance of contact infection. Almost everyone has learned through actual experience that disease may be contracted by personal contact with the sick. From what has been said about carriers, the student will understand that an infection may also be acquired by contact with a healthy person. It is further important to realize that it is by personal contact (through contaminated hands, freshly soiled objects, and droplet or air-borne infection) that the great majority of infections of all kinds (except insect-borne diseases) are transmitted. Epidemics of typhoid fever and other intestinal diseases, caused by contaminated milk or water, are so spectacular that we tend to forget how important a rôle *contact infection* plays in the spread of the very same diseases. As a matter of fact, in intestinal infections, as well as in measles, influenza, meningitis, and

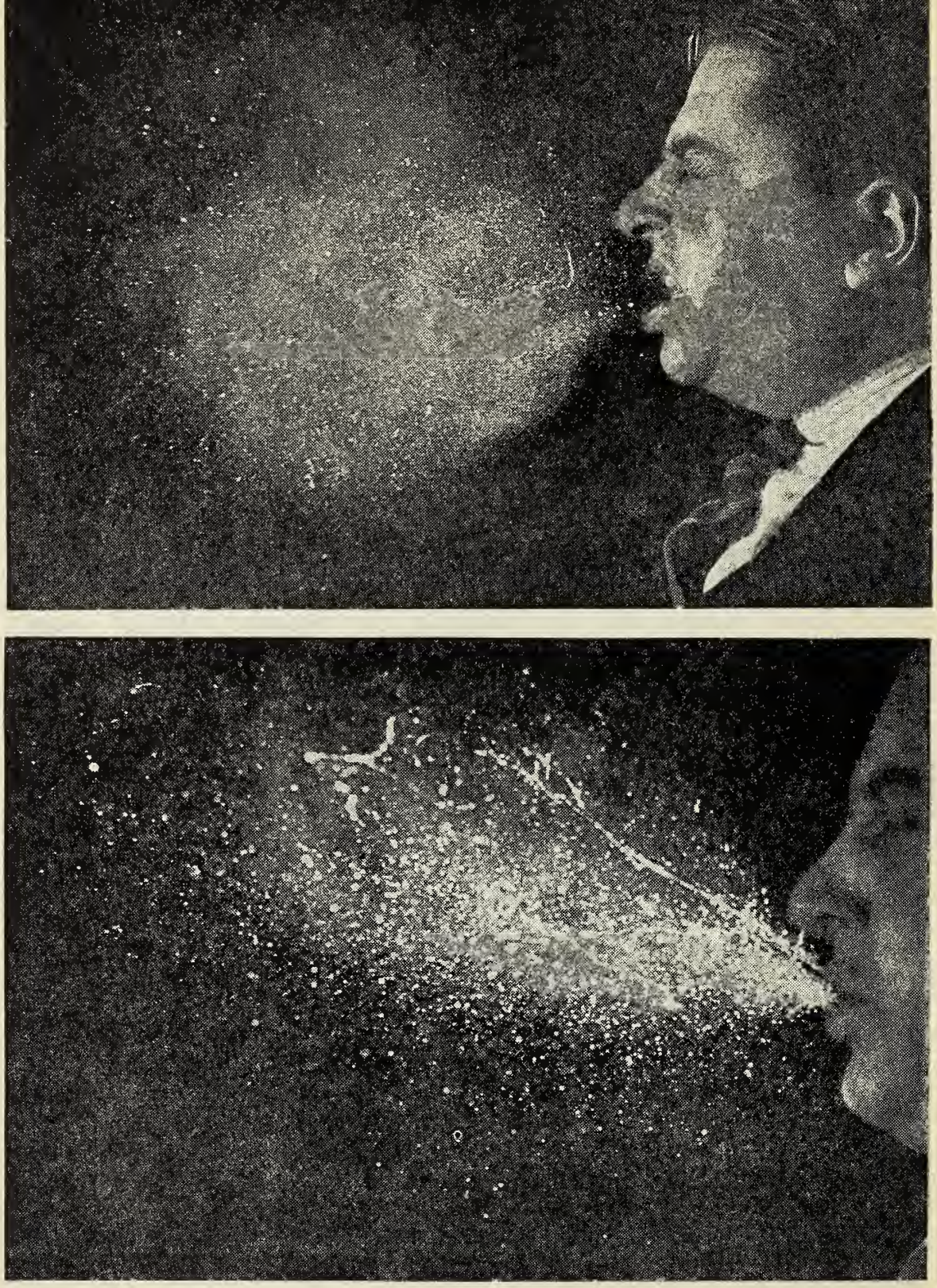


Fig. 64. Pictures made by high-speed photography showing the atomization of droplets into the air during a sneeze. *Upper photograph*: a violent, unstifled sneeze. Note that most of the material comes from the mouth, rather than the nostrils. *Lower photograph*: an unstifled sneeze of a person with a head cold. Note the larger-sized droplets and the strings of mucus. These photographs make clear why germs in the upper respiratory tract are so easily spread from person to person through sneezing or coughing. (From Jennison, M. W.: "Atomizing of Mouth and Nose Secretions into the Air as Revealed by High-speed Photography," *Aerobiology*, American Association for the Advancement of Science, Washington, D. C., 1942.)

other common diseases, the methods of germ transfer discussed above play the principal part.

Numerous instances might be given of epidemics in which the causative organisms were transmitted solely through personal contact. Naturally such outbreaks are likely to be most frequent in groups of persons crowded together in schools, institutions, or army camps, and so it is not surprising to find that diseases like measles, diphtheria, and epidemic meningitis are most common among school children and soldiers in camp. In a *hospital* there is a community of persons brought into unusually close contact, and the danger of the spread of infection by personal contact from patient to nurse, or from nurse to patient, is very real. In the days before aseptic methods were used, hospital attendants often innocently carried infection from patient to patient, usually by way of contaminated hands, and frequently themselves contracted serious illnesses. Now contact infection within the hospital is prevented by cleanliness and the conscientious practice of aseptic technique.

Personal hygiene versus public sanitation in the control of communicable diseases. It will be clear from the foregoing discussion that the maintenance of our individual health, and incidentally the public health, depends in large part upon how well we practice personal cleanliness, and personal hygiene in general. The measures taken by public authorities to purify the water supply, safeguard the purity of milk and other foods, dispose of sewage and other wastes, destroy insects, improve housing, and otherwise to promote sanitation are also important—indeed they are indispensable—but they have a more limited effect in directly preventing communicable diseases. Unfortunately it is not true, as many laymen are prone to believe, that if only sanitary facilities could be perfected everywhere, most of our ills would vanish. No amount of public sanitation has any direct effect upon the prevalence of smallpox, or influenza, for example, or upon the great host of other diseases commonly spread by personal contact. It took the human family a long time to learn the saving virtue of personal cleanliness, but now every nurse, doctor, and health worker seeks to promote by every possible means the practice of personal hygiene, physical and mental, in all its aspects, as the most direct way of preventing disease.

REFERENCES

Aerobiology. Lancaster, Pa.: Science Press Printing Co., 1942.

Boyd, M. F.: *Preventive Medicine*. Philadelphia: W. B. Saunders Co., 7th Ed., 1945.

Edward, D. G.: "Hemolytic Streptococci in the Dust of Hospital Wards, and Their Relationship to Infection," *J. Hyg.*, 1944, 43:256.

Jennison, M. W. and Turner, C. E.: "The Origin of Droplet and Air-Borne Infections," *Trained Nurse and Hosp. Rev.*, Mar. 1941, 106:186.

Perkins, J. E.: "Evaluation of Methods to Control Air-Borne Infections," *Am. J. Pub. Health*, Sept. 1945, 35:891.

Rooks, R., Cralley, L. J. and Barnes, M. E.: "Hospital Masks: Their Bacterial Filtering Efficiency and Resistance to Air Flow," *Pub. Health Rep.*, July 11, 1941, 56:1411.

Rosenau, M. J.: *Preventive Medicine and Hygiene*. New York: D. Appleton-Century Co., 6th Ed., 1935.

Smith, Geddes: *Plague On Us*. New York: The Commonwealth Fund, 1941.

REVIEW QUESTIONS—CHAPTER XVIII

1. Contrast older ideas with present knowledge concerning the sources of infection and the spread of disease.
2. Why is it that a pure water and milk supply and good sanitary conditions are not sufficient, in themselves, to prevent the spread of all communicable diseases?
3. State concisely what are the true sources of infection.
4. What three classes of persons carry germs? Explain the danger of carriers and of unrecognized cases as sources of infection.
5. Name seven common methods of germ transfer, and indicate which are the more important.
6. What is meant by congenital infection? Name one disease often transmitted in this way.
7. What importance has the soil as a source of infection?
8. What are four forms of personal contact by which germs may be spread? Give examples of diseases transmitted by these means. Define *fomite, droplet infection.* Discuss the importance of personal contact as a method of germ transfer.
9. Explain how infection may be air-borne in enclosed spaces. What are "droplet nuclei"?
10. Compare personal hygiene and public sanitation as factors in controlling communicable diseases.

CHAPTER XIX

INFECTION THROUGH CONTAMINATED WATER, MILK, AND OTHER FOODS INSECT-BORNE INFECTION

WATER-BORNE INFECTION

Water is found on the earth in the form of: (1) *rain water,* (2) *ground water,* and (3) *surface water.*

Rain water contains few bacteria, and if the cistern or other container in which it is caught is clean, it is quite safe to drink. A cistern should be so constructed that neither surface water nor ground water can get into it.

Ground water in the *deeper layers* of the earth contains no dangerous germs and few bacteria of any kind, because the organisms have been filtered out as the water passed downward through the soil. When this deep ground water is brought to the surface by means of drilled artesian wells, an excellent supply of pure water is usually obtained. Natural springs which come from a considerable depth in the earth may also contain pure water. Both wells and springs, however, must be protected from pollution at the surface of the ground.

The shallow, dug well is dangerous. Unless it is properly constructed, it is liable to be polluted with waste matter from a near-by privy or farmyard (Fig. 65).

It should be remembered that shallow springs may be as dangerous as shallow wells, and for the same reasons.

Surface water, in the form of streams, ponds, or lakes, will naturally contain many harmless bacteria from the soil. In addition, a stream or lake in the neighborhood of a populous community is almost certain to be polluted with intestinal bacteria and contaminated with the germs of intestinal diseases. This results from the almost universal practice of dumping sewage into the nearest body

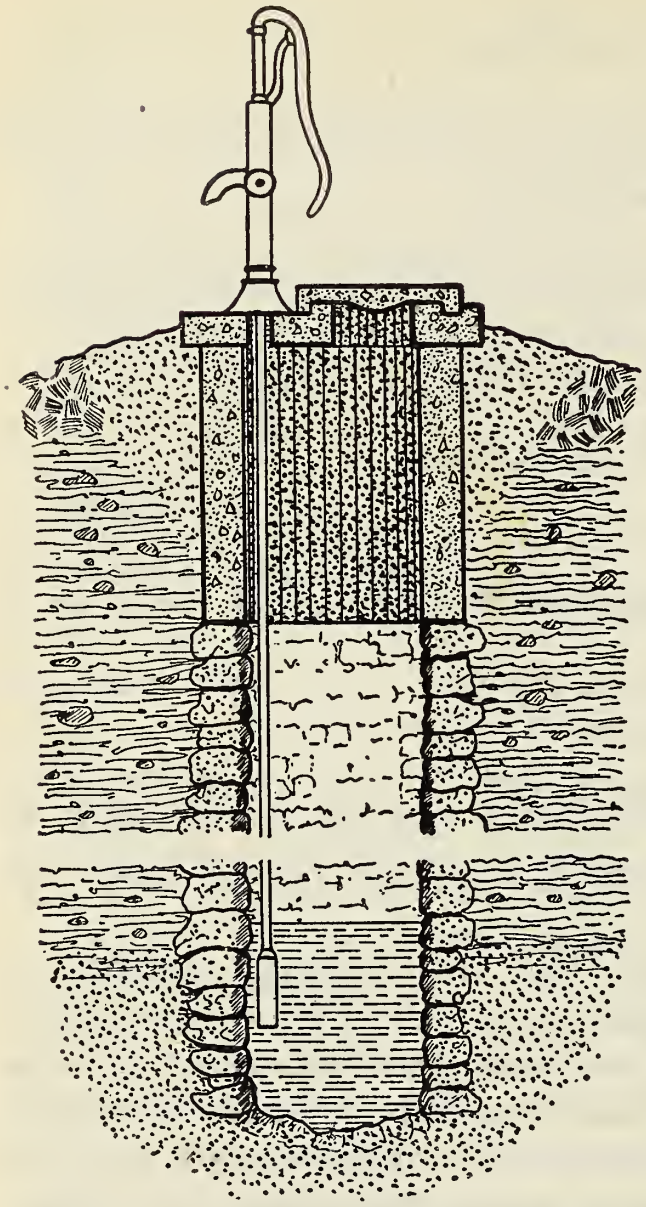


FIG. 65. A safe shallow well. The land drains away from the well. The base of the pump makes a water-tight connection with the concrete platform. The concrete construction of the upper part of the well, and the sand around it, protect against contamination from the surface of the ground and from seepage through the upper layers of the soil. The only water that can get into such a well comes from the deeper layers of water-bearing sand or gravel.

of water. It is often necessary for a community to obtain its water supply from the same river or lake which receives the sewage of its city or neighboring cities. It is, therefore, only in rare instances that surface water can be used as a source of public water supply without employing some method of artificial purification.

Diseases contracted from contaminated water. The kinds of illness which may be acquired directly from contaminated water include not only diseases of the alimentary tract but other types of infection as well. The specific bacterial diseases which may be water-borne are *cholera, typhoid fever, paratyphoid fever,* and *dysentery. Weil's disease* (infectious jaundice) may be contracted by swallowing water containing the causative spirochetes. Perhaps *poliomyelitis* should be added to this list.

The life of disease germs in water is short, and they probably never multiply there. They are likely to remain alive longer in water containing a considerable amount of organic matter, and in water that is cool. Typhoid bacilli will live in polluted water for about one week. When epidemics are caused by contaminated water, most

It must be remembered that the appearance of the water to the naked eye does not give any reliable indication of its sanitary quality. A sparkling, clear water may nevertheless contain the germs of disease.

The *microscopic examination,* reveals the numbers and kinds of algae, protozoa, and other larger forms of microscopic life, and the nature of amorphous matter in the water. This examination often helps to explain objectionable odors and tastes which are due to the presence of large numbers of certain kinds of algae or protozoa. Copper sulphate is added to the water in reservoirs to destroy these algae.

The *physical and chemical examination* includes the determination of such qualities as the color, turbidity, odor, temperature, and hardness, the content of free ammonia, nitrates, nitrites, and chlorine, and tests for poisonous minerals, such as lead. The presence of certain nitrogenous compounds in abundance suggests recent pollution of the water with sewage.

Bacteriological examination of water. This includes: (1) *a quantitative analysis,* or *determination of the total count,* that is, *the number of bacteria per cubic centimeter,* and (2) *a qualitative analysis,* or *tests for organisms of the coliform group, whose presence indicates intestinal pollution.* In all this work, the *standard methods of water analysis* worked out by the American Public Health Association and the American Water Works Association are closely followed.

Quantitative analysis. The total count. The general method of counting bacteria as outlined on page 173, is followed in determining the number of bacteria in water. A series of agar or gelatin plates is made from 1 cc or a portion of dilutions of the water samples. These plates are incubated for 24 hours at 37° C or 48 hours at room temperature. Then, with the aid of a magnifying lens, a count is made of the colonies which have developed. Each colony is assumed to represent a single organism in the original sample. The approximate *number of living bacteria in every cubic centimeter of the original sample* (*total count*) is arrived at by taking the average of the colony counts on all the plates.

The total count is of very little significance in judging the sanitary quality of water. It is not so much the number as the kind of bacteria present that is important in water analysis. The main question is, are organisms of intestinal origin to be found in the water? This question can be answered only through the qualitative analysis outlined below.

Qualitative analysis. Escherichia coli as the index of pollution. Qualitative analysis consists in tests for the presence of *Escherichia coli* and

related organisms of the colon-aerogenes (coliform) group. These bacteria are normal inhabitants of the intestine of man and animals, and if they are abundant in a water sample, this is strong evidence that intestinal wastes have polluted the water. *No attempt is made* in routine water analysis *to demonstrate typhoid bacilli* or other disease germs in the water. It is very hard to isolate them, even when the water is known to be contaminated, and for practical purposes it is not necessary, since the presence of considerable numbers of the more easily cultivated colon organisms is sufficient proof that the water is unsafe to drink.

Tests for the coliform bacilli are conducted in three stages: (1) the *presumptive test,* (2) the *confirmed test,* and (3) the *completed test.*

The *presumptive test* consists in the inoculation of *lactose broth fermentation tubes* with different amounts of the water sample, usually 0.1 cc, 1 cc, and 10 cc. If the closed arm of the fermentation tube contains gas, after 24 hours of incubation, this constitutes a *positive presumptive test.* The absence of gas-formation after 48 hours of incubation is a *negative test.* Appearance of gas on 48 hours of incubation, but not after 24 hours, is a *doubtful test,* and the presence of members of the colon-aerogenes group must be confirmed.

The *confirmed test* requires transfers to be made from the original lactose broth tubes in which gas has formed, either to certain specified liquid media—such as lactose broth containing brilliant green and bile—or to plates of Endo agar medium or eosin-methylene blue medium.

The formation of gas within 48 hours at 37° C in the special broth media, or the appearance on the streaked plates of colonies typical of those formed by known strains of *Escherichia coli* on these media, confirms the presumption that the gas-production in the original lactose fermentation tubes was due to the presence of bacilli of the colon-aerogenes group.

The *completed test* is accomplished when pure cultures, isolated from colon-like colonies on the plates, are shown to contain Gram-negative, non-sporebearing bacilli, which form gas in lactose broth.

By using measured amounts of the water sample for the original inoculation of the fermentation tubes, it is possible to arrive at an *estimate* of the number of colon bacilli per cubic centimenter. Thus, if 0.1 cc, and larger amounts, give a positive presumptive test (gas in lactose broth in 24 hours), it may be assumed that there are 10 colon bacilli to every cubic centimeter of the sample.

No attempt is made in routine water analysis to distinguish between *Escherichia coli* of intestinal origin and its close cousin *Aerobacter aerogenes,* which is much less likely to be of fecal origin.

Qualities of a good water supply. A good water supply will be *sufficient in quantity* so that it may be freely used by all through-

out the year. The water will be *free from all pollution with human or animal excreta,* and, therefore, *free from the germs of intestinal disease.* This necessitates the artificial purification of the water in most communities.

In addition, good water will possess certain desirable physical and chemical properties which will tend to encourage its use for drinking and other purposes. It will be cold, clear, colorless, odorless, and have an agreeable taste. It will not be too hard, and it will be free of lead and other poisonous chemicals.

Artificial purification of public water supplies. Artificial purification may include the following processes: (1) *aeration, clarification, and sedimentation,* (2) *filtration,* and (3) *chlorination.*

The water filters in use are of two kinds: (1) *the slow sand filters* and (2) *the rapid or mechanical sand filters.*

Slow sand filters are employed where the raw water is free from much turbidity, and preliminary clarification is not required. The filters consist of large, shallow, watertight basins containing a bed of filtering material. This is made up of sand and gravel of different degrees of fineness, beginning with coarse gravel at the bottom and finishing at the top with fine sand (Fig. 66). The water is allowed to gravitate slowly downward through this bed of sand, and the filtered, purified water is collected in drains beneath the filter.

The process of filtration is not a simple straining. Its efficiency depends upon the formation of a gelatinous scum over the grains of fine sand in the upper layers of the filter. As the water slowly passes through, bacteria are caught in the scum as in a trap. Harmless saprophytic organisms become established in this sticky matter over the sand, and effectively destroy parasitic bacteria that may be in the water. All pathogenic organisms and about 99% of all bacteria are removed.

Rapid or mechanical sand filters are used where the water is originally muddy and colored, and where chemical coagulants are necessary. The filter bed is very much the same as in the slow sand filter. But *alum* is added to the water just before filtration, and the heavy, flocculent, jelly-like precipitate (aluminum hydroxide), which then forms, settles as a layer over the surface of the sand and serves instead of the naturally developed scum on the slow sand filter. The water is passed through the filter rapidly, and in this case the process is really a mechanical straining. These filters remove from 95 to 99% of all bacteria.

Chlorination. Although both the slow and the mechanical sand filters will yield a safe water when properly operated, there is always a possibility of failure. It has become the practice, therefore, in most communi-

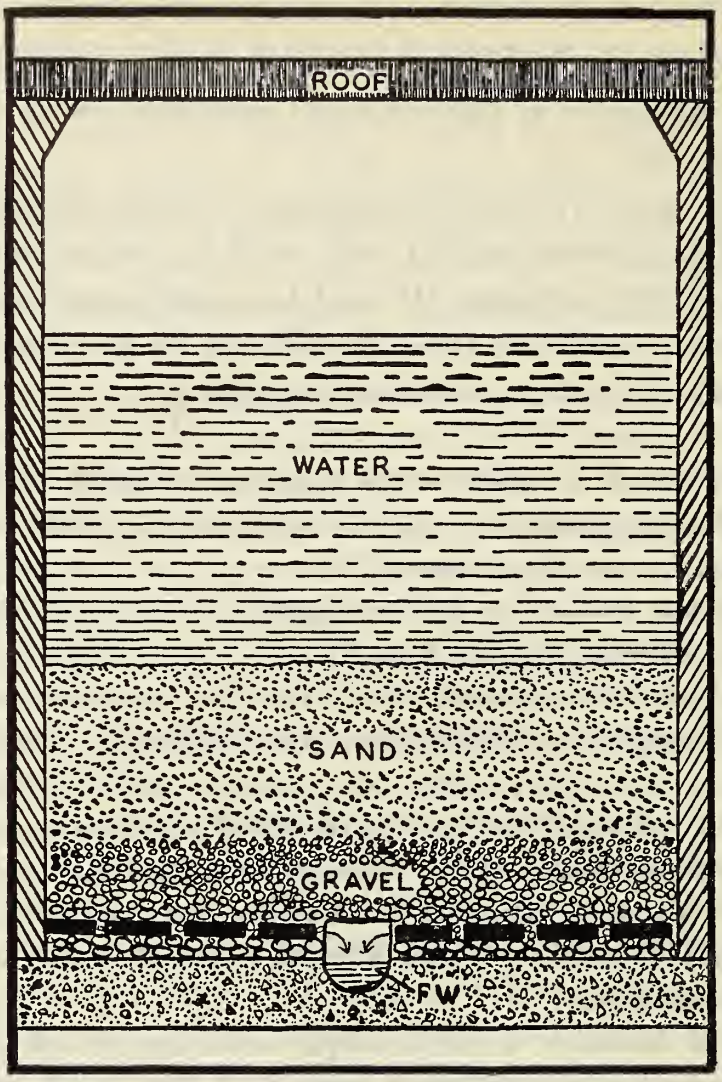


FIG. 66. A cross section of a slow sand waterfilter. The roof is necessary in colder climates to prevent freezing of the water in winter. FW: filtered water conduit.

ties, to take the further precaution of adding a chemical disinfectant to the water after filtration, and just before it is distributed to the mains. *Chlorine,* in liquid or gaseous form, is the agent used. The amounts employed vary from 0.3 to 1.0 part of chlorine per million parts of water.

MILK-BORNE INFECTION

It is hardly necessary to say that an abundant supply of pure, clean, cow's milk is essential in modern life. But milk is potentially our most dangerous food. It is difficult to get pure milk and to keep it pure. Infection through contaminated milk is common. Prevention of milk-borne disease is one of the most important, and one of the most difficult, problems of public health.

There are peculiar circumstances which make milk so liable to act as a source of disease. Milk is the only standard article of diet, obtained from animal sources, which is ordinarily consumed in the raw state (not cooked). Enormous quantities of milk and milk products—ice cream, butter, and cheese—are consumed every day. The larger part of this great quantity of milk is consumed by children, and they are more susceptible than adults to infection or poisoning through the digestive tract. Since milk is opaque, it may contain a great deal of dirt without giving any evidence of this pollution to the eye. Milk is itself a good culture medium for a great variety of microorganisms, and pathogenic as well as saprophytic bacteria are able to *multiply* in it. The milk for large cities comes from hundreds of different dairies, passes through the hands of two or three different dealers, and is commonly transported for long distances, before it is delivered to the consumer. It is exposed to pollution from the moment it is drawn, and at any time during the long journey from the dairy to the table it may be contaminated with disease germs through the ignorance or carelessness of the numerous persons who are engaged in handling it. In view of all these circumstances, it is easy to see how milk may play so important a rôle in the spread of disease, and how serious is the problem of safeguarding the purity of milk supplies.

Sources of bacteria in milk. Milk as secreted in the mammary gland of the healthy cow is sterile, but it is not possible to get this milk entirely free of bacteria even when the most elaborate precautions are taken to insure cleanliness in the milking. Some bacteria are always present in the milk ducts in the teats, and these are washed out when the milk is expressed. Milk obtained in the most cleanly manner possible will contain from a few hundred to a few thousand bacteria in every cubic centimenter.

When the milking is done without any effort at cleanliness, as is so often the case on the small dairy farm, the milk is certain to be contaminated with millions of microorganisms. These organisms get into the milk from the dust and filth of the stable or barnyard, from the dirty flanks and udders of the cows, from the unwashed hands of the milkers, and from unsterilized milk pails. The only gesture of cleanliness ever made on many farms is to pass the milk through a strainer. This takes out the gross dirt—it is no novelty to find sizable masses of manure—but of course the bacteria are not removed.

Significance of the number of bacteria in milk. Freshly drawn milk, then, is never sterile, and unless great care is taken during the milking process to insure a scrupulous cleanliness, the milk is certain to be heavily inoculated with a great many microorganisms from the very start. The number of bacteria in a sample of milk at any subsequent time will depend upon: (1) the number of living organisms originally introduced during the milking, and (2) the temperature at which the milk has been kept. If the milk is cooled to about 50° F (10° C), or lower, immediately after it is drawn, and kept continuously cold, there will be only a gradual increase in the number of bacteria, because most of the organisms will multiply slowly, if at all, at this low temperature. But if the milk is not cooled at once, or is allowed to become warm at any time, the bacteria in it will increase enormously within a very short time.

Counts of the bacteria in milk samples from dairies, collecting stations, and pasteurizing plants, and in bottled milk as it is delivered, are of great value to public-health officers, because these counts indicate the sanitary conditions which have prevailed during the production and handling of the milk. If the milk supplied by a certain dairyman contains a large number of bacteria day after day, this is clear evidence of unsanitary methods. Fresh, clean milk, which has been kept cold, will have a low bacterial count; old and dirty milk will have a high count.

Danger in unsanitary methods of handling milk. Fortunately, the great majority of bacteria that get into milk are harmless saprophytes, and even very dirty milk, containing millions of these saprophytic organisms in every cubic centimeter, may be drunk by adults without appreciable harm. But such milk is, of course, undesirable. It will sour much more quickly than clean milk and, *as food for infants, dirty milk is dangerous,* even though it does not contain disease germs. It may be one of the contributing causes to the development of the severe diarrhea many infants suffer during the hot summer months.

The greatest danger in unsanitary methods of handling milk, however, lies in the opportunities such methods give for the contamination of the milk with disease germs. An ignorant or irresponsible dairyman, who does not keep his cattle free of disease, may sell milk from a sick cow, and this milk may contain germs which cause disease in human beings. Thus, some *animal diseases* may be transmitted to man through milk. More important is the possibility that

the milk may become contaminated with the germs of *human diseases.* This may happen as the result of the use of unsterilized milking utensils; or the germs may be introduced into the milk by the hands of the milker or other persons engaged in its production or distribution.

Diseases transmitted to man through milk. The important diseases of cattle which may be transmitted to human beings through milk are *tuberculosis* and *brucellosis* (undulant fever). The human diseases most frequently transmitted through contaminated milk are *typhoid fever* and *bacillary dysentery.* Less commonly, contaminated milk is responsible for infection of the upper respiratory tract of human beings with the germs of *septic sore throat, scarlet fever,* or *diphtheria.*

Examples of milk-borne disease. *Milk-borne typhoid fever.* The great majority of epidemics of typhoid fever occurring in cities in recent years have been caused by contaminated raw milk. When these epidemics are studied, circumstantial evidence usually points clearly toward the milk supplied by a particular dairy as the source of the infection. Further investigation usually results in the discovery of a typhoid bacillus *carrier,* or an unrecognized case of typhoid fever among the persons employed at that dairy. Some epidemics have been traced to contamination of the milk by a person who was nursing a typhoid patient and at the same time acting as milker. Other outbreaks have been due to water which was contaminated with typhoid bacilli, and which was used for washing milk utensils.

One of the most extensive of milk-borne typhoid epidemics occurred in 1927 in the city of Montreal, Canada.

During the period March 1 to June 28, 1927, 4,755 cases of typhoid fever were reported with 453 deaths. This is the most serious outbreak that has occurred in any large city for many years. Investigators found that neither the water supply nor sewage disposal systems were in any way responsible for the epidemic. But the evidence pointed clearly to a contaminated milk supply. It was found that a large proportion of the cases occurred in persons who had drunk the milk distributed by a certain dairy company. Just exactly how or when the germs were introduced into this milk supply was not determined. It was found, however, that many of the 1,200 to 1,500 small farms which sent milk to this dealer were very unsanitary. Open privies were used on many of the farms, sewage-polluted water was used in the milk houses, and other unsanitary practices

were common. Obviously, there were abundant opportunities for contamination of some of the milk by typhoid cases or carriers on the farms. The milk was supposed to be pasteurized, but there was inadequate supervision of pasteurizing plants. Investigators from the United States Public Health Service * concluded that the germs must have been introduced into the milk at some of the farms or collecting stations, and that these germs were given an opportunity to multiply during transportation, and that for some reason this contaminated milk was distributed to the city without being pasteurized.

A few typhoid fever epidemics have been caused by contaminated ice cream, butter, and cheese.

Milk-borne septic sore throat. This is a severe type of sore throat caused by a variety of the pathogenic hemolytic streptococci (*Streptococcus hemolyticus*). The disease may be spread by droplet infection or by other forms of personal contact, but it has frequently been carried by raw milk. Epidemics have usually been traced to contamination of the milk with streptococci from cases of sore throat among milkers. The usual course of events is about as follows: A milker infects the udder of the cow with streptococci from his own inflamed throat through his unwashed hands. The germs multiply readily in the warm milk left in the udder after milking, an abscess forms in the udder, and for a number of days thereafter all the milk obtained from this animal is heavily contaminated and may cause a septic sore throat in persons who drink it in the raw state. Thus the cow serves as a kind of incubator for the streptococci which cause sore throat in human beings.

An extensive epidemic occurred in Massachusetts in 1928.†

In the town of "K," with a population of approximately 4,000 persons, there occurred about 950 cases in the month of July, with 48 deaths. The milk supply of the town was not pasteurized. The largest number of cases were among persons who were supplied milk by Distributor A. This dealer secured his milk from several different dairies. In the milk from one of these dairies, Dairy D, streptococci were found, and at Dairy D one of the cows was found to have an acutely inflamed udder from which hemolytic streptococci were isolated. Just prior to the outbreak of septic sore throat among the users of the milk, one of the milkers at Dairy D had suffered

* "Report of the United States Public Health Service on the Montreal Typhoid Fever Situation," *Public Health Reports,* Vol. 42, 1927, p. 1893.

† LOMBARD, H. L. "Septic Sore Throat in 1928 in Massachusetts: Epidemiology." *J. Preventive Med.,* 1929, 3:81.

from a severe sore throat. Two of this man's children were also sick with sore throats. It is very probable that this man infected the udder of the cow and was primarily responsible for the epidemic.

Methods for the production of safe milk. The first requisite for the production of good milk is *healthy milk cows.* Also contributing directly to the production of good milk is the practice of a few elementary principles of *cleanliness* during the milking process. The simple procedure of washing the udders, then wiping them dry, just before milking, will in itself have a marked influence upon the number of bacteria that get into the milk. Contamination is still further reduced by the use of specially designed milk pails with narrow openings. The milking utensils should be clean, and preferably sterilized. It is even more important that the milkers themselves should be healthy, and trained to habits of cleanliness.

A good dairy will have provision for *immediate cooling of the freshly drawn milk* to a temperature below 50° F. It is exceedingly important to keep milk continuously cold from the time it is drawn to the time it is consumed. Only bottled milk (sterilized bottles) can be safe. It should be promptly delivered to the consumer in refrigerated vehicles.

Finally, experience has shown that although cleanliness in obtaining milk and care in handling it—using the methods just mentioned—help greatly toward the production of a safe milk supply, *all raw milk is nevertheless potentially dangerous.* It is not hard to see why this is so. The milk supply for most large communities is made up of a mixture of the milk from hundreds of small dairies, and any one of a thousand persons might contaminate the supply somewhere in its progress from farm to consumer. The existence everywhere of numerous healthy human carriers of disease germs makes it almost inevitable that some lots of milk will be contaminated at times. Cows may also become diseased without its being known. Consequently a further safeguard is necessary. This is found in the milk-heating process called *pasteurization.*

The milk is held in large vats, and continuously stirred, at a temperature of about 142° F (61° C) for a half hour, then from the pasteurizers it passes through cold pipes, where it is *rapidly cooled.* It then goes directly to automatic bottling machines (Fig. 67).

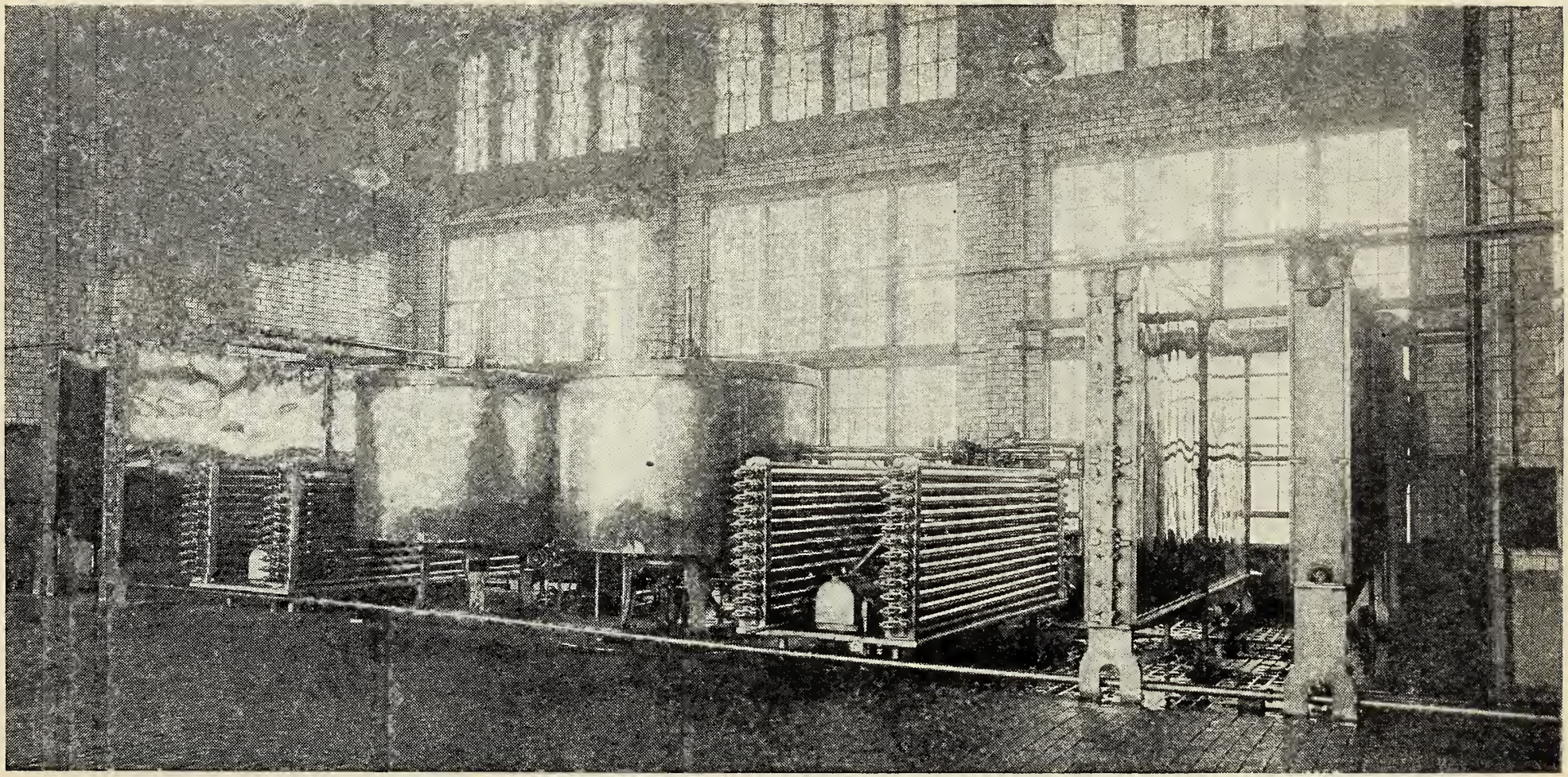


FIG. 67. A modern milk pasteurizing plant. The actual process of pasteurization goes on while the milk is held for a half-hour period in the large tanks. The milk is heated to the pasteurizing temperature (about 142° F) as it passes through the coil of pipes before it empties into the tanks. When the half-hour pasteurization period is over, the milk travels through other pipes, which cool it rapidly, and the cold milk passes directly to the bottling machines in another room. The entire process is automatic, so that the milk is protected from contamination. (Courtesy of the Pevely Dairy Co., St. Louis, Mo.)

Pasteurization of the entire milk supply of a community has been found to be a most valuable measure for the prevention of milk-borne disease.

Milk standards. Many communities in the United States have recognized, through local ordinances governing the sale of milk, two or more *grades* of milk, and have set up minimum standards which each grade must meet. The requirements are based upon conditions at the dairy farms and upon the result of chemical and bacteriological analyses of the milk. *Grade A pasteurized milk,* for example, is usually defined as milk from cows shown to be free of disease, and produced and handled so that the total bacterial count at no time exceeds 200,000 per cc. After pasteurization, and at time of delivery, this grade of milk should not contain more than 10,000 bacteria per cc.

The use of the term *Certified* as applied to milk is limited to milk produced according to the strict requirements of the American Association of Medical Milk Commissions. It is intended to be the purest milk possible to produce. Certified milk is now sold both raw and pasteurized. It is produced and handled with such care that even the raw milk can be delivered to the consumer with a bacterial count of not more than 10,000 per cc.

Infection through foods other than milk. Like milk, other foods may act as the vehicle of transfer for disease germs. Familiar dishes not infrequently become infectious or poisonous as the result of contamination with certain particular species of microbes. The acute gastro-intestinal upset popularly called "ptomaine poisoning" follows the eating of a foodstuff containing the toxic products of certain varieties of *Staphylococci,* or one contaminated with bacilli of the genus *Salmonella.* The rare disease known as *botulism* is caused by consuming canned food contaminated with *Clostridium botulinum* and containing the powerful exotoxin produced there by the organism (Chapter XXXIII). Of course, the germs of *typhoid fever* and *dysentery* may be transmitted from a carrier to other persons through uncooked or imperfectly cooked food.

The foods most liable to contain dangerous disease germs are naturally those which are ordinarily consumed in the raw state, without being heated. The most important (besides milk, butter, cheese, and ice cream) are fresh vegetables, and oysters and other shellfish.

Also, disease is not uncommonly caused by foods which have

been *partially* cooked, *but have not been heated sufficiently* to destroy all the germs or poisons present. *Thorough cooking,* however, will make foods safe to eat. When we recall the innumerable opportunities for contamination of food by carriers of disease germs among cooks and other food handlers, it is clear that the custom of cooking most foodstuffs just before eating must prevent a good deal of illness.

INSECT-BORNE INFECTION

The attention of the medical world was first attracted to insects as carriers of disease in 1893, when Smith and Kilbourne demonstrated that Texas fever, a malaria-like disease of cattle, is transmitted through the bite of ticks, these small insects acting as intermediate hosts for the germs causing the disease. Shortly after this, came the observations of Ross and others which showed that malaria is carried by mosquitoes. These discoveries led to many investigations of the rôle of arthropods as carriers of germs, and it was soon apparent that they play an important part in the spread of certain diseases.

Some general features of insect-borne infection The kinds of insects concerned in disease transmission are various: They include flies, fleas, ticks, lice, and mosquitoes (Figs. 68, 69). All types of arthropods which habitually suck the blood of human beings or animals are liable to act as *vectors* (carriers) of disease germs. In most cases, however, a particular disease is spread only by a particular kind of insect. Thus, malaria is carried only by mosquitoes of the genus *Anopheles,* while yellow fever is transmitted by *Aedes* mosquitoes, and sleeping sickness spreads only by the bites of tsetse flies, and so on. It is remarkable that the insects are not usually harmed by the germs they carry, despite the fact that in many instances these organisms multiply and undergo complicated changes as part of their life cycle of development within the bodies of their insect hosts.

Insects act in some cases merely as: (1) *agents for the mechanical transfer of germs,* but in most cases they serve as (2) *intermediate hosts for the germs,* and the sole or the chief means by which the infection is spread. In the latter instances, the germs undergo a series of changes within the body of the insect, and a definite interval must elapse after the organisms have been taken in before

these changes are complete, and the insect becomes capable of transmitting the infection. This is spoken of as *biological transmission* of disease.

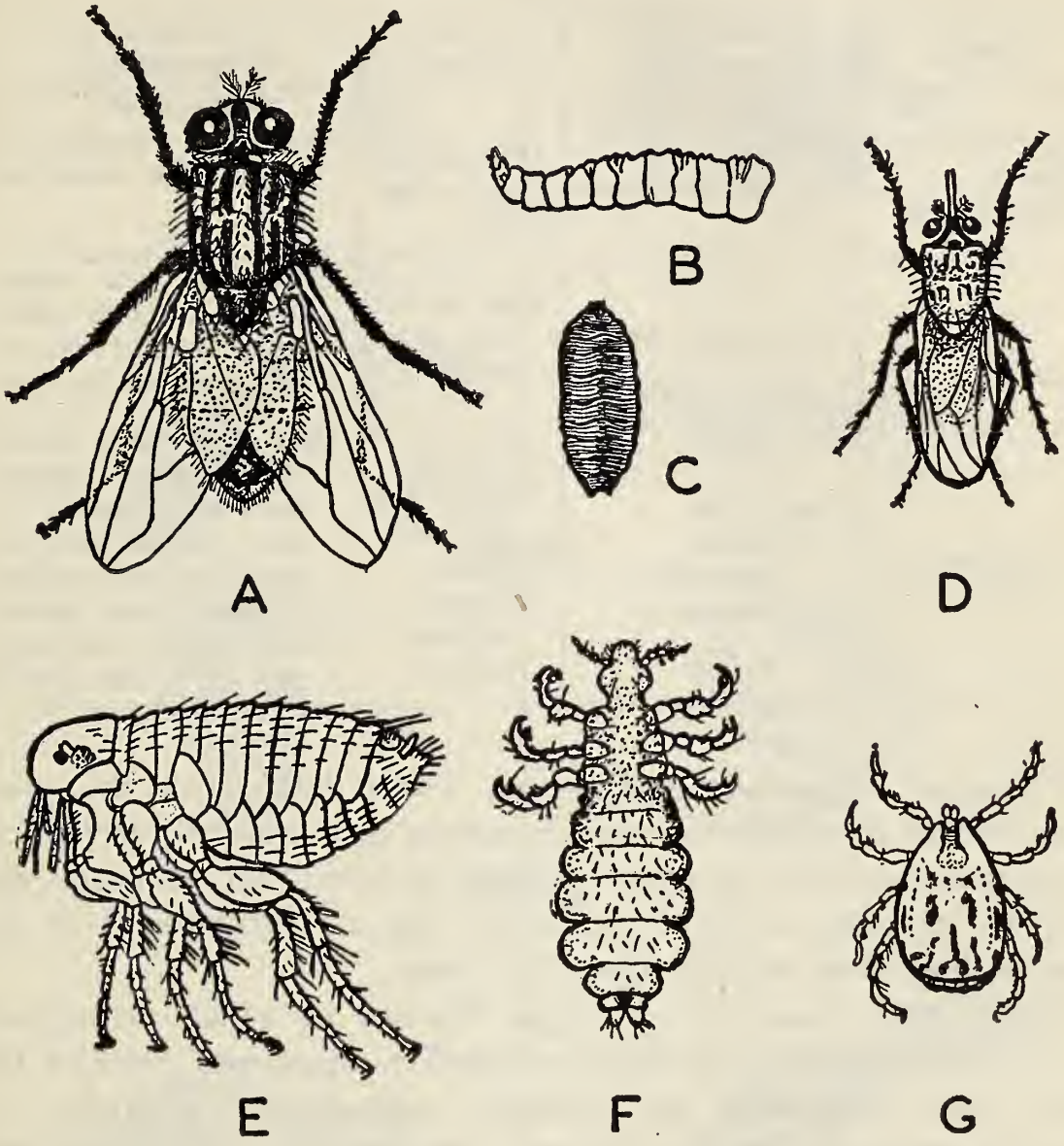


FIG. 68. Some insects that may carry germs. A: the house fly (dysentery and typhoid fever); B: the maggot, and C: the puparium of the house fly; D: the tsetse fly (African sleeping sickness); E: the Indian rat flea (plague); F: a louse (typhus); G: the wood tick (Rocky Mountain spotted fever).

Since most of the arthropod-borne diseases are spread only by certain species of insects, we naturally find these infections confined to areas in which the insect hosts abound, and where human or animal carriers of the germs are numerous. Thus, malaria is prevalent only in places where there are many persons carrying the parasites (who, therefore, act as a reservoir of the infection), and where *Anopheles* mosquitoes are always present.

TABLE XIII. Principal Insect-borne Diseases: Mechanical Transfer

DISEASE	CAUSATIVE ORGANISM	INSECT CARRIER	DETAILS OF TRANSFER
Typhoid fever Cholera Dysentery, etc.	*Eberthella typhosa* *Vibrio cholerae* *Shigella dysenteriae* etc.	House flies Cockroaches	Mechanical transfer of germs from excreta to food
Plague	*Pasteurella pestis*	Fleas of rats and other rodents	Germs in flea transferred through bites from infected rat or other rodent to man
Tularemia	*Pasteurella tularensis* (*Bacterium tularense*)	Horse flies, ticks, deer flies, and other blood-sucking insects	Deer flies may carry germs from rabbits dead of tularemia to man. Rabbit louse or wood tick may spread infection from rabbit to rabbit. Bedbugs may carry the germs

Principal insect-borne diseases. Table XIII gives the principal human diseases which may be spread as a result of *mechanical transfer* of the germs by insects. It will be noted that the germs carried in this way are *bacteria*. In no case is transmission of the disease dependent *entirely* upon any insect.

Table XIV shows the principal diseases in which arthropods act as intermediate hosts for the causative organisms. Most of the germs here concerned are protozoa, spirochetes, rickettsiae, or filtrable viruses, rather than bacteria. In most cases, these organisms multiply within the bodies of their insect hosts and also undergo certain cyclic changes. It is only after this development is complete that the disease may be transmitted to new victims by the bite of the insect.

Prevention of insect-borne infection. Successful prevention of an insect-borne disease requires an intimate knowledge of the life history and habits of the insect involved, as well as a thorough understanding of the causative organism and the nature of the disease. The most effective method of controlling this kind of disease is usually to *prevent the breeding of the arthropod vector*. Thus, malaria and yellow fever may be combated most successfully by

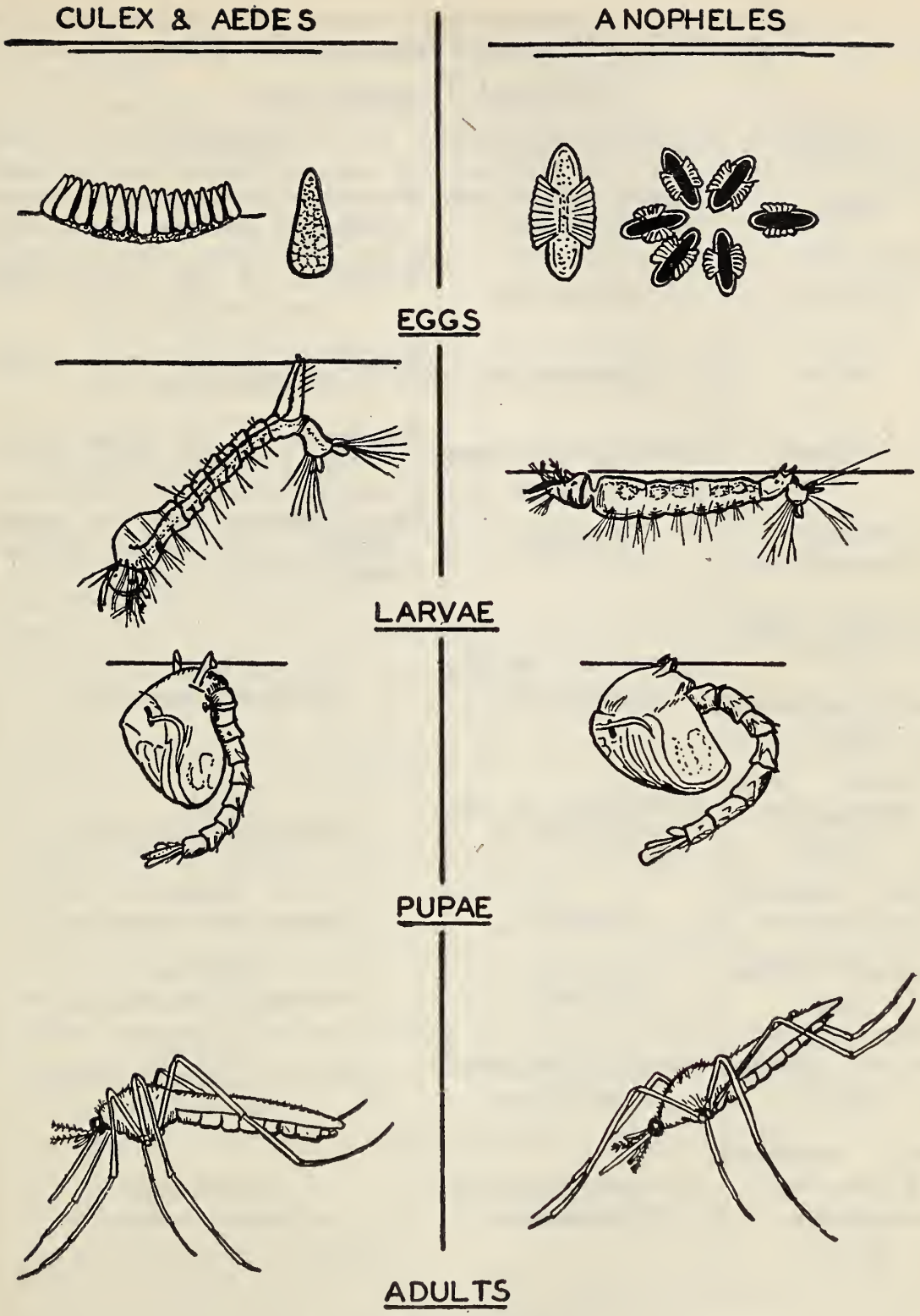


FIG. 69. Stages in the development of *Culex, Aedes,* and *Anopheles* mosquitoes.

destroying the eggs and young forms of mosquitoes, and by eliminating, so far as possible, the breeding places of these insects.

The powerful new insecticide called DDT, developed during World War II, is expected to prove of immense aid in controlling insect-borne diseases.

TABLE XIV. Principal Insect-borne Diseases: Biological Transmission

DISEASE	CAUSATIVE ORGANISM	INSECT CARRIER
Malaria	Protozoa of the genus *Plasmodium*	Mosquitoes of the genus *Anopheles* (*Anopheles quadrimaculatus*, etc.)
Yellow fever	Filtrable virus	Mosquitoes of the genus *Aedes* (*Aedes aegypti*, etc.)
Dengue	Filtrable virus	Mosquitoes of the genus *Aedes* (*Aedes aegypti*, etc.)
Filariasis	Filaria (roundworms)	Mosquitoes of the genus *Culex* (*Culex quinquefasciatus*)
European Typhus fever	Rickettsia	Lice (*Pediculus humanus*, variety *corporis*, and *capitis*) (Human body and head lice)
European relapsing fever	Spirochetes of the genus *Borrelia*	Lice (*Pediculus humanus*)
Asiatic relapsing fever		
Relapsing fever (African tick fever)	Spirochetes of the genus *Borrelia*	Ticks (*Ornithodoros moubata*)
Rocky Mountain spotted fever	Rickettsia	Ticks (*Dermacentor andersoni*)
Endemic Typhus fever	Rickettsia	Rat Fleas (*Xenopsylla cheopis*, etc.)
African sleeping sickness	Protozoa of the genus *Trypanosoma*	Tsetse fly (*Glossina palpalis; Glossina morsitans*)
South American Trypanosomiasis (Chagas' disease)	Protozoa of the genus *Trypanosoma*	Assassin bugs (*Triatoma megista*)

REFERENCES

(See also References to Chapter XVIII)

BARRON, J. L.: "Philosophy and Future of Milk Control," *Am. J. Pub. Health*, May 1944, 34:467.

DACK, G. M.: *Food Poisoning*. Chicago: Univ. of Chicago Press, 1943.

HERMS, W. B.: *Medical Entomology*. New York: The Macmillan Company, 3rd Ed., 1939.

"Milk Ordinance and Code," *U. S. Public Health Bull. #220,* 1939.
"Sanitation Manual for Public Ground Water Supplies," *Pub. Health Rep.,* Feb. 4, 1944, 59:137.
Standard Methods for the Examination of Dairy Products. New York: Am. Pub. Health Assn., New York, 8th Ed., 1941. "Corrections for 8th Edition," *Am. J. Pub. Health,* 1944, 34:303.
Standard Methods for the Examination of Water and Sewage. New York: Am. Pub. Health Assn., 8th Ed., 1936.
SUCKLING, E. V.: *Examination of Waters and Water Supplies.* Philadelphia: Blakiston Co., 1943.

REVIEW QUESTIONS—CHAPTER XIX

1. How many and what kinds of bacteria are likely to be present in: (1) rain water, (2) ground water, and (3) surface water?
2. Why are shallow wells and springs dangerous as sources of water supply? What are the essential features of a properly constructed, sanitary shallow well?
3. Why is artificial purification usually necessary before surface water can be safely used as a public water supply?
4. Name diseases which may be contracted directly from contaminated water. Discuss the life of disease germs in water and characteristic features of water-borne epidemics.
5. Describe two well-known epidemics of water-borne cholera. What other disease was frequently water-borne 30 or 40 years ago? What is the present danger of water-borne infection?
6. What must be done to determine the sanitary quality of a particular water supply? What are the purpose and value of the microscopic, the physical, and the chemical examination of water?
7. What constitutes the bacteriological analysis of water? Explain briefly the method and the purpose of: (*a*) the quantitative analysis and (*b*) the qualitative analysis. What constitutes the *presumptive test,* the *confirmed test,* and the *completed test* for coliform bacilli?
8. Describe the qualities of a good water supply.
9. What are the processes used in the artificial purification and disinfection of public water supplies?
10. Discuss the importance of a pure milk supply and give reasons why milk is so liable to be a source of infection.
11. What factors determine the number of bacteria in a sample of milk at any time after milking? Explain why bacterial counts reveal the condition under which milk has been produced and handled.
12. What dangers are associated with the use of dirty milk?
13. Name and discuss briefly two animal diseases and five human diseases which may be transmitted through milk and milk products.
14. What measures are essential for the production of safe milk?

15. What are the usual standards recommended for Grade A pasteurized milk? What is certified milk?
16. Discuss the danger of infection from foods other than milk.
17. Discuss some general features of insect-borne infection.
18. In what two ways may insects or arthropods act as carriers of germs? What is meant by biological transmission of disease by insects? Give examples.
19. Name three important diseases the germs of which may be carried mechanically by insects.
20. What is the most effective way of controlling insect-borne infections?

PART THREE

INFECTION AND RESISTANCE

CHAPTER XX

MICROBES AND DISEASE

DEFINITIONS

Infection. When living bacteria, or other microscopic organisms or viruses, *enter the tissues, multiply there, and cause injury to the body cells,* we say an **infection** has occurred.

Infection should be distinguished from *contamination.* A contaminated object is one which carries or contains disease-producing microbes. The dishes used by a patient with typhoid fever, for example, may be *contaminated* with the germs of that disease, but could not be said to be infected. Often the hands are contaminated without being infected.

Infectious disease. *Any disease resulting directly from an infection with a microscopic organism* is called an *infectious disease.* Thus tuberculosis, measles, typhoid fever, pneumonia, and tetanus are all infectious diseases, though each is a distinctly different kind of illness, because the cause of the disease in each case is an infection with a microbe.

Sometimes the word *infectious* is used in a more special sense to mean "liable to be communicated," thus having the same significance as *communicable.* For the sake of clarity of thought, however, it is better to use *infectious* only in the general sense first given above.

A distinction should be made between the *infection itself,* that is, the entrance of the germ and the injury to the body tissues, and the *clinical disease* it may bring about. The infection always occurs some hours or days, and often weeks and even months, before symptoms of the disease appear. For example, it is usually about ten days after typhoid germs have entered the intestine before the

disease sets in. There are also some cases in which an infection causes so little damage that no symptoms are produced. For example, tubercle bacilli infect a small area of the lungs in many individuals who never become actually ill with tuberculosis. It is necessary, therefore, to distinguish between tuberculous infection and tuberculous disease.

Communicable disease. A communicable disease is *one which may be transmitted naturally from one individual to another.* The germs concerned in each case have the capacity to pass in some way from one person to another during the everyday comings and goings of men.

A few of the infectious diseases are not naturally communicable. Tetanus (lockjaw), for example, caused by infection of a wound by *Clostridium tetani,* is not communicable, because this organism has no natural means of getting from the infected wound to a similar wound in another person. Therefore, cases of tetanus do not arise by contact with other cases.

The great majority of infectious diseases are communicable, however, though by no means all in the same degree. Mumps, measles, smallpox, and influenza, for example, are highly communicable; that is to say, the germs of these diseases pass readily from one person to another. Many other diseases do not spread so easily. In general, the diseases most readily communicated are those in which the microbes attack the upper respiratory tract, and are transmitted to other persons through the discharges from the nose, mouth, and throat.

Since even the diseases which are not naturally communicable can be transmitted by artificial transfer of the germs, the expression *communicable diseases* is generally used to include all the infectious diseases.

Contagious disease. The word *contagious* is sometimes used to mean the same as *communicable.* But *contagious* refers to *direct contact,* and, in a strict sense, a contagious disease is *one which is acquired only through personal contact.* Such diseases as measles, mumps, chickenpox, and influenza are properly called contagious, because they are always communicated by personal contact with the patient. Typhoid fever, on the other hand, should not be called contagious because there is little danger of contracting the disease through a single contact with a patient, whereas the germs are often transmitted through contaminated water or milk or by other remote

means. This distinction in the use of the term contagious cannot be carried out consistently, however, and it is best to avoid the word entirely. *Communicable* is much to be preferred, for its meaning is clear.

Endemic, epidemic, pandemic diseases. A disease is said to be *endemic* ("with the people") when a small number of cases occur constantly among the population of a community. Thus, measles is endemic everywhere in the United States. Cholera is endemic in India, but not in this country.

When an endemic disease flares up, and an unusually large number of cases develop within a certain community within a short time, we say the disease has become *epidemic* ("or over the people"). When an epidemic becomes very widespread it is spoken of as a *pandemic* ("affecting all the people"). During 1917–1919 influenza was at first epidemic in certain places, then became pandemic, spreading over virtually the entire world.

KINDS OF PATHOGENIC MICROBES

The **pathogenic** (disease-producing) **microbes** which we encounter in connection with illness in human beings and animals have one outstanding characteristic—they are capable of developing (though in some cases to a limited degree only) *within the tissues* of the living body; i.e., they can cause *infection*. But these pathogenic organisms are not all capable of infecting the body with the same ease or in the same circumstances. For practical purposes, it is useful to distinguish between two classes of disease-producing microbes: (1) the "opportunists," and (2) the "true pathogens."

Infection by "opportunists." *The opportunists* are those organisms capable of producing disease only when given a special opportunity to enter the body tissues through an accidental injury to the skin or mucous membranes, or when the natural resistance to infection is abnormally low. The germs of this kind do not invade the tissues of healthy individuals, but if they are introduced mechanically, through a wound, they may produce a severe or even fatal infection.

Tetanus is an example of an opportunist infection. The spores of the tetanus germ exist in the soil and become widely distributed over all sorts of dirty objects. The bacillus is a saprophyte. It has no power to invade the body by itself. But it may be carried

mechanically, with dirt, into an accidental wound. If conditions in the wound are favorable, it may multiply there to a limited extent. In so doing it secretes a powerful exotoxin which causes the fatal disease we call tetanus.

Among the bacteria inhabiting the healthy skin and mucous membranes there are many species which must be classed as opportunists. The outstanding example is the *Staphylococcus aureus*. This organism does no harm on the intact skin. But if it is given an opportunity to enter the deeper tissues through a break in the skin, an infection will result.

There are many examples of opportunist infections in persons whose natural resistance has been reduced by preëxisting disease or other abnormal condition. Infections of more or less severe character in the mouth, throat, lungs, appendix, urinogenital organs, and elsewhere are often caused by organisms which are ordinarily harmless, but which have become pathogenic when a local injury, or very low vitality in the individual, permits them to pass into susceptible tissues.

Infection by "true pathogens." In contrast to the opportunists, the *true pathogens* are able to invade the tissues of healthy individuals through some inherent power of their own. There are only a few species of true pathogens. Each of them brings about a characteristic illness in one victim after another.

Each of the most common and familiar *communicable diseases*, such as diphtheria, tuberculosis, typhoid fever, and syphilis is caused by a particular kind of true pathogen which will always produce the same kind of disease in susceptible persons.

Differences. No hard and fast line can be drawn between an opportunist on the one hand and a true pathogen on the other. But is is often helpful to make this distinction. Opportunist infections differ in a number of ways from the characteristic diseases caused by the true pathogens.

One difference is that opportunist infections are not ordinarily communicable. From the point of view of the individual, opportunist infections are very important, and must be guarded against by careful disinfection of wounds, and by efforts to avoid unhealthful habits of living, and weakening diseases which may lower resistance to infection. But from the point of view of public health, they are much less important, since they cause diseases which do not ordinarily spread to other persons. Another important difference, which

holds in most cases, is that recovery from opportunist infections, such as an appendicitis or secondary bronchopneumonia, does not usually make an individual more resistant to a similar infection, whereas a person who has survived an attack of most of the common specific diseases, such as typhoid fever, is rendered immune to that disease for a considerable period.

FACTORS GOVERNING THE START AND THE COURSE OF AN INFECTION

There is no microbe that will always produce disease in all kinds of living things. Most germs are pathogenic for human beings only, or for certain species of animals or plants only. The disease-producing power of a microorganism must always be thought of in relation to some particular *host* * (man, animal, or plant).

Furthermore, an organism which is pathogenic for some human beings or some individual animals will not necessarily produce disease in other persons or in other animals of the same species. Many factors determine whether or not a pathogenic organism can bring about an infection in any particular case. *The mere presence of the germ on the body surfaces is not necessarily followed by the development of the disease.*

There are four principal factors which determine the start and the course of an infection. These are: (1) *the place where the organisms enter the tissues,* (2) *the number, or dose, of organisms entering,* (3) *the virulence of the organisms,* and (4) *the resistance of the individual host.*

Place where the organisms enter the tissues. The particular tissues with which a germ first comes into contact, that is, its *path of entrance* into the body, often determines whether or not an infection will take place. The germs of typhoid fever and other intestinal infections can produce the typical disease only when they reach the intestines through the mouth. On the other hand, the germs of diphtheria, meningitis, gonorrhea, and other diseases may be swallowed without harm. If typhoid bacilli were rubbed into the injured skin, they would cause only a slight inflammation and typical typhoid fever would not be brought about; but if anthrax bacilli

* The *host* is the person or the particular animal or plant which supports a parasite. The host "entertains" bacteria or other parasitic organisms, as it were, at its own expense.

were rubbed in, they would produce a characteristic local lesion. Each of the true pathogens invades the body by a particular route, and unless it reaches the accustomed path of entrance, infection may not occur.

Number, or dose, of organisms entering. Obviously, the greater the number of germs entering, other factors being equal, the greater the likelihood that infection will occur, and the more severe the resulting disease is likely to be. The normal, healthy body possesses some natural defenses against invasion by germs, and the dose of microbes entering must be sufficient to overcome this resistance. The more powerful the germs and the more susceptible the individual, the smaller the dose required. It has been claimed that a single anthrax bacillus can cause anthrax in a very susceptible white mouse. The matter of dose probably has an important influence in many cases of human infection. Tuberculosis, for example, is not usually acquired by adults solely from occasional contacts with a tuberculous patient, but it is often contracted by persons who live intimately for some time with an individual having the active disease, probably because in these circumstances they cannot avoid exposure to a heavy dose of the germs.

Virulence of the organisms. Virulence is a general term meaning *the disease-producing power of an organism.* Virulence may be thought of as a property of a pathogenic organism itself, as something inherent in the nature of the microbe. A virulent organism is one that can: (1) *invade the tissues of the body* or (2) *poison the body.* Most pathogenic organisms can grow readily within certain regions of the body, at least, and can poison the tissues as well.

Toxin-production by bacteria. Of obvious importance in relation to virulence are the *toxins* formed by pathogenic bacteria. As we have pointed out previously these poisonous substances may be classed in two categories: (1) the *exotoxins,* which are soluble and diffuse out of the intact bacterial cells into the surrounding culture medium or tissue, and (2) the *endotoxins* which are contained within the bodies of the organisms, and are released only when the cells are disintegrated.

(1) *Exotoxins.* These are apparently products of the metabolism of the growing germs, and, since they are soluble in water, they appear in solution in the medium in which the organisms are growing. If the culture is passed through a bacteriological filter, a sterile, germ-free filtrate is obtained which contains the toxin in

solution. This is the way an exotoxin is secured for study. When we say diphtheria toxin, tetanus toxin, etc., we usually refer to nothing more than the *sterile filtrate from a broth culture* of the germs (Fig. 71).

The exotoxin formed by any one kind of organism is *specific,* and differs from the toxin produced by any other species. All the exotoxins, however, have certain fundamental properties in common which distinguish them from endotoxins, and from ordinary chemical poisons. As explained above, they are all products of living germs and can be separated, by filtration, from the organisms themselves. When introduced into the body of a susceptible person or animal, an exotoxin will reproduce most of the characteristic symptoms and lesions of the natural infection with the germs from which the toxin originated. Thus, if the exotoxin of tetanus bacillus is injected into the body, it will injure the nerves and produce paralysis, just as in the natural disease of tetanus. The poisonous effect of toxins, however, is not manifested until a certain interval has passed after their introduction—the incubation period. This is in contrast to the chemical poisons—for example strychnine—which act immediately. Finally, a most important property of all exotoxins is that, whenever they are introduced into the body, they stimulate the formation of *specific neutralizing antibodies called antitoxins.* Antitoxin are thus antibodies of a special kind.

There are only a few pathogenic bacteria that develop powerful exotoxins. The most important are *Corynebacterium diphtheriae* (diphtheria), *Clostridium tetani* (tetanus), *Clostridium botulinum* (botulism), *Streptococcus hemolyticus* (scarlet fever), *Clostridium perfringens* (gas gangrene), and *Shigella dysenteriae* (Shiga dysentery).

Tests for the presence of an exotoxin in the filtrate of a culture of any organism must be carried out, of course, by the inoculation of susceptible laboratory animals; and to measure the action of these toxins and their corresponding antitoxins, so-called *protection tests* are carried out (Chapter XXIV). The virulence of an organism like the diphtheria bacillus depends directly upon its power to form the characteristic diphtheria exotoxin. Hence, tests for the presence of this toxin in cultures suspected to contain diphtheria bacilli are generally called *virulence tests* (Chapter XXXI).

(2) *Endotoxins.* Endotoxins are not as well understood as the exotoxins. They are best regarded as *poisonous elements in the*

chemical structure of the cells themselves, which are liberated only after partial decomposition of the cell-protein. When organisms of the endotoxin-producing type are grown in liquid media, they do *not* excrete toxin into the broth, and the germ-free broth has little or no poisonous action. But if the organisms themselves are collected and their bodies disintegrated in some manner, *the dead, disrupted cells can be shown to be poisonous.* Endotoxins are not specific for each kind of organism, but probably in all cases owe their toxic action to a similar chemical grouping.

The endotoxins seem to be a part of the cell-substance of the bacteria containing them. It has been shown that certain lipoid-carbohydrate complexes which can be isolated chemically from the typhoid bacilli and similar organisms have the characteristics of endotoxin. These complexes are antigenic, that is, they lead to antibody formation when inoculated by themselves into animals. They are often referred to as "Boivin antigens," after their discoverer.

Endotoxic material is able to stimulate antibody formation since it is made up principally of disintegrated protoplasmic substance. However, endotoxins differ sharply from exotoxins, in that *they do not stimulate the formation of the particular type of neutralizing antibody that we call an antitoxin.*

Practically all disease germs develop endotoxin in their bodies, and *this type of toxin is the only one produced by most species.* The organisms of typhoid fever, dysentery, cholera, gonorrhea and meningitis are examples of bacteria with considerable amounts of endotoxin.

Invasiveness of virulent microbes. An extraordinary power to invade healthy tissues, and to multiply freely therein, is a conspicuous property of many virulent organisms. A full explanation of this power cannot be given. No one can say exactly what makes a parasite parasitic. However, some of the properties associated with virulence of invasive character are understood.

Capsules. Nearly all organisms that show any considerable degree of invasiveness possess capsules. We have already mentioned the relation of capsules to the pathogenicity of pneumococci. In this species, the capsule appears to function as a definite protective mechanism against the defensive activity of the infected host. The encapsulated cocci are not readily disposed of by the phagocytic leukocytes of the body. On the other hand, pneumococci without

capsules are quickly attacked and destroyed by these phagocytes. It is now thought that all species of invasive bacteria develop at least a small amount of capsular material around themselves while they are within the body tissues. Even such organisms as the gonococcus and the meningococcus, which ordinarily are not capsulated, have at times been observed to have a capsule, when examined in pus or spinal fluid. It is clear, therefore, that the ability to form capsules is one of the most important properties associated with virulence of the invasive type.

Minor products and properties apparently contributing to virulence. Another property associated with invasiveness is the power to form mildly injurious products which apparently help the organisms to spread through the tissues. The most important of these products have been named: (1) *hemolysin,* (2) *leukocidin,* (3) *coagulase factor,* (4) *fibrinolysin,* and (5) *spreading factor.* Each of these is formed by a considerable number of common pathogenic bacteria; some invasive species can produce all of them.

Hemolysins are substances that bring about the hemolysis, or dissolution of red blood cells. Leukocidins are substances that kill the white blood cells (polymorphonuclear leukocytes) which play so prominent a part in local defensive reactions against infection. The coagulase factor is a substance, elaborated by virulent staphylococci and some other bacteria, that causes citrated blood plasma to gel (coagulate) promptly. In natural infections it probably hastens the formation of blood clots in the small blood vessels in the infected area. Fibrinolysin has an action opposite to that of the coagulase factor, for it brings about a rapid dissolution of blood clots. It is thought to play an important part in facilitating the spread within the body of certain highly invasive organisms, such as the hemolytic streptococci. The spreading factor is a substance that has a remarkable effect in increasing the permeability of the skin to toxins and other inanimate materials, and also accelerates the spread of living bacteria through the tissues. It is often called the Duran-Reynals factor, after its discoverer. It is probably identical with *hyaluronidase,* an enzyme capable of dissolving mucin-like substances.

Doubtless, there are numerous miscellaneous factors that contribute to the virulence possessed by this or that kind of pathogenic microbe. Each variety may be thought of as having its own peculiar set of surface structures, metabolic habits, and enzymatic activities that combine to confer upon it a certain disease-producing power.

Degrees of virulence and variation in virulence. Some species of pathogenic bacteria are naturally more virulent than others. Streptococci, for example, always tend to produce a more widespread and dangerous infection than staphylococci. It is a serious matter when the eyes are infected with the gonococcus, because this is a very virulent germ, whereas an inflammation caused by a less powerful organism is not greatly to be feared.

Virulence differs in degree not merely with different species of disease germs, but in different cultures or strains of the same kind of germ. In other words, virulence is not a constant property, but varies in degree according to the condition of the particular strain of organism concerned.

Decrease of virulence. A pathogenic organism tends to *decrease* in virulence when it is removed from its natural environment in the bodies of man or animals and forced to live under artificial conditions in laboratory culture media, and particularly if it is exposed to abnormal temperatures, to drying, or to other unfavorable conditions. A strain that has been weakened in virulence is said to be *attenuated.* Artificial methods of reducing virulence are of some importance in connection with the preparation of vaccines for the prevention of anthrax, rabies, and other diseases.

Sometimes the continual passage of a germ or virus through one species of animal will reduce its virulence for other species. Cowpox virus (used for vaccination against smallpox) is smallpox virus which has been reduced in virulence for human beings by cultivation in the tissues of the cow.

Increase of virulence. The virulence of any strain of pathogenic bacteria is *increased* when the organisms are passed frequently from one susceptible individual to another. For example, the virulence of some strains of pneumococci for white mice may be increased many times over by passing the organism directly from one mouse to another through a series of five or six animals.

There are two facts concerning virulence which have an important practical bearing on methods of preventing communicable diseases:

(1) *an organism is, as a rule, most virulent when freshly discharged from the body,* and (2) *virulence tends to be increased when germs are rapidly passed from one person to another.*

Resistance of the individual host. The final factor which determines whether or not an infection will take place, and the severity of the disease if it does occur, is the degree and character of the

resistance offered to the invading organisms by the individual host. Once an infection has started, the body tissues do not submit to injury without protest. On the contrary, after a variable time, there begins a more or less profound reaction. This reaction is a sign that the body is defending itself against the harmful effects of the germs, and it is the attempt to get rid of the offending microbes, to repair the damage they have caused, and to neutralize or destroy their poisons, that brings about the signs and symptoms of the clinical disease.

Summary. The relationship between the factors of infection which have just been outlined may be expressed in the formula:

$$D = \frac{NV}{R}$$

D means *disease,* N means *number* (of the germs), V means *virulence* (of the germs), and R means *resistance* (of the host). The formula signifies that, in any case of infection, it is the total disease-producing power of the organisms (which depends upon their number and virulence), balanced against the resistance of the infected individual, that determines the nature and severity of the disease.

GENERAL CHARACTERISTICS OF INFECTIOUS DISEASES

Varieties of infection. *Acute or chronic infection.* An *acute* infection is one which runs a rapid and severe course, then terminates rather abruptly. Lobar pneumonia is a typical acute disease. It begins suddenly, the patient is very ill for a few days, then, if death does not occur, recovery is usually rapid. The majority of the common infectious diseases are typically acute.

Chronic diseases are those which run a slow course, usually with comparatively mild symptoms, over a period of weeks, months, or years. Tuberculosis in adults is in most cases a typical chronic infection. So, also, are leprosy and syphilis. *Often an infection at first acute, later becomes chronic.* Gonorrhea, for example, begins as an acute inflammation of the urethra, but if not adequately treated, it later becomes a chronic infection involving the deeper parts.

Primary or secondary infection. By the *primary* infection is meant the first or original infection which makes a person ill. A *secondary* infection is one which follows as a complication of the original disease. Secondary infections are often due to organisms

of the "opportunist" type. These infections are frequently very serious, for the patient is in a weakened condition. One of the commonest causes of death is an infection of the lungs (bronchopneumonia) which follows diseases due primarily to other germs, as in influenza, measles, and whooping cough. Secondary infections of the middle ear, mastoid, meninges, lungs, or other parts, are often more to be feared in cases of measles, scarlet fever, and many other diseases, than the primary infection itself.

Local or generalized infection. A *local* infection is one confined to one spot or area in the body. A boil is a local infection. Infections are said to be *generalized* when they involve the whole body. A generalized infection, such as is sometimes caused by the staphylococci, in which there are many separate abscesses located in various parts of the body, is called *pyemia.* When bacteria are present in the circulating blood, as, for example, in the early stages of typhoid fever, the condition is called *bacteremia. Septicemia* is a term used by physicians to designate an infection which involves the blood stream. In many septicemias, bacteria are actually multiplying in the circulating blood; this is the condition called "blood poisoning" in popular language. There is no sharp distinction between the terms *septicemia* and *bacteremia.* When the body is poisoned by a toxin carried about by the blood, as in diphtheria, the condition is called *toxemia.*

Focal infection. This term is applied to a local infection which acts as a *focus,* or center, from which germs spread to set up infection in other parts of the body. Abscesses about the teeth and chronic inflammation of the tonsils, and similar conditions, are common focal infections. From these centers of infection, germs may enter the lymph or blood stream and be carried about. This is one of the ways in which microbes may reach the joints, and the heart valves, and set up arthritis and heart disease.

Latent infection. An infection is said to be *latent* when it is not progressive, but is quiescent, dormant, hidden. There may be no symptoms whatever, although the germs are still present in the body. In the course of syphilis and tuberculosis, there are often long periods during which the infection remains latent and the patient is free of symptoms, and yet the presence of living germs in the body is manifested some time later, when signs and symptoms of the disease again appear. There are similar latent periods in brucellosis and in tularemia.

Specific and nonspecific infection. A *specific* infection is one caused in all cases by a particular bacterium, such as syphilis, tuberculosis, and other infections with true pathogens. Many infections, as, for example, inflammations of wounds, and of the lungs and middle ear, are *nonspecific* in character, the germs present depending upon just what opportunists happen to get in.

Mixed infections. An infection is said to be *mixed* when microbes of two or more kinds are responsible. Most opportunist infections are mixed, two or three different species of organisms being present in the infected tissues.

An outstanding example of a disease caused by two different organisms apparently operating in symbiosis is Vincent's angina (Fig. 80). Such mixed infections are probably more common than is generally realized. In many diseases for which the primary cause is a filtrable *virus,* such as influenza and common colds, certain bacteria are likewise present, and the activities of these accompanying bacteria account in large part for the usual course of the illness.

Typical course of infectious diseases. During the course of any infectious disease, there are three more or less distinct phases. Before the disease actually begins there is: (1) the *period of incubation;* this is followed by (2) the *period of illness,* which merges into (3) the *period of convalescence,* during which the patient recovers his normal health.

Incubation period. This is an important feature of all germ diseases. The incubation period is defined as *the interval of time between the entrance of the germs into the body and the appearance of symptoms of the disease.* Even though the organisms are very virulent, and the dose large, there is always a lapse of some hours, days, or weeks, after infection has occurred, before the illness begins. Just what happens during the incubation period is not entirely understood. In some cases, the length of the period seems to be a measure of the time necessary for the germ or virus to reach the particular tissues in the body on which it exerts its harmful effects. Thus, the symptoms of rabies do not develop until the virus reaches the central nervous system, and the long incubation period in this disease represents the time required for the virus to travel from the place where it is introduced by the bite of the mad dog to the spinal cord and brain. In other diseases, as in typhoid fever, there is a rapid increase of the germs and at the same time a gradually increasing reaction on the part of the body during

the incubation period, and it is only when the germs have become sufficiently numerous and the reaction against them sufficiently great, that the symptoms of illness appear. Doubtless, other factors operate to explain the incubation period in different diseases.

The *length* of the incubation period is characteristic in each disease, and within certain limits is remarkably constant. As might be expected, the period is relatively short in those diseases (e.g., diphtheria, botulism, staphylococcus food poisoning) in which the causative germ forms a strong exotoxin. In typhoid fever the period is ten to fourteen days, in diphtheria two to four days, in mumps two to three weeks, and in rabies one to three months or more.

It is important to remember that, in many cases, an infected individual may transmit the disease to others during the incubation period, and since he is still up and about, he may spread the infection to many persons.

Period of illness. A continuous or intermittent *fever* occurs during the period of illness in nearly all germ diseases. The character of the fever is constant in each kind of disease. It is thought that fever, unless it goes too high, may be of benefit in helping the body to resist germs.

Period of convalescence. This is the period of recovery after the symptoms have disappeared. Convalescence may begin, as often happens, for example, in typhoid fever, *despite the fact that virulent germs are still being discharged from the body;* and the convalescent patient may be an important source of infection.

REFERENCES

Baker, A. C.: "Typical Epidemic Series," *Am. J. Trop. Med.*, 1943, 23:559.
Dubos, R. J.: *The Bacterial Cell.* Cambridge, Mass.: Harvard Univ. Press, 1946.
Duran, F.-Reynals: "Tissue Permeability and the Spreading Factors in Infection. A Contribution to the Host-Parasite Problem," *Bact. Rev.*, 1942, 6:197.
Eaton, M. D.: "Recent Chemical Investigations of Bacterial Toxins," *Bact. Rev.*, June 1938, 2:3.
Gay, F. D. and Associates: *Agents of Disease and Host Resistance.* Springfield, Ill.: Chas. C Thomas, 1935.
Hill, J.: *Germs and the Man.* New York: G. P. Putnam's Sons, 1940.
Smith, T.: *Parasitism and Disease.* Princeton, N. J.: Princeton Univ. Press., 1934.

REVIEW QUESTIONS—CHAPTER XX

1. Define and explain the meaning of the following terms: (*a*) *infection,* (*b*) *contamination,* (*c*) *infectious disease,* (*d*) *communicable disease,*

(*e*) *contagious disease,* (*f*) *endemic disease,* (*g*) *epidemic disease,* (*h*) *pandemic disease.*

2. What is one outstanding characteristic of pathogenic bacteria? Name two classes of disease-producing bacteria.
3. Define opportunists, and explain under what conditions they may produce disease.
4. Give an example of a saprophytic organism which may be classed as an opportunist. Give examples of opportunist infections caused by organisms naturally present on the body surfaces.
5. Define true pathogens. Explain how they differ from opportunists. Name some diseases caused by them.
6. State four principal factors which govern the start and the course of an infection.
7. Illustrate the importance of: (*a*) the path of entrance, and (*b*) the number of germs entering the tissues.
8. Define *virulence.* On what general properties does virulence depend?
9. Compare *exotoxins* with *endotoxins.* Outline the general properties of exotoxins. What is special about the antibody formed in response to an exotoxin? Give examples of bacteria that produce powerful exotoxins.
10. Outline the general properties of endotoxins. Name some bacteria that contain considerable amounts of endotoxin.
11. What structure is possessed by most invasive bacteria when they are within the body? Give an example to illustrate the relation between capsules and virulence.
12. Name, and describe briefly, five mildly injurious products that may be formed by invasive strains of bacteria.
13. Do all disease germs naturally possess the same degree of virulence? Illustrate. Under what circumstances may virulence of an organism be: (*a*) decreased? (*b*) increased? Define *attenuated.*
14. Give two facts of practical importance about virulence.
15. What is meant by the *host*? Give a formula showing the relationship between the four factors of infection.
16. Define and illustrate the following terms as they are used in describing infectious diseases: (*a*) *acute,* (*b*) *chronic,* (*c*) *primary,* (*d*) *secondary,* (*e*) *local,* (*f*) *generalized,* (*g*) *pyemia,* (*h*) *bacteremia,* (*i*) *septicemia,* (*j*) *toxemia,* (*k*) *focal,* (*l*) *latent,* (*m*) *specific,* (*n*) *nonspecific,* (*o*) *mixed.*
17. Name three phases in the course of infectious diseases. Define and discuss the nature and importance of the incubation period.
18. What clinical symptom characterizes the period of illness? Under what circumstances may a convalescent patient be a source of infection for other persons?

CHAPTER XXI

REACTION OF THE BODY TO INFECTION: CELLULAR DEFENSE

Principal factors in the body defense against infection. Resistance to germ diseases depends upon several interrelated factors. In general, there are three lines of defense: (1) *external* body defenses, (2) *nonspecific internal* defenses, and (3) *specific internal* defenses, acting against the particular microbe concerned through *antibodies*. Explanation of the first two of these is the principal concern of this chapter.

External defenses. When the skin is intact, it will prevent the entrance of the great majority of disease germs. The healthy mucous membranes also resist the passage of most microbes, although they are more easily penetrated than the skin. This resistance offered by the skin and the mucous membranes is successful, of course, only so long as it keeps infectious agents out of the body tissues; once an infection has begun, the external defenses do not count any more. Nevertheless they are important, and resistance to infectious disease in general is increased by cleanliness and good nutrition, which help to keep these body surface-coverings in the best state of health.

Many of the natural secretions of the body help to keep out germs. Thus, the eyes are protected by the fluid which bathes them. This is made clear when these secretions dry up, as may happen when the diet is for a long time deficient in Vitamin A. Under these circumstances, the eyes are always infected, and by germs which would be harmless in the normal eye. The high acid content of the stomach secretions sometimes serves to kill microbes which would otherwise pass into the intestines and infect them; one is less likely to be infected with typhoid bacilli if they are swallowed immediately *after* a meal. The normal vaginal secretions are acid; they inhibit the development of many bacteria.

Certain mechanical features of the body tend to protect against

infection. This is most strikingly shown in the anatomy of the nose and the deeper parts of the respiratory tract. The nasal cavities are so constructed that they catch dirt and bacteria as in a trap. The walls of the trachea and bronchi are lined with cells having tiny cilia, which are in continuous motion, sweeping dirt and germs upward toward the mouth (Fig. 83). This must certainly help to protect the lungs from infection. We learn the value of this arrangement when the cilia are injured, as by a poisonous gas, for then pneumonia almost inevitably follows.

Nonspecific internal defenses. The internal defensive mechanisms that operate against invading microbes of all sorts are of great significance, and are truly indispensable.

Bactericidal power of normal blood and other body fluids. Among the nonspecific defenses must be counted the real, though limited, power of normal blood to destroy germs. If we mix the fresh blood serum of a normal, healthy person with a suspension of one of the common bacteria, such as *Escherichia coli,* and then make a count of the number of living bacteria in that suspension as compared with the same suspension before the serum was added, we find that the serum has destroyed a certain number of the organisms. By inoculating germs of low virulence into the peritoneal cavity of animals, and watching the changes that take place there, it is possible to show that the normal peritoneal fluid has a similar bactericidal power.

This capacity of the normal body fluids to kill germs is feeble, however, and can have little value in protecting us against frankly virulent, invasive germs. It is nevertheless probable that potentially pathogenic bacteria get into the blood stream in small numbers more often than is commonly thought, through minute injuries in the skin or the mucous membranes, chronically inflamed tonsils, etc., and if it were not for this bactericidal power of the blood, infection would be much more frequent.

Cellular reactions, inflammation, and phagocytosis. All-important in the body defense are the activities of certain body *cells* and the associated inflammatory reactions. These cells are of two principal kinds: (1) the polymorphonuclear leukocytes of the blood and (2) the large, fixed or wandering mononuclear cells, or macrophages, of the blood and tissues. Both of these types of cells play a prominent part in the local inflammation that usually accompanies an infection, and also they contribute to defense more directly by

engulfing many of the invading microorganisms into their own cytoplasm—the process called *phagocytosis*. Description of this *cellular response* of the infected host is the purpose of the following paragraphs.

Local reaction to infection; inflammation. When germs have passed through the body surfaces into the deeper tissues, they usually stimulate a more or less intense local reaction at the site of the infection. In response to the injury caused by the bacteria, or their poisons, the process called *inflammation* sets in. *Inflammation* may be defined as the *local reaction caused by any agent that injures the tissues.* A blow, an irritating chemical substance, or foreign body, or other injurious condition, may bring about an inflammatory reaction. But the pathogenic bacteria are the most common causes of inflammation.

The inflammatory process is defensive. It is an attempt to localize the disease process, to eliminate injurious agents, to neutralize and destroy poisons, and to repair damaged tissues. Hence the inflammatory reaction is an important factor in the body defense against germs.

Types of inflammatory reaction; exudates. *Usually, in inflamed tissues, there is an abnormal accumulation of fluids and of cells from the blood and other tissues.* This material is called the *exudate.* The character of the exudate varies somewhat with the part of the body affected, but it is principally determined by the kind of germ causing the infection. The inflammatory process differs in intensity in different infections, and the exudate differs in amount and in composition.

Special forms of inflammation. In tuberculosis, there is a peculiar reaction resulting in the formation of little hard masses of infected tissue called *tubercles.* This is so constant a feature of tuberculous infection that a tentative diagnosis of tuberculosis may be made whenever tubercles are seen, although the final diagnosis must depend upon finding the tubercle bacilli.

In a few infections, such as tuberculosis of the pleura, a *serous exudate* forms. This is a clear fluid, containing few cells of any kind.

In diphtheria, the exudate takes the form of a tough, grayish-white, leather-like membrane, covering the inflamed tonsil, or other part. This is called a *membranous exudate.*

The acute inflammatory reaction; purulent exudates. The most common result of an *acute* bacterial infection is the formation of an

exudate consisting mainly of *polymorphonuclear leukocytes* (the white blood cells with many-shaped nuclei). These cells are attracted to the infected region, wandering there from neighboring blood vessels. When, through the action of proteolytic enzymes from these leukocytes, the exudate becomes partially liquefied it is called a *purulent exudate* or *pus.* Pus is made up of small amounts of fluid from the blood or lymph vessels, some fibrin and red blood cells, and masses of inflammatory body cells, mostly polymorphonuclear leukocytes. These latter cells are such prominent constituents of pus that they are commonly called *pus cells* (Fig. 94). The large mononuclear cells, called *macrophages,* derived from the blood or tissues, are also present, especially in the latter stages of the inflammatory reaction.

Cellular reactions in subacute or chronic inflammations. Where injury to the tissues is relatively slight, these reactions are characterized by an accumulation of *lymphocytes,* or other *mononuclear cells,* rather than by the presence of polymorphonuclear leukocytes. Pathologists thus recognize several varieties of inflammatory cells which contribute to the defense of the body against germs.

Phagocytosis. The protective action of acute inflammatory exudates is due principally to the special powers of the polymorphonuclear leukocytes and macrophages. These cells can engulf (take into their own cytoplasm) foreign particles of all kinds, including disease germs. The *leukocytes* circulate in the blood stream, but they have the power of independent motion (ameboid motion), and they are able to migrate out from the capillaries to a point in the tissues where they are attracted by some particle of foreign material—a group of germs, for example. Once in contact with the germs, the leukocytes take many of them into their own substance, just as an ameba surrounds and engulfs a particle of food. When pus is examined under the microscope, many of the organisms are seen to be *within the cytoplasm of the pus cells* (Fig. 94). The engulfed bacteria are sometimes destroyed then and there within the leukocyte, or they may be carried away to their eventual destruction with the death of the cell itself. The *macrophages* not only engulf bacteria, but the leukocytes as well. Metchnikoff, who first called attention to the remarkable action of these cells, named them *phagocytes* (eating cells): from this is derived the term *phagocytosis.*

The power of phagocytosis is not confined to the polymorpho-

nuclear leukocytes and wandering macrophages, but is possessed in different degrees also by a variety of other body cells, some of which are *fixed* in the tissues. Among the most actively phagocytic of the latter are the cells lining the capillaries and sinuses in the liver (Kupffer cells), spleen, bone marrow, and lymph nodes. All these belong to the so-called *reticulo-endothelial system.*

As we have indicated, the wandering phagocytic cells of the large mononuclear types, known collectively as *macrophages,* to distinguish them from the smaller leukocytes (*microphages*), are of great importance. The macrophages are the scavengers that finally clear away cellular débris, bacteria, the polymorphonuclear leukocytes and all, when the infection has been conquered.

Example of an inflammation with pus-formation; history of a boil. The familiar boil, caused by the *Staphylococcus aureus,* is a good example of an acute inflammatory process characterized by the accumulation of pus at the site of infection. Figure 70 illustrates the stages in the development of this inflammation. The process begins a few hours after the staphylococci have entered the deeper layers of the skin and multiplied there. The first stage of the reaction is marked by an enlargement of the capillaries in the region and, therefore, an increased flow of blood through the infected aera. The leukocytes, circulating through these distended vessels, are attracted to the spot where the bacteria are growing. This attraction is probably due to chemical substances which diffuse out from the mass of organisms and injured tissue. At first, the leukocytes pass more slowly, then they appear to stick on the walls of the blood vessels opposite the infected part. Finally, the attraction becomes so great that they force their way through the thin walls of the swollen capillaries and travel by ameboid motion toward the germs. Soon there is an accumulation of a great many of these cells in the inflamed tissues, along with some fluid from the blood and surrounding tissues.

After a time, this pus becomes concentrated at one point under the skin, and can be seen as a yellowish "head" protruding above the surface. The skin in the region of the boil now shows all the cardinal signs of inflammation—it is reddened, hot, swollen, and painful. The redness and heat are due to the increased amount of blood there, the swelling is caused by the accumulated exudate, and the pain is due to the pressure of this exudate upon the nerves.

The boil is a typical *abscess*—that is, *a localized accumulation*

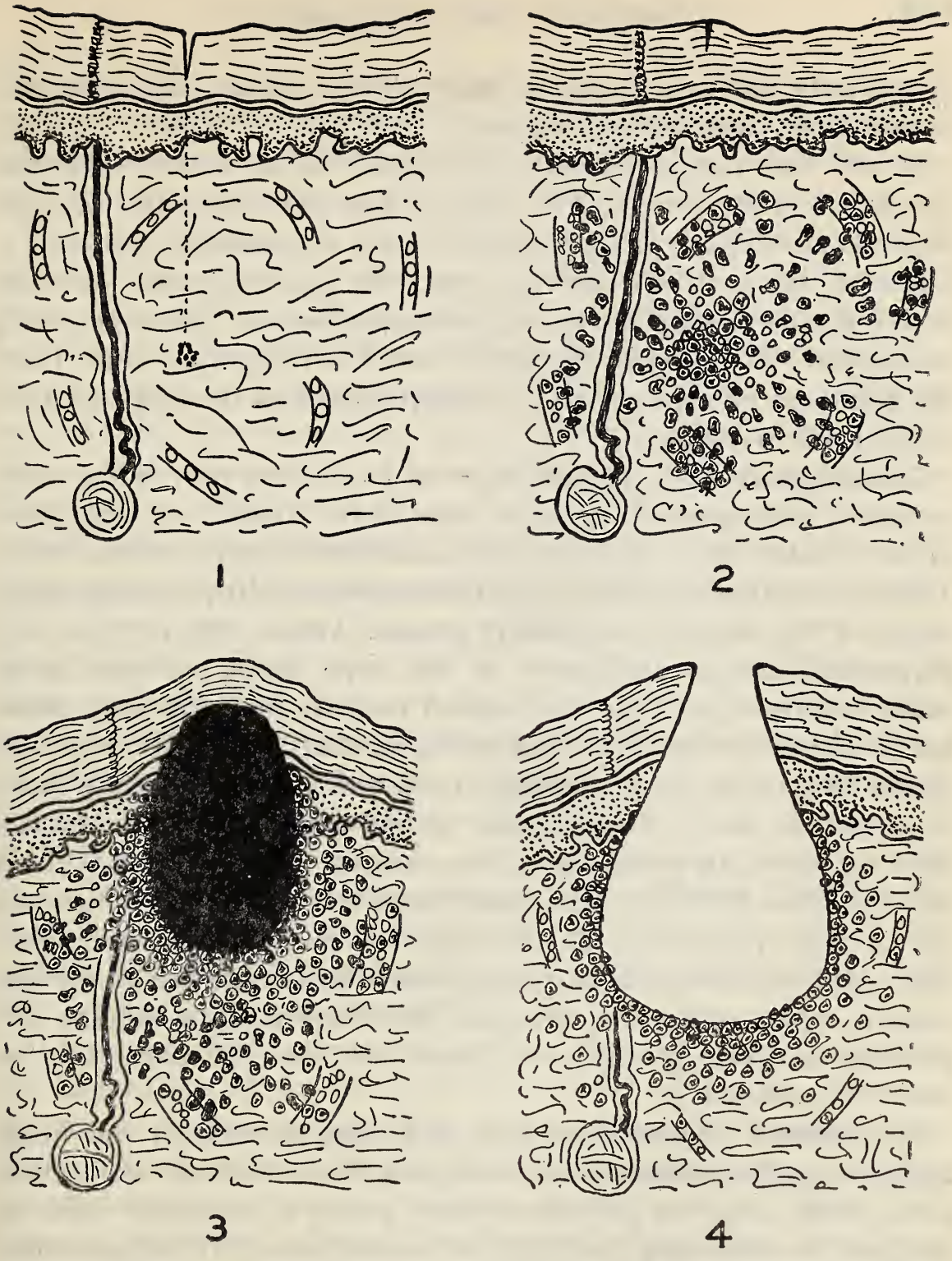


FIG. 70. The "history of a boil." 1. A cut-section of the normal skin just after it has been pierced by a dirty pin, carrying in staphylococci.

2. The early stages of inflammation; migration of leukocytes from swollen capillaries toward the infected area. The skin is becoming red, swollen and painful.

3. The accumulation of leukocytes has continued and now there is a mass of purulent exudate about the germs. The boil has "come to a head" and the semi-liquid pus can be seen through the thin cuticle over it.

4. The boil has finally ruptured and the pus has escaped, carrying with it the staphylococci. The migration of leukocytes has ceased and the capillaries are returning to their normal size. New tissue will grow in. (Redrawn and adapted from Emerson and Taylor's *Essentials of Medicine,* J. B. Lippincott Company, Philadelphia, 14th Ed., 1940.)

of pus held within a pocket or cavity in the tissues formed by the destruction of the normal tissues.

In the boil, further changes soon occur. Most of the leukocytes die, much of the surrounding tissue is also destroyed, and the pus becomes semiliquid. If the pressure of the accumulated exudate is sufficient, the boil now ruptures, and the pus oozes out, carrying with it a mass of dead tissue, and the staphylococci. The same result is achieved if the head of the boil is lanced at the proper time. Thus the germs are eliminated, and the deeper parts of the body are protected from invasion.

Leukocytosis. The accumulation of pus in any part of the body is usually accompanied by an *increase in the number of leukocytes in the circulating blood.* This is the condition known as *leukocytosis.* There are normally 5,000 to 8,000 white blood cells per cubic millimeter of the blood, in a healthy person. About 70% of these are polymorphonuclear leukocytes of the type found in pus. Most acute infections give rise to a marked increase in the *relative* number of polymorphonuclear leukocytes, so that these cells may constitute 90% of all the white blood cells, and at the same time there is an increase in the *total* number of leukocytes present in the circulating blood. In pneumonia, for example, there is a leukocytosis with 20,000 or 30,000 or more white blood cells per cubic millimeter, and a high proportion of these are of the polymorphonuclear type. Also, an increased percentage of the leukocytes are of the less mature types, indicating that the blood-cell-forming organs are pouring relatively young white blood cells into the circulation to meet the emergency.

Importance of pus-formation and phagocytosis in the body defense against infection. It is obvious, from the story of the boil given above, that the capacity to form pus is of very real value to the body in defending itself against staphylococci. It happens that these organisms have an especially marked tendency to form localized abscesses similar to a boil in any part of the body they may invade, and pus-formation is so characteristic a reaction to staphylococcus infection that these germs are commonly called *pyogenic cocci* (pus-generating cocci). A few other germs are also markedly pyogenic. In pneumonia, gonorrhea, and meningitis, for example, most of the symptoms are really due to the accumulation of pus in the infected tissues, and at the same time the phagocytic action of the pus cells is an important factor in the body defense.

In many other infections actual pus formation does not occur,

although more or less sizable accumulations of inflammatory cells appear in infected areas. One fundamentally important effect of this cellular reaction is to help *localize* the infection, and reduce the liability of a more serious generalized invasion of the body.

Metchnikoff believed that the leukocytes and other phagocytic cells were responsible for the entire phenomenon of immunity to germs. It would be difficult indeed to overstate their importance. It is always the phagocytes, particularly the macrophages, that finally dispose of invading bacteria. There is a close parallelism between the vigor of phagocytosis and the degree of resistance displayed. Whereas microbes of low virulence are readily captured and destroyed by phagocytes, the more highly virulent kinds are resistant; hence, their virulence is accounted for in part by their antiphagocytic power. The longer the invading bacteria have to multiply before there is an effective cellular response, the more severe the infection is likely to be. On the other hand, if mobilization of phagocytic cells in the infected area is accelerated, as by heat or counterirritation, or if rapid multiplication of the germs is inhibited by the presence of a bacteriostatic substance (such as a sulfonamide drug) so that the phagocytes have an early advantage, the infection is likely to be stopped quickly. Any influence that interferes with the cellular reaction, or phagocytosis, favors infection. This is especially noted in patients suffering from diseases of the blood-cell-forming organs, such as leukemia or agranulocytosis. These patients always have severe secondary infections.

It must be said, however, that in most virus infections, in diseases like diphtheria where the body is poisoned by a toxin, in spirochetal infections such as syphilis, and in some other common germ diseases, local cellular reactions are slight, and phagocytosis does *not* play an important part in defense. Moreover, even in the frankly pyogenic infections, where phagocytosis is most active, the leukocytes do not act entirely alone, for the bacteria are first made susceptible to the phagocytes *by antibodies called opsonins* present in the blood serum and other body fluids. This will be explained in the following chapter.

Finally, there is nothing specific about the action of phagocytes (since they may take up several different kinds of disease germs with equal readiness) and their activities do not result in any permanent immunity. It follows that the specific and lasting resistance that many persons have toward certain infectious agents cannot be dependent on phagocytosis. Instead, it is the development of *anti-*

bodies that accounts for increased resistance toward specific kinds of microbes or toxins.

REFERENCES

Cannon, P. R., Chase, W. E. and Wissler, R. W.: "The Relationship of the Protein Reserves to Antibody Production," *J. Immunol.*, 1943, 47:133.

Cottingham, E. and Mills, C. A.: "Influence of Environmental Temperature and Vitamin Deficiency Upon Phagocytic Functions," *J. Immunol.*, Dec. 1943, 47:493.

Jordan, E. O. and Burrows, W.: *Textbook of Bacteriology*. Philadelphia: W. B. Saunders Co., 14th Ed., Revised, 1945.

Lurie, M. B.: "Heredity, Constitution and Tuberculosis; Experimental Study," *Am. Rev. Tuber., Suppl.*, 1941, 44:1.

Menkin, V.: *The Dynamics of Inflammation*. New York: The Macmillan Co., 1940.

Perla, D. and Marmorston, J.: *Natural Resistance and Clinical Medicine*. Boston: Little, Brown & Co., 1941.

Rich, A. R. and McKee, C. M.: "Pathogenicity of Avirulent Pneumococci for Animals Deprived of Leucocytes," *Bull. Johns Hopkins Hosp.*, 1939, 64:434.

Thompson, R.: "Lysozyme and Its Relation to the Antibacterial Properties of Various Tissues and Secretions," *Arch. Path.*, 1940, 30:1096.

REVIEW QUESTIONS—CHAPTER XXI

1. What are the three "lines of defense" against infection?
2. Discuss the external defense of the body against infection.
3. What importance has the bactericidal power of normal blood?
4. Define *inflammation*. What is the purpose of the inflammatory process?
5. What disease germ causes the formation of tubercles? Define *exudate*. Give examples of infections resulting in: (*a*) a serous exudate, (*b*) a membranous exudate.
6. What is the usual character of the acute inflammatory reaction? How does the cellular reaction differ in less acute inflammations?
7. What is meant by *purulent exudate, pus, pus cell, polymorphonuclear leukocyte, lymphocyte, phagocyte, macrophage, phagocytosis*?
8. Describe phagocytosis. What types of body cells have phagocytic power?
9. Trace the history of a boil. Define *abscess*.
10. What is meant by *leukocytosis*?
11. What are *pyogenic* organisms? What relation has pus-formation to the typical symptoms accompanying pyogenic infections?
12. Name one desirable general effect of inflammatory reactions.
13. Discuss the importance of the cellular response, and phagocytosis, in the body defense against infection.
14. Give reasons why the cellular reaction cannot be considered responsible for all the phenomena of immunity.

CHAPTER XXII

ANTIGENS AND ANTIBODIES: ANTIGEN-ANTIBODY REACTIONS

Development of specific resistance. Recovery from most germ diseases is accompanied by deep-seated physiological changes in the whole body, as a result of which the individual becomes resistant *to the particular organism* causing the illness. It is clear that some reaction goes on between the germ and the tissues of the infected host during the course of the disease, so that the individual, at first susceptible, becomes, at the time of recovery, highly resistant toward that particular infectious agent. Everyone is familiar with the fact that this newly acquired immunity often persists for years, and that a person who has recovered from typhoid fever, measles, and other common infections, is not likely to have the same disease again.

The physiological processes which underlie immunity are exceedingly complex, and by no means entirely understood. It has been found, however, that the development of a specific resistance is always associated with the appearance, first in the infected tissues, then in the circulating blood, of so-called *antibodies.* These antibodies are *capable of reacting specifically with the particular organism, or toxin, which caused their formation.* Thus, a person infected with typhoid bacilli develops antibodies which act upon these organisms, and a person poisoned by the toxin of the diphtheria bacillus develops an antibody which acts upon this toxin, and so on.

Antigens. It is important to realize that this antibody formation is not a unique process occurring only in the diseased person. On the contrary, *the body forms specific antibodies* not only when it is invaded by bacteria or poisoned by their toxins, but also *whenever foreign protein material of any kind is introduced into the tissues.* Antibody formation is, therefore, a general biological

phenomenon. It is one of the ways in which living tissues respond to the presence of foreign matter. *It happens that, as a general rule, the antibodies formed when we are infected, or artificially vaccinated with microbes, help to protect the body against the organisms or their toxins, and these antibodies are to a large extent responsible for our resistance to infectious diseases.*

Any substance which, when introduced into the tissues, will stimulate the formation of antibodies, is called an *antigen,* or, more properly, a *complete antigen.* Practically all *proteins* are complete antigens. Baterial cells and toxins, of course, contain much protein. Carbohydrates and other nonprotein materials usually are not able to stimulate antibody formation when injected *by themselves.* When combined with protein, however, they often determine the specific character of the antibody which the whole compound produces. They also may react with this antibody in the test tube and in the animal body. Such substances, which cannot cause antibody formation when injected alone, yet react *in vitro* and *in vivo* with antibodies produced by complete antigens of which they are a part, are called *partial antigens,* or *haptens.* An example of a hapten is the carbohydrate present in the capsule of a pneumococcus.

Antigen-antibody reactions. Since antibodies often become abundant in the circulating blood, the usual way of securing them for study is to bleed the immunized individual, allow the blood to clot, and collect the blood *serum*—the clear, yellowish fluid that separates from the clotted blood. The antibodies will be present in such a serum, which is more properly referred to as an "antiserum," or an "immune serum." However, there is no way of proving the presence of the antibodies, or of determining their "strength" or amount, by ordinary physical or chemical tests. The antibodies can be demonstrated only by bringing the antiserum containing them into contact, in some way, with the particular antigen (bacterial cell, toxin, foreign protein, or whatnot) which caused their formation. The result will be a reaction—an *antigen-antibody reaction*—which will vary in character according to the particular conditions under which the two reacting substances are brought together.

Serological reactions. There are several kinds of antigen-antibody tests that may be performed *in vitro.* These are generally called *serological tests,* since they are carried out with serum (antiserum). These tests are named according to the nature of the changes observed in the antigen-antibody mixtures. Also, the antibodies partic-

ipating in each of the different types of reactions are referred to by distinctive names. The *in vitro* tests are tabulated in Table XV.

In vivo antigen-antibody reactions. Many of the test-tube reactions listed above are highly sensitive, and will detect very small concentrations of antibody. But *in vivo* tests are still more sensitive.

TABLE XV. *In Vitro* Antigen-Antibody Tests

Nature of the Antigen	*Antibodies Called*	*Test Called*	*Nature of the Reaction*
Exotoxins ("Toxins")	antitoxins	flocculation	A flocculent precipitate occurs when toxin and antitoxin are mixed in certain proportions.
Intact cells (bacteria, red blood cells, etc.)	agglutinins	agglutination	Cells are clumped, and may fall out of suspension.
	opsonins	opsonic index determination, or oposono-cytophagic test	Cells are made more susceptible to phagocytosis by leukocytes.
Extracts or solutions made from bacteria, etc., containing complete antigens or the hapten portions therefrom, or other colloidal solutions (such as egg white, foreign serum, etc., etc.)	precipitins	precipitation	A fine precipitate appears when the clear antigen solution is layered over, or mixed, with the clear antiserum.
Intact cells (bacteria, red blood cells, viruses, etc.)	bacteriolysins cytolysins hemolysins	bacteriolysis cytolysis hemolysis	The cells are killed, and sometimes actually dissolved (lysed), when mixed with antiserum in the presence of complement.
	"complement-fixing antibodies"	complement-fixation tests	Tests for the presence or absence of specific antibody in an unknown serum, by determining ability of a mixture of the serum and antigen to fix complement.

Specific antigen-antibody reactions can often be elicited *in the living body* of the immunized or sensitized individual when the antibodies that must be responsible cannot be demonstrated in the circulating blood by any test-tube method. The kinds of *in vivo* tests include: (1) *protection tests* and (2) *hypersensitivity tests.* The latter are carried out in human beings in the form of *skin tests* (for example, tuberculin tests), while in experimental animals more severe or fatal *anaphylactic reactions* are produced, as well. These procedures will be described later.

Nature and origin of antibodies. Immunologists have concluded that antibodies consist of nothing more nor less than particles of *serum globulin*. These globulin antibody-particles are altered *from their normal physicochemical make-up in a specific way because they were formed by the body cells in the presence of a particular foreign substance—the antigen. Their specialized structure makes them capable of combining specifically with that antigen.*

Antibodies are formed locally, in the tissues in actual contact with the antigen, and reach a certain concentration there before they appear in demonstrable amount in the circulating blood. They also disappear from the blood before they do from the tissues. Possibly, all actively metabolizing body cells can form antibodies, but it is suspected that the principal antibody-making cells are those of the reticulo-endothelial system—the system that includes the same cells that are responsible for the cellular defensive reactions described in the preceding chapter. Recent evidence points to *lymphocytes* as important antibody-formers.

It must not be imagined that the antibodies differently named (Table XV) are different substances. It is now generally agreed that the phenomena of bacteriolysis, agglutination, and so on, may all be brought about by a single antibody. Which one of these reactions is observed in a particular test or experiment depends upon the physical state of the antigen and the conditions under which the serum and the antigen are brought together.

THE PRINCIPAL ANTIGEN-ANTIBODY REACTIONS

Toxin-antitoxin reactions. The kind of antibody called antitoxin was first demonstrated by Behring and Kitasato in connection with their early studies of resistance to tetanus and diphtheria (1890). They found that animals which had survived a series of injections of small amounts of tetanus toxin had become very resistant to this poison. In attempting to find an explanation of this immunity, they were naturally led to examine the blood of the resistant animals. They found that *the blood serum of animals immune to tetanus contained something which neutralized the poisonous action of tetanus toxin*. The substance or element in the serum responsible for this action they called *tetanus antitoxin*.

Behring showed that resistance to diphtheria, as well as to tetanus, depends upon an antitoxin—in this case *diphtheria anti-*

toxin, which specifically neutralizes the *diphtheria* exotoxin, the poison secreted by the diphtheria bacillus.

It has since been found that all exotoxins, including those of scarlet fever streptococci, toxigenic staphylococci, dysentery and gas gangrene bacilli, etc., stimulate the body to form specific neutralizing antitoxins. Also, poisons of animal or plant origin, such as those of the scorpion and the black-widow spider, snake venom, and ricin (a poisonous substance in the castor-oil bean), are capable of causing the production of specific antitoxins when injected into animals. The otherwise fatal poisoning that follows the bite of certain snakes can be prevented by the injection of a serum containing antitoxins (antivenins) which will neutralize the deadly venom of these snakes.

Antitoxins (and antivenins) are antibodies of a special kind, because they are developed only in response to the presence in the body tissues of an exotoxin, venom, or similar poison, and because they have the power of specifically *neutralizing* those poisons, thus making them harmless. Toxins and their respective antitoxins may combine in varying proportions, and the mixtures may still be poisonous in some degree, but when enough antitoxin is present to combine with all the active toxin, the mixture is neutralized, and entirely harmless.

Just how the antitoxin acts to neutralize the toxin is not known. The toxin *is not destroyed* by mixture with antitoxin, for such mixtures can be dissociated and active toxin can be recovered. It is known that the antibody molecules are adsorbed upon the surface of the toxin molecules, and it has been suggested that the antitoxic serum simply covers the poisonous radicals of the toxin so that they cannot reach the body tissues.

Antitoxins are regularly studied by *in vivo* protection tests (Fig. 71). The amount of antitoxin in a serum is determined by inoculating animals (usually guinea pigs) with the serum after mixture with different amounts of the corresponding toxin, thus testing the capacity of the antitoxin to protect against the poison. The protective power of the serum is expressed in terms of the antitoxin *units* it is found to contain.

When toxin and antitoxin are mixed in test tubes in certain proportions, a flocculent precipitate occurs, and this precipitate appears *earliest* in that tube containing closest to a neutral mixture. Preliminary measurements of the antitoxic content of an unknown

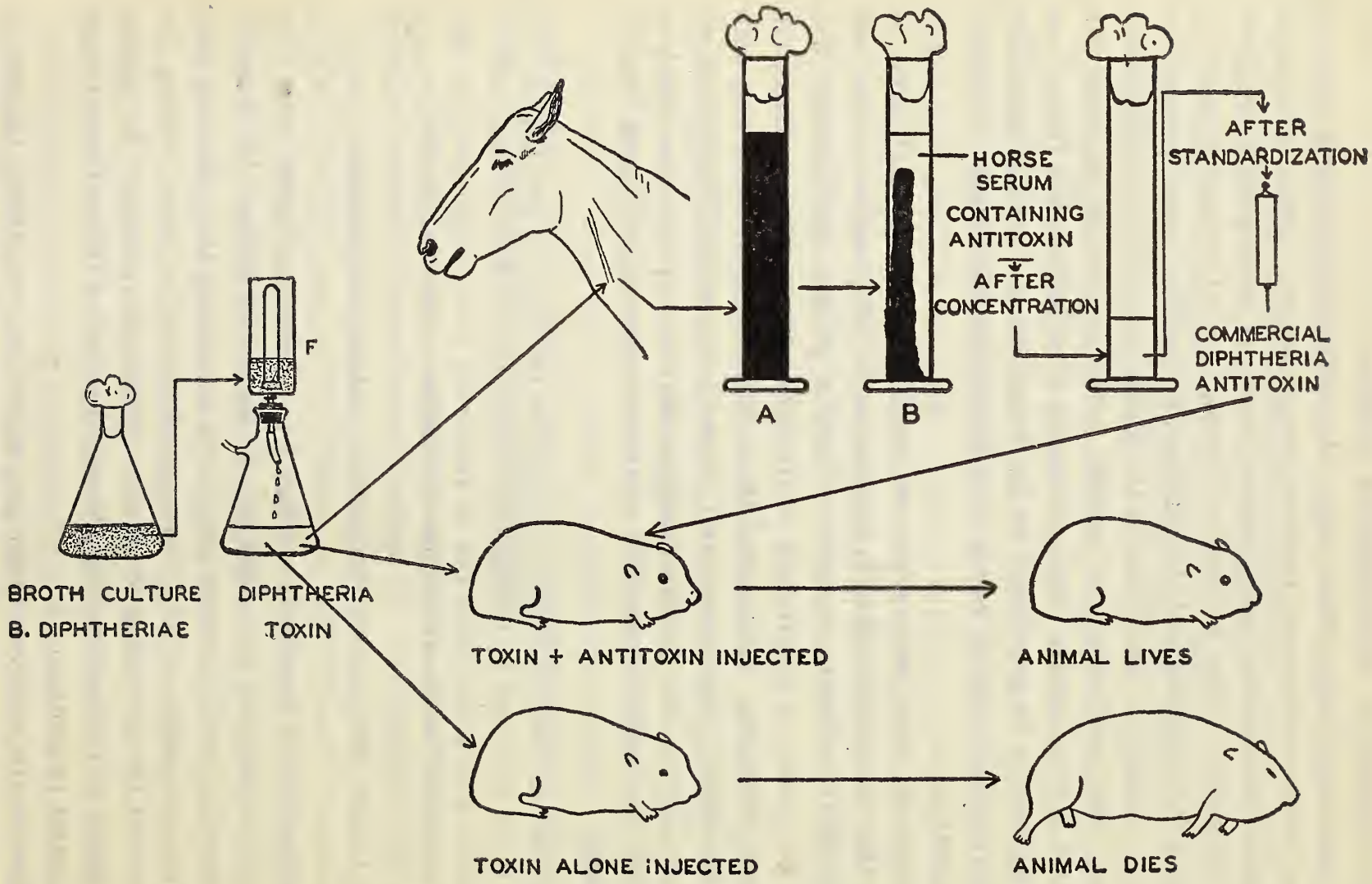


FIG. 71. Legend on opposite page.

antitoxin serum may thus be made by means of this so-called *flocculation test,* before beginning animal protection tests.

The careful standardization of commercial antitoxins by these methods is, of course, of great practical importance. Only by having a standardized product can the physician know the proper doses to use.

There are two widely used skin tests which are based upon toxin-antitoxin reactions: the *Dick test* and the *Schick test.* These are designed to determine the susceptibility of human beings to scarlet fever and to diphtheria, respectively. In each case an intradermal injection into the forearm is made of a minute amount of toxin (in the Dick test, the exotoxin of the scarlet fever streptococcus, and in the Schick test the exotoxin of the diphtheria bacillus). If an inflammation develops at the site of injection, the individual is obviously susceptible to the toxin, and likely to have the natural disease, whereas, if no reaction occurs in the skin (negative test), the person must have neutralized the toxin—that is, he must possess the necessary specific antitoxin to make the injected toxin harmless. An individual showing negative tests is resistant, and not likely to have typical scarlet fever or diphtheria (Chapters XXVI and XXVII).

Bacteriolysis and specific hemolysis; complement fixation. The experiments of Pfeiffer (1894) were among the first to demonstrate the occurrence of antibodies acting directly upon bacterial cells. Pfeiffer found that when virulent cholera spirilla were inoculated into the peritoneal cavity of guinea pigs immune to this organism, the spirilla rapidly became swollen, then granular, and finally disappeared altogether. In other words, he showed that the peritoneal fluid of immune animals had the power to destroy cholera spirilla. He further demonstrated that this power was possessed to a very limited extent only by normal animals, but was developed to high degree during the course of a cholera infection, or as a result

---

FIG. 71. Diagram illustrating the production and action of antitoxin, using diphtheria antitoxin as an example. At the left is shown how diphtheria *toxin* is secured. After *C. diphtheriae* has grown in broth for several days, the culture is passed through a filter (F), and *the clear, sterile filtrate constitutes diphtheria toxin.* When this is injected repeatedly into the horse, the animal responds by the formation of a specific *antitoxin,* and this becomes abundant in the blood of the animal. To secure the antitoxin the horse is bled, and from the blood (A), after it has been allowed to clot (B), the clear blood *serum* is separated. This serum, after concentration, purification, and standardization is put upon the market as diphtheria antitoxin. The action of this antitoxin in protecting against the diphtheria toxin is demonstrated by animal tests, as illustrated.

of a series of injections of the spirilla. The destructive action of the peritoneal fluid of the immune animals was evidently due to antibodies developed by the guinea pigs in response to the presence of the germs. The antibody concerned here was appropriately called a *bacteriolysin,* meaning a substance which causes the lysis (dissolution, destruction) of bacterial cells.

Metchnikoff, Bordet, and others soon showed that the bacteriolytic power of the blood and body fluids of animals immune to cholera and other germs can be demonstrated in test-tube laboratory experiments, as well as in the animal body (Fig. 72:1). They found that bacteriolysis depends upon the interaction of two elements with the bacterium: (1) *the specific antibody* (bacteriolysin) and (2) *an unstable element normally present in the blood* and other body fluids of human beings and animals, which is now called *complement.* The bacteriolysin is produced in large amount when an individual becomes immune. Its action is *specific,* that is, it can combine only with the kind of bacterium which caused its formation. Complement, on the other hand, is naturally present in blood and other body fluids of normal individuals, and is not increased in amount by immunization. Complement is the active element in causing bacteriolysis, but it can do so only after the antibody (bacteriolysin) has combined with the bacterial cell and prepared it ("sensitized" it) for the action of the complement.

Bacteriolysis, resulting from this combined action of antibody and complement, plays an important part in the resistance to many bacterial diseases. It is probably the chief mechanism by which the blood stream is cleared of the invading microbes during the process of recovery from pneumonia, typhoid fever, and other diseases.

In connection with their test-tube studies of bacteriolysis, Bordet and others found that *red blood cells* were hemolyzed by complement after they were mixed with a specific red-blood-cell-antibody (*hemolysin*). Just as complement is the agent that brings about bacteriolysis of the organism in the serum of individuals immune to a particular bacterium, so also it is the active element that causes, in an exactly similar way, the disintegration (hemolysis) of red blood cells in the serum of an animal immunized to these cells. In each case, the complement acts only after the antigen (bacterial cells or red blood cells) has been "sensitized" by combination with its specific antibody (bacteriolysin or specific hemolysin).

Thus,

bacterial cells + bacteriolysin + complement = bacteriolysis
(antigen) (antibody)
red blood cells + hemolysin + complement = hemolysis
(antigen) (antibody)

When hemolysis of red blood cells occurs in a test tube, the original opaque suspension of erythrocytes becomes changed to a crystal-clear, transparent red solution—a change which is easily appreciated by the naked eye. Hence, the addition of sensitized red blood cells (i.e., r.b.c. + r.b.c. antibody) to a solution is a good way to find out whether there is any free complement present. If so, the cells will be hemolyzed, and this can be detected by simple inspection of the tubes.

Diagnostic complement-fixation tests. Complement becomes firmly adsorbed, or *fixed,* upon antigen-antibody complexes. If the antigen consists of bacterial cells, these cells are first coated with the specific antibody, then these sensitized bacteria adsorb complement, thus removing it from the solution. If the antigen is an unformed substance, like egg-white, a precipitate occurs when the specific antibody combines with it, and then this precipitate fixes complement.

Diagnostic complement-fixation tests are designed to utilize this phenomenon of complement fixation to determine whether or not a patient's serum contains a particular antibody. To make the test, the antigen (for example, a suspension of a certain pathogenic organism) is mixed in suitable proportions, in a test tube, with the patient's serum (which may or may not contain the antibody being tested for), and with complement (usually supplied by fresh guinea-pig serum) (Fig. 72:2). This mixture is incubated at 37° C for an hour. During this time, if the patient's serum contains an antibody specific for the germs, combination occurs and, as a result, the complement is adsorbed, or fixed. However, if the patient's serum lacks the specific antibody, there will be no combination, and the complement will remain free. But there is no visible change in the appearance of the mixture in the test tube to show what may have taken place. The next step, then, is to add to the mixture something which will indicate whether or not free complement is present. The indicator used is a suspension of red blood cells plus *their* specific antibody (often called the hemolytic amboceptor). Measured amounts of this suspension are added, and the mixture is incubated again. Now, if complement is free in the mixture, it will

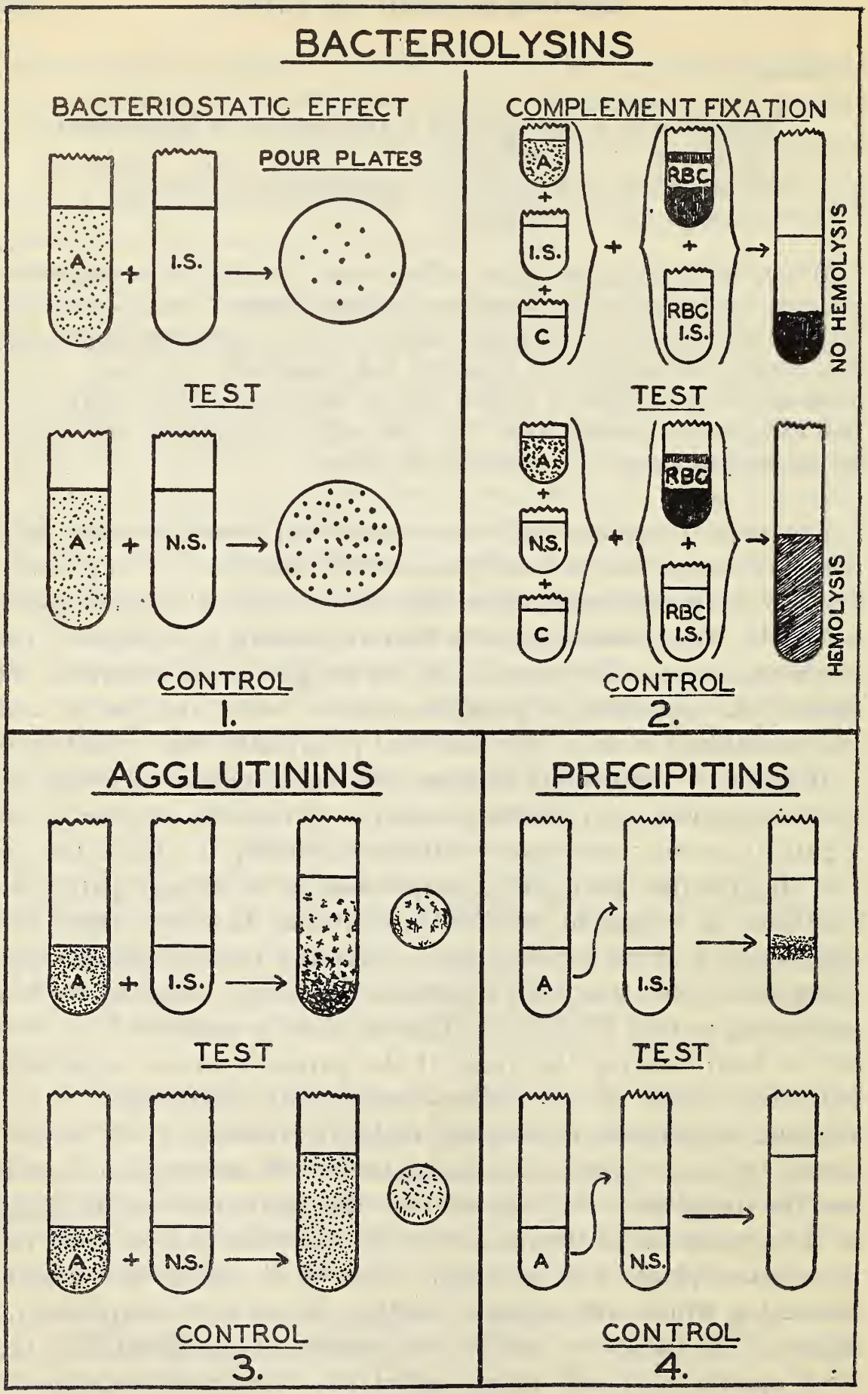


Fig. 72. Legend on opposite page.

cause hemolysis of the added red blood cells, which produces an easily visible change in the appearance of the mixture. If, on the contrary, all the complement was fixed during the first incubation, so that none is now available, the red blood cells will remain unchanged. *Complete hemolysis* means *a negative reaction,* i.e., the antibody tested for is not present in the patient's serum. *No hemolysis* means a *strongly positive reaction* (usually reported as four plus), i.e., there was sufficient antibody present in the patient's serum to fix all the complement. *Partial hemolysis* means a *weakly positive reaction,* i.e., some antibody was present in the patient's serum, but not enough to absorb all the complement.

The *Wassermann test for syphilis* is a complement-fixation test.

FIG. 72. Diagrams illustrating the action of antibodies when functioning as bacteriolysins, agglutinins, and precipitins.

Diagram 1. When a suspension of bacteria (A) is mixed with the *fresh* blood serum of an animal immune to the organisms, i.e., unheated immune serum (I.S.), and agar plates are then poured from the mixture, the resulting growth shows that many of the organisms in A have been killed or, at least, prevented from growing (bacteriostatic effect), since in this plate there are fewer colonies than in the control plate made from the same suspension mixed with normal serum (N.S.). This effect of the immune serum is due to the antibody called *bacteriolysin.* The action of the bacteriolysin, however, depends upon the presence of *complement* in the serum. This is destroyed by heat; hence the serum must be fresh and unheated.

Diagram 2. When a suspension of bacteria (A) is mixed with its specific immune serum (I.S.) which has been heated to remove its natural content of complement, and then a measured quantity of complement (C), (in the form of fresh guinea pig serum) is added, the antibody in the immune serum combines with the organisms and *to this combination the complement becomes bound, or "fixed."* To demonstrate this fixation of the complement, a mixture of a red blood cell suspension (R.B.C.), and an immune serum specific for red blood cells (R.B.C.I.S.) is added. Hemolysis of the red blood cells, for which complement is necessary, does not occur, because there is no free complement there. In the control, on the contrary, using normal serum (N.S.), the complement is not fixed, and so is free to act when the red blood cell mixture is added, and hemolysis of the cells occurs.

Diagram 3. When a specific immune serum (I.S.) is added to a suspension of a bacteria (A), the organisms clump together into visible masses which may fall out of suspension. This phenomenon of agglutination is due to the antibody called *agglutinin.* Normal serum (N.S.) has no effect. At the right, the microscopic appearance of the mixtures is illustrated.

Diagram 4. When a clear solution of a certain bacterium or other protein matter is added to the serum of an animal immunized to that particular bacterium, or protein (I.S.), a precipitate occurs. This is due to the antibody called *precipitin.* The reaction is most striking when the mixture of protein solution and immune serum is made in such a way that one liquid is stratified over the other, the precipitate forming at the junction of the two. Normal serum (N.S.) has no effect.

The antigen preparation used is not a suspension of the causative germ, however, but a colloidal solution containing lipoids extracted from muscle, which has been found to be superior as an antigen for detection of the particular antibody formed by the syphilitic patient.

Complement-fixation tests are also used, to a limited extent, in the laboratory diagnosis of various other infections, including those caused by protozoa, fungi, rickettsiae and viruses, as well as by spirochetes and ordinary bacteria. These tests are rather complicated, and require much skill for their proper performance, but they can be made very sensitive so that they will detect the presence of antibody when all other test-tube methods fail. They are consequently used extensively in experimental work, particularly in the study of viruses.

Agglutination reactions. Shortly after the discovery of bacteriolysins, Gruber and Durham called attention to another antibody reaction. They noticed that when suspensions of certain bacteria were mixed in a test tube with the serum of animals immunized against these bacteria, the organisms clumped together into visible masses which finally settled to the bottom of the tube. The same phenomenon of clumping of the organisms could be seen in drops of a mixture of serum and bacteria under the microscope (Fig. 72:3). They spoke of the clumping as *agglutination;* the organisms were said to be *agglutinated.* The specific antibody in the serum responsible for this phenomenon was named *agglutinin.*

The mechanism of agglutination is best understood by realizing that suspensions of bacteria act like colloidal solutions. The individual cells, like the particles of a true colloid, are kept separate and in suspension by the repelling effect of the electric charge that they all carry. On addition of a specific antiserum, the cells become coated with the antibody globulin. This causes a reduction in the repellent electric charge, and at the same time probably makes the cells somewhat sticky so that they tend to cling together. When the surface electric charge on the bacteria is lowered below a critical potential, the cells then actually form clumps, and fall out of suspension if the clumps get heavy enough.

Agglutination requires the presence of electrolytes, and the cells are always suspended in salt solution. They may be living or dead. Commonly the bacteria are grown on solid media, then suspended in salt solution and killed by addition of about 0.2% formalin. The antiserum need not be fresh, for complement plays no part in agglutination. The agglutinating serum is often heated to 56° C, to destroy any complement in it, and it

may be preserved by the addition of merthiolate, or by other means. *Microscopic agglutination tests* are carried out by mixing the antigen suspension and antiserum on ordinary glass slides or in hanging drops, while *macroscopic* tests are performed by mixing and incubating the reagents in small test tubes.

The *amount* of agglutinin in an antiserum is measured by making a series of dilutions of the serum with salt solution, and testing each dilution for its agglutinating power against the same antigen suspension. The highest dilution at which agglutination takes place is said to be the agglutinin *titer* of the serum. Thus, if agglutination still occurs when the final dilution of the serum is 1:5000, but not at higher dilutions, the serum is said to have an agglutinating titer of 1:5000. In typhoid fever and other human diseases, the titer of agglutinins in the patient's serum is relatively low (commonly less than 1:500), but by the artificial immunization of animals it is often possible to secure an agglutinating serum active in a dilution as high as 1:10,000 or more.

Agglutinated bacteria are not necessarily killed, but there is no doubt that when invading organisms are clumped within the body, they are more likely to fall prey to phagocytes.

Diagnostic agglutination tests. A simple procedure which sometimes helps to make a diagnosis of typhoid fever is the so-called Widal test. In this test, we put some of the patient's serum in contact with a suspension of *known* typhoid bacilli. If the bacilli are agglutinated, the *patient's serum must contain antibodies* (*agglutinins*) *for these organisms.* Since antibodies are specific, reacting only with the germs which caused their formation, this means that the individual has been infected, or vaccinated, with typhoid bacilli some time previously, or else he is now infected with them. Of course, if he has symptoms of typhoid fever, the latter is probably the case. If we make the same test a day or two later, and repeat at intervals, and find that the typhoid antibodies are increasing in amount in the serum, this is positive proof that the case is one of typhoid fever, because the only way in which a person could manufacture these particular antibodies is by being infected (or vaccinated) with this particular antigen (typhoid bacillus).

Similarly in other diseases, a diagnosis may be made by demonstrating agglutinins for the causative organism in the patient's blood, even though the germs themselves cannot be isolated or identified. Among the infections in which laboratory diagnosis is

commonly made on the basis of such agglutination tests are (besides typhoid fever), brucellosis (undulant fever), tularemia, and typhus fever.

It is possible, of course, to use agglutination and other antigen-antibody reactions not only for demonstrating the presence of a particular antibody in a patient's blood, but also as a means of *identifying unknown organisms*. If, for example, a pure culture is isolated from a patient, and is suspected to be typhoid bacilli, the identity of the organisms can be established by mixing a suspension of them with an antiserum known to contain typhoid antibodies. If agglutination occurs, under properly controlled conditions, the germs must be typhoid bacilli.

Precipitation (or flocculation) reactions. In 1897, Kraus described the most striking of all test-tube antibody reactions. He found that when a clear solution or extract of bacterial cells, or any clear fluid containing some of the protein matter from those bacteria, is mixed with clear serum from animals immune to these organisms, a *precipitate* occurs. This reaction was attributed to an antibody appropriately called a *precipitin*.

Precipitation tests are delicate and highly sensitive antigen-antibody reactions; they may be positive in very high dilutions. Non-protein haptens, as well as solutions of complete antigens, give strong precipitation with specific antisera.

The mechanism of the precipitation or flocculation that occurs in these tests is essentially the same as that operating in agglutination tests. In precipitin reactions, however, the antigen preparation used is in a different physical state. Whereas a visibly cloudy suspension of intact cells (bacteria, red blood cells, etc.) constitutes the antigen in agglutination reactions, precipitin tests are carried out with cell extracts or other solutions that appear clear to the naked eye. Actually, however, these latter preparations are not true solutions, but rather colloidal solutions in which the antigenic substance is dispersed in invisible particles. When antiserum is added, these colloidal antigen particles become coated with antibody globulin, and then, through the operation of forces similar to those bringing about agglutination of intact cells, these particles come together to form small aggregates which become visible to the eye as a fine precipitate.

The ring-test method (Fig. 72:4), in which the diluted antigen is layered over the antiserum in tiny, small-bore test tubes, is widely used. The only reagents are the clear antigen solution and the clear

antiserum. To titrate the precipitins in the serum, the same general procedures are followed as in titration of agglutinins, except that, instead of diluting the antiserum, it is customary to keep this reagent constant (undiluted, or diluted only slightly) and to *dilute the antigen solution.* Precipitin titers (in terms of the final antigen dilution) are commonly as high as 1:50,000 with antiserum from artificially immunized animals; and indeed, positive reactions at the 1:1,000,000 dilution have been reported. This ilustrates the great sensitiveness of precipitin reactions. (Complement-fixation tests, however, are even more sensitive.)

Diagnostic precipitin tests. In routine diagnosis, precipitin reactions are less commonly used than agglutination tests. They are useful, however, in a number of circumstances in which other antigen-antibody tests are not readily employed. For example, the spinal fluid from a patient with meningococcus meningitis will give a positive precipitin test with known antimeningococcus serum, and this test may insure the proper diagnosis when microscopic and cultural studies of the fluid are not conclusive. Other examples will be mentioned in later discussions of particular infectious diseases.

Forensic precipitin tests. Precipitin reactions are especially useful in *identifying unknown protein materials.* For example, by precipitin tests it is easy to determine whether a blood spot is of human or animal origin. It is only necessary to have in the laboratory an antiserum *known to contain antibodies for human blood.* (Such a serum may be prepared by immunizing rabbits with human blood.) If a solution of the unknown blood gives a precipitate when mixed with this antiserum, and not in control preparations, it must be human blood. Such tests are widely employed in crime-detection laboratories and in other phases of medico-legal work.

Opsonic action of antiserum. We have previously mentioned that the engulfing of microbes by leukocytes (phagocytosis) is not accomplished through the unaided efforts of the phagocytes alone, but occurs in the presence of blood or other body fluids. The importance of these fluids in furthering phagocytosis was noticed as early as 1895 by Denys and LeClef, but was first studied extensively by Wright and his associates, beginning in 1903. These investigators showed that the blood serum of normal men and animals, when added to a mixture of washed leukocytes and bacteria, had a slight effect in aiding the engulfing of the organisms by the leukocytes, but that the blood serum from an *immune* individual contained this

phagocytosis-promoting property in a far greater degree. It was shown that this effect of an immune serum is specific, and its action is upon the bacteria, preparing them for phagocytosis, rather than directly upon the leukocytes. Wright named the phagocyte-helping substances in the serum *opsonins,* a word derived from the Greek meaning "to prepare food for, to cater to." Opsonins, then, are antibodies that prepare or sensitize cells so that they are made especially susceptible to phagocytosis.

The importance of these antibodies in defense, when the tissues are invaded by virulent bacteria, is obvious. The opsonins assist the phagocytes in the necessary process of capturing and removing the harmful microbes.

The *opsonic power* of an immune serum may be estimated by mixing a measured amount of the serum with leukocytes and with a dilute suspension of staphylococci (or other bacteria). The amount of phagocytosis in this mixture is compared with the amount in a similar mixture prepared in the same way, but containing only sterile *normal* serum. Smears of the mixtures are made after they have been incubated for a time, and, by counting the number of bacteria engulfed by 100 leukocytes in each mixture, the phagocytic activity may be expressed numerically, and the relative opsonic power of the immune serum over that of the normal serum may be stated in a figure called the *opsonic index.* The practical value of opsonic-index determinations is limited, however, because of the technical difficulties; and unless the tests are performed by experts, in special circumstances, little significance can be attached to them.

The *opsono-cytophagic test* is a modified form of opsonic-index determination. As used in connection with the study of resistance to brucellosis, the test consists in counting the number of *Brucella* organisms found within the leukocytes in a smear of the patient's own blood, after mixing and incubating the bacteria with the blood sample for 30 minutes. A relatively high average count of phagocytized bacilli per leukocyte (40 or more) is interpreted to mean that the patient has *Brucella* antibodies and is in some degree immune.

Protection tests. The most significant special property of an antiserum, of course, is the ability it may have actually to protect a susceptible animal against illness or death, as demonstrated by well-controlled *in vivo* protection tests. The general method of per-

forming protection tests has already been outlined above. The sterile antiserum and the active bacterium, virus, or toxin concerned are mixed, the mixture is allowed to incubate for a short time, and then it is inoculated into fully susceptible animals of an appropriate species. Specific protective action is indicated when animals receiving such mixtures survive, while control animals, injected with the same material, *but without that particular antiserum,* succumb to the infection.

Such tests have a wide use in determining the identity of unknown viruses; in these tests a known specific protective antiserum is mixed with material thought to contain a certain virus. Protection tests are also employed to standardize antitoxins, therapeutic antipneumococcus serum, and other serums intended for treatment of human disease. Often no direct correlation is found between the concentration of antibody in a particular serum, as measured by test-tube reactions, and the protective power shown by *in vivo* tests. There is usually fairly good agreement with the results of complement-fixation tests, however. As we have noted above, these are the most sensitive of the ordinary *in vitro* tests.

Skin tests. Another type of antigen-antibody reaction is that produced when a minute amount of a preparation containing a microorganism (or a hapten derived from it), or other antigenic substance (such as ragweed pollen, horse serum, etc., etc.), is introduced into the skin of a person or animal that has specific antibodies for that antigen in the tissues. A local inflammatory reaction occurs, because of the injury to the skin which is secondary to the combination of the antigen and antibody. This "positive skin reaction" is a so-called *allergic reaction;* the individual is said to be "sensitive" to the antigen.

Such skin tests are of great help in identifying the particular pollen, foodstuff, etc., to which human beings who suffer from hay fever, asthma, and other clinical forms of *hypersensitivity* (or *allergy*) have become sensitized. Also, they are important as a means of determining those persons who are sensitive to horse or rabbit serum, in order that dangerous reactions may be avoided when injections of antitoxins and other therapeutic antiserums are to be made. A specific hypersensitivity develops toward the causative germ, or some of its products, in the course of most infectious diseases, especially the more chronic infections, like tuberculosis. Allergic skin tests may therefore be useful in diagnosis of past or

present infection. Best known and most widely employed of the diagnostic skin tests are the *tuberculin test* (for tuberculosis) and the *Frei test* (for lymphogranuloma venereum).

Anaphylactic reactions in animals. When guinea pigs or other animals are given injections of any antigenic substance, they become sensitized. If, now, they are reinoculated rapidly, at the proper time, with a sizable dose of that same antigen they may be thrown into a severe or fatal *anaphylactic shock*. This is a consequence of the rapid, injurious changes in the body that occur as secondary phenomena following the combination of the injected antigen with its specific antibody. This dramatic type of antigen-antibody reaction is so interesting and important that it is discussed below in a separate chapter (Chapter XXIV).

THE HUMAN-BLOOD GROUPS

Discovery and nomenclature of the four main blood groups. Landsteiner, in 1900, discovered the phenomenon of *iso-agglutination,* when he found that the serum of certain human beings would agglutinate the red blood cells of other persons. Through his work and that of his associates, it was established (1902) that all human beings may be typed into one of four blood groups. In 1907 Jansky made the first definite classification of the blood groups, assigning them the numbers I, II, III, and IV. Again Moss, in 1910, independently studied the blood types, and arranged them in a similar way, but unfortunately his group I corresponded with group IV of Jansky, and vice versa. The existence of the contrary nomenclature has been a source of confusion and because of this scientific workers have agreed to name the groups O, A, B, and AB, according to the antigenic content of the respective red blood cells. This classification, known as the Landsteiner, or International, nomenclature, is now universally used in scientific publications. It is the only scheme that the student need learn, for it is also the one now employed by well-informed physicians.

The basis for the International nomenclature is supplied by the work of Landsteiner, who showed that iso-agglutination is due to the reaction between two agglutinins or antibodies (a, b) that may be present in the serum, and two corresponding agglutinogens or antigens (A, B) that may be present in the erythrocytes. A person's red blood cells may contain one, both, or neither of the agglutino-

gens. The serum of an individual never contains agglutinins for his own cells, but does contain agglutinins corresponding to the absent agglutinogens. Thus, the blood of a group O individual is represented by the formula O(a, b), meaning neither agglutinogen in the blood cells, both agglutinins in the serum. The formulae for the other groups are, then, A(b), B(a), and AB(o). In the following table, the interreactions between the cells and sera and the approximate frequency of occurrence of the four blood groups (or *types*, as they are commonly called) are shown.

TABLE XVI. The Human Blood Groups (or Types)

BLOOD TYPE	REACTIONS (+ = AGGLUTINATION) CELLS OF TYPE	SERUM OF TYPE O	A	B	AB	PER CENT OCCURRENCE IN ADULTS (U. S.)*
O	O	—	—	—	—	45
A	A	+	—	+	—	41
B	B	+	+	—	—	10
AB	AB	+	+	+	—	4

* As determined by Snyder, from Wiener, A. S.: *Blood Groups and Transfusion,* Chas. C Thomas, Springfield, Ill., 3rd Ed., 1943.

Technique of blood-typing. Several different methods may be used for determining the blood group, but the following is the technique most widely employed in hospital laboratories.

(It is first necessary to secure a quantity of serum from middle-aged persons known to be of Type A, and also from persons known to be of Type B. Preserve the sera by the addition of 1:10,000 merthiolate, and keep in the refrigerator. These sera should cause prompt agglutination in a dilution of 1:8 or higher. Typing sera in fluid or powdered form are available commercially.)

1. Disinfect and puncture the finger of the individual to be typed, and collect 3 or 4 drops of blood in a small test tube containing about 1.0 cc of physiological salt solution.

2. Thoroughly clean a microscopic slide, then mark off a large circle on each end of the slide with wax crayon. Label the left circle *anti-A* and the right circle *anti-B*.

3. Place a good-sized drop of known anti-A serum in the circle marked *anti-A,* and a similar drop of known anti-B serum in the other circle. Now add to each drop an equal amount of the unknown cell suspension (well-shaken).

4. Rock the slide to mix the reagents. After about 5 minutes, place a cover glass over each drop, and examine under the microscope with the low-power objective. Reserve final decision for about 15 minutes. If results are doubtful, repeat, and use in addition a control preparation, consisting of a drop of the unknown cell suspension mixed with a drop of saline.

5. Determine the group of the unknown blood by checking the results against the table of reactions given above (Table XVI).

If no agglutination occurs in either test drop, the blood is of group O. If agglutination occurs in both test drops, the blood is of group AB. Agglutination with anti-A serum, but not with anti-B serum, indicates that the blood is of group A. Agglutination with anti-B serum, but not with anti-A serum, indicates that the blood is of group B.

True agglutination must be carefully distinguished from false clumping and from mere rouleaux formation.

Further individual differences in human bloods. The discovery of the chief blood groups made possible the safe transfusion of whole blood from one individual to another—a life-saving measure in many cases of serious illness. But untoward reactions following transfusions are not entirely avoided merely by using donors of the same blood type as the recipient. It is necessary to perform *direct matching* of prospective donors' cells with the patient's serum, or *cross-matching,* i.e., testing the patient's cells against the donors' sera. Explanation for intragroup transfusion reactions, and other facts of great value, have resulted from further studies of individual differences in human bloods.

It has been found that in some bloods the agglutinogen A has a character somewhat different from that in others; hence, this antigenic factor is now subdivided into two: A_1 and A_2. Moreover, when various samples of bloods were injected into rabbits, Landsteiner and Levine discovered other antigenic factors in the human blood cells, distinct from A and B. The two most important factors thus found were called M and N (letters chosen from the word *immune,* since discovery was made by immunization of animals). Most recently, by immunization of rhesus monkeys and guinea pigs with human bloods, still another antigenic factor of much practical importance was demonstrated. This is called the Rh factor (from *rhesus,* the species of monkey first used). Thus, the principal, distinctive antigens (agglutinogens) that may be present in human red blood cells include A_1, A_2, B, M, N, and Rh.

The composition of a person's blood with respect to these factors is determined by heredity; each of these agglutinogens is inherited as a simple Mendelian dominant.

The M and N factors. All human bloods possess at least one of these factors, or both. M-type persons have the M agglutinogen in their red blood cells, but not the N. N-type individuals have the N factor, but lack the M. MN-type people possess both antigens. Within each of the four main blood groups (O, A, B, AB) there is about the same distribution of persons of the M, N, and MN types.

The principal practical value of knowledge of these types is in connection with problems of disputed parentage and other medico-legal matters.

The Rh factor and its clinical importance. Landsteiner and Wiener, who first demonstrated the Rh factor in 1940, found it to be present in the blood of about 85% of white individuals, irrespective of the main blood groups or of the MN types. Such persons are said to be Rh-positive, while the remaining approximately 15% are Rh-negative. The further studies of Levine and of Wiener soon led to findings of dramatic interest. It was shown that many cases of serious hemolytic reactions following repeated transfusions, previously unexplained, are due to the blood-cell destruction that may follow mixture of an Rh+ blood with one containing a high titer of anti-Rh iso-antibodies.

Of still greater importance was the demonstration that iso-immunization against the Rh factor *during pregnancy* plays an essential rôle in causation of erythroblastosis fetalis (hemorrhagic disease of the newborn). In the typical case, the mother is Rh-negative, the father is Rh-positive, and the fetus is Rh-positive, having inherited the Rh factor from the father. Now, if fetal blood enters the mother's circulation (presumably through some defect in the placenta), it incites the production of Rh iso-antibodies. These filter back through the placenta into the circulation of the fetus. This results in a specific antigen-antibody reaction between the fetal red blood cells and these Rh-antibodies, and, unfortunately, in the hemolysis of these cells. This blood-cell destruction may be so great that the fetus dies and is stillborn. Often, however, the baby is born alive only to show, at once, manifestations of more or less severe hemorrhagic disease. Death may occur in a few hours; or, sometimes, a spontaneous recovery occurs.

Today, part of the routine examination of a pregnant woman is a test for Rh. If the prospective mother is Rh-negative, and the husband is Rh-positive, a potential danger exists. Even so, the first and second babies are almost always quite normal; it is in later pregnancies that difficulties may appear. But the physician can now anticipate possible trouble, and give enlightened advice. He will be prepared to give a transfusion of Rh-negative blood to the baby.

REFERENCES

Boyd, W. C.: *Fundamentals of Immunology*. New York: Interscience Publishers, Inc., 1943; "Rh Blood Factors: An Orientation Review," *Arch. Path.*, 1945, 40:114.

Landsteiner, K.: *The Specificity of Serological Reactions*. Cambridge, Mass.: Harvard Univ. Press, revised ed., 1946; "Individual Differences in Human Blood," *Science*, 1931, 73:403.

Kolmer, J. A. and Tuft, L.: *Clinical Immunology, Biotherapy and Chemotherapy*. Philadelphia: W. B. Saunders Co., 1941.

Sherwood, N. P.: *Immunology*. St. Louis: C. V. Mosby Co., 2nd Ed., 1941.

Wiener, A. S.: *Blood Groups and Transfusion*. Springfield, Ill.: Chas. C Thomas, 3rd Ed., 1943.

Wiener, A. S. and Karowe, H. E.: "Diagrammatic Representation of the Human Blood-Group Reactions," *J. Immun.*, July 1944, 49:51.

Zinsser, H., Enders, J. F. and Fothergill, L. D.: *Immunity Principles, and Application in Medicine and Public Health*. New York: The Macmillan Co., 1939.

REVIEW QUESTIONS—CHAPTER XXII

1. What accounts for the development of specific resistance toward a particular germ or toxin? Discuss *antibody-formation* as a general biological phenomenon, and its particular significance in relation to immunity.
2. Define and give examples of a *complete antigen* and of a *partial antigen* (*hapten*).
3. What is meant by *antiserum* (or *immune serum*), *antigen-antibody reactions*? Define: *in vivo, in vitro.*
4. Give the names generally applied to the antibodies concerned in the various *in vitro* antigen-antibody reactions, and the names of the tests themselves.
5. Name two kinds of *in vivo* antigen-antibody reactions, and two varieties of hypersensitivity tests.
6. Discuss the nature and origin of antibodies. Are the antibodies that have been differently named actually different substances?

7. Describe the discovery of antitoxin. In what sense may antitoxins be considered antibodies of a special kind? What can be said of the mechanism by which toxins are neutralized?
8. Mention two ways in which the antitoxin in an antiserum can be measured and standardized.
9. Name and describe briefly two skin tests based upon toxin-antitoxin reactions.
10. Describe the phenomenon of specific bacteriolysis. Name the reagents necessary, and explain how they react with a bacterium to bring about its death or lysis. Define *complement.* What is the probable importance of bacteriolysis in body defense against infection?
11. Explain how it happens that the addition of red blood cells plus an antibody (hemolysin) specific for them is a good way to determine whether there is any free complement present in a test tube.
12. Explain the principle of complement-fixation tests. Outline the essential steps in performing such a test, and indicate the significance of the results finally observed. Give examples of the practical use of such tests. In what way does the Wassermann test differ from other complement-fixation tests?
13. Describe agglutination. How may the clumping of cells in immune serum be explained?
14. How are agglutination tests carried out in the laboratory? How is the amount of agglutinin in an antiserum determined? Define: *titer* of agglutinins.
15. In what way may agglutination aid the body defense? Give examples of the use of agglutination reactions for diagnostic purposes.
16. Describe the phenomenon of specific precipitation (or flocculation). What is the mechanism that accounts for the appearance of the precipitate?
17. How are precipitins tested for, and measured, in the laboratory? Are precipitation tests more, or less, sensitive than agglutination? than complement fixation?
18. How may precipitation tests be used in practical diagnosis? in medico-legal work?
19. Explain how phagocytosis is aided, in the presence of immune serum, by antibody. What name is given to this antibody? What is the probable importance of opsonic action in body defense against infection?
20. How may the opsonic power of an immune serum be estimated? Define *opsonic index, opsono-cytophagic test.*
21. Explain how protection tests are performed, and give examples of their practical use.
22. What is the mechanism of allergic skin tests? Give examples of their practical use.

23. What severe reaction may follow an antigen-antibody reaction in a sensitized animal?
24. What is the composition of the blood with respect to the blood factors A and B in each of the four main groups? What happens when blood cells of Groups O, A, B, and AB are mixed with serum from persons belonging to each of these groups?
25. Outline a method for determining the blood group. What is meant by *direct matching* and *cross-matching* of bloods?
26. What additional antigenic factors may be present in human red blood cells, besides the A and B factors?
27. How were the factors M and N discovered? How are they distributed in human blood, and what is their importance?
28. Explain how the factor Rh was discovered. Discuss the clinical importance of the hemolytic reactions that may follow mixture of Rh-positive red blood cells with a blood containing Rh antibodies.

CHAPTER XXIII

NATURAL AND ACQUIRED IMMUNITY

In the previous chapter we have described some of the important reactions of the body which are associated with resistance to infection. We shall now consider immunity in a wider sense. Under what circumstances are human beings immune to our common infectious diseases? How can this immunity be purposely acquired?

Immunity. The word *immune* is unfortunately used in two senses. It is sometimes employed to mean *not susceptible.* Practically the only instance, however, in which the term can be properly used in this absolute sense is in the case of the immunity which depends upon differences in species. Thus, human beings are not susceptible (*i.e.,* they are absolutely immune) to hog cholera, because they are humans and not hogs. *But absolute immunity is unknown against any infection to which the species is naturally susceptible.* When we say an individual is immune, we usually mean merely that he has a *relatively increased resistance toward some particular pathogenic organism.* He is simply more resistant, that is to say, less susceptible, than normal, nonimmune, fully susceptible individuals.

For convenience in discussing this subject, the outline of immunity given below will be followed.

AN OUTLINE OF IMMUNITY

I. *Natural Immunity* (immunity at birth)
  (*a*) Acquired from mother before birth (congenital immunity)
  (*b*) Inherited
    (1) *Species immunity* (differences in susceptibility of various species)
    (2) *Racial and group immunity* (differences in susceptibility of different races and groups of the same species)

(3) *Individual immunity* (differences in susceptibility of different individuals of the same race)

II. *Acquired Immunity* (immunity acquired during life)

(*a*) By recovering from the disease, or by carrying the germs without being ill

(*b*) Artificially, by use of:

(1) *vaccines* (active immunization)

(2) *immune serums* (passive immunization)

Natural immunity. What is called *natural immunity* depends upon factors which are *inborn,* and part of the heritage of the race to which an individual belongs. This must be distinguished from *acquired immunity,* by which is meant the immunity developed as the result of contact with germs during the life of an individual.

(a) *Congenital immunity from the mother.* For a few months after birth, many infants show a resistance toward certain diseases because of antibodies acquired from the mother during uterine life. For example, babies often have considerable amounts of diphtheria antitoxin in their blood passed to them from the blood of their mothers, who were immune to diphtheria. Similarly, infants may possess a specific resistance to other diseases to which the mother was immune. This form of natural immunity is not of great practical importance, however. It usually disappears by the end of the first year of life.

(b) *Inherited natural immunity.* Apart from this transient immunity acquired from the mother, individuals inherit as part of their constitution, in common with other members of their race and species, varying degrees of natural resistance toward infection. If we take any particular disease, we find that there are marked differences in susceptibility to that disease in certain species of animals as compared with other animal species, or with human beings, and also there are differences in susceptibility among different races and groups of the same species, and among the individuals of the same race.

(1) *Species immunity* (*differences in susceptibility of various species*). In general, the cold-blooded animals (fish, frogs, turtles, etc.) are not susceptible to diseases common to warm-blooded animals. The body temperature seems to be one factor in this resistance. For example, the temperature of fowls is higher than that of human beings (about 42° C), and they are normally resistant to the organ-

isms of plague and anthrax. But Pasteur showed, in one of his dramatic experiments, that a hen chilled by immersion in water until the body temperature is lowered becomes susceptible to anthrax. Differences in the body temperature, however, can hardly account for natural species immunity in all cases. There must be other factors, not as yet fully understood.

The differences in susceptibility of different species to tuberculosis germs is especially interesting. Fish have a form of tuberculosis, but the tubercle bacilli which cause the disease in fish will not infect human beings, nor will the human tubercle germs infect fish. Hens and other birds have a spontaneous disease much like tuberculosis in man, but the avian tubercle bacilli probably never infect human beings. Man is susceptible to the type of tubercle bacilli which infect cattle (bovine type), but much less so than to the human type of the germ. Both rabbits and guinea pigs are very susceptible to the bovine type, but rabbits are much more resistant than guinea pigs to the human type.

Human beings are not susceptible to some diseases of lower animals, such as chicken cholera. There are a number of diseases, however, which are primarily animal infections, but which may be transmitted to man. Among the most important are *anthrax* (cattle, sheep, horses), *glanders* (horses), *plague* (rodents), *undulant fever* (goats, cattle, swine), *bovine tuberculosis* (cattle), *tularemia* (rabbits), and *rabies* (dogs, cats).

There are many diseases of human beings that do not naturally occur in animals. Examples are *syphilis, gonorrhea, cholera, typhoid fever, influenza, measles, mumps,* and *poliomyelitis.* In the case of some of these diseases, the artificial inoculation of the germs into certain animals will cause development of the disease, but usually the animals are much more resistant to the infection than human beings are. It is an interesting fact that animals closely related to man in the evolutionary scale—chimpanzees and other anthropoid apes—are more likely to be susceptible to human diseases than other animal species. It has been found possible to reproduce experimentally only in these animals such characteristic human diseases as measles, typhoid fever, and poliomyelitis.

(2) *Racial and group immunity* (*differences in susceptibility of different races and groups of the same species*). Among animals there are several examples of what appears to be a racial immunity. Thus the field mouse is very susceptible to glanders, but the white

mouse is immune. Algerian sheep are resistant to anthrax, while common sheep are susceptible.

Among human races there are also differences in susceptibility to common infections, but these are not so marked. Negroes are more resistant to some skin diseases than white people, but they appear to be considerably more susceptible to venereal diseases, pneumonia, and tuberculosis. It may be that differences in mode of life, in hygienic conditions in the homes, and in opportunities for infection explain to a large extent these apparent differences in susceptibility.

A race of people that have been exposed to a disease continuously for a very long time is likely to have a considerable degree of natural immunity to that disease, because only the more resistant stock has survived, and a kind of balance has been effected between the virulence of the germs and the resistance of their hosts. This probably explains why the Caucasian race in general, and Jewish people in particular, are more resistant to tuberculosis, for example, than Negroes and Indians.

When an infectious disease is first introduced into an aboriginal race, it takes a heavy toll—witness the havoc wrought by tuberculosis and syphilis among Eskimos and American Indians. In the course of time, however, the disease tends to become less severe, and it may eventually reach the same level of incidence and severity as it has among people of other races, with whom the disease has been endemic for long periods. When Captain Cook, on one of his famous voyages, touched at the South Sea Islands, the natives contracted measles from a member of his crew. Apparently this disease had never occurred there before. It spread rapidly, causing severe illness, and killed a large proportion of the native population. Now, however, measles is no more severe in the South Sea Islands than elsewhere. The people there now inherit a degree of natural resistance to measles which the aborigines did not have.

The group of people inhabiting any particular region show a certain degree of natural immunity to diseases that are common there. This *group immunity* is characteristic of the people as a whole, without regard to race. If we count all human beings in civilized countries in one group, we can say that this group has a considerable degree of natural resistance to many common infections, such as smallpox, syphilis, and tuberculosis. Furthermore, this immunity is always tending to increase. All the diseases just men-

tioned were more severe one hundred years or more ago than they are now. During this time, many of the more susceptible individuals have died out, and also the most virulent of the germs have been eliminated with their hosts, leaving at the present time a population of relatively resistant individuals and less virulent strains of the organisms. In other words, mankind as a whole is always tending toward a state of balance with the microbes which assail us. Among the people in any one community—comprising a local group—this *man-microbe balance* under normal conditions keeps the communicable diseases at a certain more or less constant level of frequency and severity in that community.

(3) *Individual inherited immunity* (*differences in susceptibility of individuals of the same race*). Apart from any specific immunity toward any particular infectious agent that they may happen to possess, individuals vary in their capacity to resist our common germ diseases. Some individuals seem to be naturally more susceptible than other to skin disorders, common colds, and other familiar diseases. It is also a well-known fact that the natural resistance of the same individual varies from time to time.

These differences cannot be fully explained, but some of the factors which influence natural resistance are easily understood. As pointed out in the previous chapter, many of the mechanical features of the body—for example, the anatomical structure of the nose and deeper respiratory tract—help to keep out germs. Obviously, physical imperfections, such as a deviated nasal septum, flat chest, and the like, tend to increase the liability to infection. In more subtle ways, our normal resistance is affected by the physiological state of the body. Any infection is likely to be more severe, for example, during pregnancy. As might be expected, natural resistance is lowered by malnutrition, particularly when the diet lacks some needed vitamin; by fatigue associated with overexertion and worry; by exposure to cold and wet; by alcoholism; and by preëxisting weakening disease, such as cancer, diabetes, leukemia, measles, influenza, or tuberculosis. Age is also an important factor, the greatest susceptibility being among the very young (1 to 10 years) and among the very old.

The normal bactericidal power of the blood of different individuals doubtless varies, and probably it varies in the same individual from time to time. Some persons are able to mobilize their natural body defenses (inflammatory reaction, phagocytes) more

promptly than others. There is also a marked difference in the rapidity and amount of antibody formation.

Acquired immunity; importance as compared with natural immunity. The natural resistance to infection which we have been discussing is doubtless at its highest level in an individual without physicial defects or physiological disturbances, that is to say, in an individual in a state of perfect health. But it must be remembered that this natural inherited immunity is not sufficient in itself to protect against most disease germs. The organisms of typhoid fever, influenza, syphilis, and other common diseases attack the physically strong and robust individual as readily as they do the weakling. Physical culture itself cannot make the body immune to infection.

Instead, successful resistance against infectious diseases depends upon the development of a *specific immunity,* associated with the appearance of antibodies which act upon the causative organism or its toxin. This *specific resistance is acquired* as a result of a natural infection, or by one of the artificial methods of immunization described below.

(a) *Immunity acquired by recovery from germ diseases.* Everyone is familiar with the fact that a lasting immunity follows recovery from certain diseases—for example, smallpox, measles, mumps, whooping cough, poliomyelitis, plague, typhoid fever, and yellow fever. In most cases, this immunity appears to persist for the lifetime of the individual. Second attacks of some of these diseases may occur, but this is very rare.

On the other hand, there is only a temporary resistance following pneumonia, influenza, gonorrhea, staphylococcus, and streptococcus infections, and a few other diseases.

In *syphilis* a person is resistant to a second attack as long as the original infection is still present. If an individual does become reinfected, this is generally regarded as proof that the first infection had been cured.

In *tuberculosis* the situation is similar. Here, again, the infected individual develops a considerable degree of resistance, but if the infection is fully eliminated, he becomes, in time, as susceptible as ever.

Immunity acquired by carriers. Many adults have a specific immunity to diphtheria, typhoid fever, and other diseases, though they have never suffered, to their knowledge, an attack of the disease. It is highly probable, however, that this immunity is in

every case acquired (not inborn), as a consequence of some unconscious contact with the germ. They have had a slight, unrecognized illness, or they have been a *carrier* of the organism. In the latter case, the germ, or toxin, must have gotten into the tissues in sufficient amount to stimulate the development of specific antibodies, though noticeable symptoms may never have been produced. The development of immunity by carriers is generally regarded as the explanation of the fact that, as a rule, there are more people immune to diphtheria, for example, among the adult population in our crowded cities than in country districts. Country people are less often exposed to the germs, for they have fewer contacts with other individuals; there are fewer carriers, and therefore, fewer persons become immune.

(b) *Artificially acquired immunity. Active and passive immunization.* There are two ways in which immunity may be acquired by artificial means: (1) by the introduction of a *vaccine,* or (2) by the injection of an *immune serum.* The former brings about *active immunization,* and the latter *passive immunization.*

(1) *Vaccines* (*active immunization*). The dead or weakened germ, or toxin, may be introduced into the body in the form of a *vaccine,* for the purpose of stimulating an individual to develop specific antibodies. The process of producing immunity in this way is called *active immunization.* The individual is said to have an *active immunity,* because this immunity is the result of his own activity in manufacturing antibodies. (The resistance which follows a natural infection is an active immunity.) In general, the immunity induced by active immunization is of a high degree and tends to be lasting.

(2) *Serums* (*passive immunization*). A temporary immunity may be acquired by injecting into the body an *immune serum,* i.e., the blood serum of an immune animal or man. Production of immunity in this way is called *passive immunization.* The individual is said to have a *passive immunity,* because he took no active part in the development of his resistant state, but merely has received an injection of a serum *already containing the antibodies desired.* Passive immunity is temporary, because the injected serum, being foreign to the body, is soon eliminated.

Vaccines; vaccination. We have previously explained how the term *vaccine,* which, in a strict sense, refers only to the cowpox virus as prepared for use in immunizing against smallpox, came to be applied in a general sense to any material used for active

immunization. *Vaccination* and *active immunization* now have essentially the same meaning.

Whatever its nature or origin, *a vaccine always contains a microorganism (killed or attenuated) or a toxin (in some way rendered harmless), and it is always introduced into the body for the purpose of stimulating the formation of specific antibodies and the development of a state of active immunity.*

Bacterial vaccines. A bacterial vaccine is merely a suspension of a culture of bacteria in physiological salt solution. In the commonly employed vaccines, such as that used for prevention of typhoid fever, the bacteria are *killed* by heating to about 60° C. In some similar vaccines the organisms are killed by the addition of formalin in a concentration of about 0.2%.

Sometimes vaccines are made not from a single bacterium, but from mixtures of several different species, such as staphylococci, pneumococci, and other organisms commonly associated with respiratory inflammations. Such a vaccine is called a *mixed vaccine.*

When bacterial vaccines are made from laboratory stock cultures, they are called *stock vaccines.* Better results are usually secured by the use of so-called *autogenous vaccines.* These are vaccines made from cultures of bacteria freshly isolated from the patient to be treated. Thus, an autogenous vaccine for furunculosis would be prepared by first cultivating the staphylococci from the boil, then making a vaccine from this same culture.

Other types of vaccines. The vaccines used for the prevention of smallpox, rabies, and yellow fever are examples of vaccines containing *viruses* whose virulence for human beings has been reduced by artificial adaptation to animal hosts, or by treatment with some physical or chemical agents.

The vaccines for typhus and other rickettsial diseases consist of suspension of the *rickettsiae* harvested from cultures in the yolk sac of embryo chicks, and killed by formalin.

Still another type of vaccine material is that employed for active immunization against diphtheria, tetanus and scarlet fever. Since, in these diseases resistance depends upon a specific *antitoxin,* active immunity is induced by injections of the respective *exotoxins* or of *toxin-antitoxin mixtures* or *toxoids* (plain or alum-precipitated). A toxoid is the harmless, nonpoisonous product secured by adding about 0.4% of formalin to the original toxin.

Use of vaccines. Vaccines are occasionally tried for the treat-

ment of various infectious diseases, but their therapeutic value is uncertain. Vaccines have their greatest usefulness as *prophylactic* agents for the active immunization of well persons or animals *before* exposure to certain diseases, or before symptoms begin.

The human infections at present most successfully controlled by artificial active (prophylactic) immunization are *typhoid and paratyphoid fevers, whooping cough, smallpox, rabies, diphtheria, tetanus, scarlet fever, typhus, Rocky Mountain spotted fever,* and *yellow fever.* Also, a definitely increased protection against *encephalitis, cholera,* and *plague* is known to follow injections of vaccines made from the causative organisms. Vaccines for prophylaxis of bacillary dysentery, influenza, pneumonia, tuberculosis, and some other diseases have been tried recently, but the practical effectiveness of these preparations remains uncertain.

Vaccination for children. Active immunization against smallpox, diphtheria and tetanus should be recommended for *every child.* These immunizations are best administered at about the age of eleven or twelve months, though the smallpox vaccination may be given earlier. The tetanus and diphtheria toxoids may be mixed, and injected together from the same syringe. The success of the immunization against diphtheria should be checked by a Schick test about six months later. If this test is positive, additional injections of diphtheria toxoid should be given. At the age of five or six years, when the youngster is about to go to school, revaccination for smallpox is advisable. Also, the Schick test may be tried again, and in case it is still positive, further injections of diphtheria toxoid are indicated.

Consideration should be given, also, to the advisability of vaccinating infants (6–9 months old) against whooping cough, and possibly against scarlet fever (at 2–4 years of age), and typhoid fever (at 8–12 years of age).

Nature of immune serums; differences between serums and vaccines. An immune serum is the blood serum of an individual (animal or man) highly immune to an infectious disease. It contains, already formed, *antibodies* for the microorganism or toxin of that disease. In concentrated, purified state, it is used for the passive immunization of another individual.

The student should be careful to avoid the indiscriminate use of the words *vaccine* and *serum.* A "serum" (by which we mean an antiserum or immune serum) is literally *blood serum,* but a

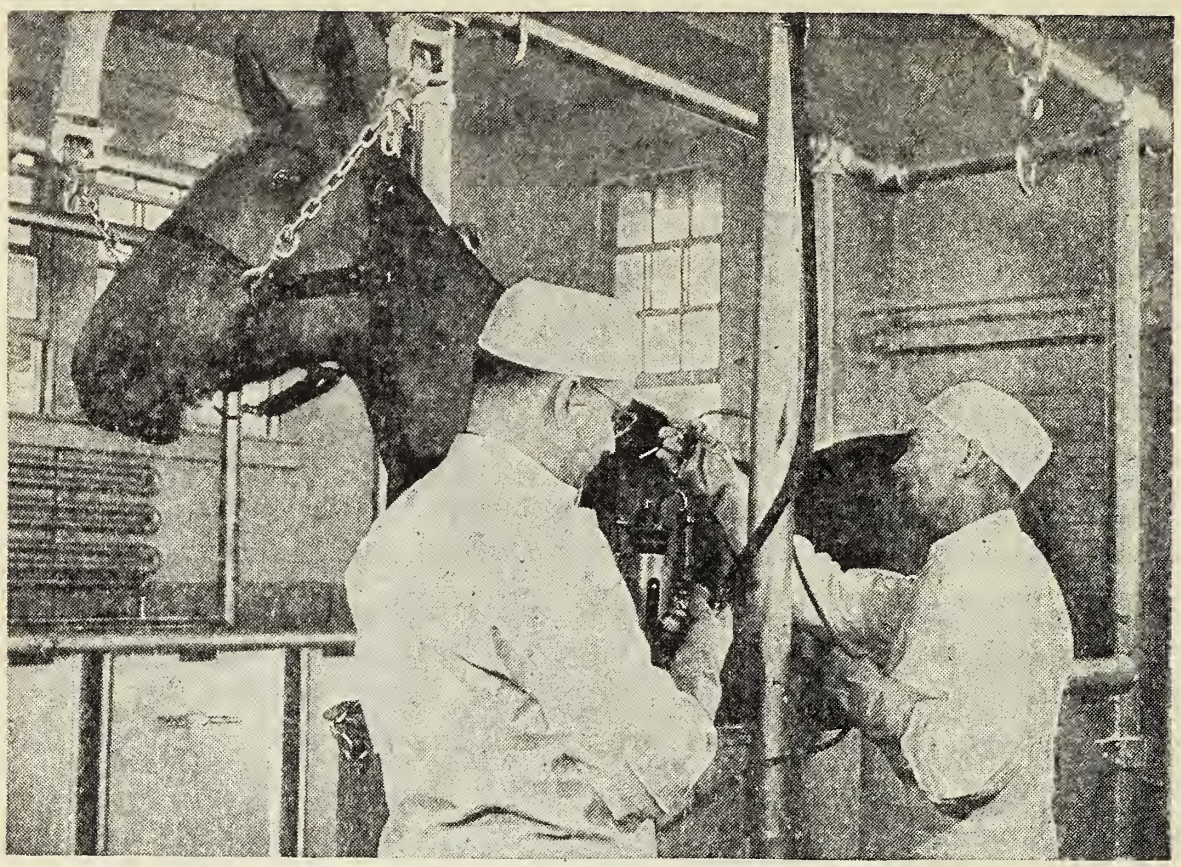


FIG. 73. Inoculation of a horse for the production of immune serum. This animal is being used for the making of diphtheria antitoxin. An accurately measured dose of diphtheria toxin (or toxoid) is being injected intramuscularly. (Reproduced by courtesy of Parke, Davis & Co.)

vaccine is a totally different substance, and is used with a different purpose. The essential constituent of a vaccine is the germ or toxin in it which acts as an *antigen* and stimulates the formation of specific antibodies. The essential constituents of an immune serum are the *antibodies it contains.* Vaccines are principally used for the *prevention* of disease through the production of a slowly developing, but lasting, active immunity in healthy persons before they are exposed to the infection. Serums, on the other hand, are of greatest value for the *treatment* of acute disease, although they are also useful in some instances for the prevention of illness when an immediate protection is needed. The immunity produced by an injection of an immune serum is of brief duration—a few weeks at most—because the foreign serum, together with the antibodies it contains, is soon eliminated from the body.

Commercial immune serums. Most of the immune serums commonly used are prepared commercially by the immunization of

horses (Fig. 73), except those for treatment of pneumonia and influenzal meningitis, which are now made from rabbits.

Antitoxin serums (for example, diphtheria and tetanus antitoxin) are manufactured by inoculating the horses with increasing doses of the respective *toxoids* and *exotoxins* only (not the bacteria), and the only type of antibody in the serum is the specific antitoxin. When, after several injections during weeks or months, the horse has developed a high content of antibodies in his blood, the animal is bled from the jugular vein, with strict precautions to avoid contaminations. The clear blood serum, or plasma, is then collected (Fig. 74).

The serum, or plasma, is now purified and concentrated by chemically separating from it the globulin portion which contains most of the antibodies, and discarding the remainder. This process eliminates a large proportion of the native horse protein and concentrates the antibodies, so that a much smaller amount of the concentrated serum will be needed to get the same results. The only harmful reactions which may follow the injection of serum are due to sensitiveness to this foreign (horse) protein, and therefore it is very important to reduce this element of the serum as much as possible. Further purification of antitoxic serums by a partial digestion of the horse proteins with certain proteolytic enzymes is now practiced. The serum is finally made sterile by filtration, and a small amount of a chemical preservative is usually added. It is then standardized, in order that the physician may know the dose he may need to give.

Antibacterial serums are prepared in a similar way by immunizing the horses (or rabbits) with pure cultures of the bacteria. These serums contain no antitoxin, but specific antibodies which act directly upon the germs.

In the United States, antitoxin and other immune serums, and similar biological products, such as smallpox vaccine, diphtheria toxin for Schick tests, scarlet fever toxin for Dick tests, tuberculin, etc., are manufactured only in establishments licensed by the Government, and under the supervision of the United States Public Health Service (National Institute of Health, Washington, D. C.). Frequent inspections of the plants and their methods are made, to insure the reliability and safety of their products.

The principal serums in common use are the *antitoxins* for *tetanus, diphtheria,* and *scarlet fever. Antibacterial serums* for *pneumonia* and *epidemic meningitis* are also available, and recently

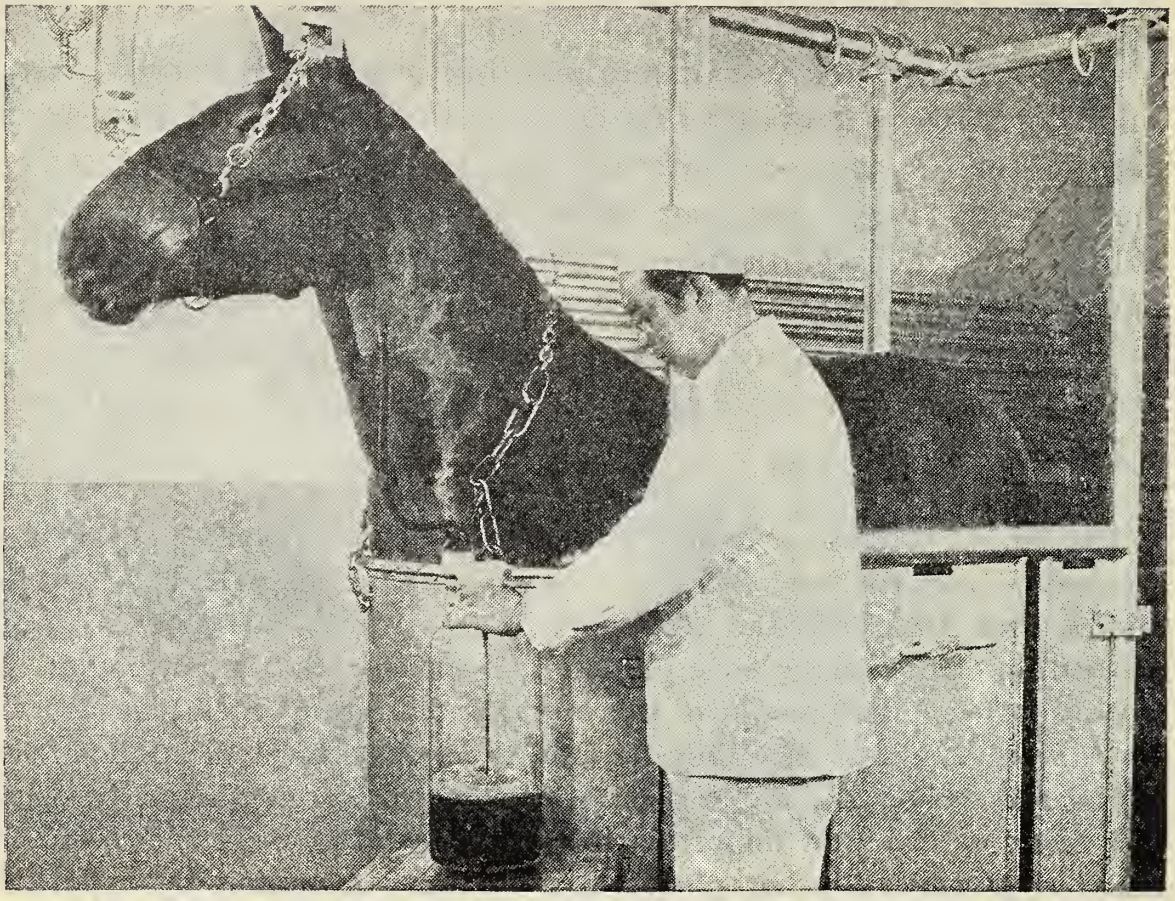


FIG. 74. Collection of blood from an immunized animal. A sterile needle is inserted into the jugular vein, and the blood is allowed to run into a tall glass bottle containing sterile sodium oxalate. Note the careful aseptic technique employed. The oxalate prevents clotting of the blood, and the blood cells settle out on standing. The overlying clear plasma (with its contained antitoxin or other antibodies) is then siphoned off, and after it has been concentrated, purified, and standardized it is ready for use in the treatment of human beings. (Reproduced by courtesy of Parke, Davis & Co.)

patients with so-called influenzal meningitis (due to *H. influenzae,* type b) have been successfully treated by a specific antiserum. Serums have also been used with varying success for the treatment of staphylococcus and streptococcus infections, the Shiga type of bacillary dysentery, anthrax, plague, gas gangrene, and some other diseases. The advent of the sulfonamide drugs and the newer antibiotics has greatly reduced the need for antibacterial serums.

Convalescent serum. For prevention or treatment of a few human infectious diseases, it is now a common practice to use the *blood serum of another person who is convalescing* (or has recently recovered) from that disease. Such serum is spoken of as *convalescent serum* (Fig. 75). *Measles* and *scarlet fever* are the diseases in which

convalescent serum has been most often employed. When serum from a child just recovering from measles is given to a child just coming down with measles, the disease is made much milder; or, if the serum is given early enough, it may prevent the illness entirely. Convalescent serum may be used either for the prophylaxis or therapy of scarlet fever.

Precautions in the use of serum. The dangers associated with injection of commercial serums arise entirely from the fact that the serum itself—without regard to the antitoxin or other anti-bodies it may contain—is protein material (usually horse serum) which is foreign to the human body, and some individuals may have a *hypersensitiveness to this foreign protein.* The injection of serum into an individual naturally very sensitive to horse protein may produce severe shock or even sudden death. But cases of natural hypersensitiveness of high degree are rare. Such an extreme hypersensitivity is always acquired unknowingly by natural absorption of horse dander or other forms of horse protein, rather than as a result of a previous inoculation of horse serum. A person may acquire a certain degree of sensitiveness, however, as a result of a previous injection of serum, so that if at a later period (two weeks or more after the first injection) he must receive a second injection, there is danger of a reaction. In the majority of individuals, including those not abnormally sensitive, there develops, after the injection of a *large amount* of serum, a condition known as *serum sickness.* This is not serious and does no permanent harm. These phenomena of hypersensitivity are discussed more fully in the following chapter.

FIG. 75. Drawing showing method of bleeding from the veins at the bend of the elbow, by use of a hypodermic syringe. (Redrawn from Todd and Sanford, *Clinical Diagnosis by Laboratory Methods,* Philadelphia: W. B. Saunders Co., 8th Ed., 1935.)

The possibility that the patient is hypersensitive must be taken into account whenever serum is to be administered, but this does not mean that serum treatment cannot be given when it is really needed. There are various ways by which the existence of a sensitive state can be detected before any damage is done, and also there are ways of administering serum safely even to sensitive individuals.

REFERENCES

Holt, R. L. and Hitchens, A. P.: "Typhoid Vaccine: Technique of Its Preparation at Army Medical School," *Pub. Health Rept.,* June 25, 1937, 52:829.

Levinson, S. O., Milzer, A., Shaughnessy, H. J., Neal, J. L. and Oppenheimer, F.: "A New Method for the Production of Potent Inactivated Vaccines with Ultra-Violet Irradiation—II. Sterilization of Bacteria and Immunization with Rabies and St. Louis Encephalitis Vaccines," *J. Immunol.,* June 1945, 50:317.

Long, A. P.: "Immunizations in the United States Army," *Am. J. Pub. Health,* 1944, 34:27.

Longfellow, D. and Luippold, G. F.: "Typhoid Vaccine Studies; Revaccination and Duration of Immunity," *Am. J. Pub. Health,* Nov. 1940, 30:1311.

Luippold, G. F. "Typhoid Vaccine Studies—IX. Intracutaneous versus Subcutaneous Vaccination for Initial Immunization," *Am. J. Pub. Health,* Nov. 1944, 34:1151.

"Multiple Antigens for Active Immunization. Report of Study Committee on Multiple Antigens." Am Pub. Health Assn., *Am. J. Pub. Health,* 1944, 34:452.

REVIEW QUESTIONS—CHAPTER XXIII

1. Define *immunity, natural immunity, acquired immunity.*
2. Discuss and give an example of congenital natural immunity.
3. What is meant by species immunity? Give examples of differences in susceptibility among animal species.
4. Name seven animal diseases transmissible to man. Name eight human diseases that do not occur in animals. What kinds of animals, in general, are most susceptible to human diseases?
5. Give examples of racial immunity among: (*a*) animals; (*b*) human races.
6. Discuss immunity shown by a group of people considered as a whole.
7. Discuss the differences in susceptibility shown by different individuals. What explanation can be offered for some of these differences?
8. What is the importance of acquired immunity, as compared with natural (inborn) immunity?
9. Discuss, and give examples of, immunity acquired by recovery from germ diseases, and immunity acquired by carriers.
10. In what two ways may immunity be acquired artificially? Define: (*a*) *active immunization,* (*b*) *passive immunization.*
11. What was the original meaning of the word *vaccine,* and how did it come to have its present use? Describe the significant content of a vaccine and its purpose.
12. Define *vaccination, bacterial vaccine, mixed vaccine, stock vaccine, autogenous vaccine.* Name other types of vaccines.
13. Name eleven diseases successfully prevented by prophylactic vaccination (active immunization). Mention other diseases for prevention of which vaccines are sometimes used with good effect.

14. What are the three diseases against which every child should be vaccinated? What other immunizing procedures should be considered?
15. Explain clearly the differences in the nature, use, and purpose of: (1) vaccines and (2) serums.
16. Outline briefly how commercial antitoxins and antibacterial serums are prepared. What is the purpose of purifying, concentrating, and standardizing the serum? Name six immune serums widely used in prevention or in treatment of common diseases. Name other immune serums of more limited use or value.
17. What is *convalescent serum*? Name two diseases in which it may be successfully used.
18. Explain the dangers associated with the injection of foreign serum into the body.

CHAPTER XXIV

ANAPHYLAXIS OR HYPERSENSITIVENESS

Importance of anaphylactic phenomena. Closely associated with immunity is the interesting and important condition called *anaphylaxis* or *hypersensitiveness*. The term *anaphylaxis* means, literally, the "contrary of protection." It was originally coined to signify the condition opposite to *prophylaxis,* which means "protected" (by preventive methods). It is not true, however, that the anaphylactic individual is without protection—only that, in addition to any powers of resistance toward bacteria or other harmful agents he may have, he is in a state of abnormal susceptibility, or sensitivity, i.e., he is *hypersensitive* toward a germ or its products, or toward a foreign substance of some other kind.

Anaphylaxis is a subject of much theoretical interest because of its relationship to immunity. It is also a matter of great practical importance, because there are several common forms of illness in human beings which are due to a condition of hypersensitiveness. Some individuals, for example, are naturally hypersensitive to the pollen of the ragweed, or other plants, and suffer annually an attack of hay fever or asthma when these pollens are abundant in the air. Other individuals are sensitive to certain foods, or drugs, or other substances; and some skin disorders, gastrointestinal disturbances, and other pathological conditions are anaphylactic in nature. In the course of various infections, hypersensitiveness of the tissues probably accounts for many of the characteristic lesions and symptoms of the disease. Of the greatest importance from the practical point of view are the anaphylactic reactions which may follow the deliberate injection into human beings of foreign substances, such as vaccines, and particularly serums.

In order to understand hypersensitiveness in man, it will be necessary, first, to describe the principal features of anaphylaxis as observed in laboratory animals.

Although hypersensitiveness in animals had been noted by sev-

eral workers some time before, the subject gained particular attention as a result of the observations of Theobald Smith (1904). In the course of some studies of diphtheria antitoxin, Smith accidentally discovered that guinea pigs which had received an injection of the antitoxic (horse) serum *became, after an interval of about twelve days or more, highly susceptible to a second injection of plain horse serum.* Within a few minutes after the second injection of the serum, the animals became acutely ill, with characteristic symptoms, and most of them died in a very short time. They suffered what is now called an acute *anaphylactic shock.*

Careful studies of this remarkable occurrence, and of other forms of anaphylactic reactions in animals, have since been made by a great many investigators, and the most important features of anaphylaxis are now well known, although authorities are not yet agreed as to the correct theoretical explanation of the phenomenon.

Principal features of anaphylactic reactions in animals. The most important facts concerning anaphylaxis as it has been observed in laboratory animals are summarized in the following paragraphs:

(1) A state of hypersensitiveness can be induced in animals by the injection of *any foreign protein material, i.e., any antigen*—for example, perfectly harmless substances like egg-white, milk, or blood serum, as well as bacteria, etc., etc. (The injection of an antigenic substance leads, of course, to the development of specific *antibodies.*)

(2) An anaphylactic reaction may follow the second (or later) injection of the particular protein, *whenever the interval between injections is ten to twelve days or more.* The animal does not become hypersensitive until an interval of about ten days has passed since the first or sensitizing dose was given.

(3) Hypersensitiveness is highly *specific,* just as immunity is specific. An animal sensitized to one protein reacts only to subsequent inoculation of that identical protein, or to the specific (hapten) portion of the antigen, and not to other substances.

(4) A single injection of a very minute amount of a foreign protein may suffice to produce a highly sensitive state in some animals, notably the guinea pig. Rabbits and some other animals are not so easily sensitized. Among individual animals of the same kind, there are marked differences in the ease with which they can be sensitized. Once an animal has become hypersensitive to a certain substance, it will remain so for months and years, and

probably for the rest of its life. However, the degree of sensitiveness becomes gradually less after the first month or two.

(5) A relatively large amount of the antigen must be inoculated into the hypersensitive animal in order to produce a typical anaphylactic shock—perhaps 100 times as much as was required to sensitize. *Smaller amounts may cause only a slight reaction, or none at all.*

The rate at which the foreign substance enters the circulating blood is also of very great importance in determining the severity of the reaction. *The acute and severe anaphylactic reactions are usually brought about by a sudden introduction into the circulation of a considerable amount of the foreign material.*

(6) It follows, from the statements just made, that anaphylactic reactions can usually be prevented if the specific antigen is introduced into the hypersusceptible animal in such a way that it reaches the general circulation *slowly and in very small amounts.* By repeated injections of this kind a hypersensitive animal may be rendered temporarily refractory—it is said to be *desensitized.* Thus, if the sensitive animal is first given an injection of a very minute amount intradermally, or subcutaneously, so that the material will be absorbed only slowly, then at half-hour intervals further injections are made, it is possible to introduce, without producing anaphylactic reactions, a total amount far in excess of the amount necessary to kill the animal if injected in a single dose.

(7) Again, if an animal survives an anaphylactic attack, it becomes resistant to further injections of the same antigen for a variable period thereafter, though it eventually becomes sensitive again. This temporary lack of sensitiveness following recovery from an anaphylactic reaction is spoken of as *antianaphylaxis.*

(8) *Passive hypersensitiveness* can be induced in a normal animal, just as passive immunity is conferred, by injecting into the normal animal some of the blood serum of the hypersensitive animal. As a rule, in guinea pigs, but *not* in mice or rabbits, a period of a few hours must pass before the injected animal becomes sensitive. If, then, an injection of the specific substance is given, this passively sensitized animal will show a typical anaphylactic reaction.

(9) The *symptoms* of anaphylaxis are always the same in animals of the same species, irrespective of the nature of the substance to which the animal is sensitive. But the anaphylactic reaction differs in different species of animals. It differs according to the anatomi-

cal and physiological make-up of the animal, not according to the chemical nature of the sensitizing substance.

In the *guinea pig,* the symptoms of acute anaphylactic shock usually begin within a minute or two after the material to which the animal is hypersensitive has been injected. The animal becomes very restless and agitated, and soon it is apparent that it is having a great deal of difficulty in breathing. It rubs its nose, coughs, and often rises on its hind legs or makes a series of jumps in a violent effort to get air. Within a few minutes the animal is no longer able to stand; it falls upon its side, gasping for breath. Sometimes a slow recovery takes place, but usually convulsions set in and breathing soon ceases. The heart continues to beat for several minutes after breathing has stopped. On autopsy, the lungs are found distended and filled with air. This is due to the fact that the smooth muscle tissue about the bronchi and bronchioles has contracted, so that the passages for air are almost completely closed off. The devitalized air in the lungs could not be expelled and fresh air could not be drawn in, and as the inevitable result of this obstruction to respiration, the animal suffocates.

In *rabbits,* respiratory symptoms are not prominent, and death is not due to suffocation but is explained by a spasmodic constriction of the walls of the pulmonary arteries, causing a rapid dilatation of the right side of the heart, and sudden heart failure.

In *dogs,* the symptoms are more varied, but are referable principally to the digestive tract. There is a marked fall of blood pressure, and a collection and stagnation of blood in the tissues, especially in the liver.

(10) A prominent feature of the acute anaphylactic reaction is the spasmodic contraction of *smooth* (involuntary) *muscles.* It happens that, in guinea pigs, the bronchi and bronchioles are especially rich in this smooth muscle tissue. This explains why anaphylactic shock in these animals is characterized principally by difficulties in respiration and eventual suffocation. In other animals the smooth muscle is less abundant about the bronchi. It is probable that the differences in the symptoms and lesions of acute anaphylaxis as observed in different species of animals may be due principally to differences in the amount and distribution of smooth muscle tissue in the various parts of the body.

The fact that in the hypersensitive animal the smooth muscles are sensitized, and will be thrown into spasmodic contraction when

the specific antigen reaches them, can be demonstrated by experiments upon a piece of such smooth muscle tissue (the uterus, or a strip of the intestine, for example) taken from the body of the animal. If the uterus of a hypersensitive guinea pig is removed and immediately suspended in a bath of warm physiological salt solution, it will contract vigorously when a little of the substance to which the animal was sensitized is added to the bath.

(11) A significant contribution to our understanding of anaphylaxis was made by Dale, when he showed that a substance called *histamine* will cause the symptoms of acute anaphylaxis when injected by itself into normal animals. Histamine is derived from the amino acid, histidine. It may be formed during the decomposition of protein material. It is probable that many of the most characteristic features of acute anaphylactic shock are the result of the liberation of a *histamine-like substance* in the body fluids or cells during the anaphylactic reaction.

(12) Anaphylactic shock can be prevented, and the symptoms of anaphylaxis can be relieved, by the use of drugs such as atropine, epinephrin (adrenalin), and ephedrine, which help to maintain blood circulation and tend to inhibit spasmodic contraction of smooth muscles, especially those of the bronchi.

HYPERSENSITIVENESS IN HUMAN BEINGS

The manifestations of hypersensitiveness in man differ in a number of respects from the typical anaphylactic reactions experimentally produced in laboratory animals, as above described. These differences led some of the earlier workers in this field to believe that true anaphylaxis does not occur in human beings. It is now well established, however, that, although very severe or fatal anaphylactic shock in man is rare, the sensitiveness shown by many individuals toward pollens, foods, drugs, and other substances, the reactions following injection of serum, and other forms of human hypersensitiveness are all fundamentally of the same nature as anaphylaxis in animals.

Allergy. On account of the disagreement among scientists as to the real nature of human hypersensitiveness, much confusion in terminology has arisen. One term now widely employed with reference to hypersensitiveness in man is *allergy*. This term is derived from the Greek *allos,* other or different, and *ergon,* work or action,

and means literally the *different or altered power to react.* Thus, the hypersensitive individual is said to be *allergic,* because he reacts in a different way from the normal individual toward foreign matter of some kind. He will show an *allergic reaction* when exposed to contact with the substance to which he is sensitive.

If the term were used in its literal sense, *allergy* would include *immunity,* as well as hypersensitiveness, because the immune individual also reacts toward foreign substances in a different way from the normal individual. In clinical medicine, however, physicians use the word *allergy* to mean the same as *hypersensitiveness,* and with special reference to the hypersensitivity observed in human beings.

Common forms of human allergy. Human beings may show a natural hypersensitiveness to a great variety of substances. Just how this sensitiveness originates is not entirely understood. Undoubtedly, an *inherited tendency to become sensitized* plays an important part. Probably the hypersensitiveness arises in susceptible individuals as a result of absorption of foreign substances through the mucous membranes of the respiratory or digestive tract, or through the skin, and allergic reactions occur when the same substances are absorbed at a later time in the same way.

One class of substances to which many individuals are hypersensitive includes the *pollens* of certain weeds, grasses and other plants. These pollens are of the type that are borne about by the winds, and become abundant in the air at certain seasons. So-called *hay fever* is most commonly due to hypersensitiveness to ragweed pollen. When the pollen grains are inhaled, they cause a more or less acute coryza, somewhat like an ordinary cold, and may produce symptoms of *asthma.* This distressing condition is often successfully prevented by a process of partial desensitization before the season begins. This is accomplished by a series of subcutaneous injections of small amounts of a highly diluted extract of the pollen given at intervals of a few days. The condition of partial *antianaphylaxis* thus produced is not permanent, and the individual must submit to the injections every year.

Asthmatic symptoms are caused in some individuals by the inhalation of particles of feathers, hair, or dandruff of certain animals, or by breathing in various other substances which may be present in the air.

Everyone is familiar with the fact that some persons cannot eat

certain *foods* without developing a more or less severe illness, which may be characterized by gastrointestinal disorders, a skin rash, or asthmatic symptoms.

Skin rashes or other disturbances sometimes follow the taking of a certain *drug,* such as iodine, quinine, or mercury. These substances are not proteins and are not antigenic in themselves. It is believed, however, that some peculiar protein product of the tissues, which forms when the drug is taken frequently, acts as an antigen to produce specific antibodies, and so may bring about a state of hypersensitiveness.

Just as in the experimental anaphylaxis in animals, the symptoms of these allergic reactions in man are essentially the same, no matter what the nature of the sensitizing material may be. The symptoms are relieved by the same agents (adrenalin, ephedrine, etc.) that serve to prevent anaphylactic reactions in animals. Sometimes the hypersensitiveness almost entirely disappears when abnormal conditions of the mucous membranes—caused, for example, by a deviated nasal septum or chronically inflamed tonsils—are corrected, and some forms of food allergy may vanish as a person grows older. Presumably this improvement is due to a decrease in the amount of the foreign protein absorbed.

Anaphylactic reactions following injection of vaccines. When several injections of a vaccine are given over a considerable period, as, for example, in the preventive vaccination against rabies, the local inflammation about the site of the inoculations following the later injections is undoubtedly due to the fact that the tissues have become sensitized to the vaccine. These local reactions, however, are not serious.

More marked local or general anaphylactic responses sometimes develop when an individual who has once been vaccinated is revaccinated with the same vaccine some months, or even some years, later. Thus, a person who has received the usual three injections of typhoid vaccine will often show an almost immediate reaction of more or less severity if he later takes the vaccine subcutaneously a second time. These reactions pass quickly, however, and there are no serious consequences.

An interesting example of a modified response following a second inoculation of a vaccine is the so-called accelerated reaction which often occurs when an individual is revaccinated against smallpox some years after the first "take." Instead of the slow development

of the vaccination sore, characteristic of the primary vaccination, which usually does not reach its height for about ten days, the accelerated reaction runs a more rapid course and is completed within seven or eight days (Fig. 131).

Anaphylactic reactions following injection of serum. Of the greatest practical importance are the anaphylactic reactions which may develop as a result of the inoculation of a foreign serum, such as a commercial antitoxin, into human beings. Of course, these reactions are not due to the antitoxin, or other antibodies the serum may contain, but are caused by a sensitiveness to the serum itself. They usually take the form of so-called *serum sickness,* but rarely there occurs a severe, or even fatal, *anaphylactic shock.*

Serum sickness. Most commonly, reactions appear only after an interval of 6–12 days and are mild in character. This delayed response to the injection of serum is called *serum sickness.* A peculiarity of this condition is that it may develop not only in persons who have been sensitized by a previous injection of serum, but in a large majority of perfectly normal individuals after receiving an injection of a considerable amount (100 cc or more) of a serum for the first time. Symptoms include a skin eruption and usually some fever, and tenderness and stiffness of the joints. There are no permanent ill effects. This delayed response is thought to be due to the reaction between the portion of the injected serum still present in the body and the antibodies to the serum itself which are actively forming as a result of the injection.

Acute anaphylactic shock. Whereas the mild serum reactions above described are common, acute anaphylactic shock is an extremely rare occurrence. Nevertheless it may occur, though only one individual in many thousands may be so hypersensitive as to be liable to develop so violent a reaction. Practically all the reported cases of sudden death following a serum injection have occurred in persons *naturally* highly hypersensitive to horse dandruff or other forms of horse protein—a hypersensitivity developed as a result of natural absorption of the horse antigen, probably through the respiratory tract—and the shock developed *immediately after the first injection of the serum.*

Desensitization of individuals before injection of serum. Physicians make it a practice to precede the injection of the full dose of any serum by an *intradermal* inoculation of a very minute amount (say 0.02 cc) of diluted serum as a test for sensitivity. The

unsensitive individual will show at the point of inoculation a small wheal, which will disappear rapidly, but in the sensitive persons there will be a marked inflammatory reaction. Instead of this skin test, the *ophthalmic* test may be employed, in which a little of the dilute serum is instilled into one eye. Again, an inflammatory reaction develops at once if the individual is hypersensitive. These preliminary tests are especially important in the case of patients who give a history of sensitiveness to horse protein in any form, patients with asthma from any cause, and individuals who have received previous injections of horse serum.

If the patient proves to be hypersensitive by a skin test, it is still possible to give him the benefit of serum treatment. It is only necessary that the physician exercise great care to administer the serum *very slowly* so as to avoid an explosive anaphylactic reaction. This is accomplished by giving very small amounts of the diluted serum subcutaneously, at half-hour intervals, until one or two cubic centimeters of serum have been injected without harm. It is then usually safe to inject the remainder of the dose. A positive eye reaction, on the other hand, is generally regarded as contraindicating altogether the injection of the serum.

Hypersensitiveness in infectious diseases; skin reactions and their use in diagnosis. Persons may become hypersensitive to bacteria, just as to other protein substances, and in the course of infectious diseases a general or localized hypersensitiveness may develop toward some substance contained in, or produced by, the bacterial cell. This is especially likely to occur in infections which are chronic, like tuberculosis. There is no doubt that many of the characteristic symptoms and pathological changes in many diseases are in part anaphylactic in nature. The hypersensitiveness which develops as a result of infection is sometimes utilized as a basis for diagnosis. If an extract containing the antigenic protein fraction or the hapten of the infecting organism is introduced into the skin, there will be a specific local reaction in the infected individual, but not in the normal individual.

The most widely used of such allergic skin tests is the *tuberculin test* for tuberculosis. Tuberculin is a product prepared from tuberculosis bacilli, and individuals infected with these germs become hypersensitive to it. When a small amount of tuberculin is injected or rubbed into the outer skin of a person who is infected, or at some previous time *was* infected with tuberculosis bacilli, a marked

local reaction occurs. A noninfected person does not give this reaction because his tissues lack the necessary antibodies.

Relation between hypersensitiveness and immunity. The same antibodies responsible for the condition of immunity are also concerned in the phenomenon of anaphylaxis. Though the effect upon the body as a whole is quite different, the fundamental occurrence in anaphylaxis, as well as in immunity, is the combination of the foreign protein (antigen) with its specific antibody, either in the circulating blood or in the body cells or both.

The difference in the effects produced by the union of antigen and antibody in the immune individual, on the one hand, and the hypersensitive individual, on the other hand, may be explained by the difference in the *speed* with which secondary physicochemical changes occur following this combination. The antigen-antibody union is merely the event that sets off a series of injurious changes, which are especially rapid and profound in the hypersensitive individual, resulting in what we recognize as an allergic or anaphylactic reaction. In the hypersensitive state, toxic products are formed in relatively large amounts within an extremely short space of time. These products probably include poisons of the nature of histamine or related substances. It appears that more poison forms *within a limited time* than can be assimilated without harm, and the symptoms of local allergic inflammation or general anaphylactic shock appear.

REFERENCES

Bronfenbrenner, J.: "The Mechanism of Desensitization," *An. Allergy,* Nov.–Dec. 1944, 2:472; "The Allergic State and Its Relation to Hypersensitiveness and Resistance," *Am. Rev. Tuberculosis,* 1937, 36:293.

Burdon, K. L.: "Anaphylaxis," *Science,* Oct. 1, 1937, 86:306.

Kojis, F. G.: "Serum Sickness and Anaphylaxis," *Am. J. Dis. of Children,* 1942, 69:93–143, 313–350.

Ratner, B.: *Allergy, Anaphylaxis and Immunotherapy.* Baltimore: Williams & Wilkins Co., 1943.

Sulzberger, M. B.: *Dermatologic Allergy.* Springfield, Ill.: Chas. C Thomas, 1940.

Urbach, E.: *Allergy.* New York: Grune & Stratton, 1943.

Vaughan, W. T.: *Practice of Allergy.* St. Louis: C. V. Mosby Co., 1939.

REVIEW QUESTIONS—CHAPTER XXIV

1. Give a literal definition of *anaphylaxis.* In a more general sense, what characterizes the anaphylactic state? What were the circumstances in which acute anaphylactic shock in animals was first observed?

2. Summarize the principal features of experimental anaphylaxis in animals (twelve main points).
3. Explain clearly what is meant by *desensitize, antianaphylaxis, passive hypersensitiveness.*
4. Give a literal definition of *allergy.* Explain the common use of the term.
5. Discuss briefly common forms of human allergy.
6. Under what circumstances may anaphylactic reactions follow the injection of vaccines? Give examples.
7. Why are anaphylactic reactions following injections of serum into human beings of great practical importance? What two types of reactions may occur?
8. Explain the nature of serum sickness. Under what circumstances is a mild serum sickness likely to develop? What circumstances may make the reaction more severe? Are there any serious or permanent ill effects from serum sickness?
9. Under what circumstances may a fatal anaphylactic shock occur following a serum injection?
10. Describe methods of testing for hypersensitiveness to serum. When are such tests advisable? What is the general procedure for giving serum treatment safely to a hypersensitive individual?
11. What importance have the phenomena of hypersensitiveness in connection with infectious diseases? How may this hypersensitiveness be used as a basis for diagnosis? Give an example.
12. Discuss briefly the relation between hypersensitiveness and immunity and a possible explanation of anaphylaxis.

PART FOUR

MICROBIOLOGY OF IMPORTANT INFECTIOUS DISEASES

CHAPTER XXV

INFECTIONS OF THE SKIN. STAPHYLOCOCCUS INFECTIONS

Bacteria on the normal skin. The surface of the skin of human beings is never quite free of bacteria, whether it be clean or dirty. Of course, if it is not clean, a great number and variety of micro-organisms may be present, including, perhaps, yeasts and other fungi, or protozoa, as well as bacteria. The hands, especially, are liable to acquire a large and varied population of both harmless and disease-producing organisms by contact with dust and unclean objects of all sorts, and the fingers are liable to be contaminated with germ-laden saliva or excreta.

These accidental contaminations of the skin, however, do not concern us here. The important fact is that *even the perfectly clean skin constantly harbors bacteria.* There are a few kinds of organisms which seem to multiply freely upon the healthy skin. These bacteria are to be found not merely on the superficial layers, but also in the deeper parts, especially about the hair follicles and sebaceous glands, and in the ducts of the sweat glands. Ordinary washing does not remove all these organisms, and even prolonged scrubbing will not sterilize the skin.

The most common types of these bacteria found constantly on the clean skin are Gram-positive cocci, and the majority of these belong to a single group—the *staphylococci.* In certain regions of the body, several other types of bacteria are usually present. About the external genitals and anal region, for example, an organism almost always to be found is a harmless acid-fast bacillus called the *Mycobacterium smegmatis.* Similarly, on the skin lining the external opening of the ear, nonpathogenic *diphtheroids* and acid-fast bacilli which live in the ear wax are often present, as well as the usual staphylococci.

Skin diseases. Physicians are familiar with a great many dis-

orders of the skin. The greatest number and variety of these diseases occur in the *tropics,* but they are common in temperate countries, also. Some of the most prevalent are manifestations of *hypersensitivity.* Many others are infectious in nature, that is, they are the result of the invasion of the skin by microorganisms of one kind or another. Some are due to fungi, others to protozoa (Chapters XLI and XLII). Those caused by viruses or bacteria are discussed below.

Virus infections. Several kinds of filtrable viruses cause characteristic skin lesions. Among the more prevalent of these are *smallpox* (discussed in Chapter XXXIX), *chicken pox* or *varicella, herpes zoster, herpes febrilis,* and *common warts.*

Chicken pox (*varicella*) appears to be due to a virus, which is present in the bacteria-free fluid from the vesicles. When appropriately stained, the virus particles or elementary bodies may be seen in this fluid, and characteristic intranuclear inclusion bodies are found in the affected epithelial cells.

Herpes zoster is a disease occurring almost entirely in adults. It is often called "shingles." There are various respiratory or digestive disturbances. The characteristic manifestation, however, is the appearance of a skin rash, which soon becomes changed to a series of large vesicles, beginning at the points where infected cutaneous nerves come to the surface, and spreading along the course of these nerves. There are accompanying neuralgic pains. The vesicles finally dry up and become covered with shingle-like scabs.

Elementary bodies may be found in the vesicular fluid and there are intranuclear inclusions in the tissue cells. The virus has been shown by immunological tests to be similar to, though apparently not entirely identical with, the varicella virus. Clinically, cases of chicken pox in children have often been seen after contact with a person having herpes zoster.

Herpes febrilis (often called simply "herpes") is entirely distinct from herpes zoster. It usually manifests itself as small vesicles about the mouth or nose of patients who are suffering from severe colds, pneumonia, or other acute febrile disease. These lesions are often called "cold sores" or "fever blisters." The disease is known as *herpes simplex* when similar vesicles appear without the presence of fever. Herpetic eruptions on the genitalia are not uncommon, and herpes infection of the cornea of the eye is a fairly frequent and serious condition.

A filtrable virus is always present in the local vesicles, and sometimes can be recovered from other tissues. The affected tissue cells contain intranuclear inclusion bodies, and in the vesicular fluid the virus is in the form of stainable elementary bodies. In tissue cultures the virus grows readily. Herpes virus may cause a brain infection (encephalitis) in experimentally

inoculated rabbits or mice, and is capable also of infecting the human brain, but it is not the usual causative agent of human encephalitis.

Common warts are caused by a virus which can be recovered by filtering ground-up wart material. Inclusion bodies in the affected skin cells have been described, but the specific relation of these bodies to the virus is uncertain.

Specific bacterial diseases with lesions in the skin. The skin is involved, either primarily or secondarily, in several important germ diseases. In one of the principal clinical forms of *leprosy,* hard nodular swellings appear in the skin, principally upon the face. The organisms of *tuberculosis* sometimes localize upon the skin. The commonest form of skin tuberculosis is called *lupus.* The earliest lesion in *syphilis* is a hard sore called the chancre, which appears at the spot on the body where some minute break in the mucous membrane or skin has permitted the spirochete to enter. *Yaws,* a tropical disease resembling syphilis, also has characteristic lesions upon the skin. In the course of *typhoid fever,* the germs of this disease may localize in the so-called "rose-spots" just under the skin. Similar skin lesions may appear in patients with a septicemia due to *meningococci.* Human beings are sometimes infected with the germs of *anthrax* through a break in the skin. A sore develops called a "malignant pustule." When the organisms of *glanders* or *tularemia* get through the skin, a characteristic inflammatory lesion appears at the place of entrance.

Streptococcus infections. A variety of *hemolytic streptococcus* causes *erysipelas,* a peculiar skin disease characterized by an acute, spreading inflammation of the superficial lymphatic vessels of the skin. This infection is liable to occur especially in old persons, or in individuals whose general resistance has been weakened by a pre-existing disease. Fever and other symptoms arise from *toxin* formed by the organisms. The patient must be isolated, because the streptococci in the discharge from the skin lesions are dangerous to other persons, and would likely cause a fatal septicemia if introduced into a wound.

Rarely, streptococci produce a localized *abscess in the skin,* but usually these cocci are so virulent that, once through the skin, they immediately invade the deeper tissues, causing an acute spreading infection of the subcutaneous tissues, called *cellulitis,* with finally, an invasion of the lymphatics and the blood, or an acute *septicemia* without any localization in the skin or elsewhere. The numerous

infections which may be caused by virulent streptococci are considered fully in the following chapter.

Staphylococcus infections. The most frequent of all bacterial skin infections is the familiar boil, or *furuncle,* caused by staphylococci. A description of the pathogenic staphylococci and of their important part in human disease is presented in the following section.

SKIN INFECTIONS AND OTHER DISEASES CAUSED BY STAPHYLOCOCCI

The pathogenic staphylococci. *Species and their distribution.* There are only two species of staphylococci which are important in connection with disease: *Staphylococcus albus* and *Staphylococcus aureus.* These species names refer to the color of the growth of these organisms on agar media. Cultures of the *albus* species are white, while the *aureus* species forms an orange or golden-yellow pigment.

The staphylococci are constant inhabitants of the mucous membranes, as well as the skin, of human beings and animals. Since they are rather hardy organisms, and especially resistant to drying, they may often be found in the dust of inhabited rooms. The *albus* type is usually abundant there, and upon the clean skin and mucous membranes. The *aureus* species, on the other hand, is the kind found most commonly associated with pathological conditions.

Morphology and staining. The individual staphylococci are tiny spheres, slightly less than one micron in diameter. In smears, they are characteristically clustered together in irregular-shaped clumps; the cells in these clumps form a mosaic-like pattern (Fig. 76). Sometimes in smears from pus or pure cultures they appear arranged in groups of two or four cells, or in short chains, and it is often difficult to be certain, from microscopic examination alone, whether or not the organism is a staphylococcus.

They are readily colored with the common stains, and are Gram-positive, although usually some Gram-negative cells are seen in older cultures.

Physiological properties and biochemical activities. All varieties of staphylococci grow luxuriantly upon simple culture media, and they multiply at room temperature, as well as at body temperature. They develop best in the presence of abundant atmospheric oxygen, but will also grow in partially anaerobic conditions.

The staphylococci are unusually resistant organisms. They remain alive in laboratory cultures without transplantation, much longer than most disease germs. When dried on paper or cloth, they may be found alive after many weeks. They are also less readily killed by chemical disinfectants than many other pathogenic bacteria.

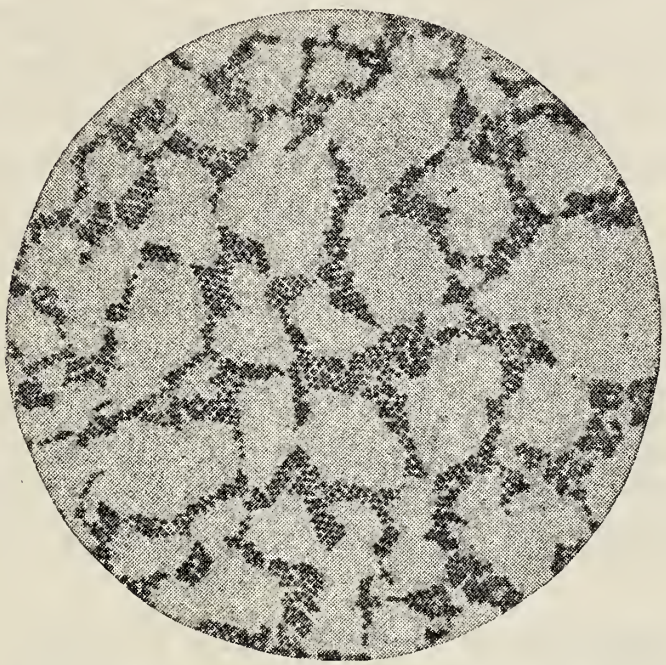


FIG. 76. Smear of a pure culture of *staphylococci* showing the typical mosaic arrangement of the groups of cocci. (Reproduced from *Experimental Bacteriology*, Volume I, by Kolle and Hetsch, by permission of George Allen & Unwin Ltd.)

Staphylococci ferment a number of common sugars, with acid formation. Pathogenic strains usually ferment mannitol. Also, most of these strains liquefy gelatin.

Special properties of virulent staphylococci; toxin production. The virulence of staphylococci seems to depend largely upon their capacity to produce the coagulase factor and other mildly injurious substances. Some varieties form true exotoxins. Virulent strains are almost invariably hemolytic (forming filtrable *hemolysins*); they can destroy leukocytes (by action of *leukocidin*); they can dissolve fibrin clots (through their *fibrinolysin*); and they can coagulate citrated plasma (through action of the *coagulase factor*). The *plasma coagulase test,* described below, is the one laboratory procedure most consistently positive with pathogenic staphylococci and negative with nonpathogenic strains. Many virulent staphylococci also form the Duran-Reynals' *spreading factor.*

Moreover, certain pathogenic cultures of *Staphylococcus aureus*

elaborate true *exotoxins*. The sterile filtrate from cultures of these toxigenic strains has: (1) a hemolytic action, causing the hemolysis of red blood cells, (2) capacity to cause death and destruction (necrosis) of the skin of rabbits and other animals, when inoculated intradermally, and (3) power to kill rabbits and mice almost instantly when inoculated intravenously. The part played by these toxic principles in human staphylococcus infections is uncertain, but probably it is not as great as might be expected from these marked effects on animals. Since the staphylococcus toxin is an exotoxin, it is possible to prepare from it a specific *toxoid* (by adding about 0.3% formalin) and an *antitoxin* (by immunizing horses or rabbits).

Food-poisoning staphylococci. Apart from the toxic substances mentioned above, some strains of *Staphylococcus aureus* may form another sort of toxin, *enterotoxin*. A filtrate from a culture of a staphylococcus of this kind produces gastrointestinal symptoms, when swallowed by human beings. An overgrowth of these staphylococci in cream-filled pastries and similar foods has been responsible for numerous outbreaks of acute *food poisoning* (Chapter XXXIII).

Disease production by staphylococci. The fact that staphylococci of varying degrees of virulence are constantly present on the skin and mucous membranes, as well as in common materials all around us, is sufficient explanation for the great frequency with which these organisms are found associated with inflammations in various parts of the body.

Furunculosis. When a cut or abrasion of the skin permits the staphylococci to penetrate, the usual result is the rapid development of a small pustule (or pimple) or a larger abscess commonly called a *boil,* or *furuncle*. This disease condition is called *furunculosis*. The process by which a boil is formed has already been described (p. 316). The rapid accumulation of purulent exudate about the invading staphylococci is the characteristic reaction of the body to the presence of these organisms. Although many other pathogenic bacteria incite pus-formation, this reaction is always marked in the case of staphylococcus infection of any part of the body. For this reason, the staphylococci are the outstanding examples of *pyogenic* (pus-forming) *cocci*.

The fact that a furuncle is the usual result of the entrance of staphylococci, indicates that most human beings possess a considerable resistance against these organisms, and that the cocci are

usually of rather low virulence, as compared, for example, with the streptococci.

A more severe disease, with invasion of the deeper parts of the body, may occur when the individual is infected with an unusually virulent strain, or when the general resistance of the body is abnormally low. Under these conditions, instead of a single localized boil, a considerable area of the deeper tissues of the skin may be invaded, with perhaps several accumulations of pus reaching to the skin surface. This is called a *carbuncle.*

Another common form of staphylococcus infection is *paronychia,* infection of the nail bed on the fingers or toes.

Osteomyelitis—inflammation of the periosteum and of the soft parts of the bones—is often caused by staphylococci. This infection may be due to contamination of a compound-fracture wound from outside, but commonly it arises from bacteria in the blood stream which localize on a bone that has suffered some kind of mechanical injury. The inflammation may be acute, and may soon develop into a septicemia. Often, however, staphylococci of low virulence cause a chronic osteomyelitis which may persist for months.

Sometimes the staphylococci reach the blood stream from an original point of infection in considerable numbers, but do not produce a septicemia at once. Instead, the organisms localize in various internal tissues, as, for example, in the bones, joints, kidneys, lungs, and liver. Wherever they lodge and begin to multiply, an abscess is formed which is essentially the same as a boil. There may be many of these internal foci of infection, all acutely inflamed at the same time, and the patient may be very ill. This is the condition known as *pyemia.*

Any one of these clinical forms of staphylococcus infections may terminate in a fatal *septicemia.* Boils or carbuncles about the nose or lips are especially liable to spread rapidly, the infection extending to the venous sinuses in the membranes covering the brain (dura mater), with consequent clotting of the blood in these sinuses (venous sinus thrombosis). Transfer of infected blood clots from these veins to the lungs and escape of the staphylococci into the general circulation may end in death by "blood poisoning," i.e., by an overwhelming septicemia.

Occasionally, the staphylococci which get into a break in the skin are so virulent, or the patient's resistance is so low, that septicemia develops at once. In young children, a rapid and severe

generalized staphylococcus sepsis is not uncommon, and before the days of modern chemotherapy it was almost invariably fatal.

Staphylococci are usually regarded as the cause of a contagious skin infection occurring most commonly in young children and called *impetigo contagiosum*. The disease is characterized by boil-like pustules on the face or other exposed parts of the body. The infection is readily transmitted by contact. Unless care is taken, it may spread rapidly among the infants in a hospital nursery.

Staphylococci are also found in a great many other disease conditions. Indeed, they are encountered more constantly than any other kind of organism in inflammatory conditions of various parts of the body. But since their virulence is generally low, they are not often the cause of severe infections, other than those already mentioned. They are regularly to be found in infected *wounds*. They are often associated with *inflammations of the bladder, kidney, or other parts of the genito-urinary tract*. Sometimes they play a leading rôle in *infections of the middle ear*, in *meningitis*, and in *bronchopneumonia* following measles or influenza.

Certain strains are capable of forming enterotoxin, and these are responsible for many cases of acute *food poisoning*.

Bacteriological diagnosis. A specimen of pus from the boil or other staphylococcus lesion is to be obtained. A direct smear of this pus may reveal Gram-positive cocci of suggestive appearance. The organisms may be few in number, however, and typical mosaic-like groupings may not be seen. Identity of the cocci can be established only by culturing the material, preferably on blood agar plates.

The great majority of pathogenic, toxigenic staphylococci will have orange or golden-yellow colonies, will be hemolytic, will decompose gelatin and ferment mannitol, and will give a positive plasma coagulase test.* Special methods must be used to identify the food-poisoning (enterotoxin-producing) strains.

---

* *Coagulase Test (Staphylococci)*

(a) Emulsify the growth from a slant culture in approximately 1.0 cc of saline.
(b) Place approximately 0.5 cc of the suspension in a tube already containing 1.5 cc of citrated human or rabbit *plasma* (diluted 1:5).
(c) Add 0.5 cc of saline to a second tube of plasma as a control.
(d) Incubate the tubes at 37° C. Observe every 15 minutes for 2 hours. A positive reaction is indicated by a clotting of the plasma, resulting in a firm clot which is not disturbed on tilting the tube. The control should show no change.

In cases of suspected staphylococcus bacteremia or septicemia, a *blood culture,* made by adding aseptically 5–10 cc of the patient's own blood to infusion broth or agar, should be prepared. Great care should be taken, in securing the blood sample, to avoid accidental contamination of the specimen with cocci from the skin.

Immunity. Resistance to staphylococci seems to depend almost entirely upon the phagocytic action and general defensive effect of the cellular body-reaction. It is not permanently increased by recovery from an infection. On the contrary, one attack of furunculosis may shortly be followed by another. Persons who have had staphylococcus infections may show a somewhat higher titer of staphylococcus antitoxin in their blood serum than other persons, but this does not appear to offer much protection.

Specific treatment. It has been found helpful, in some cases of furunculosis, *to treat* the patient with an *autogenous vaccine,* that is, a vaccine made up of the staphylococci cultivated from the patient's own lesions. Sometimes such a vaccine brings about a rapid cure; in other cases it is not effective.

In selected cases of severe staphylococcus infection, good results have followed judicious use of staphylococcus *toxoid* as a means of stimulating resistance. Treatment with staphylococcus *antitoxin* has also a limited value.

Modern methods of *chemotherapy* are highly successful in most cases of staphylococcus infection.

REFERENCES

Blair, J. E.: "The Pathogenic Staphylococci," *Bact. Rev.,* 1939, 3:97.

Chapman, G. H.: "The Reliability of Bromthymol-blue Lactose Agar and Bactophenol-red Agar Mannitol Agar for the Isolation of Pathogenic Staphylococci," *J. Bact.,* Nov. 1944, 48:555.

Julianelle, L. A.: "Experimental Staphylococcus Infection and Passive Protection," *J. Immunol.,* Mar. 1944, 48:135.

Kleigh, B. and Blair, J. E.: "Rôle of Toxin and the Use of Antitoxin in Systemic Staphylococcic Infections," *Arch. Surg.,* 1943, 46:548.

Miles, A. A., Williams, R. E. O. and Clayton-Cooper, B.: "The Carriage of *Staphylococcus (pyogenes) Aureus* in Man and Its Relation to Wound Infection," *J. Path. and Bact.,* Oct. 1944, 56:513.

Reid, J. D. and Jackson, R. M.: "An Improved Method of Determining the Coagulase Activity of Staphylococci by Means of the Plasma Agar Plate," *J. Lab. and Clin. Med.,* Feb. 1945, 30:155.

REVIEW QUESTIONS—CHAPTER XXV

1. What kinds of bacteria are likely to be found on the normal skin? What can be said of the prevalence of skin diseases?
2. Name five virus infections involving the skin. Describe briefly *chicken pox, herpes zoster, herpes febrilis, and common warts.*
3. Name nine bacterial diseases with characteristic lesions of the skin.
4. What is the cause of erysipelas? Describe this infection. What other forms of infection may follow invasion of the skin by streptococci?
5. Name, and characterize briefly, the principal species of the pathogenic staphylococci. Where are these organisms naturally found?
6. Describe the morphology and staining, and the principal physiological properties and biochemical activities, of staphylococci.
7. What toxic substances may be formed by virulent staphylococci? What is the importance of the so-called *enterotoxin*?
8. Describe the commonest form of infection caused by staphylococci.
9. Name, and describe briefly, at least eight other forms of staphylococcus infection.
10. How may a bacteriological diagnosis of a staphylococcus infection be made? What is the significance of a positive coagulase test?
11. What is the basis of successful resistance against virulent staphylococci? What may be said concerning the value of specific treatment of staphylococcus infections?

CHAPTER XXVI

INFECTIONS OF THE MOUTH AND THROAT. STREPTOCOCCUS INFECTIONS. SCARLET FEVER. VINCENT'S ANGINA

INFECTIONS OF THE MOUTH AND THROAT

Normal flora of the mouth and throat. There are no regions of the body which normally support a more abundant and varied population of microbes than the mouth and throat. The moist, warm mucous membrane furnishes excellent conditions for the life of all types of microbes. Bacteria thrive especially well in remnants of food, and in the débris of dead epithelial cells, about the teeth. The peculiar anatomy of the mouth cavity and throat affords sheltered living places for numerous types of anaerobic as well as aerobic bacteria. Anaerobic organisms are especially abundant about the gum margins, in crevices between the teeth, and in the deep folds, or crypts, in the surface of the tonsils.

Microorganisms in the mouth. A list of the microbes which have been cultivated at one time or another from the human *mouth* reads like a catalogue of all the main groups of microorganisms. Numerous species of cocci, bacilli, and spirilla, and also several kinds of higher bacteria, spirochetes, yeasts, and mold-like organisms, as well as amebae and other protozoa, may be present.

In the mouth of nearly every person there are places where the gums are not anatomically perfect—they do not meet the teeth with a knife edge, but instead, the gum margin is blunted and easily loosened from the teeth. If an inoculating needle is passed along this blunted margin, or forced between the gum and the tooth, and if the material thus secured is examined under the microscope, a seething mass of bacteria is revealed (Fig. 77).

Conspicuous among these organisms are the *spirochetes* called *Treponema microdentium,* and larger, coarser forms, similar to if

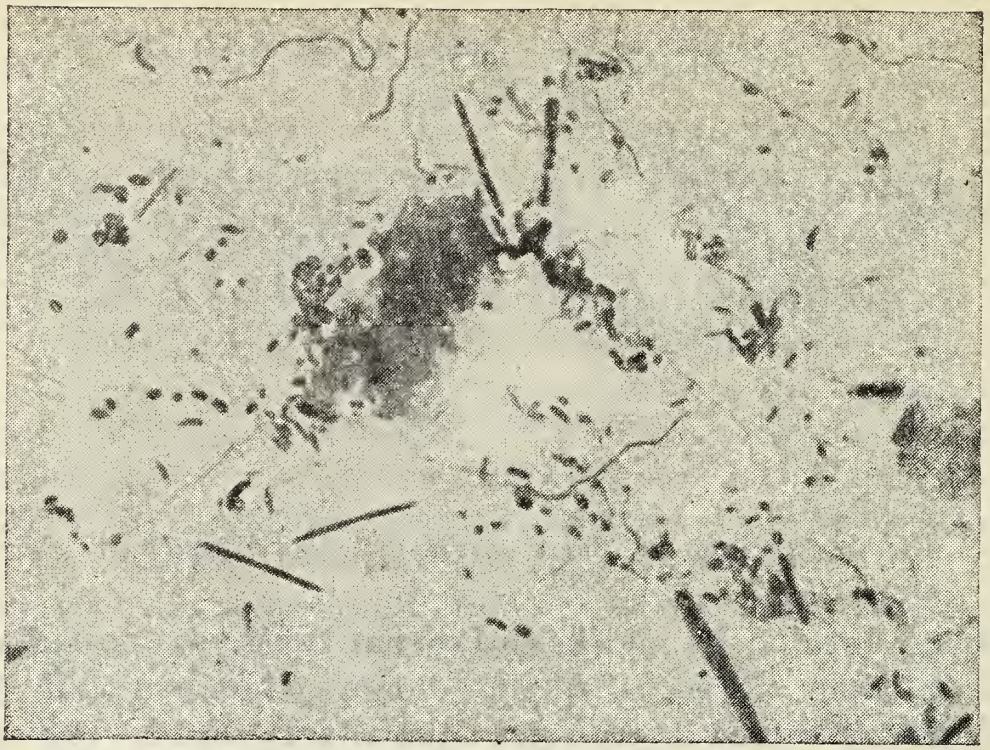


FIG. 77. A direct smear from the margin of the gum in a human mouth, showing spirochetes, fusiform bacilli, and other organisms.

not identical with *Borrelia vincenti*. Also present are the slender *fusiform bacilli* (*Fusobacterium fusiformis*). Both of these groups of bacteria are *anaerobic*. In cultures from such material, one of the most abundant organisms is the small Gram-negative anaerobic diplobacillus named *Bacterium melaninogenicum* (*Bacteroides melaninogenicus*) (Fig. 78). The number of these bacteria in the mouth depends upon the degree of cleanliness of the teeth, as well as upon the anatomical condition of the gum margin. A similar flora is present in the tonsillar crypts. It is important to realize that these very organisms in the normal mouth are often found associated with disease, such as ulcerations in the mouth or throat and abscesses about the head and neck. The origin of lung abscesses, particularly those following an operation in which ether anesthesia was used, may often be traced to bacteria from the mouth, and the anaerobic types mentioned above are usually abundant. These facts make clear the great importance of good mouth hygiene.

Microorganisms in the pharynx and nasopharynx. When blood agar plates are streaked with swabbings from the healthy *throat*, the most numerous colonies that develop consist of Gram-negative diplococci (*Neisseria catarrhalis* and related members of this genus),

the green-colony-type streptococci (*Streptococcus salivarius*), and staphylococci (*Staphylococcus albus* and *aureus*). Often present also are colonies of pneumococci (*Diplococcus pneumoniae*), and of diphtheroids (*Corynebacterium xerose* and related varieties). The composition of the bacterial flora in any one person's throat varies from time to time, and changes under the influence of weather and other factors that affect the mucous membranes. The varieties of bacteria that predominate at any particular time are likely to be the same as those that are most abundant in the throats of intimate associates at that time, and the dominant species may differ from place to place and from season to season. Many of these organisms found normally in the pharynx, such as most of the Gram-negative diplococci, are entirely harmless, but others, like the streptococci, are at least potentially pathogenic.

Hemolytic streptococci (*Streptococcus hemolyticus*) are *not* commonly found in the healthy throat. However, these and other dangerous germs may be present, of course, in the pharynx of healthy *carriers*. Fully virulent pneumococci, diphtheria bacilli (*Corynebacterium diphtheriae*), the organisms of epidemic meningitis (*Neisseria meningitidis*), *Hemophilus influenzae*, and many other disease-producing species may be isolated from carriers.

When the *nasopharynx*—the upper region of the throat back of the soft palate—is cultured without touching other areas, one often obtains an almost pure culture, representing the predominating species of bacteria present. Thus, the culture may be an almost pure growth of a hemolytic streptococcus, or a green-colony streptococcus, or the meningococcus. Culturing the nasopharynx is therefore a most useful procedure, especially when carriers of virulent microbes are being sought.

Diseases of the gums and teeth. Despite the large population of microbes in the mouth, the gums, though frequently injured, are rarely infected. On the other hand, *caries or decay of the teeth, abscesses about the teeth,* and *pyorrhea* are common diseases.

The cause of caries of the teeth is not certainly known. A very prevalent idea is that acid, developed as a result of the growth of aciduric bacteria in particles of food caught between the teeth, eats through the outer surface (enamel) of the teeth, thus exposing the softer dentine beneath. As the decay goes on, a hole appears which must be filled by the dentist if the tooth is to be saved. However,

FIG. 78. *Bacterioides melaninogenicus.* A: appearance of a mixed culture on a blood agar slant from the gum margin of a patient with pyorrhea, after 14 days' incubation. The originally opaque red medium has become almost colorless and transparent, and the black pigment formed from the hemoglobin by *Bact. melaninogenicus* is obvious. The organisms are growing characteristically between the agar and the glass at the base of the tube. B: a pure culture, showing the tiny diplo-coccobacilli.

some authorities do not agree with this idea of the origin of caries, but assign great importance to the influence of faulty diet.

Nearly everyone suffers at some time from an "ulcerated tooth" or a milder form of toothache. These conditions are due to an acute or chronic infection about the root of a tooth, or in the root canal of the tooth. Several types of bacteria may be responsible for the inflammation, including staphylococci, and other organisms found normally in the mouth, but probably the majority of cases are due to the *Streptococcus salivarius*, the green-colony variety of streptococcus.

In the condition known as *pyorrhea alveolaris* there is a chronic inflammation, with pus-formation about the roots of the teeth and a slow destruction of the alveolar bone, so that the teeth eventually become loosened. The cause of this rather common disease is unknown. In the pockets of ill-smelling pus about the affected teeth, a great many bacteria of different kinds are present, but their relation to the cause of the condition has never been clearly established.

Infections of the mouth, pharynx, and tonsils. *Thrush* is a disease of the mouth sometimes seen in children, and caused by a yeast-like organism. A form of this fungous infection, involving the vagina, occurs also in adult females (Chapter XLI).

Inflammation of the pharynx (*pharyngitis,* or sore throat) is too familiar to require description. It is not always caused by the same germs. In a majority of acute cases, however, the predominating organisms in a sore throat are *streptococci* of the hemolytic variety. The same is true of the various clinical forms of *tonsillitis.* Streptococci also cause the throat inflammation in *scarlet fever,* and the milk-borne disease called *septic sore throat.* These and other streptococcus infections are discussed below.

Aside from these conditions, there are two common and important *specific* infections which produce characteristic lesions in the mouth or throat, namely *Vincent's angina* and *diphtheria.* The germ of *syphilis* also sometimes infects the mouth or tonsils, causing lesions which must be distinguished from those of Vincent's angina or diphtheria.

STREPTOCOCCUS INFECTIONS

General properties of the parasitic streptococci. The genus *Streptococcus* contains the many varieties of spherical bacteria that grow habitually in chain formations. Within this large group there are two subdivisions made up of purely saprophytic varieties: (1) the harmless sour-milk streptococci of the *lactic group* and (2) the *enterococci,* found in the normal intestinal contents. The two remaining subdivisions of the aerobic streptococci—(3) the pyogenic, *hemolytic streptococci,* and (4) those of the *viridans group*—contain the parasitic varieties in which we are primarily interested.

Morphology and staining. These organisms are tiny spherical bodies, usually less than one micron in diameter. They are arranged characteristically in chains, like a string of beads. Often the chain is made up of pairs of cocci very close together, separated by a considerable space from adjacent pairs, so that the chain appears as a series of diplococci end to end. The length of the chain varies with the conditions of growth, and also to some extent with the variety of streptococcus. Virulent hemolytic streptococci may develop a capsule.

All the parasitic streptococci are Gram-positive, though they are easily decolorized unless special care is taken in performing the stain.

Physiological properties. The streptococci do not grow as readily on artificial culture media as the staphylococci, but will develop

well on media enriched with blood or blood serum. Even on these media, however, their growth is comparatively delicate, and their colonies are small. They multiply best at body temperature.

The streptococci die off rather quickly in laboratory cultures, but they appear to survive for considerable periods outside of the body. They may remain alive in pus or in sputum or similar materials for hours, and they have been recovered from the dust of hospital wards. They multiply readily in milk, and milk may serve as the vehicle by which the germs are spread, as in epidemics of septic sore throat, and sometimes in scarlet fever outbreaks.

Products of growth; toxins. Virulent streptococci, like staphylococci, form a number of products which enhance their disease-producing power. The *hemolysin* of the hemolytic streptococci is definitely associated with virulence. Most pathogenic strains also produce *fibrinolysin*, and *leukocidin;* some form the Duran-Reynals' *spreading factor.*

Moreover, the strains associated with scarlet fever and erysipelas, at least, and probably others, manufacture true *exotoxins* which are poisonous to the body as a whole. These toxins are not as powerful as those of the diphtheria or tetanus germs, but nevertheless they are sufficiently potent to weaken the patient considerably, so that the streptococci are enabled to invade the tissues more extensively.

Grouping of parasitic streptococci according to their action on blood agar. Bacteriologists have found it difficult to classify the many varieties and subvarieties of parasitic streptococci which are found in the mouth and throat and in connection with disease. It is not clear which types of these organisms should be regarded as separate species. For practical purposes, however, it has been found most useful to group these streptococci *according to their action upon the blood in blood agar media.* On this basis three groups are recognized: (1) the *hemolytic* or *beta-type* streptococci, whose colonies on blood agar are surrounded by a clear, transparent zone in which the red blood cells are destroyed and there is complete decolorization of the hemoglobin (Fig. 79); (2) the *green-colony, viridans* or *alpha-type* streptococci, whose colonies are surrounded by a zone in which the red color of the hemoglobin has been changed to a green or brownish green, while the blood cells remain virtually intact; and (3) the *indifferent, anhemolytic* or *gamma-type* streptococci, whose colonies produce no visible change

in the medium. Often colonies of the green (*alpha*) type show at the periphery a narrow transparent zone of partial "hemolysis," in which there is a more or less complete decolorization and destruction of red blood cells, but the term "hemolytic streptococci" is by custom applied only to organisms of the first-mentioned, *beta* type.

Hemolytic (beta type) streptococci. The name *Streptococcus hemolyticus* is generally applied to all strains of the hemolytic (beta type) streptococci. It is now certain that the same variety of hemolytic streptococcus may be responsible for a typical case of scarlet fever in one person, or for erysipelas, or septic sore throat, or other less well-defined clinical disease, in other persons. Hence the older specific names, such as *Streptococcus scarlatinae, Streptococcus erysipelatis,* and *Streptococcus epidemicus* are no longer considered justified.

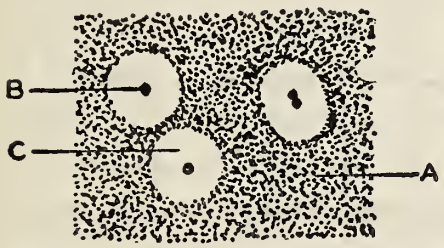


FIG. 79. Appearance of colonies of *Streptococcus hemolyticus* on blood agar. A: the opaque medium containing intact red blood cells; B: the colony; C: the clear transparent zone about the colony, in which "hemolysis" has occurred. The hemoglobin is completely decolorized and the red blood cells have disappeared.

Differentiation within the group of hemolytic streptococci is based primarily upon immunological methods, utilizing mainly a precipitin test technique developed by Lancefield. The hemolytic streptococci pathogenic for *man* fall almost always into the Lancefield's *Group A*. There are occasional cases of human infection, however, with streptococcus strains classified in Group C. Some of the special characteristics of the hemolytic streptococci are summarized in Table XVII.

Viridans (alpha type) streptococci. These organisms—the *green-colony streptococci*—have long been known under the name *Streptococcus viridans*. According to the more recent nomenclature, however, the cocci of this kind found in association with human beings are more properly called *Streptococcus salivarius*. There are numerous varieties; different strains may differ markedly in disease-producing power. But unfortunately these varieties cannot be classified into definite serological groups, like the Lancefield groups among the hemolytic streptococci. Instead, each strain tends to have an antigenic make-up peculiar to itself, bearing no relation to its origin or virulence.

Streptococci of the alpha type may be confused with pneumo-

TABLE XVII. Characteristics of the Principal Varieties of Hemolytic Streptococci

SPECIES OR VARIETIES	LANCEFIELD'S GROUP	FERMENTATION OF						HYDROLYSIS OF SODIUM HIPPURATE	HUMAN FIBRINOLYSIN	NATURALLY PATHOGENIC FOR
		LACTOSE	MANNITOL	SALICIN	TREHALOSE	SORBITOL	INULIN			
Streptococcus pyogenes (*Str. hemolyticus*)	A	+*	+	+	+*	—*	—	—	+	Man (but occasionally infect cattle, monkeys, rabbits, mice)
Streptococcus mastitidis	B	+*	—	+	+	—	—	+	—	Cattle
Streptococcus equi	C	—	—	+	—	—	—	—	—	Horses
"Animal pyogenes"	C	+	—	+	—	+	—	—	—	Hogs, sheep, various other animals
"Human C"	C	±	—	±	+	—	—	—	±	Man

* Indicates that occasional variations occur.

cocci (*Diplococcus pneumoniae*), since both of these organisms are often cultured from the same materials, have a similar morphology, and form alpha-type blood agar colonies. Differentiation depends principally upon the fact that pneumococci are soluble in bile, while streptococci are not.

Viridans-type streptococci found in the mouth and intestinal tract of horses and of cows are called *Streptococcus equinus* and *Streptococcus bovis,* respectively. Some of the special properties of the viridans streptococci are shown in Table XVIII.

Disease production by streptococci. *Hemolytic streptococcus infections.* Hemolytic streptococci are sometimes associated with infections of mild character, but included among these organisms are highly virulent strains, and by far the largest proportion of all serious streptococcus diseases are caused by these hemolytic cocci. They may be found in the "normal" throat, but they are not commonly encountered except in connection with some pathological condition.

Hemolytic streptococci are the organisms most to be feared as the cause of infection of surgical or accidental *wounds,* whether superficial or deep, because, of all the common bacteria, they are the most liable to invade the deeper tissues to produce a grave *cellulitis,* or severe *osteomyelitis,* and all streptococcus infections tend to terminate in a fatal *septicemia.* Many instances are known in which surgeons or pathologists have developed septicemia as a result of pricking their fingers during an operation or an autopsy on a streptococcus case. At least one member of practically every class of nurses develops a serious streptococcus finger infection at some time during the period in training school. Extraordinary care must be exercised in handling pus or other material containing virulent streptococci.

Hemolytic streptococci are the frequent cause of acute *otitis media* (inflammation of the middle ear). This inflammation may extend to the mastoid region (*mastoiditis*) and sometimes leads to *meningitis.*

These streptococci cause most cases of *acute sore throat, tonsillitis,* and *peritonsillar abscess,* and, under the special circumstances previously described, are responsible for the milk-borne epidemic infection, *septic sore throat.* The severe, and often fatal, *bronchopneumonia* which commonly follows an attack of measles, influenza, or other weakening disease is often caused by streptococci.

TABLE XVIII. Properties of Viridans-type Streptococci

SPECIES OR VARIETY	GROWTH AT 45° C	SURVIVAL AT 60° C, 30 MIN.	FERMENTATION OF				STARCH HYDROLYZED	SOLUBILITY IN BILE	PATHOGENICITY
			LACTOSE	MANNITOL	SALICIN	INULIN			
Streptococcus salivarius (*Str. viridans*)	—*	—	+	—	+	—*	—	—	Cause a variety of infections (mostly subacute) in man. Virulence generally less than that of hemolytic streptococci
Streptococcus equinus	+	±	—	—	+	—*	±	—	Probably not pathogenic
Streptococcus bovis	+	+	+	—*	+	—*	+	—	May cause mastitis in cows; and other animal infections. Generally of low virulence

* Occasionally +.

Infection of the uterus and blood after childbirth (*puerperal fever*) is frequently due to hemolytic streptococci introduced from without. The patient usually dies with a septicemia.

Scarlet fever and *erysipelas* are the outstanding examples of hemolytic streptococcus infections with characteristic clinical features. These diseases are caused by streptococci that are both toxic and invasive. In erysipelas, there is a spread of the infection through the superficial lymphatic vessels of the skin, and a marked reddening of the affected area. This inflammation is due, in part, to the erythrogenic exotoxin formed by the germs. In scarlet fever, an exotoxin causes a characteristic reddening of the whole skin.

Viridans-type streptococcus infections. The alpha-type, green-colony streptococci are constantly present in the healthy mouth and throat. Most strains are pathogenic, but their virulence is low, as compared with that of the hemolytic varieties. They are therefore more commonly found in less severe infections. They do not form the filtrable hemolysins, nor fibrinolysins, which contribute to the virulence of the hemolytic streptococci, nor do they manufacture the skin-reddening exotoxins characteristic of the scarlet fever and erysipelas hemolytic strains.

The viridans streptococci are frequently responsible for *tooth abscesses,* and may be present in *otitis media,* and in infections of the accessory *sinuses* of the nose, or of the *appendix,* or *gall bladder.*

Streptococcus salivarius is often the cause of the comparatively rare, but important, disease called *subacute bacterial endocarditis.* This is an infection of the valves inside the heart, in which so-called vegetations, consisting of an exudate enclosing masses of streptococci, accumulate on the delicate valvular tissue. The organisms escape intermittently into the general circulation, so that cultures from the blood at these times will contain the organisms; but usually a true septicemia does not develop until just before death. Before the days of penicillin a fatal outcome was always to be expected within a few weeks or months, but now some patients are being saved through large doses of this antibiotic.

The endocarditis is initiated when streptococci in the circulating blood localize and set up an inflammation upon heart valves which have previously been damaged by rheumatic fever or by congenital heart disease. It is liable to occur in persons with such vulnerable heart tissue, after the extraction of an infected tooth, or following tonsillectomy, or as a consequence of some other circumstance which

permits large numbers of the green-colony streptococci to enter the blood stream all at once.

Rheumatic fever. This severe, crippling disease ranks not far below tuberculosis and syphilis as a major cause of acute and chronic illness, especially among children and young adults. Most patients suffer from a wandering *arthritis,* with swelling and reddening of the larger joints; many develop some form of *chorea,* a nervous disorder; and, most important, a high proportion of rheumatic patients show definite signs of *injury to the heart.* There is a marked tendency for repeated attacks to occur in the same individual, with consequent severe cardiac damage. Epidemics have frequently been noted among groups of children living together in institutions, and among young recruits in military camps. In the United States, the disease is most prevalent among the poorer classes of the population in crowded cities, particularly in the cities of the North and Northwest sections of this country, where severely cold winter weather is the rule. In recent years, there has been increasing realization of the importance of rheumatic fever as a public-health problem.

The true cause of this disease is still not established beyond question. There is much evidence, however, that an infection of the upper respiratory tract (often a mild tonsillitis or pharyngitis) caused by virulent (toxigenic) hemolytic streptococci is the significant event which leads directly to the subsequent appearance of rheumatic symptoms. The epidemics observed have followed outbreaks of streptococcus tonsillitis, and an obvious connection between the primary attack (and also the recurrent attacks) and a preceding scarlet fever, or other form of streptococcus infection of the nose and throat, has been noted many times. Not all rheumatic fever patients give a history of a preceding acute upper respiratory-tract infection, but it is not unlikely that virulent streptococci may have been present in abnormal numbers in these persons at some time just prior to the illness, even though there were no obvious clinical signs of their presence. Often two or more members of the same family develop rheumatic fever, and there is good reason to believe that an inherited susceptibility plays an important rôle. This susceptibility, and various environmental factors, such as poor nutrition and exposure in inclement weather to repeated upper-respiratory-tract infections, seem to be necessary contributing factors.

The exact rôle of the streptococci is uncertain. They may be merely secondary to the real etiologic agent, which so far has eluded

discovery. Some investigators believe that poisons from the cocci directly injure the heart and other tissues of susceptible young persons. The concept most widely supported at the present time, however, is the belief that the manifestations of rheumatic fever are primarily the result of a state of hypersensitivity to the proteins or toxins of streptococci developed following previous infection with these organisms. At any rate, there is every reason to believe that more effective control of streptococcus infections of the respiratory tract, through improved environmental conditions and better personal hygiene, would result in a reduction in the incidence of rheumatic fever.

Bacteriological diagnosis of streptococcus infections. Microscopic examination of *smears* made directly from the inflamed throat or other part, or from the discharges from the lesions, usually show numerous Gram-positive cocci in short or long chains, and there is little question about the presence of streptococci. *Cultures* must be made, however, to determine definitely whether or not these organisms are present, and if so, to what variety they belong. The material is streaked upon plates of infusion agar enriched with horse, rabbit, or human blood; or, better, pour plates are made. Examination of the growth on these plates will permit recognition of streptococcus colonies of the beta, alpha, or gamma types. The latter are generally regarded as of no medical importance. If cocci are isolated from green (alpha type) colonies, a bile solubility test is indicated. Glucose brain broth is an excellent medium for the primary cultivation of streptococci from any source; the original growth may later be plated on blood agar. In cases of suspected bacteremia or septicemia, *blood cultures,* made by adding 5–10 cc of the patient's blood aseptically to 100 cc of sterile broth or agar, are prepared.

It is important to remember that many pathogenic streptococci prefer a low-oxygen tension when first isolated, and may grow out better under anaerobic conditions than on the surface of aerobic cultures. Hence, some of the primary cultures should be incubated anaerobically, and some may be placed in an atmosphere of 10% carbon dioxide. Provision for development under different degrees of oxygen tension is especially needed when cultures are being made from the blood in cases of bacterial endocarditis, for these streptococci often show a decided preference for partial anaerobiosis.

Immunity. Except in the case of scarlet fever, recovery from streptococcus infections does not lead to a lasting immunity. Even

after scarlet fever, the resistance of the recovered patient is effective only toward the erythrogenic *toxin* of the scarlet-fever streptococci and protects little, if at all, against the organisms themselves. As in the case of staphylococci, effective resistance to invasive streptococci seems to depend principally upon the cellular defenses (phagocytosis). Again with the exception of scarlet fever, neither vaccines nor immune serums are widely used, and the results of attempts at the specific prophylaxis or therapy of the various other forms of streptococcus infection have not been promising on the whole.

Fortunately, the sulfa drugs and newer antibiotics have proved to be extraordinarily effective against streptococci.

SCARLET FEVER

Present conception of the cause of scarlet fever and the nature of immunity to the disease. Although certain Russian investigators really demonstrated the truth of the matter as early as 1907, and many other workers contributed important evidence, it was principally the work of Dick and Dick, beginning in 1923, which finally made clear the true rôle of streptococci in scarlet fever. It has been established that these organisms produce an *exotoxin*, which, when absorbed into the blood, causes the fever and other toxic symptoms, and the skin rash, characteristic of scarlet fever. Even though the streptococci remain localized in the throat (as they do in mild cases), the poison they secrete is carried throughout the body by the circulating blood. Scarlet fever is much like diphtheria, because in this disease, also, the germ causes a local throat inflammation, while its toxin poisons the whole body.

The scarlet-fever-streptococcus toxin, however, is not so destructive as the poison of the diphtheria bacillus, and by no means as powerful as the tetanus toxin, so that the direct effect of the poison on the body is relatively unimportant. The greatest danger arises from the tendency of the streptococci themselves to spread from the throat to set up serious inflammation in the middle ear, neck glands, or elsewhere. When death occurs, there is a streptococcus septicemia.

Persons who recover from scarlet fever have in their blood and tissues an *antitoxin* which specifically neutralizes the scarlet-fever toxin. So long as this antitoxin is present, the individual remains immune to the disease. It is important to remember, however, that

although such persons are resistant to scarlet-fever *toxin*, and so are not likely again to develop the typical disease, they have *no lasting immunity to the streptococci themselves*, and may later be reinfected with scarlet-fever streptococci. When so reinfected, such an individual may transmit typical scarlet fever to susceptible individuals. It follows, then, that the same streptococci may produce in some persons merely a local throat inflammation of more or less severity, and in others a typical scarlet fever, depending upon whether or not the individual has scarlet-fever antitoxin in his blood.

How scarlet fever spreads. The germs of scarlet fever are present in the nose and throat discharges and in the pus from infected ears, glands, etc. They are usually transmitted by contact directly from person to person, or more rarely, through contaminated *unpasteurized* milk. The streptococci are present in the nose and throat from the very beginning of symptoms, throughout the disease, and *for weeks after convalescence*. Most health departments require the quarantine of scarlet-fever patients in uncomplicated cases for at least four weeks. Even after this long period, the individual may still be able to transmit the disease to others. If there are complications in the form of infected ears or other local inflammations, the patient must be isolated until these have healed and all abnormal discharges have ceased. The "scales" from the peeling skin have nothing to do with the spread of the infection.

The Dick test for susceptibility to scarlet fever. Susceptibility to scarlet fever may be determined by the injection, *intradermally*, of a small amount of diluted scarlet-fever-streptococcus *toxin*. The test, called the *Dick test*, after the workers who devised it, is the same in method and principle as the Schick test used for determining susceptibility to diphtheria toxin. The test is made by the injection, into the skin of the forearm, of 0.1 cc of an especially prepared toxin solution. The amount of poison in this injection is one "skin-test dose," by which is meant the *smallest amount of the toxin which will give a typical reaction in fully susceptible individuals.*

If the individual is *susceptible*—i.e., does not have scarlet-fever antitoxin in his tissues—a characteristic red area of inflammation develops around the test injection, reaching its height in from eighteen to twenty-four hours, and often disappearing within thirty-six hours. This *positive reaction* is due to the direct action of

the poison on the cells of the skin. If the individual is *immune*—i.e., if he has scarlet-fever antitoxin in his tissues—this antitoxin neutralizes the injected poison, so that there is no change at the site of injection and the test is *negative.*

The results of Dick tests upon many individuals have indicated that the majority of young children, especially those in the five-to-ten-years age group, are susceptible to scarlet fever, whereas most adults are immune. This is in accordance with the fact that the greatest number of cases occur in children.

Scarlet-fever antitoxin. Antitoxin serum for the treatment of scarlet fever has been prepared commercially by immunizing horses with the toxin alone or with whole cultures containing the organisms as well as the poison. The strength of the antitoxin is determined by the number of skin-test doses of the toxin it will neutralize. *The unit of antitoxin is taken as the least amount which will neutralize fifty skin-test doses of scarlet-fever-streptococcus toxin.*

When this antitoxin is injected early in the course of severe scarlet fever, the patient's condition is usually remarkably improved within a few hours. The antitoxin has little direct effect upon the streptococci in the throat, but relief from the poison seems to give the body increased power to battle with the germs, so that complicating infections of the middle ear, etc., are usually avoided. Good results may also be obtained by the use of blood from persons convalescent from the disease, in place of commercial horse-serum antitoxin.

If scarlet-fever antitoxin is injected intradermally into a reddened area of the patient's skin, there will occur a definite and permanent *blanching* of the skin rash at that point within a few hours. This so-called *Schultz-Charlton reaction* is a useful diagnostic test, for the blanching will occur only if the patient actually has scarlet fever.

Prevention of scarlet fever; active immunization. The occurrence of many mild cases, never recognized as scarlet fever, and the existence of healthy carriers of scarlet-fever streptococci, make it very difficult to control this disease. The most strict and careful isolation technique is required to prevent the spread of infection, and the isolation must be prolonged until the organisms have been eliminated. Early diagnosis and the prompt use of antitoxin will greatly reduce the severity of the disease.

Nurses and others who are obliged to come into contact with scarlet-fever patients are especially liable to be infected, and serious efforts have been made to develop for such persons successful methods of active immunization. Nurses in most infectious-disease hospitals are now given some form of vaccination, if Dick tests show that they are susceptible. The method of immunization most widely adopted consists in the subcutaneous injection of three or more graded doses of diluted scarlet-fever-streptococcus *toxin*. Within a short period after these injections, the Dick test should become negative, indicating that immunity to the toxin has been established. Not all persons in any group will be successfully immunized by the same series of injections, but usually at least 85% become Dick-negative. The duration of this immunity is uncertain, but it probably lasts for several years.

There are several factors that seriously limit the practical usefulness of this immunization procedure. The inoculation of unaltered toxin often results in severe local or general reactions. The necessity of giving so many injections, perhaps with loss of time from duty after each dose, is an unfortunate feature. Nevertheless, the practice of testing by the Dick test all members of the nursing staff and other attendants, and of following up with the active immunization of the susceptible persons, has been found worth while in many hospitals, as a means of reducing, if not eliminating, cases of typical scarlet fever among the hospital personnel. Some of the difficulties have been lessened by the use of intradermal rather than subcutaneous injections, and particularly by the employment of purified toxin preparations.

VINCENT'S ANGINA AND RELATED INFECTIONS

Clinical features of Vincent's infections. The condition known as *Vincent's angina* is an acute infection of the tonsil, or neighboring parts of the throat, characterized by the appearance of a pseudomembranous inflammation followed by ulceration. The same infection may be localized on the gums (Vincent's gingivitis) or in the mouth (Vincent's stomatitis). The disease may occur in varying degrees of severity. In the most typical cases there develops a deep ulcer covered with a foul-smelling, grayish-yellow exudate on one tonsil only. In some cases, however, particularly in children, the infection may be more extensive, with numerous deep ulcerations over the whole mouth and throat.

The adult patient is usually not severely ill, and aside from some malaise and difficulty in swallowing, may have no complaints. The infection usually yields promptly to treatment with penicillin.

The typical Vincent's angina, with ulceration of the tonsil, is a comparatively rare disease. But mild cases of gingivitis, with swollen, tender, or bleeding gums, and with or without the formation of a definite membrane or ulcer, are frequent; and this form of Vincent's infection is probably much more common than is generally realized.

Vincent's angina is not ordinarily communicable. The occasional cases which occur among the general population usually cannot be traced to any preëxisting case, and they do not give rise to new cases. Under unusual conditions, however, such as may prevail among soldiers in the field or in camp, apparently the infection may become transmissible, and many of the men in the same group may have the disease at the same time. Possibly this may be explained, however, not by assuming spread of the disease from man to man, but rather by the occurrence of a similar state of lowered resistance on the part of many individuals in the group concerned. A very striking feature of the disease is its frequent association with such a state of heightened susceptibility. The typical tonsillar ulceration often occurs in persons whose resistance has been lowered by other diseases, such as scarlet fever, measles, tuberculosis, diabetes, pellagra (nicotinic acid deficiency), mercurial poisoning, scurvy, and especially *leukemia* and other blood disorders.

Microorganisms associated with Vincent's infections. Plaut (1894) and Vincent (1898) first called attention to the constant appearance of certain peculiar microorganisms in the lesions of the infection later called Vincent's angina. These organisms are of two kinds: (1) so-called *fusiform bacilli*—spindle-shaped or cigar-shaped rods with a thick center and pointed ends, often appearing in pairs, and (2) *spirochetes*—pale-staining coiled threads with shallow and irregular curves. When a smear is made directly from the lesion, early in the course of the infection, almost no other bacteria are to be seen. There are very few pus cells (Fig. 80). The same striking picture is found in smears made somewhat later, at the time of deep ulceration, except that at this time there are likely to be a considerable number of other organisms present.

We pointed out, in the earlier part of this chapter, that fusiform bacilli and spirochetes of similar appearance are to be found about

the gum margins and tonsillar crypts in healthy persons. Many authors have suggested that Vincent's angina may be caused by these organisms normally present, when, for some reason, the resistance of the individual is lowered to the extent that such organisms become invasive. The spirochetes probably have the chief rôle in causing the destruction of tissue and the advancement of the lesion, though possibly not in initiating the infection. Both the fusiform bacilli and spirochetes are *anaerobic* organisms, and both are difficult to cultivate and study.

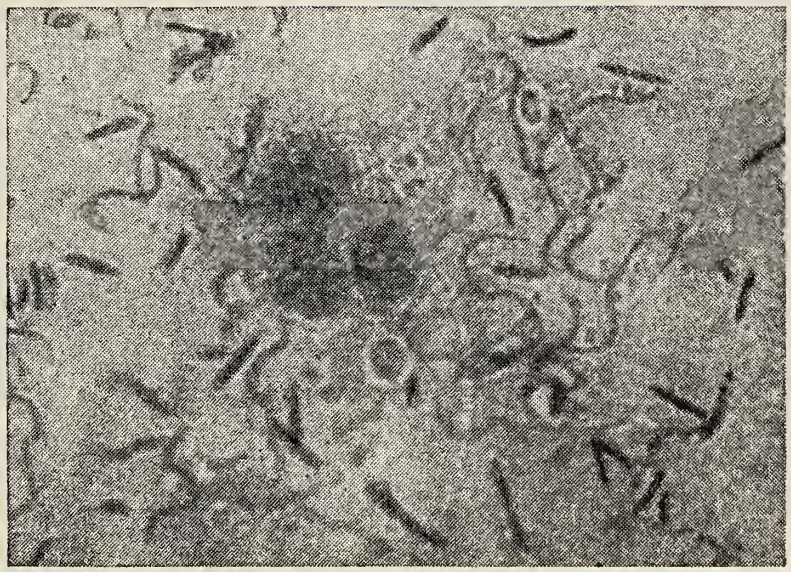


FIG. 80. Smear from the ulcerating lesion of Vincent's angina, stained with gentian violet. A positive diagnosis of this disease is made when this characteristic mixture of fusiform bacilli and spirochetes is seen in a direct smear.

The *spirochetes* have not been satisfactorily classified, and it is doubtful whether they have ever been cultivated in pure culture. They are generally called *Borrelia vincenti.*

The *fusiform bacilli* are non-sporebearing, Gram-negative rods, with ends which taper into a more or less sharp point. They can be cultivated on blood agar under anaerobic conditions. They show no distinctive chemical activities, and do not appear to be pathogenic for animals, at least not when in pure culture. There are probably several varieties of fusiform bacilli, but they have not been described with sufficient completeness to warrant recognition as separate species. The name most commonly applied to the best known variety is *Fusobacterium fusiformis* (Fig. 81).

Bacteriological diagnosis. The laboratory diagnosis of Vincent's infection depends entirely upon the finding of the typical mixture

of many fusiform bacilli and spirochetes in *direct smears* from the lesions. These smears are best stained with a strong stain, preferably gentian violet, or carbol fuchsin, in order to be sure that the pale-staining spirochetes will not be overlooked. At present, no attempt is made to cultivate the organisms or to identify them any more definitely than by their appearance under the microscope.

Differential diagnosis between Vincent's angina, diphtheria, and syphilis. The pseudomembrane which forms in the early stages of a simple Vincent's angina may not be distinguishable, by inspection alone, from the lesion of diphtheria; and the ulceration which later develops resembles the ulcer which may be caused by the syphilis germ. Furthermore, a Vincent's infection may be *superimposed* as a complication upon either a primary diphtheritic or a primary syphilitic infection. In such cases, a direct smear from the lesion, stained by gentian violet or carbol fuchsin would not reveal the syphilis spirochete, even if present, because it would not stain; and if diphtheria bacilli were present, they would probably be overlooked. Hence, unless other tests were made, the infection might be diagnosed as a simple Vincent's angina, and the patient would not receive the necessary treatment and care. Deaths from diphtheria have occurred in persons denied antitoxin because their infection was thought to be an uncomplicated Vincent's angina.

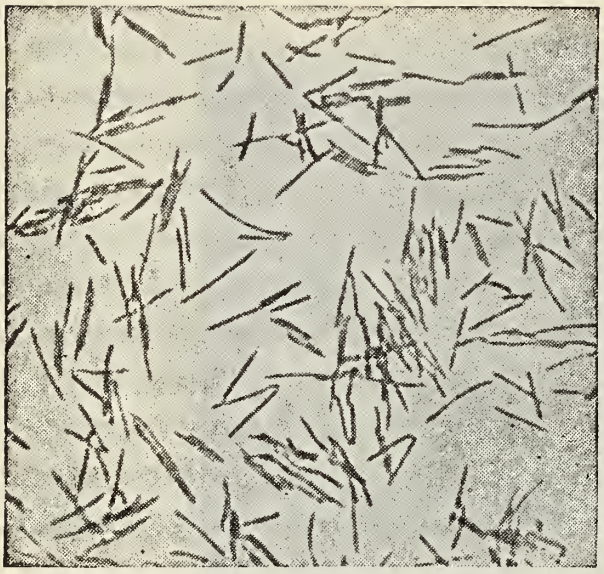


FIG. 81. A pure culture of fusiform bacilli (*Fusobacterium fusiformis*). (From Varney, P. L., *J. Bact.*, 13:275, April, 1927.)

The possibility of confusion in diagnosis between these diseases requires that careful laboratory tests be carried out whenever a patient presents a pseudomembranous or ulcerating lesion in the throat. In addition to direct smears, to determine the presence or absence of Vincent's organisms, cultures should always be made on Loeffler's serum medium, or tellurite medium, and examined for diphtheria bacilli. The likelihood of syphilitic infection must be considered, and blood may be secured for a Wassermann test.

Other infections in which fusiform bacilli and spirochetes are abundant. Fusiform bacilli and spirochetes of the same appearance as those in the typical Vincent's infections of the throat and mouth are frequently associated with abscesses, ulcerations, and gangrenous processes in other parts of the body. These disease conditions are generally classed together under the heading of *fusospirochetosis,* or *fusospirochetal infection.* The relation of these two microbes to the cause of the condition is not always clear, for they are usually mixed with many other organisms. Nevertheless, there is no question about their importance in maintaining and extending the lesions. They probably enter the tissues, which have in some way become vulnerable to their attack, from their normal location on the gum margins and in the tonsillar crypts, or on the genitals. They appear to play an especially prominent part in many cases of lung abscess and bronchiectasis, brain abscess, and ulcerative and gangrenous lesions about the genitals. They are also abundant in pyorrhea, and sometimes in conjunctivitis, otitis media, and appendicitis. Human bites on the hands are frequently infected with these microbes.

REFERENCES

Streptococcus Infections; Scarlet Fever

Boisvert, P. L., Darrow, D. C., Powers, G. F. and Trask, J. D.: "Streptococcosis in Children," *Am. J. Dis. Children,* 1942, 64:516.

Commission on Acute Respiratory Diseases: "Rôle of Beta-Hemolytic Streptococci in Common Respiratory Disease," *Am. J. Pub. Health,* July 1945, 35:675.

Graham, H. C.: "Scarlet Fever Immunization: An Evaluation of Some Methods of Immunization over a 15-year Period," *So. Med. J.,* Feb. 1942, 35:132.

Kuttner, A. G. and Krumwiede, E.: "Observations on Epidemiology of Streptococcal Pharyngitis and the Relation of Streptococcus Carriers to the Occurrence of Outbreaks," *J. Clin. Invest.,* Mar. 1944, 23:139.

Lancefield, R. C. and Stewart, W. A.: "Studies on the Antigenic Composition of Group A Hemolytic Streptococci," *J. Exper. Med.,* Jan. 1944, 79:79.

Rubenstein, A. D. and Foley, G. E.: "Scarlet Fever in Schoolrooms," *Am. J. Pub. Health,* Sept. 1945, 35:905.

Schwentker, F. F., Hodes, H. L., Kingsland, L. C., Jr., Chenoweth, B. M. and Peck, J. L.: "Streptococcal Infections in a Naval Training Station," *Am. J. Pub. Health,* Dec. 1943, 33:1454.

Sherman, J. M.: "The Streptococci," *Bact. Rev.,* 1937, 1:1.

Rheumatic Fever

Boisvert, P. L., Dawson, M. H., Schwentker, F. F. and Trask, J. D.: "Epidemic Rheumatic Fever," *An. Int. Med.,* July 1943, 19:107.

Coburn, A. F. and Moore, L. V.: "Rheumatic Fever—Nutrition as Conditioning Factor," *Am. J. Dis. Children,* May 1943, 65:744.

Green, C. A.: "Hemolytic Streptococcal Infections and Acute Rheumatism," *An. Rheumat. Dis.,* May 1942, 3:4.

HENCH, P. S. and OTHERS: "Rheumatic Fever—Review," *An. Int. Med.,* Dec. 1941, 15:1002.

ROBINSON, J. J.: "Pathogenesis of Rheumatic Fever in Relation to Streptococci Toxin Injury," *Arch. Pediat.,* Jan. 1944, 61:6.

TERRY, E. M.: "Rheumatic Fever and the Nurse," *Am. J. Nurs.,* Dec. 1943, 43:1083.

WASSON, V. P. and BROWN, E. E.: "Immunity to Rheumatic Fever," *J. Pediat.,* July 1943, 23:24.

WEDUM, A. G. and WEDUM, B. G.: "Rheumatic Fever in Cincinnati in Relation to Rentals, Crowding, Density of Population, and Negroes," *Am. J. Pub. Health,* Oct. 1944, 34:1065.

Vincent's Infections

BOLAND, F. K.: "Fusospirochetal Infection of Extremities," *J.A.M.A.,* 1941, 116:127.

DEAN, H. T. and SINGLETON, D. C.: "Vincent's Infection—A Wartime Disease: Preliminary Considerations on the Epidemiology of Ulcerative Gingivostomatitis," *Am. J. Pub. Health,* May 1945, 35:433.

FOLEY, G. and ROSEBURY, T.: "Comparative Infectivity for Guinea Pigs of Fusospirochetal Exudates from Different Diseases," *J. Dent. Research,* Aug. 1942, 21:375–378.

HAMPP, E. G.: "Vincent's Infection—A Wartime Disease: Observations on the Oral Spirochetal Flora Present in Vincent's Infection," *Am. J. Pub. Health,* May 1945, 35:441.

HINE, M. K. and BERRY, G. P.: "Morphologic and Cultural Studies of Genus *Fusiformis*," *J. Bact.,* Nov. 1937, 34:441.

SPAULDING, E. A. and RETTGER, L. F.: "*Fusobacterium Genus*," *J. Bact.,* 1937, 34:535–549.

VARNEY, P. L.: "The Serological Classification of Fusiform Bacilli," *J. Bact.,* 1927, 13:275.

REVIEW QUESTIONS—CHAPTER XXVI

1. Why do the mouth and throat offer particularly good conditions for the growth of microorganisms? What aerobic organisms are usually found in cultures from the normal mouth and throat? Mention some pathogenic organisms that may be present.
2. Describe the bacteria usually found on the gum margins and in the tonsillar crypts.
3. Discuss the bacteriology of: (1) caries of the teeth, (2) tooth abscesses, (3) pyorrhea.
4. Name a common mouth infection caused by a yeast-like organism. What germ is most often the cause of pharyngitis and tonsillitis? Name two other forms of throat infection caused by the same type of organism. Name three important specific infections of the throat and mouth.
5. Name four subdivisions within the genus *Streptococcus.* Describe the general properties of the parasitic streptococci. What poisonous products may they form?
6. Describe how parasitic streptococci are grouped according to their action on blood agar.

7. Name and describe the principal varieties of hemolytic streptococci. What are the *Lancefield groups*? Which serological group includes the great majority of the hemolytic streptococci pathogenic for man?
8. Name and describe the principal varieties of viridans (alpha type) streptococci.
9. Name at least twelve forms of infection that may be caused by hemolytic streptococci.
10. Name at least four infections commonly due to viridans streptococci. Describe the form of endocarditis often caused by these organisms.
11. Discuss the probable relationship between streptococcus infections and rheumatic fever.
12. Outline procedures used in making a bacteriological diagnosis of streptococcus diseases. What further steps should be taken after isolation of: (1) a hemolytic (beta type) streptococcus, (2) a viridans (alpha type) streptococcus?
13. Discuss immunity to streptococcus infections. What seems to be the basis of effective body resistance against invasive streptococci?
14. Explain the present-day conception of the cause of scarlet fever and the nature of immunity to the disease.
15. How does scarlet fever spread? Why is a long period of quarantine necessary?
16. Explain the purpose and technique of the *Dick test*. Exactly what is the origin of the material injected?
17. What types of reaction may occur in the Dick test and what does each mean with respect to immunity to scarlet fever? What do the results of many Dick tests show as to the resistance of the general population to scarlet fever?
18. Explain the: (*a*) preparation and (*b*) standardization of scarlet-fever antitoxin. What is a unit of this antitoxin? What is its value in treating scarlet fever? What other serum may be used in the place of commercial antitoxin? What is the *Schultz-Charlton reaction*?
19. Discuss the prevention of scarlet fever. Explain the method of active immunization, and discuss its value and limitations.
20. Describe the clinical features of typical Vincent's angina, gingivitis, and stomatitis. Is Vincent's angina communicable?
21. Describe the organisms found in the lesion of Vincent's angina. Explain how a bacteriological diagnosis of Vincent's infection is made.
22. What two infections may be confused with, or complicated by, Vincent's angina? State clearly what steps are necessary to make a correct diagnosis.
23. Name other infections in which fusiform bacilli and spirochetes are abundant.

CHAPTER XXVII

DIPHTHERIA

Diphtheria was once among the most dreaded of all diseases. Now we have an almost complete mastery of it. We know its cause (*Corynebacterium diphtheriae*), we have a specific remedy (diphtheria antitoxin), a means of determining susceptibility (Schick test), and successful methods for making individuals immune to the disease (active immunization with toxoid).

The clinical disease. The disease of diphtheria is due partly to the local disturbance in the throat, or other parts of the upper respiratory tract, caused by the growth of the germs themselves in these places, but principally to the poisoning of vital internal organs by a powerful *exotoxin* secreted by the organisms. The illness begins after an *incubation period of two to five days.* The bacilli rarely penetrate deep into the body; their growth is ordinarily confined to the superficial layers of the mucous membrane—usually to a small area of the throat or nose—where they cause a peculiar kind of inflammation characterized by the formation of a tough, grayish-white *pseudomembrane.* The leathery character of this membrane gave origin to the name *diphtheria,* which comes from the Greek word meaning *leather.*

In the days before antitoxin was widely used, severe cases of diphtheria were common in which this membranous inflammation extended over the whole surface of the throat, down into the larynx, and even into the trachea below. This caused the condition known as "membranous croup." Frequently, the membrane formation in the air passages caused death by suffocation. Today this severe form of diphtheria is rare in adults, but it still occurs occasionally in children. It is often necessary, in these cases, to make an opening into the windpipe, through the neck (tracheotomy), or to place a tube in the larynx, in order to assure a passage for air.

Prompt diagnosis and early use of antitoxin have almost banished these grave forms of diphtheria, and now in most cases the local

inflammation in the throat is not in itself a serious matter. Indeed, in some instances there may be no visible membrane at all. *The chief damage is done by the poison absorbed from the growing germs.* This toxin, carried about by the blood, injures important tissues, especially certain nerves, and the heart muscle. In some cases not effectively treated with antitoxin, paralysis of various muscles may develop, as much as a month or more after the acute illness has subsided.

Diphtheria, then, is essentially a toxemia, and *resistance to the disease depends upon the presence of diphtheria antitoxin in the blood and tissues.*

Corynebacterium diphtheriae. The bacillus of diphtheria was first described by Klebs in 1883, but it was Loeffler, in 1884, who isolated the organism in pure culture and later proved it to be the sole cause of the disease.

Morphology and staining. The diphtheria bacillus is one of the few pathogenic bacteria that can be recognized with some degree of certainty merely by its appearance under the microscope. The organisms are slender rods, often slightly curved, showing a characteristic irregularity, both in form and in staining. They tend to lie in groups, in which all the organisms are parallel or nearly parallel. In regard to staining, three types may be recognized: (1) the granular forms, in which deeply colored metachromatic granules appear at the ends or near the center of the rods, (2) the "barred forms," which seem to be crossed by bands or bars of material which does not stain, and (3) even-staining forms (Fig. 82).

These various forms of the diphtheria bacillus may occur in the same culture, and there is no proof that any particular form is more virulent than another.

The characteristic irregularity in staining is best brought out when smears are colored with Loeffler's methylene blue, toluidine blue, or some one of the special stains designed to demonstrate metachromatic granules. The bacilli are Gram-positive. They have no spores or capsules, and are nonmotile.

Physiological properties. Diphtheria bacilli grow best in the presence of abundant oxygen and at body temperature. They will live even on plain agar and broth, but they develop more luxuriantly on media enriched with blood or blood serum. The most widely used medium for diphtheria bacilli is the one devised originally by Loeffler, called *Loeffler's serum medium.* This is made by mixing

three parts of beef serum with one part of a 1% glucose infusion broth. The mixture is coagulated by heat in tubes held in a slanting position, so as to make a solid medium in the form of slants. For many years this medium was used exclusively for primary cultures from the nose and throat for the diagnosis of diphtheria and the

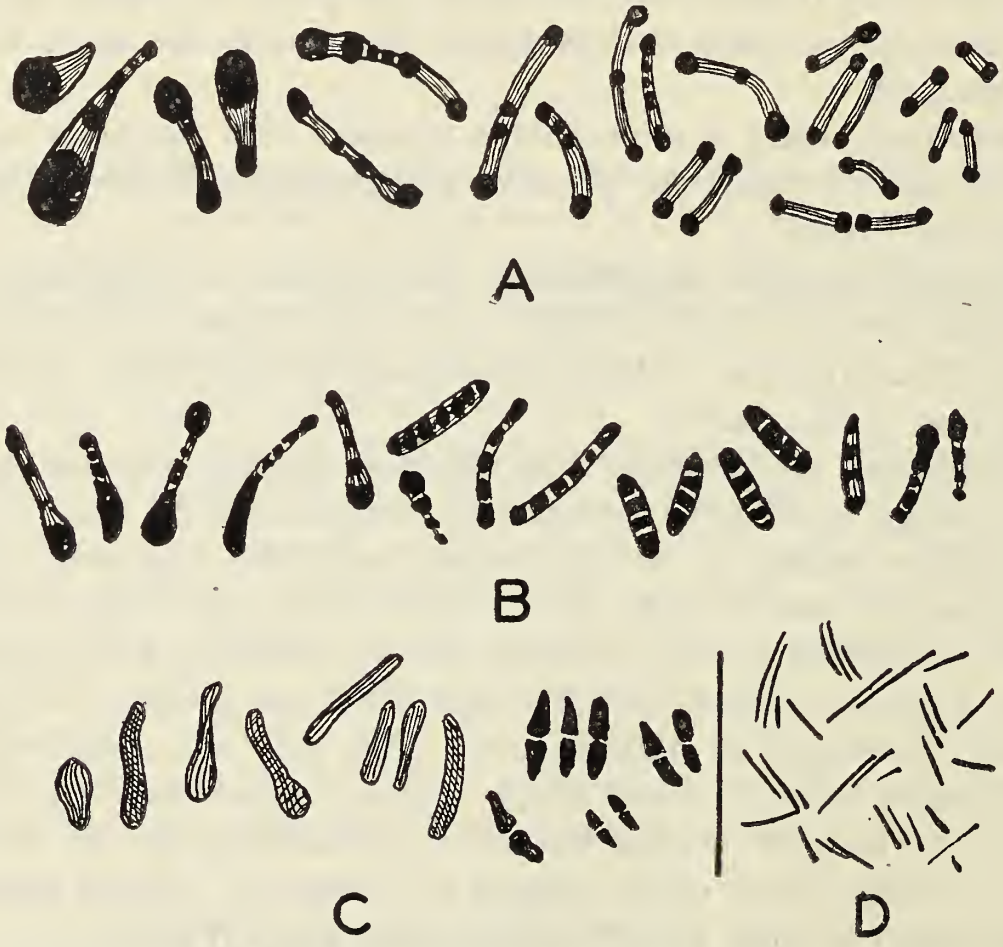


FIG. 82. *Corynebacterium diphtheriae.* A: granular forms; B: barred forms; C: even-staining forms. D: sketch to show the characteristic parallel arrangement of the bacilli in a smear.

detection of carriers of the germ. Recently, some laboratories employ an additional medium, consisting of an infusion blood agar to which has been added 0.04% potassium tellurite.

Since the diphtheria bacilli have no spores, they are killed rapidly by boiling or by the usual chemical disinfectants. They have considerable resistance to drying, however, and *may remain alive for many weeks on dried bits of the pseudomembrane* from an infected throat.

Diphtheria toxin and toxoid. The most important property of the diphtheria bacillus is its capacity to form the powerful *exotoxin* which is responsible for the toxemia in diphtheria. When virulent

organisms are grown for several days in a suitable liquid medium, a large amount of this peculiar poison is developed. By passing such a culture through a bacteriological filter, one secures a clear, sterile filtrate which contains the toxin in solution. The poison in this filtrate can be concentrated somewhat, and has been shown to be protein-like. But the purified toxin is not generally available, and when we say "diphtheria toxin," ordinarily we are referring to nothing more than a sterile filtrate containing the toxin obtained as indicated above (Fig. 71).

Diphtheria toxin is very poisonous to horses, dogs, cats, rabbits and guinea pigs, as well as to man. To measure the strength of the poison, *injections are made subcutaneously into guinea pigs* to determine the *smallest amount which, on the average, will kill guinea pigs weighing 250 grams in from four to five days.* This amount is called the *minimum lethal dose* (M.L.D.). Most virulent diphtheria bacilli will yield a toxin so strong that as little as 0.002 cc contains one M.L.D. for a guinea pig. Another measure for diphtheria toxin is the so-called *L+ ("L plus") dose.* The L+ dose is the *least amount of toxin which, when mixed with one unit of antitoxin and injected subcutaneously into guinea pigs weighing 250 grams, will, on the average, kill the animals on the fourth day.* The L+ dose of diphtheria toxin is usually about 0.15 cc.

It is important to remember that not all strains of diphtheria bacilli produce toxin. Such *nonvirulent strains* are often found in the throats of carriers.

Toxoid. Diphtheria toxin is unstable, and, when exposed to air, light, moderate heat, and certain chemicals it changes into what is called *toxoid.* In this state it is *nonpoisonous,* but *still capable of combining with antitoxin,* and *it will still stimulate the formation of diphtheria antitoxin* when injected into the body.

Diphtheria toxoid, prepared by adding formalin to fresh toxin, is now in wide use for the active immunization of horses for the preparation of commercial diphtheria antitoxin, and also for the immunization of human beings. About 0.4% of formalin is added to the toxic diphtheria-culture filtrate, and the mixture is allowed to stand at 37° C until it loses its poisonous properties—a slow process that may require several months for completion.

How diphtheria spreads. The germs of diphtheria are conveyed from person to person by way of fingers, or articles, such as eating utensils, toys, pencils, or handkerchiefs, contaminated with nasal

discharges and saliva, or through droplets expelled from the throat and nose during sneezing or coughing. The disease spreads most rapidly among school children and persons in crowded places. In diphtheria, as also in scarlet fever, typhoid, and other diseases, *mild, unrecognized cases* of the infection and *healthy carriers* of virulent germs are the commonest sources of new cases. Occasionally epidemics of diphtheria have been traced to contaminated raw *milk*.

Carriers of diphtheria bacilli are numerous, especially in groups of people who live in close quarters, as in tenements, boarding schools and other institutions, army camps, and the like, and among hospital attendants, nurses, and doctors who come in frequent contact with cases of the disease. The bacilli tend to remain in the throat for several weeks after the patient's recovery from diphtheria; some persons become chronic carriers, harboring the germs for months or years. Also, individuals who have never had any symptoms of diphtheria may carry the germs. Sometimes the organisms thus carried are not toxin-producers, and therefore are not dangerous to others, but often fully virulent bacilli are carried for months by individuals who are themselves immune, though constituting a source of infection for other (susceptible) persons. It is sometimes extremely difficult to get rid of the germs in the throats of these chronic carriers, but when pathological conditions of the nose and throat, such as deviated nasal septum or inflamed tonsils, are corrected, the diphtheria bacilli usually disappear.

Bacteriological diagnosis, and detection of carriers. While the final decision as to whether or not a patient has diphtheria, and needs antitoxin, must rest with the physician, laboratory tests are of great aid in the diagnosis of this disease. Bacteriological diagnosis depends upon the finding of virulent diphtheria bacilli in cultures from the nose and throat. Carriers are detected by the same means. These cultures can be made so easily, and they give such reliable information, that *they should never be omitted in any case of sore throat which might possibly be diphtheria.* It should be remembered that there are cases of diphtheritic infection of the throat without fever or other obvious symptoms, and without membrane formation. Furthermore, membranous inflammations resembling the lesion of diphtheria may be caused by other organisms, such as streptococci and the germs of Vincent's angina. In these clinically doubtful cases, a definite diagnosis must depend upon laboratory

cultures. It is exceedingly important that diagnosis of diphtheria be made as *early as possible,* in order that the patient may have the benefit of receiving antitoxin before the poison has done serious damage to the body.

Presumptive diagnosis. Two steps are required to make a presumptive bacteriological diagnosis of diphtheria: (1) cultures must be made from the throat and nose, on *Loeffler's serum medium,* and incubated at 37.5° C for from twelve to eighteen hours, and then (2) a smear must be made from the growth on the medium, stained with methylene blue, toluidine blue, or some special stain, and examined for the presence of organisms resembling diphtheria bacilli. If bacilli having the characteristic irregular form and staining, and parallel arrangement of *Corynebacterium diphtheriae,* are present, the bacteriologist will report the culture as *positive.* If the culture was made from a patient having the clinical symptoms of diphtheria, it is not necessary to go further to establish the diagnosis, and it may be assumed that the organisms seen in the smear are, in truth, virulent diphtheria bacilli. Thus, in typical cases of diphtheria, this presumptive diagnosis, which is sufficient for all practical purposes, can be reached within twelve hours after the culture is made.

Cultures from carriers and convalescents. When the organisms are less abundant, as in the case of a healthy carrier or a convalescent, it is helpful to inoculate, with the throat and nose swabs, plates of *tellurite blood agar,* as well as Loeffler's serum slants. Colonies of diphtheria bacilli are recognizable on the tellurite medium after 24 to 48 hours' incubation.

Final diagnosis. Virulence tests. In order to establish a diagnosis of diphtheria beyond doubt it is necessary to identify definitely the diphtheria bacilli in the primary cultures, and to prove that they are virulent; i.e., to show that they produce diphtheria toxin. In case the positive cultures come from a healthy carrier, it is essential that these further tests be carried out, because it would be unfair and useless to require the isolation of the individual if the organisms he is harboring are not virulent. In order to *prove* that the diphtheria-*like* organism in a primary mixed culture is actually a virulent diphtheria bacillus, and not a nonvirulent variant, or a harmless diphtheroid, *virulence tests* must be conducted on animals.

The simplest way to do this is to inoculate a small amount of a

suspension of the organisms from the original Loeffler's medium culture or tellurite plate *intradermally* into each of two guinea pigs, one of which has been protected by a previous inoculation of about 250 units of diphtheria antitoxin. If the culture contains virulent diphtheria bacilli, a characteristic inflammation will appear about the site of the intradermal inoculation in the unprotected animal, while no reaction will occur in the animal that received antitoxin.

Another, and better, way to perform a virulence test is first to isolate the suspected diphtheria bacillus in pure culture, then inoculate guinea pigs *subcutaneously* with this pure culture. If the organism is a virulent diphtheria bacillus, an unprotected animal will die within from three to five days, while an animal protected by antitoxin will remain well.

Precautions in making nose and throat cultures. Nurses are often called upon to make from the patient the original cultures on which the whole procedure of bacteriological diagnosis depends. It is essential that these cultures be made properly. The following are the principal points to be borne in mind:

(1) Have at hand *small, sterile, cotton swabs,* and tubes of *fresh, sterile Loeffler's medium slants.* Do not use old, dried-up medium. Do not use ordinary blood agar.

(2) For nose cultures, pass a swab straight back into one nostril, remove, and, with the usual aseptic precautions, roll the swab gently over the *entire* surface of a slant. *Label culture carefully,* and *place in a 37.5° C incubator at once.*

(3) For a throat culture, get the patient into a good light, or use a flashlight to *illuminate the throat well, so that you can see exactly what you are doing.* Hold the tongue down, if necessary, with a tongue depressor or spoon handle. Be careful that the patient does not cough directly into your face.

(4) Pass a swab over *the inflamed part of the throat only,* and remove quickly *without touching it to the cheeks, tongue or lips.* Try to get a bit of the membrane, if one is present; otherwise, swab the reddened areas. It is essential to avoid picking up many bacteria from other places, because these organisms might overgrow and crowd out the diphtheria bacilli in the culture. In case the swab is thus contaminated, or in case you are not sure that you reached the inflamed part, it is better to repeat the procedure with a new swab.

(5) Using aseptic precautions, roll the swab gently on a slant so as to *inoculate the entire surface of the medium.* It is better to use separate

slants for the throat and nose cultures. *Label each culture carefully,* and *see that the culture is placed in an incubator (37.5° C) as soon as possible.*

It is desirable to place the swabs themselves in a sterile tube and send them to the diagnostic laboratory. The technicians may wish to use them for inoculation of tellurite blood agar plates.

Diphtheria antitoxin. *Preparation and standardization.* Diphtheria antitoxin is prepared commercially by inoculating healthy horses repeatedly with increasing amounts of diphtheria *toxin* (not the bacilli). The injections are continued during several months, until the blood of the animals contains a large amount of antitoxin. The horses are then bled from the jugular vein, and the blood is allowed to stand until a firm clot has formed. Then the clear, fluid *serum,* which has exuded from the clot, is collected. Sometimes the citrated *plasma,* freed of blood cells by centrifugation, is collected instead of serum. This crude horse serum or plasma contains the desired antitoxin, but before it is put on the market for use in human disease, the serum is greatly concentrated—to remove most of the inert constituents—purified, and carefully tested to be sure it is free of germs. It is then standardized, in order that its antitoxin content may be known.

Manufacturers of antitoxin compare their product with a *standard antitoxin* kept in the laboratories of the National Institute of Health, at Bethesda, Maryland (near Washington, D. C.). First, a *toxin* is standardized by tests against this standard antitoxin; then, by use of this freshly tested toxin, the protective power of the unknown antitoxin for guinea pigs is determined. *One unit of commercial diphtheria antitoxin is defined as the least amount of the serum which, when mixed with an L+ dose of the standardized toxin and injected subcutaneously, will, on the average, preserve the life of guinea pigs weighing 250 grams for four full days.* Commercial antitoxin usually contains about 500 units per cubic centimeter.

Prophylactic and therapeutic use. Susceptible persons who have been exposed to diphtheria may be given immediate protection by the subcutaneous injection of about 1,000 units of antitoxin, and thus avoid an attack of the disease. The prophylactic use of antitoxin in this way is now rarely necessary, however.

In the *treatment* of diphtheria, antitoxin is invaluable. When injected into the body, it combines with any diphtheria toxin it

encounters, and neutralizes it, thus preventing poisoning of vital tissues. It stops the progress of the disease, and permits the patient to recover. At the present time, most of the deaths from diphtheria occur in persons who receive antitoxin too late, or not at all, for it has been demonstrated repeatedly that when antitoxin is given in sufficient amounts on the first day of illness, recovery is practically certain. The great importance of early diagnosis is obvious.

Schick test for susceptibility to diphtheria. This test, first perfected by Schick, in 1913, is really a test for the presence of diphtheria antitoxin in the tissues. It is at the same time a test for susceptibility (or immunity) to diphtheria, since resistance to this disease depends principally upon antitoxin. The procedure consists in the inoculation of a minute amount of diluted diphtheria *toxin* intradermally into the skin of the forearm.

Toxin prepared commercially for the Schick test is supplied in diluted form, ready for use. The toxin solution contains *an amount of toxin in each Schick-test dose* (0.1 cc) *equivalent to 1/50 of the M.L.D. for a guinea pig.* Of this toxin solution, 0.1 cc is injected *intradermally* into the flexor surface of one forearm. For a *control,* a like amount of solution of *the same toxin, which has been heated to 75° C or boiled for one hour,* is injected intradermally into the opposite arm. This heating destroys the toxin, but does not change other substances in the solution which might cause an allergic reaction.

The changes occurring at the sites of inoculation are carefully watched; the final reading should be made in four or five days. The following types of reaction may be observed:

(*a*) *Negative Schick test.* No reaction in either arm. The injected toxin has been neutralized by the antitoxin in the blood and tissues.

(*b*) *Positive Schick test.* No reaction in the control arm. In the test arm, a red flush appears after from twenty-four to thirty-six hours, reaching its maximum development about the fourth day. At this time there is a circular circumscribed area, ½ cm in diameter, slightly raised above the general surface of the skin. This inflammatory reaction is caused by the injurious effect of the toxin upon the skin. It fades slowly, leaving an area of brownish pigmentation.

(*c*) *Negative Schick test with pseudoreaction.* An inflammatory reaction, less sharply circumscribed than a true positive reaction, develops *equally on both arms within twenty-four hours.* It fades rapidly, and is usually gone by the fourth day. This reaction is an *allergic* reaction, and is due to sensitiveness to some constituent of the toxin solution.

(*d*) *Positive Schick test with pseudoreaction.* The control arm shows the changes described under (*c*)—the pseudoreaction. During the first twenty-four hours, the test arm shows the same type of changes, and the reaction is almost indistinguishable from that in the control arm. But after this time, the reaction in the test arm continues to develop, while that in the control arm begins to fade. By the fourth day, the difference between the two arms is usually quite definite though sometimes the correct interpretation of the test remains uncertain.

Experience has shown that a *negative* Schick test indicates the presence in the individual of sufficient antitoxin to *insure immunity against diphtheria* under ordinary circumstances.

The results of Schick tests upon thousands of persons have largely explained why diphtheria occurs so often in children under the age of ten, but so rarely in adults. It has been found that 80% or more of adults are immune. It has also been shown that the largest proportion of negative Schick tests—i.e., the greatest percentage of immune individuals—is found among groups of persons who live in close contact with one another. Under these circumstances, the diphtheria bacilli are easily passed from person to person and there are likely to be many carriers. These *carriers immunize themselves,* i.e., develop antitoxin as the result of the absorption of small amounts of toxin from the germs in their throats. This is the bright side of the carrier problem. Most adults are resistant because they have carried about virulent diphtheria bacilli, at some time or other, long enough to build up an immunity. It is easy to understand, therefore, why diphtheria is most frequent and most severe in young children.

Active immunization against diphtheria. To make individuals actively immune to diphtheria, it is necessary to stimulate their tissues to form diphtheria antitoxin. But in order to do this, the toxin must be safely introduced into the body. Diphtheria toxin is too dangerous to be used directly for the immunization of human beings without reducing its poisonous properties in some way. Two kinds of vaccine have been found useful: (1) *Toxin-antitoxin mixtures* and (2) *toxoids* (plain or alum-precipitated).

Several different methods of preparing diphtheria *toxin-antitoxin* mixtures have been used but, in any case, the product always contains *a slight excess of free, unneutralized toxin.* When the mixture is injected, dissociation of the toxin-antitoxin combination begins, and the free toxin acts as the stimulus for formation of antitoxin

by the *individual's own body cells*—in other words, it starts the development of an *active immunity* against diphtheria.

The immunization of children with diphtheria toxin-antitoxin mixtures was practiced widely in the early 1920's, but at the present time, this kind of vaccine is seldom used, since toxoid preparations are generally superior.

Diphtheria toxoid, either *plain* or mixed with alum (*alum precipitated toxoid*), is now the preferred immunizing agent for routine use with children. It stimulates the development of a strong immunity in about 95% of individuals receiving the usual course of two or three injections. This is a better result than is usually secured with the toxin-antitoxin method. Toxoid has the further advantage of containing no antitoxic serum, and therefore it cannot sensitize the individual to horse serum. In young (pre-school) children (the persons most in need of immunization), neither the plain nor the alum-precipitated toxoid excites any appreciable reaction. Older children and adults, however, are often hypersensitive to proteins in the toxoids and, to avoid disagreeable local or general reactions, a preliminary skin test with the diluted toxoid should be made. Then, if sensitivity does exist, the injections will have to be made with caution, and the usual doses may have to be reduced. It is in these cases that it may be advisable to use toxin-antitoxin instead of toxoid. The alum-precipitated toxoid is more efficient than the plain toxoid, for, when injected subcutaneously, the material remains for several weeks in the injected area, and from this depot the toxoid is slowly absorbed. Thus, the stimulus to antitoxin formation continues for a long period. A single injection of alum-precipitated toxoid may result in as high an antitoxin production as two or more injections of plain toxoid.

There is ample evidence that active immunization against diphtheria is highly successful in preventing the disease, although the vaccination may not protect every single individual. In communities where a considerable proportion of children have been immunized, the incidence of diphtheria among them has been very markedly reduced. All children should be given the vaccine at about the age of 1 year.

It is important to remember that the immunity is not fully developed immediately after the injections have been given; it becomes slowly stronger during succeeding weeks. The success of the immunization should be checked by Schick tests about *6 months*

later, and again when the 5- or 6-year-old child is about to enter school. In case either of these tests is positive, more injections of the toxoid should be made.

Active immunization against diphtheria is an especially valuable protection for nurses and doctors, who must necessarily come into contact with many cases of the disease. In former years, it was not at all uncommon for as many as 20% of the nurses in infectious-disease hospitals to develop diphtheria in the course of a year. This is now a rare occurrence in those hospitals where the routine immunization of all susceptible (Schick-positive) members of the nursing staff is practiced.

Summary of the practical methods for the prevention and control of diphtheria. Diphtheria is typically a disease of schools and institutions where young children are herded together. The practical application of all measures for controlling diphtheria may be illustrated by the methods which would be used in the case of an epidemic in such an institution.

Any case of sore throat among the students is suspected as possibly diphtheria, and nose and throat cultures on Loeffler's medium are made at once. If the cultures show diphtheria-like bacilli, and the student has had suspicious symptoms, a diagnosis of diphtheria is made. Then certain definite procedures are carried out to care for the patient, and to protect his associates in the school and the community from the disease.

The patient is isolated, and he receives at once a sufficient dose of diphtheria antitoxin. The isolation is continued for about sixteen days, or until successive throat and nose cultures are negative for diphtheria bacilli.

Other students, with whom the patient was in immediate contact during the week before his illness, are also segregated at once, and antitoxin is given them if they show any signs of coming down with the disease.

Nose and throat cultures are made from every individual in the school. If carriers are found, they are isolated until freed of the bacilli. Repeated cultures may have to be made and virulence tests performed upon the positive cultures from carriers, to determine definitely the origin of the epidemic.

Schick tests are performed at once upon every individual. Those found susceptible are carefully watched, and cultures are made again if any signs of infection develop. Those found to be Schick-

negative are immune, and, so long as they are not carriers, do not need to be isolated.

Active immunization of the susceptible individuals with toxoid should begin at once.

To avoid future epidemics the opportunities for the spread of germs should be reduced as much as possible by encouraging the development of personal cleanliness among the students. Carriers could be detected by cultures when they enter the institution. Schick tests could be conducted routinely and susceptible persons could be actively immunized.

REFERENCES

ANDERSON, A.: "Diphtheria Immunization. Observations on Prophylactics and Duration of Immunity," *Pub. Health Rep.,* Sept. 1944, 57:131.

FROBISHER, M., JR.: "Recent Advances in Diphtheriology," *Am. J. Med. Sci.,* 1938, 195:417; "Properties of Strains of *C. Diphtheriae* Obtained from Various Parts of the United States," *Am. J. Pub. Health,* 1942, 32:708; "The Etiology of Malignant Diphtheria," *Am. J. Pub. Health,* 1943, 33:1244.

HAMILTON, P. M. and KNOUF, E. G.: "Combined Immunization Against Diphtheria, Tetanus and Pertussis," *J. Pediat.,* Sept. 1944, 25:236.

KELLOGG, D. K. and WENDE, R. D.: "Use of a Potassium Tellurite Medium in the Detection of *Corynebacterium Diphtheriae," Am. J. Pub. Health,* 1946, 36:739.

MCLEOD, J. W.: "The Types Mitis, Intermedius and Gravis of *Corynebacterium Diphtheriae,*" *Bact. Rev.,* 1943, 7:1.

MORTON, H. E.: "*Corynebacterium Diphtheriae.* A Correlation of the Recorded Variations within the Species," *Bact. Rev.,* 1940, 4:177.

PAPPENHEIMER, A. M., JR.: "Studies on Diphtheria Toxin and Its Reaction with Antitoxin," *J. Bact.,* Mar. 1942, 43:273.

REVIEW QUESTIONS—CHAPTER XXVII

1. Describe the clinical features of diphtheria, and explain their cause. What is the origin of the term *diphtheria*?
2. Describe the morphological and staining characteristics of *Corynebacterium diphtheriae.*
3. Name the medium most commonly used, for the growth of diphtheria bacilli in the laboratory and give its composition. What peculiar advantages has this medium?
4. Discuss the resistance of diphtheria bacilli outside of the body.
5. Explain exactly what is meant by "diphtheria toxin." Define the terms *M.L.D.* and *L+ dose* with reference to this toxin. Are all strains of diphtheria bacilli toxin-producers?
6. Explain the preparation and properties of diphtheria *toxoid.*

7. Explain how diphtheria spreads. Discuss the importance of carriers of the bacilli.
8. Outline the necessary procedures for making a *presumptive* bacteriological diagnosis of diphtheria. Why is it important to have an early diagnosis? Under what circumstances may a presumptive diagnosis be sufficient for practical purposes? When is a final diagnosis by virulence tests essential?
9. Outline the procedures necessary to make a *final* diagnosis of diphtheria and to prove the virulence of the organisms.
10. Outline concisely precautions to be taken in making nose and throat cultures. Explain why these precautions are essential.
11. Describe briefly: (*a*) the preparation and (*b*) the standardization of commercial diphtheria antitoxin. Define a unit of diphtheria antitoxin.
12. Discuss the use and value of antitoxin in the treatment and prevention of diphtheria.
13. Explain precisely what is meant by a Schick test, and how the test is carried out.
14. What types of reaction may occur in a Schick test? What is the meaning of each with respect to immunity, and why? What do the results of Schick tests show in regard to immunity among the general population?
15. Describe the preparations and the methods used for producing active immunity to diphtheria.
16. Discuss the practical value of active immunization against diphtheria. What test should be performed, and when, to prove the immunization successful?
17. Outline the practical steps that might be taken in the event of an outbreak of diphtheria in a boarding school.

CHAPTER XXVIII

INFECTIONS OF THE RESPIRATORY TRACT. COMMON COLDS. VIRUS DISEASES. WHOOPING COUGH

Anatomy of the respiratory tract, and its influence upon infection. The respiratory tract may be thought of as made up of three sections: (1) an upper part, including the *nose* and the accessory *sinuses,* and the *nasopharynx,* (2) an intermediate part—the *pharynx*—which serves as a passage for food on its way to the esophagus and stomach, as well as for air on its way to the lungs, and which contains the *tonsils,* and (3) a lower part, including the *larynx, trachea, bronchi,* and *lungs.*

The great importance of natural nose-breathing in reducing the possibility of respiratory infections, and in preserving the general health, is strikingly shown by the long list of troubles which follow when adenoids, deviated nasal septum, or other pathological conditions make an individual a habitual mouth-breather (Fig. 83). When the inspired air passes first through the nose, it is made moist and warm, and almost entirely free of dust and germs. It seems that only by this route can the air reach the lungs in proper condition. Mouth-breathing often leads to chronic sore throat, and obstructions in the nasal passages greatly increase the tendency toward infection of the whole respiratory tract.

The nose, and the accessory sinuses, and also the trachea and bronchi are lined with *ciliated epithelium* (Fig. 84). The cilia are constantly acting to clear the whole bronchial tree of dirt and germs by sweeping mucus up to the mouth, where it may be swallowed or expectorated. The action of these cilia probably is an important factor in protecting the lungs from infection.

Normal flora of the nasal passages and upper respiratory tract. In the nasal passages there are fewer bacteria normally present than in the mouth and throat. Blood-agar-plate cultures, streaked with swabbings from the nose, usually differ strikingly in

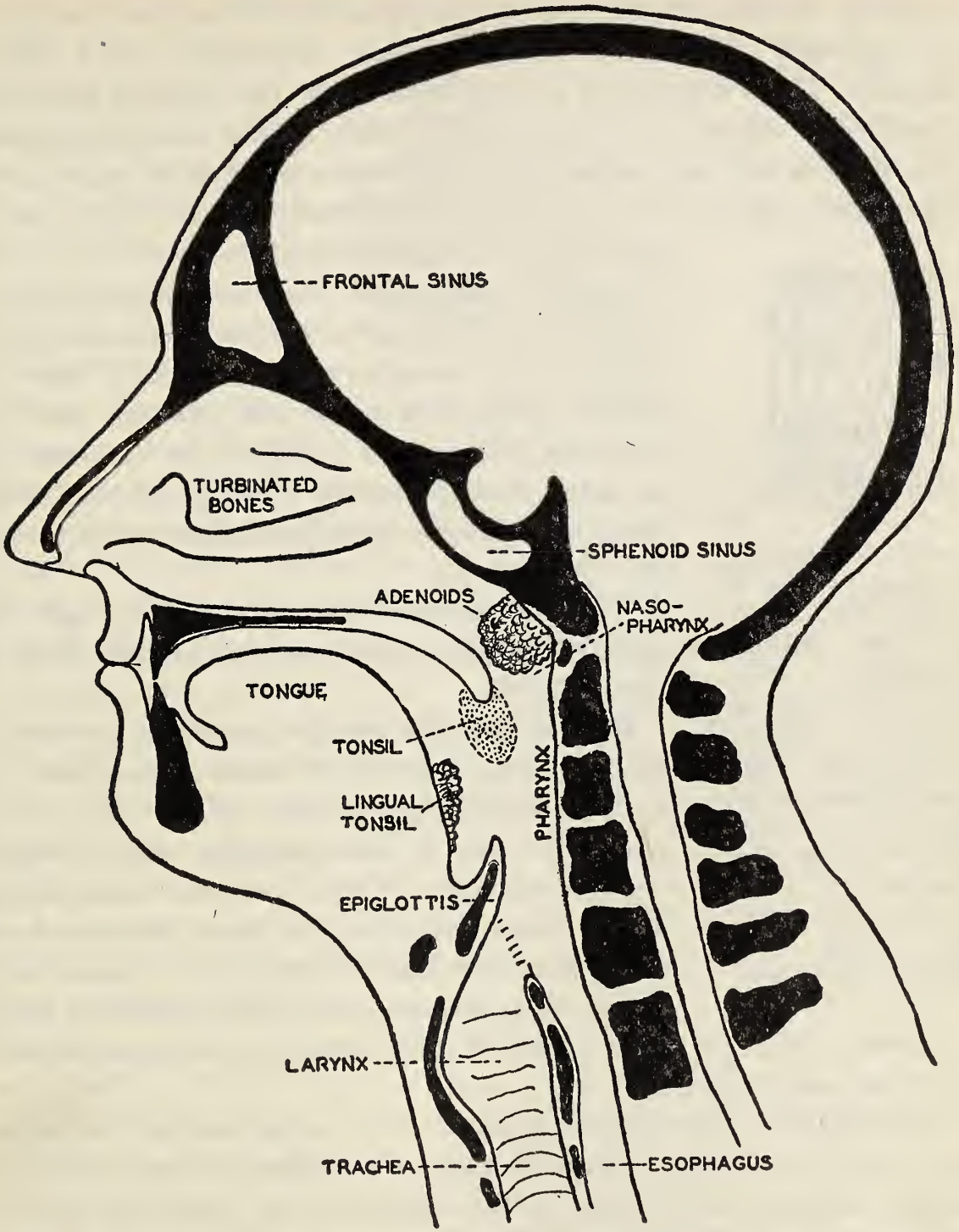


FIG. 83. Vertical section through the head and neck, showing the principal anatomical features of the upper respiratory tract.

appearance from plates similarly inoculated from the throat of the same person. The nose cultures are likely to show a conspicuous number of relatively large, white colonies surrounded by a wide zone of hemolysis. These are colonies of a hemolytic *Staphylococcus albus*—cocci which have no special pathologic significance, so far as we know. Usually present in such cultures, also, are some of the

harmless varieties of Gram-negative diplococci (*Neisseria*), indifferent (gamma) and green-colony (alpha) streptococci, and diphtheroids. These organisms seem to constitute the normal bacterial population. About half of the individuals in the general population of cities may be harboring in their noses potentially pathogenic organisms, such as one or more types of pneumococci (*Diplococcus pneumoniae*), and *Hemophilus influenzae;* and a smaller proportion may be carrying *Streptococcus hemolyticus* or *Corynebacterium diphtheriae.* The *nasopharynx* is especially likely to harbor pathogens, if any are present, and the bacteriologist, when looking for carriers of meningococci (*Neisseria meningitidis*) or other dangerous germs, takes particular pains to culture this area.

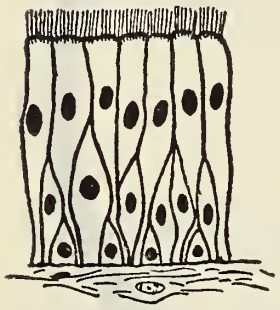


FIG. 84. Section through the wall of a bronchus, showing the ciliated epithelial cells. These cilia are in constant motion, carrying dirt and bacteria upward toward the mouth. The walls of the trachea and nasal sinuses are similarly lined with ciliated epithelium.

The sinuses are normally sterile, and the healthy trachea and bronchi contain few, if any, bacteria.

Common colds. Studies among factory workers, students, and others have shown that by far the most frequent cause of illness, year in and year out, is inflammation of the upper respiratory tract. "Colds," in one form or another, cause more loss of time from work or study than any other ailment. Few individuals escape an occasional cold, and many have three or more every year. The common cold can fairly be regarded as a kind of modern plague.

No single infectious agent appears to be responsible for all colds, nor for all the clinical manifestations of the disease. Some colds are highly communicable, and spread rapidly from person to person by contact, especially through droplet infection. The symptoms appear after an incubation period of from 48–72 hours. Colds of this type are *probably caused primarily by a filtrable virus.* The studies of Dochez and others have shown that this virus is distinct from that of epidemic influenza. Infection is readily passed to other persons during the earliest stage, when comparatively few bacteria are present, and before the nose becomes blocked by pus. The cold virus has been shown to be contained in the watery nasal discharges collected at this time. Soon secondary infection begins, caused by

the potentially pathogenic bacteria—the opportunists—present originally in the respiratory tract (or introduced from without along with the virus), and the discharges become purulent in character. Presumably, the effect of the virus is to weaken tissue resistance, so that these bacteria can multiply abnormally, producing acute inflammation. Prominent among the bacteria likely thus to complicate colds are more or less highly virulent strains of pneumococci, staphylococci, hemolytic streptococci, and *Hemophilus influenzae.* These are the organisms most liable to spread the inflammation to the ears or other parts.

Many observations suggest, however, that this cold virus is not the cause of all "colds." There are numerous individuals who live in a delicate state of balance, so far as upper respiratory tract infections are concerned. Slight lowering of their level of resistance, induced by extra fatigue, by exposure to cold and wet, by local drying or chilling of the mucous membranes of nose and throat, and similar circumstances, results, within three or four days, in the appearance of a sore throat or other symptoms of a "cold." The microbes concerned in this type of cold are, most likely, bacteria of the kinds mentioned above, the predominant species depending upon the relative virulence of the several potentially pathogenic varieties that happen to be present. Colds of this sort are not so readily communicable. They are likely to be more frequent, or more severe, in persons who are chronic carriers of virulent bacteria, such as streptococci, in infected tonsils or other foci, or who have anatomical defects of the nasal passages, or who are subject to recurrent allergic rhinitis.

A nurse who develops an acute respiratory infection ought to be relieved from duty at once, as a measure of protection for herself as well as for her patients.

The avoidance of colds is a matter of real importance to the nurse. She should *try to avoid infection,* by keeping away from persons with colds, and she should *try to avoid* excessive fatigue, exposure, and other *predisposing causes of colds.* An excellent rule, though perhaps hard to follow, is *to go to bed as soon as one feels a cold coming on.* This would prevent spreading of the infection and, at the same time, would help toward a rapid recovery.

No well-defined immunity develops after recovery from a cold, although an increased resistance to the cold virus may persist for a few weeks. Mixed vaccines, containing several of the common

pathogenic organisms found in respiratory infections, have been tried extensively for the prevention and treatment of colds, but all the evidence indicates that they are of no value. Whatever benefit may be noticed from vaccine injections in individual cases should be attributed to a nonspecific stimulation of general resistance. Cold-vaccine preparations taken by mouth have, at most, only a psychological effect.

Other forms of respiratory infection. *Sinusitis.* When infection extends from the nose into one of the sinuses and sets up an inflammation there, with pus-formation, we have the rather common disease called sinusitis. The condition may have harmful effects in many ways upon the whole body. Any one of the several germs we have mentioned in connection with colds, or a mixture of them, may be responsible for sinus infection.

Tonsillitis. We have already mentioned that most cases of acute tonsillitis are caused by hemolytic streptococci. We have also noted that the germs of diphtheria, Vincent's angina, and syphilis frequently produce lesions on the tonsils. The tonsils are often the seat of a chronic inflammation and a source of trouble for the whole body. They may act as a focus from which germs are carried to the joints, or to the heart or other organs. The removal of chronically infected tonsils is often followed by a marked improvement in general health, as well as a reduction in the frequency of infections of the respiratory tract.

Laryngitis. Often infection spreads downward from the throat into the larynx. The walls of the larynx are rigid, and cannot expand, so that when any considerable amount of pus and fluid collects there may be a serious interference with the breathing. We then have the condition called *croup.* The importance of laryngeal diphtheria has already been discussed. The larynx is sometimes the site of infection with the germs of syphilis or of tuberculosis.

Bronchitis. "Cold in the chest" may occur as part of general acute inflammation of the respiratory tract, and may be a complication of measles, influenza, or some other disease. There is always the possibility that it may lead to pneumonia. The organisms most likely to be responsible are those already mentioned as commonly present in colds. Chronic bronchitis is common in old persons.

Bronchiectasis. Sometimes, as a result of chronic bronchitis, the wall of a bronchus is weakened at one place, and it bulges out there to form a sac-like cavity (Fig. 85). In these cavities, masses of bac-

teria of both aerobic and anaerobic types, and of various species, accumulate and grow. Staphylococci, streptococci, other aerobes, and the anaerobic fusiform bacilli and spirochetes (from the mouth) are often numerous in the foul-smelling sputum expectorated by the unfortunate patient.

Lung abscess. A localized inflammation in the lung tissue may occur as a complication of various disease conditions. Such abscesses, however, most commonly develop following aspiration of foreign material into the lung, which sometimes occurs during an operation, or after pneumonia. These postoperative lung abscesses are apparently due to bacteria sucked into the lungs from the mouth. The pus which accumulates in them contains a great variety of organisms, but conspicuous among them, in many cases, are the anaerobic mouth bacteria, including *Bacterium melaninogenicum,* fusiform bacilli, and spirochetes. The infection often clears up spontaneously, but sometimes persists for months, and, unless effective surgical treatment can be given, it may eventually lead to a fatal broncho-pneumonia.

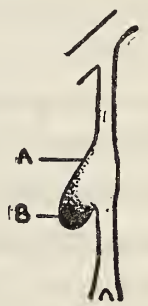


FIG. 85. Diagram to illustrate the condition called *bronchiectasis.* At A the walls of the bronchus have become weakened and have dilated to form a sac-like cavity. B: mass of pus and bacteria which collects in the cavity.

Specific diseases of the respiratory tract. In addition to the disease conditions already mentioned, there are several important specific infections which primarily involve the respiratory tract. We have already discussed *scarlet fever, septic sore throat, diphtheria, Vincent's angina,* and *common colds.* Lung infection may be caused by *Actinomyces, Aspergillus, Blastomyces* or other fungi (Chapter XI). There remain to be considered *measles, mumps, psittacosis, influenza, whooping cough, pneumonia,* and *tuberculosis.* Of these, the first four are *virus* diseases.

Measles. Measles is a highly communicable disease, characterized by catarrhal symptoms (resembling an ordinary cold), fever, the early appearance of a characteristic eruption on the mucous membrane of the cheeks and lips (Koplik spots), and later a skin rash. It is an exceedingly common disease all over the world. It spreads rapidly, and seems to attack every susceptible person, of whatever age, who is exposed to it. Most cases occur in children, because the majority of adults have already had the disease and are immune.

The incubation period averages about ten days. The infection is spread most readily toward the end of this period, during the four or five days before and after the appearance of the characteristic rash.

The cause of this disease has been shown to be a specific *filtrable virus*. It has been found that this virus is present in the blood, and also in the mouth and nose secretions, early in the disease.

Uncomplicated measles is not a particularly severe disease, but, like influenza, it seems to reduce markedly the normal resistance toward respiratory infection, so that secondary inflammation of the middle ear or other parts, due to staphylococci, streptococci, or other organisms, is liable to occur. The most dangerous complication is bronchopneumonia, and this is responsible for most of the deaths. It is important to remember that this serious bronchopneumonia may spread to other measles patients in a crowded hospital ward, unless isolation technique is carried out carefully.

Good results are obtained by the use of *convalescent serum* in the prevention and treatment of measles. If given early in the incubation period, it will entirely prevent the disease. If given later, it may greatly reduce the severity of the infection and help to prevent serious complications. Extracts from normal human placental tissue, known as *immune globulin* (human), have been used instead of convalescent serum, with apparently equal success. Active immunization of children by injection of measles virus weakened in its virulence for human beings by cultivation on chick embryo membranes is a possible future development.

Mumps. This disease (also called *parotitis*) is characterized by inflammation and swelling of the salivary glands, especially the parotid glands on the side of the face. Actually, the infection is often generalized, and the involvement of the salivary glands is only one of the prominent clinical manifestations. Mumps has an incubation period of from two to three weeks. During this time, while the patient is still up and about, the infection is readily passed to other persons; hence it is hard to prevent the spread of the disease. The infection is transmitted by contact with mouth and nose secretions.

Epidemics of mumps spread with great rapidity, and few susceptible persons escape. It is chiefly a disease of children in schools and institutions, but may occur in epidemic form among young adults who are crowded together, as in army camps. It is usually not

severe in children, but may be serious in older persons, particularly in males when the inflammation involves the testicles (orchitis). An encephalitis is a rather frequent manifestation of mumps. Recovery usually leads to a lasting immunity. Convalescent serum appears to have a limited prophylactic value.

The cause of mumps is a *filtrable virus.* This virus has not been cultivated successfully, and not much is known about it as yet. However, typical mumps has been reproduced in monkeys by injecting into the salivary glands of these animals a filtrate of the saliva from human patients.

Psittacosis. This is primarily a disease of parrots, parrakeets, love birds, canaries or pigeons, but it is readily transmitted to human beings. Occasionally the infection is spread from one person to another. The disease in man is serious, and often fatal, especially in older persons. After an incubation period of about two weeks, fever, prostration, and other signs of a generalized infection appear, and the lungs become involved in an acute form of pneumonia. The individual who recovers has a strong immunity.

The disease is highly contagious, and there have been extensive outbreaks among family groups, among workers and visitors in pet shops, and among bacteriologists studying the infection. Possibly there are more human cases of mild psittacosis infection than is generally realized. Patients should be rigidly isolated, and nurses should be especially careful to be well-masked when caring for those who are coughing.

The cause of psittacosis is a *filtrable virus.* This virus is present in the nasal secretions, as well as in the patient's blood, and with these materials the infection may be reproduced in mice and other laboratory animals.

Laboratory diagnosis can be made by inoculating into mice the patient's sputum, or blood taken during the first week of illness. Characteristic pathological changes occur in these animals, and the typical *elementary bodies* of the psittacosis virus (the so-called L.C.L. bodies) can be found in smears from the spleen. The blood serum of the recovered patient will give a positive complement-fixation test with an appropriate psittacosis antigen.

Influenza. During 1914–1919, influenza became pandemic, spreading over the whole world. In a few months the disease destroyed more lives than were sacrificed on the battlefields during the four years of the war. It is still a frequent cause of illness, and public-

health workers are eager to find effective methods of prevention. The infection is evidently communicated through personal contact.

Influenza often produces only a slight inflammation in the respiratory tract, but the disease is characterized by a sudden and profound prostration. As a result of this weakness, serious or fatal complications frequently follow. When death occurs, it is due to secondary infection; usually there is a bronchopneumonia.

The virus of influenza. In 1892, Pfeiffer isolated from the sputum of influenza patients the bacillus called *Hemophilus influenzae.* He regarded this "influenza bacillus" as the cause of the disease, and this was the general belief until recently. It has now been established that the primary cause of influenza is a *filtrable virus* and that "influenza bacilli" and other bacteria play only a secondary (though often a significant) rôle in bringing about the usual clinical disease. The *Hemophilus influenzae* is independently pathogenic, and a disease germ of considerable medical importance, as we shall see below, but *not* the specific infectious agent that initiates the disease we call influenza.

Although experimental results suggesting that influenza is a virus disease were reported previously by several other investigators, it was the work of Smith, Andrews, and Laidlaw, in England (1933), with human influenza, and of Shope, in the United States (1931), with swine influenza that laid the foundation for the fruitful studies of influenza virus now going on. The English workers succeeded in producing in ferrets an influenza-like disease by inoculating into their noses filtrates of the nasal or pharyngeal washings from human influenza patients. The disease was readily passed from ferret to ferret by intranasal inoculations or by natural contact. Serum from animals that recovered, and from human convalescents, neutralized the virus. These findings were soon confirmed by Francis and by other workers in the United States, and numerous strains of influenza virus have now been isolated in different parts of the world.

It has been found that the majority of strains of the virus, now encountered in human influenza cases, fall into one of two distinct serological types—*Type A* or *Type B.* Probably other serological varieties also exist. The type of virus causing any particular epidemic is usually found to be the same in all the cases, but sometimes both "influenza A" and "influenza B" are prevalent at the same time in the same community. Recently it has been found possible to isolate the causative virus directly from patients by inocu-

lation of unfiltered nasal washings onto the chorio-allantoic membrane of chick embryos. The influenza viruses exist as ovoid particles having diameters of from 70 to 100 mμ (Fig. 86).

Immunity in influenza. Immunity in man following influenza appears to be transient, since repeated attacks are likely to occur in successive years. It may be, however, that the resistance is actu-

FIG. 86. Electron micrograph showing particles of influenza virus in a preparation of influenza vaccine. The specimen was coated with a thin layer of gold before the photograph was taken; by this special technique the particles in the preparation cast a clear shadow and one can see their shape more clearly. Magnification is 35,000 times. It can be seen that the individual virus particles are spheres about 100 mμ in diameter. (Courtesy of Dr. W. M. Stanley, Rockefeller Institute for Medical Research, Princeton, N. J.)

ally more lasting, but is effective only against a particular strain of virus, and not against other strains that may appear in subsequent epidemics.

Resistance seems to depend not only upon a sufficient concentration of antibodies in the circulating blood, but also upon a sufficiently strong virus-inactivating capacity on the part of the nasal secretions, that is, at the natural port of entry of the virus.

Vaccines prepared from the killed viruses after cultivation in chick embryos are now being tried on a large scale. Encouraging results have recently been reported by Francis and his collaborators.

Hemophilus influenzae. The "influenza bacillus" is a small, somewhat pleomorphic, Gram-negative organism. It often appears

as extremely short rods, but also may have the form of long slender filaments. It is nonmotile, and has no spores. Capsules have been demonstrated on some strains. It grows best aerobically, and at 37° C.

This organism is a so-called *hemophilic* (blood-loving) *bacillus,* because it will not grow in the absence of blood, unless the essential growth-accessory factors present in blood are supplied from another source. These are the so-called V and X factors previously mentioned. Blood agar medium (especially the kind called chocolate blood agar) is commonly used in the laboratory. The growth is delicate, and cultures die unless frequently transplanted.

The *Hemophilus influenzae* may be found in the healthy throat and nose, but it is potentially pathogenic, and of considerable medical importance. Aside from its prominent part in the bronchopneumonia and other complications of *influenza,* it commonly appears as a secondary invader in other disease conditions, such as the *pneumonia* following measles. It may cause *conjunctivitis, sinusitis, otitis media,* or *endocarditis.*

The most important infection, however, for which this bacillus *alone* may be responsible is a form of *meningitis* in young children —so-called *influenzal meningitis.* The "influenza bacilli" are found in pure culture in the cerebrospinal fluid. Pittman has classified these bacilli serologically, and has shown that the great majority of the strains found in meningitis cases belong to a single type—Type b (Chapter XXX).

Whooping cough. This disease is an acute bronchitis caused by a small bacillus, first described by Bordet and Gengou in 1906, called *Hemophilus pertussis.* After an incubation period of about two weeks, the disease begins like an ordinary cold; then, in about a week, the characteristic "whooping" cough begins. The whoop is caused by the effort of the child to recover his breath after a long paroxysm of coughing. The only result of this violent coughing may be the expectoration of a little mass of very sticky sputum. The disease often continues for a month or more. As in the case of other children's diseases, the greatest danger arises from complications, especially bronchopneumonia. Whooping cough is more dangerous than is generally realized. It causes more deaths than measles or scarlet fever, especially in infants. The disease spreads easily among children through personal contact. A lasting immunity is acquired by those who recover.

Hemophilus pertussis. This organism is a small, somewhat oval-shaped aerobic, Gram-negative bacillus. Smooth forms may be capsulated. It resembles the *Hemophilus influenzae,* but is a distinctly different germ. It is found in enormous numbers in the sticky sputum from cases of whooping cough. A special medium devised by Bordet and Gengou, consisting of a 1% glycerine agar containing crushed potato, mixed with an equal amount of rabbit or human blood, is used to secure the initial growth of the organism from the sputum. The bacillus grows slowly and feebly, and attempts to isolate it often fail. It is somewhat easier to obtain on so-called cough-plates. These are made by holding Petri plates of Bordet-Gengou medium 4 or 5 inches from the mouth of the patient while he coughs. The plates are then incubated in the usual way, and examined for colonies of *Hemophilus pertussis* in about forty-eight hours.

Once isolated, the pertussis bacillus grows fairly readily on media enriched with blood or blood serum, and after a few generations may grow even on plain agar. In this respect it differs notably from *Hemophilus influenzae,* which does not adjust itself to plain media, and requires the accessory growth factors X and V. The whooping cough bacillus differs also in its failure to ferment carbohydrates, form indol, or reduce nitrates.

The pertussis bacilli have no marked poisonous properties; they seem to exert their harmful effects by growing in masses on the walls of the larynx and trachea, and especially in the bronchi and bronchioles, causing the formation of a thick mucopurulent exudate.

Virulent, freshly isolated strains are usually in the smooth (S) phase of growth (spoken of, in this connection, as Phase I) and are serologically homogeneous. They quickly dissociate, however, into progressively rougher (R) phases, unless carefully nursed along on appropriate media. This fact is of practical importance, since vaccines, to be successful, must be made from smooth (Phase I) cultures.

Active immunization. The seriousness of whooping cough in very young children has prompted vigorous efforts, in recent years, to develop a satisfactory method for artificial active immunization; and it is now well established that vaccines prepared from Phase I *H. pertussis* (killed by phenol), according to methods largely worked out by Sauer and his associates, are effective for this purpose. Three subcutaneous injections, at intervals of approximately three weeks,

constitute a scheme widely used. This vaccine may be combined with diphtheria toxoid and tetanus toxoid, all three antigens being inoculated simultaneously. Whooping cough vaccine should be given to infants at the age of about seven months.

REFERENCES

Common Colds

COMMISSION ON ACUTE RESPIRATORY DISEASES: "Endemic Exudative Pharyngitis and Tonsillitis; Etiology and Clinical Characteristics," *J.A.M.A.*, Aug. 26, 1944, 125:1163.

MCGEE, L. C., ANDES, J. E., PLUME, C. A., SUCCASUMA, N. J. and HINTON, S. H.: "'Cold Vaccines' and the Incidence of the Common Cold," *J.A.M.A.*, Feb. 26, 1944, 124:555.

Measles

MORIS, E. P. and OTHERS: "Immunity—Results of Chance and Planned Exposure to Unmodified Measles Virus in Children Previously Inoculated with Egg-Passage Measles Virus," *J. Pediat.*, Jan. 1943, 22:17.

STOKES, J., JR. and OTHERS: "Studies on Measles—IV. Results Following Inoculation of Children with Egg-Passage Measles Virus," *J. Pediat.*, Jan. 1943, 22:1.

Mumps

ENDERS, J. F.: "Parotitis Immunity," *An. Int. Med.*, June 1943, 18:1015.

MCGUINNERS, A. C. and GALL, E. A.: "Parotitis at Army Camps in 1943," *War Med.*, Feb. 1944, 5:95.

WORDEN, E. M.: "Epidemic Parotitis: Analysis of 250 Cases in Male Adults," *Can. Med. Assoc. J.*, Jan. 1944, 50:47.

Psittacosis

BECK, M. D., EATON, M. D., and O'DONNELL, R.: "Further Laboratory Studies on Classification of Psittacosis-Like Agents," *J. Exper. Med.*, Jan. 1944, 79:56.

SMADEL, J. E., WALL, M. J. and GREGG, A.: "An Outbreak of Psittacosis in Pigeons, Involving the Production of Inclusion Bodies, and Transfer of the Disease to Man," *J. Exper. Med.*, Sept. 1943, 78:189.

Influenza

FRANCIS, T., JR.: "Influenza; Methods of Study and Control," *Bull. N. Y. Acad. Med.*, July 1945, 21:337.

FRANCIS and OTHERS: "Vaccination against Influenza," *Am. J. Hyg.*, July 1945, 42:1.

KRUEGER, A. P. and OTHERS: "Experimental Human Influenza," *Am. J. Med. Sci.*, Mar. 1944, 207:306.

SHARP, D. G., TAYLOR, A. R., MCLEAN, I. W., BEARD, D., BEARD, J. W., FELLER, A. E. and DINGLE, J. H.: "Isolation and Characterization of Influenza Virus B (Lee Strain)," *Science*, Oct. 1, 1943, 98:307; *J. Immun.*, Feb. 1944, 48:129.

STANLEY, W. M.: "The Size of Influenza Virus," *J. Exper. Med.*, Mar. 1944, 79:267.

TAYLOR, A. R. and OTHERS: "Isolation and Characterization of Influenza Virus A (PR 8 Strain)," *J. Immun.*, Sept. 1943, 47:261.

RICKARD, E. R., THIGPEN, M. and CROWLEY, J. H.: "The Isolation of Influenza A Virus by the Intra-Allantoic Inoculation of Chick Embryos with Untreated Throat Washings," *J. Immun.*, 1944, 49:263.

Whooping Cough

KENDRICK, P. L.: "A Field Study of Alum-Precipitated Combined Pertussis Vaccine and Diphtheria Toxoid for Active Immunization," *Am. J. Hyg.*, 1943, 38:193.

MILLER, J. J., LEACH, C. W., SAITO, T. M. and HUMBER, J. B.: "Comparison of Nasopharyngeal Swabs and the Cough Plate in the Diagnosis of Whooping Cough and H. Pertussis Carriers," *Am. J. Pub. Health,* 1943, 33:839.

REVIEW QUESTIONS—CHAPTER XXVIII

1. Review the anatomy of the respiratory tract, and discuss its influence upon infection of these parts.
2. Name five kinds of bacteria that can normally be found in the nose or nasopharynx. Name five important pathogenic organisms that may be present.
3. Discuss common colds as to: (*a*) frequency, and importance from the point of view of both the individual and public health, (*b*) the cold virus and its rôle in causation, (*c*) organisms likely to cause complicating infections, (*d*) manner of spread, (*e*) prevention.
4. Name six other common forms of respiratory infection. Discuss the cause and nature of each.
5. Name three fungi that may cause lung infection. Name four virus infections and three bacterial diseases which primarily involve some part of the respiratory tract.
6. State briefly the characteristic clinical features of measles. Discuss its: (*a*) frequency and importance, (*b*) manner of spread, (*c*) cause, and (*d*) prevention.
7. State briefly the characteristic clinical features of mumps. Discuss its cause and manner of spread.
8. Describe briefly the chief features of psittacosis. How may laboratory diagnosis be made?
9. Discuss the clinical features and manner of spread of influenza. What is the relation of *Hemophilus influenzae* to the causation of influenza?
10. Describe the discovery of influenza virus, and the principal properties of this virus. Discuss immunity to influenza.
11. Describe the morphological and physiological properties of *Hemophilus influenzae.* In what important disease condition other than influenza may *Hemophilus influenzae* be found?
12. What are the characteristic features of whooping cough? Name and describe the causative organism. Discuss active immunization against whooping cough.

CHAPTER XXIX

PNEUMONIA

Kinds of pneumonia. By *pneumonia* is meant an inflammation of the lungs. In the commonest form, the alveoli, or air sacs, which make up the lung tissues, become filled with exudate. This disease may be *primary*—i.e., a well person may suddenly develop an acute pneumonia. In this case, the infection almost invariably involves one *entire lobe,* or two or more entire lobes of the lung. This is called *acute lobar pneumonia.* Another form of pneumonia is *secondary*—i.e., it occurs as a complication of some other disease condition. In this case, the infection usually results from an extension of an acute bronchitis into the alveoli, and the lung is involved only in scattered patches about the inflamed bronchioles. This is called *bronchopneumonia.* Finally, a kind of lung inflammation with relatively mild symptoms in most cases, and not caused by bacteria, is spoken of as *atypical pneumonia.* Often, in these cases, the interstitial tissue of the lungs is primarily involved.

Pneumonia in one form or another occurs in practically every individual at some time during the period of adolescence to old age. Lobar pneumonia is common, especially in young adult males, and, despite a marked reduction in mortality rates in recent years, it still ranks fifth among the principal causes of death in the United States.

Primary lobar pneumonia. The onset of this disease is typically abrupt. A person who is feeling quite well suddenly develops a severe chill, pain in the side, then a high fever, and goes to bed at once. An acute inflammation involving whole lobes of the lungs has set in. The alveoli become filled with an exudate, consisting at first mostly of fluid and red blood cells, then later of leukocytes (Fig. 87). The affected lobe becomes much enlarged, and is no longer a sponge of air cells, but a solid tissue, more like liver than lung. The patient is very ill, and death may occur in a few days. The majority of cases recover, however, and their recovery is usually associated with the phenomenon called the *crisis,* which occurs

from about the fourth to the tenth day. At the time of crisis, the temperature falls to normal and, within a few hours, there is a remarkable change for the better in the general condition of the patient. Convalescence then begins. The exudate in the alveoli rapidly disappears, and the lung is soon able again to function normally. The cause of the crisis in pneumonia is not certain, but

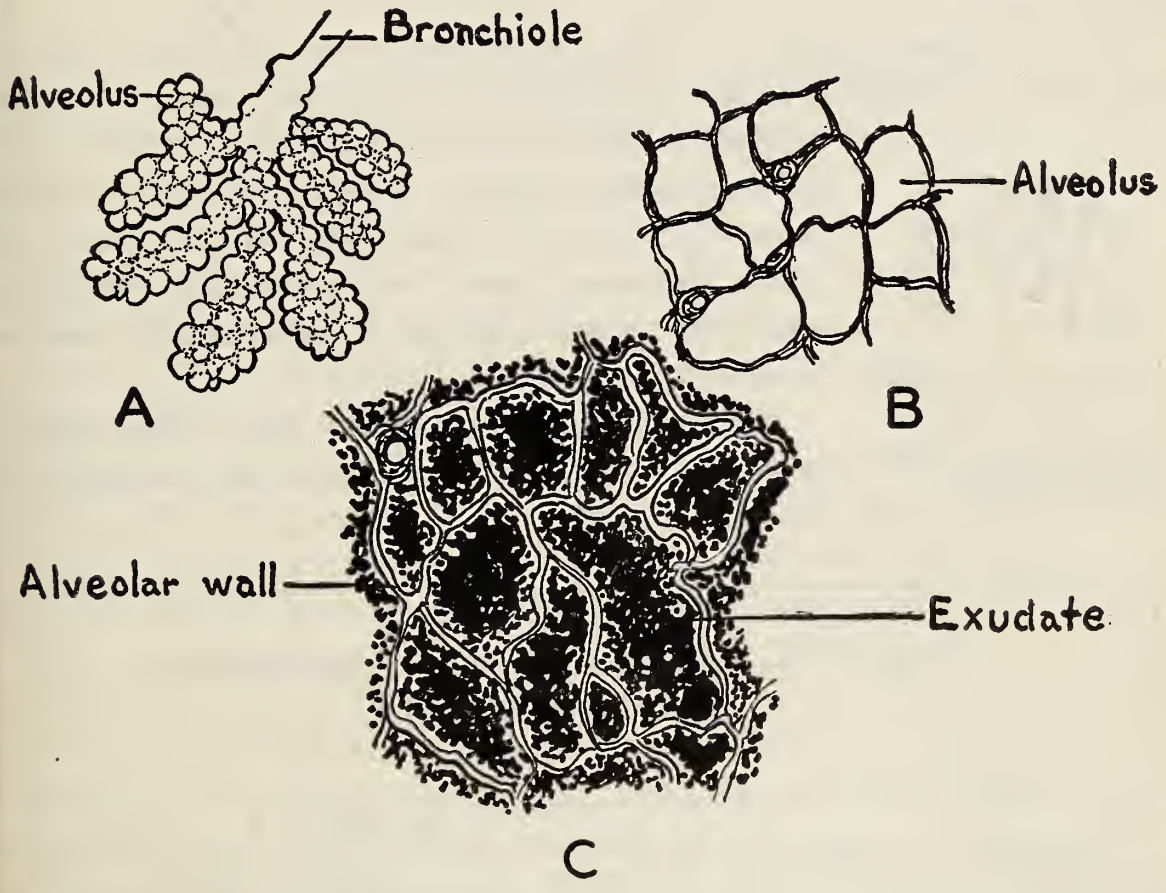


FIG. 87. A: diagrammatic representation of a terminal bronchiole and the group of tiny air sacs, or alveoli, attached. The lungs are made up of innumerable units of this nature. B: section of the normal lung, showing the thin-walled alveoli filled with air. C: section of the lung in lobar pneumonia, showing how the alveoli become solidly filled with a purulent exudate.

it is probably associated with the development by the patient of a sufficient amount of antibodies to counteract the harmful effects of the germ.

Occasionally, pneumonia is caused by *Streptococcus hemolyticus, Staphylococcus aureus, Hemophilus influenzae,* or *Klebsiella pneumoniae* (Friedländer's bacillus), but by far the greatest number of cases of acute lobar pneumonia are due to *Diplococcus pneumoniae,* the *pneumococcus.*

Diplococcus pneumoniae. *Morphology and staining.* Pneumococci are comparatively large, Gram-positive cocci, occurring in

pairs, and typically are shaped somewhat like the head of a lance or spear (Fig. 88). As seen in smears from the body tissues, each pair is surrounded by a wide capsule. The capsule is sometimes lost when the organisms are grown on artificial culture media. Occasionally, these bacteria form short chains and closely resemble the streptococci. As previously explained, only capsulated pneumococci are virulent.

Physiological properties. The pneumococci are parasitic organisms, closely adapted to the living body; hence, they require media enriched with blood or other animal fluids, and will grow readily only at body temperature. On blood agar they form a colony surrounded by a greenish-brown zone, and practically indistinguishable from that of a viridans-type streptococcus.

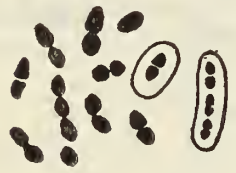


FIG. 88. The pneumococcus (*Diplococcus pneumoniae*).

The principal properties that differentiate pneumococci from streptococci are summarized in Table XIX.

TABLE XIX. Differentiation of Pneumococci from Streptococci

ORGANISMS	MORPHOLOGY	BILE SOLUBILITY	FERMENTATION OF INULIN	PATHOGENICITY FOR WHITE MICE
Pneumococci	Typically: lance-shaped diplococci, with capsules	+	+	++++
Streptococci	Typically: smaller cocci in chains, rarely capsulated	—	— (most strains)	±

The clinical symptoms in lobar pneumonia suggest that the patient is suffering from a poisoning of some kind, but the pneumococci themselves do not appear to produce toxic substances of much consequence. Virulent strains do form a hemolysin and a leukocidin, but most of their poisonous action is apparently due to endotoxin.

The specific soluble substance. The capsular substance of pneumococci contains a complex carbohydrate, which differs with each type. This carbohydrate, or *specific soluble substance,* as it is called, acts as a partial antigen, or *hapten* and, in its combination with the protein of the body of the organism, is responsible for the specific character of the antibody produced by each type. It may be separated by chemical means from a culture of the organisms. It will react specifically with an immune serum for pneumococci of the same type. During the course of lobar pneumonia, the specific soluble substance from the organisms is present in the infected lung tissue, and in the blood stream, urine, and other body fluids. In order to defend itself successfully from pneumococcus infection, it appears that the body must make sufficient anticapsular antibodies to neutralize this free specific soluble substance, as well as to react with the intact capsulated pneumococci so as to degrade them into forms which may be phagocytized or lysed.

Types of pneumococci, and their importance in causing lobar pneumonia. At present there is some confusion regarding the total number of separate serological types that should be recognized, and the best way to name them. It is still customary, however, to designate the thirty-two better-known types by Roman numerals (I–XXXII). Recent surveys have indicated that by far the majority of cases are caused by the lower-numbered types. Type I alone is responsible for at least 20%, and Type III for about 15%. Other types commonly found have been Types II, IV, V, VI, VII, VIII, XIV and XIX. Doubtless, there is considerable variation from time to time, and from place to place, in the frequency with which the various types occur in the current pneumonia cases. Some special interest attaches to Type III pneumococcus pneumonia. This type has a conspicuously large capsule, and is so virulent that as many as 33–50% of the patients may die. In any case of pneumonia, a fatal issue seems to be closely correlated with the persistence of pneumococci in the blood stream. In at least a third of lobar pneumonia cases, a bacteremia occurs; if this is transient, eventual recovery is likely; but if it is persistent, the patient is more liable to die.

Bacteriological diagnosis of lobar pneumonia. The causative germs may be discovered by means of: (1) *blood cultures* or (2) *direct smears and cultures from the sputum.*

Blood cultures. These are made, in the usual way, by taking 5 or 10 cc of blood from the patient's arm vein and introducing this directly into broth, or into agar for plates. All the organisms likely

to be responsible for the infection will grow out readily, and the bacteriologist will have little difficulty in identifying them. Usually, of course, there will be a pure growth of the pneumococcus.

Sputum smears and cultures. Except in the occasional instances of pneumonia due to other organisms, the patient's sputum will contain conspicuous numbers of the causative pneumococci. Cultivation of the pneumococci directly from the specimen may be accomplished by first washing the tenacious sputum mass in several changes of sterile saline, then streaking the washed material over blood agar plates. Isolation of pneumococci from sputum is most easily accomplished, however, by inoculating some of the washed sputum into the peritoneal cavity of a white mouse. This animal is so susceptible to pneumococci that it will die in a few hours, and in the peritoneal exudate, and the heart's blood, a pure growth of pneumococci will be found. The germs may be identified as pneumococci by tests for inulin fermentation, and for solubility in bile.*

Determination of pneumococcus types; typing by Neufeld's "Quellung" method. When it is desired to determine the type of pneumococcus causing a particular case of pneumonia, the capsule-swelling test of Neufeld is now almost universally employed. The method depends upon the fact that, when a capsulated pneumococcus comes in contact with its specific antiserum, the capsule undergoes an almost immediate enlargement, the so-called "Quellung" reaction (Fig. 89). This reaction may be tested for as follows:

A loopful of the patient's sputum is mixed in separate drops on slides with specific commercial rabbit antiserums for the different pneumococcus types. A little methylene blue is also added to each serum-sputum mixture. The drops are covered with cover glasses, or set up as hanging drops, and examined with the oil-immersion objective. When the serum and the pneumococcus are of the same type, a marked swelling of the capsule appears within from two to thirty minutes, and usually there is little doubt of the result, since comparison of the other drops, in which no capsular swelling has oc-

---

* **Bile solubility test.** Divide the young broth culture (or the saline suspension of the young organisms) to be tested into two portions (of about 2 cc each) in separate small test tubes. Add about 0.5 cc of sterile, clear ox bile or a 10% solution of sodium taurocholate to one tube, and an equal amount of salt solution to the other (control) tube. Compare the appearance of the liquids in the two tubes at once, and at intervals in the next half-hour. Usually pneumococci dissolve and the originally cloudy suspension clears up immediately, even at room temperature. Streptococcus cultures remain cloudy.

curred, will show a marked difference. Sometimes, however, the capsule-swelling test gives indeterminate results, and ordinary agglutination tests may have to be carried out on pure cultures of the pneumococci isolated from the patient.

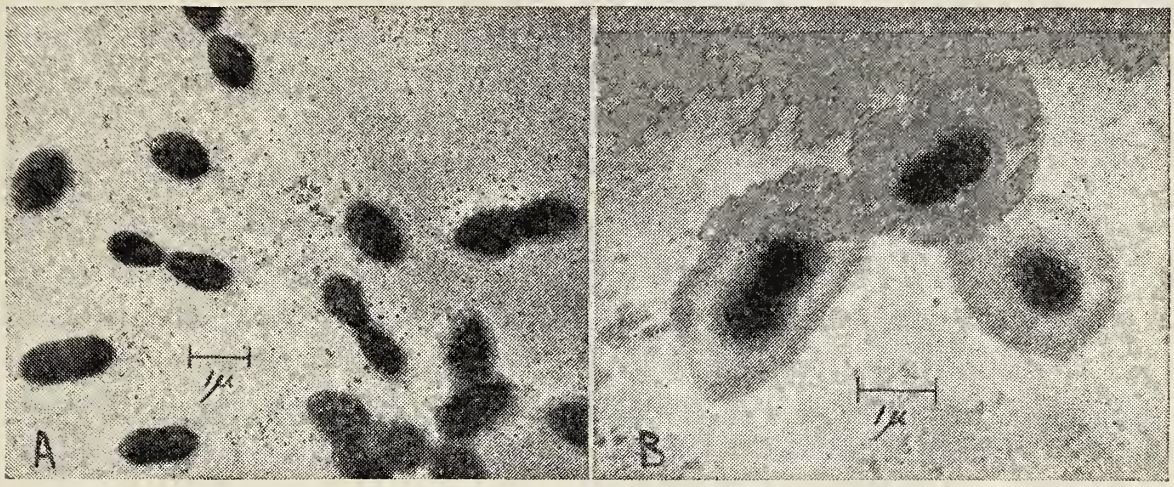


FIG. 89. Electron micrograph of pneumococci, showing the capsule-swelling (Quellung) reaction. A: Pneumococci before the application of specific antiserum; the capsules can be discerned as faint gray halos around the organisms. B: the same pneumococci three minutes after mixture with specific rabbit antiserum; the capsules are enormously swollen. (From Mudd, S., Heinmets, F., and Anderson, T. F., "The Pneumococcal Capsular Swelling Reaction, Studied with the Aid of the Electron Microscope," *J. Exper. Med.*, 78:237, Nov. 1, 1943.)

Tests for the determination of type are reliable in direct proportion to the care taken in the collection of the sputum sample. Since harmless pneumococci are likely to be present in the normal mouth, an effort must be made to exclude these, and to get a sample of sputum which really comes from the inflamed lungs. The patient's mouth should be thoroughly rinsed, and then he should be induced to raise sputum from the deeper bronchi by coughing.

It was formerly most important to determine, at the earliest possible moment, the type of pneumococcus present in each case of pneumonia so that the doctor could learn which therapeutic antiserum to administer. But at present, pneumococcus typing does not have the urgency it once had, since treatment with type-specific antiserum has been largely abandoned in favor of chemotherapy with sulfa drugs or penicillin.

Immunity in pneumonia. As in the case of staphylococcus and streptococcus infections, successful resistance to pneumococci seems to depend principally upon the cellular body defenses, including phagocytosis. Of course, type-specific antibodies that will act upon

the capsulated cocci must contribute also. Susceptibility to primary lobar pneumonia is definitely increased by circumstances that depress the general well-being, such as exposure to wet and cold, fatigue, and alcoholism. Following recovery, there is only a brief period of comparative immunity. Recently attempts to immunize persons actively have given promising results. The vaccines are made not from the whole pneumococci but from antigenic fractions of the organisms separated chemically.

Secondary pneumonia. We have already pointed out that measles, whooping cough, and influenza are especially liable to be complicated by a secondary infection of the lungs, in the form of a *bronchopneumonia,* and we have mentioned that this complication is responsible for nearly all the deaths of patients with these diseases. This type of lung infection may also complicate a great many other diseases, and, in fact, a terminal bronchopneumonia is the immediate cause of death in the great majority of cases of chronic tuberculosis, cancer, and various other pathological conditions. Though not always fatal, a secondary pneumonia is a dangerous infection, because the patient is usually greatly weakened by preëxisting disease.

No single kind of germ is responsible for all cases of secondary pneumonia, and there is a mixture of bacteria in the inflamed lung, but, in groups of cases occurring in the same place, a particular species often predominates. Thus, *Streptococcus hemolyticus* is frequently the most abundant germ, while in other groups of cases *pneumococci, staphylococci,* or *Hemophilus influenzae* may be predominant. Virulent strains of these germs, introduced by contact with a human carrier, may be present in the throat for some time before the disease begins, invading the lung only when the resistance of the patient becomes sufficiently low. Occasionally a secondary bronchopneumonia is caused by the organism responsible also for the primary infection—as, for example, the typhoid bacillus, in cases of typhoid fever.

Atypical pneumonia. The form of pneumonitis known as *primary atypical pneumonia* has been observed with increasing frequency in the United States since 1941. The work of several groups of investigators indicates that this clinical syndrome may be caused by three or more different infectious agents. A small proportion of the cases is due to lung infection with psittacosis virus or with a species of rickettsiae (*Rickettsia diaporica*), while the majority are

thought to be caused by a newly recognized virus (or by a group of viruses) still incompletely described. Further studies are needed.

Prevention of pneumonia. It is probable that primary (lobar) pneumonia does not usually occur unless the individual is for some reason less resistant than normally. But, of course, a virulent strain of pneumococcus must be present before the disease can begin, and such a strain must be acquired, through personal contact, from a human carrier. Epidemics of lobar pneumonia are rare, but they do occur among crowded populations of poorly nourished people in mines, labor camps, and the like. Crowding, especially in sleeping places, is conducive to the free spread of virulent pneumococci, and to the occurrence of pneumonia.

Patients with lobar pneumonia in the hospital should be carefully isolated. Even though another case of the disease is not likely to develop by ordinary contact, the pneumococci in the sputum, or in the nose or ear discharges, etc., must be regarded as dangerous germs. The nurse should give special attention to the disinfection of the sticky sputum and the numerous articles which will inevitably be contaminated with it.

Precautions against contact infection should be even more strict in the case of patients with secondary bronchopneumonia. Dangers of carrying virulent streptococci or pneumococci from bed to bed in a ward of influenza or measles patients should be guarded against by the wearing of gauze masks, and the faithful practice of the rules of hand disinfection.

Infections other than pneumonia caused by Diplococcus pneumoniae. Aside from their rôle in pneumonia, pneumococci are frequently the cause of serious infections of other kinds. During or following pneumonia, they may cause an *empyema* (inflammation with pus in the chest cavity), an *otitis media* or a *meningitis,* an *endocarditis* or *arthritis.* They also may be responsible for a *primary meningitis, or arthritis in children,* and they may cause severe *conjunctivitis* or other form of eye infection.

REFERENCES

Pneumococci; pneumococcus pneumonia

EDDY, B. E.: "Nomenclature of Pneumococcus Types," *Pub. Health Rep.,* Apr. 7, 1944, 59:449; and "Cross Reactions Between the Several Pneumococcic Types, and Their Significance in the Preparation of Polyvalent Antiserum," *Pub. Health Rep.,* Apr. 14, 1944, 59:485.

ROGERS, E. S., BAHLKE, A. M. and HARRIS, A. H.: "Epidemiology of Pneumococcus Pneumonia," *Am. J. Pub. Health,* 1943, 33:671.

RUMREICH, A. S. and OTHERS: "A Nation-wide Study of the Bacterial Etiology of the Pneumonias," *Pub. Health Rep.,* Jan. 22, 1943, 58:121.

SPICER, S.: "A Study of Sulfapyridine Resistance in Pneumococci," *J. Bact.,* July 1945, 50:23.

SMILLIE, W. G., CALDERONE, F. A. and ONSLOW, J. M.: "The Epidemiology of Pneumonia: The Prevalence of Pneumococci in the Throats of Homeless Men," *Am. J. Hyg.,* 1943, 37:158.

WHITE, B.: *The Biology of the Pneumococcus.* New York: The Commonwealth Fund, 1938.

Atypical pneumonia

COMMISSION ON ACUTE RESPIRATORY DISEASES. "Primary Atypical Pneumonia," *Am. J. Pub. Health,* Apr. 1944, 34:347; "Epidemiology of Atypical Pneumonia and Acute Respiratory Diseases at Fort Bragg, North Carolina," *Am. J. Pub. Health,* Apr. 1944, 34:335; "Transmission of Primary Atypical Pneumonia to Human Volunteers," Jan. 20, 1945, *J.A.M.A.,* Jan. 20, 1945, 127:146.

HORSFALL, F. L., JR., CURNEN, E. C., MIRICK, G. S., THOMAS, L. and ZIEGLER, J. E., JR.: "A Virus Recovered from Patients with Primary Atypical Pneumonia," *Science,* Mar. 26, 1943, 97:289.

REVIEW QUESTIONS—CHAPTER XXIX

1. What two types of pneumonia are there, from the anatomical point of view? What is meant by primary pneumonia, secondary pneumonia?
2. Describe the principal features of acute lobar pneumonia. Name the usual causative agent and three other bacteria that may cause primary lobar pneumonia.
3. Describe the morphology and staining, and principal physiological properties, of pneumococci. What variety of streptococcus forms a colony on blood agar similar to that of pneumococci? What are the principal differences which serve to distinguish streptococci from pneumococci?
4. Explain the part played by capsules of pneumococci in: (*a*) determining the specificity of types, (*b*) determining virulence. What is the *specific soluble substance*?
5. How are the principal types designated? Which types are the more commonly encountered in pneumonia cases? Which type is especially virulent?
6. Outline procedures used for the bacteriological diagnosis of pneumonia.
7. Outline techniques for determining the type of pneumococcus causing a particular case of pneumonia.
8. Discuss resistance to pneumococcus infections.
9. What is the importance of secondary bronchopneumonia? What organism may be responsible?

10. What is the so-called primary atypical pneumonia?
11. Discuss the prevention of pneumonia. What measures are needed to reduce the mortality from lobar pneumonia?
12. Name eight infections, other than pneumonia, that may be caused by pneumococci.

CHAPTER XXX

INFECTIONS OF THE EYES AND EARS. INTRACRANIAL INFECTIONS. MENINGITIS

INFECTIONS OF THE EYES

Normal flora of the conjunctiva. There are only a few microorganisms which make up the usual flora of the normal conjunctiva. Conspicuous among them are *diphtheroids* (*Corynebacterium xerose,* or other varieties), staphylococci, sarcinae, and alpha-type streptococci. Potentially pathogenic bacteria, such as virulent strains of *Staphylococcus aureus* or pneumococci, may be present without causing injury to the healthy conjunctiva.

Eye infections. The normal eye is protected from infection, in some degree, by the mild bactericidal power of the tears. This power is due in part to the presence, in lacrimal fluid, of a substance called *lysozyme,* which has a destructive (lytic) action on various saprophytic, and some pathogenic, bacteria. We see the importance of this natural protection when, as a result of a deficiency of Vitamin A, the secretions which normally bathe the eyeball dry up. Then we have the condition known as *xerophthalmia,* in which the ordinarily harmless bacteria of the normal conjunctiva, or others of low virulence, may cause severe inflammation.

The eyes are vulnerable to infection from the outside, brought by unclean fingers, or introduced with dirt and foreign bodies. Also, their general condition reflects the vigor of the body as a whole, and sometimes disease of the eyes is a local manifestation of a generalized illness. There may be lesions in the eyes, of course, accompanying specific infections of other parts of the body—as, for example, in smallpox, and especially often in tularemia. We shall mention here only the commoner forms of eye infection.

Bacterial infections of the eyelids, cornea, and lacrimal apparatus. A *sty* (or hordeolum) is the most frequently seen infection

of the eyelid. It begins in one of the sebaceous follicles of the eyelashes, and is most likely to develop on the lower lid, near the corner of the eye. The sty is usually associated with a subacute inflammation of the margin of the lids. The causative organism is almost invariably a virulent *Staphylococcus aureus.*

Infection of the cornea (the transparent outer wall of the eyeball) may be caused by a number of different bacteria and viruses. In the United States, *ulcerations of the cornea* are most often due to pneumococci; but many other species may be concerned, for example, *Hemophilus influenzae,* staphylococci, streptococci, or the diplobacillus of Morax-Axenfeld (*Hemophilus duplex*). A still wider variety of "opportunists" have been incriminated in other clinical forms of corneal inflammation (*keratitis*), such as dysentery bacilli, Friedländer's bacilli, and *Pseudomonas aeruginosa.* Syphilitic or tuberculous infection may be responsible for a chronic disease of the cornea.

The downward flow of the tears, with their bactericidal properties, ordinarily protects the tear ducts from infection, even if the conjunctiva is badly inflamed. Sometimes, however, especially in older persons, an acute or chronic infection of the lacrimal apparatus develops, most commonly as an extension of a sinus infection, or as a consequence of obstruction of the tear duct. The organisms concerned are again various, as would be expected in this frankly "opportunist" type of infection, and may be pneumococci, streptococci, staphylococci, "influenza bacilli," or other familiar species. Cases of obstruction of the tear duct by concretions formed by actinomyces or true fungi have been described.

Conjunctivitis. Many varieties of bacteria may cause an acute or subacute conjunctivitis. The following are examples of some of the better-known types of conjunctival infection.

Acute epidemic conjunctivitis—so-called "pink-eye"—is caused by an organism named the *Koch-Weeks bacillus,* after the men who first described it. This germ is a small, Gram-negative, hemophilic organism, and is probably identical with *Hemophilus influenzae.* "Pink-eye" is contagious, and may spread rapidly among members of a family or other group. In children, the infection may accompany or follow measles or other common diseases.

The commonest cause of so-called *catarrhal conjunctivitis* is a *pneumococcus.* A severe, acute inflammation may set in, but this rarely leads to any permanent damage to the eye. Epidemics of

pneumococcic conjunctivitis may occur among children. Cases of persistent conjunctivitis associated with sties are likely to be caused by toxigenic strains of *Staphylococcus aureus,* while chronic conjunctivitis in patients with infected tear ducts may be due to *Streptococcus hemolyticus.* Conjunctival infection, in both sporadic and epidemic form, has been attributed to the Gram-negative bacillus *Alkaligenes fecalis.* Occasionally, *fusospirochetal infection* of the eyes has been reported.

The fairly common subacute or chronic form of conjunctivitis, especially noticeable about the corners of the eyes, and known as *angular conjunctivitis,* is caused by the *Morax-Axenfeld bacillus* (*Hemophilus duplex*).

Gonorrheal ophthalmia. Inflammation of the eyes by the germ of gonorrhea (*gonorrheal ophthalmia*), and particularly the form of this disease which occurs in newborn babies (*ophthalmia neonatorum*), is the most important of the acute eye infections. Children born of mothers with gonorrhea are almost certain to develop this infection, unless preventive measures are promptly taken. The gonococci (*Neisseria gonorrheae*) get into the eyes as the baby passes through the birth canal. The organisms inflict the eye tissues with severe injury, which often results in blindness. This form of eye infection, however, is easily preventable. It is only necessary to instill a drop of a 1% silver nitrate solution into the eyes immediately after birth (Credé method). This simple treatment is now given to *every* newborn baby, whether the mother is suspected of having gonorrhea or not, and cases of gonorrheal ophthalmia in the newborn are now rare.

Occasionally, an adult develops a gonococcal infection of the eye as the result of self-inoculation with the fingers. Nurses and doctors should remember this possibility when called upon to care for a patient with gonorrhea.

Virus infections of the eyes. There are three important eye diseases caused by specific viruses: (1) *herpes infection,* (2) *trachoma,* and (3) *infectious kerato-conjunctivitis.*

Herpes infection. The same virus responsible for "cold sores" on the lips (p. 374) may infect the eyes, causing a characteristic type of ulceration of the cornea.

Trachoma. This is the most serious eye infection of virus causation; the trachoma virus affects the eyes only. Trachoma is a chronic, contagious disease which begins as a follicular conjunctivitis (granu-

lar lids), and progresses to involve the cornea, with the eventual formation of scars and other changes, resulting in partial or total blindness. The disease is common in many parts of the world, particularly in Egypt, China, and other Eastern countries. There are cases also in the United States and Canada among the Indians, and also among the native white people in certain sections. It spreads by contact from person to person in schools, asylums, and institutions, and among members of the same family.

FIG. 90. Cytoplasmic inclusions in an epithelial cell in a scraping from the infected conjunctiva of a patient with inclusion conjunctivitis. The darker, oval body at the lower left is the nucleus of the cell. The inclusion bodies nearly fill the cytoplasm of the cell around this nucleus. (Drawn from an original photograph by Dr. Charles Weiss, *Am. J. Clin. Path.*, 14:200, April, 1944.)

The virus of trachoma is present in the infected epithelial cells in the form of *elementary bodies,* and so-called *initial bodies* (Fig. 90).

Infectious kerato-conjunctivitis. This highly contagious eye infection came to public notice in the United States late in 1941, when a severe epidemic broke out among shipyard workers in San Francisco, and later in Atlantic coast yards and industrial plants. Soon it became known as "shipyard eye." Weiss states that the disease is probably identical with an epidemic eye infection previously reported from India and elsewhere. The infection spreads with great

ease by contact with the virus-laden secretions from the eyes; first-aiders have innocently transmitted it through contaminated eye droppers, and many doctors and nurses have been infected. The disease, which begins within about a week after exposure, usually feels at first like "something in the eye." There is an acute inflammation of the conjunctiva, a swelling of the lids, a watery discharge, and a more or less serious involvement of the cornea. Sulfa drugs are successfully used in treatment.

No significant bacteria are found in the infected eyes, but a specific *virus* is present, as demonstrated by Sanders and Alexander in 1943.

INFECTIONS OF THE EARS

Earache, like toothache, is a more important matter than is commonly thought. It is caused by an infection in either the external or the middle ear, and may have serious consequences.

Infections of the external auditory canal. Just as on the skin of the neck, or elsewhere, a virulent *Staphylococcus aureus* may get down into the hair follicles or sweat glands in the external ear canal and cause the formation of a small boil (furuncle). This is usually very painful, and may be accompanied by a high fever. A boil in this location is most likely to occur during the swimming season, because continued dampness in the auditory canal tends to soften the tissues there, making them more than ordinarily susceptible. Usually in a few days the furuncle ruptures and drains spontaneously.

Another form of external ear-canal infection, also commonly occurring in patients who go swimming frequently, is a low-grade fungous infection, usually caused by a species of *Aspergillus*.

Infections of the middle ear (otitis media); mastoiditis. Otitis media is the most serious and frequent form of ear infection (Fig. 91). It may be acute or chronic. It occurs most frequently in children as a complication of scarlet fever, measles, common colds, or other infections of the nose and throat, but also may occur in adults. In the case of an acute otitis media, it may be necessary to incise the eardrum to permit the exudate to escape. Otherwise, the infection may spread and cause a *mastoiditis* or a *meningitis,* and perhaps a fatal *septicemia.* Frequent, severe ear infection, or chronic otitis media, may result in deafness.

Streptococcus hemolyticus, Staphylococcus aureus, and *Diplococcus pneumoniae* are the bacteria most commonly responsible for otitis media. The hemolytic streptococci, either alone or mixed with other bacteria, have been found to be present in approximately 30% of cases, while staphylococci are encountered even more frequently—in at least 50% of all cultures made from infected ears.

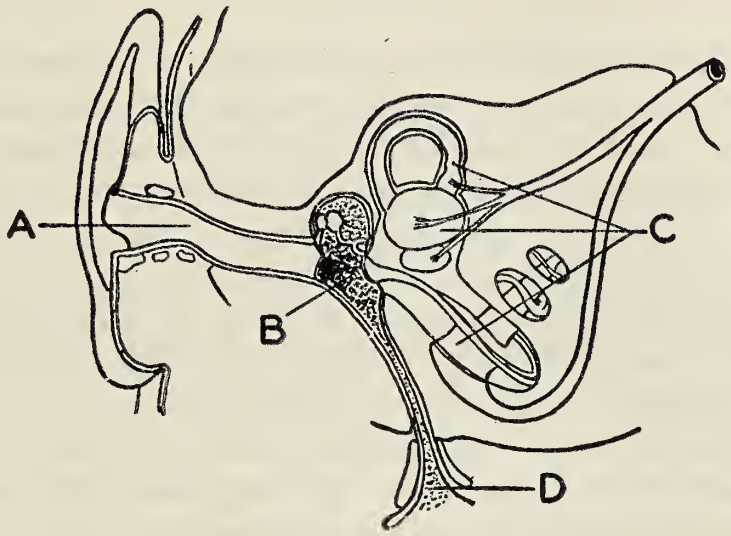


FIG. 91. Section through the ear, showing the condition in cases of acute otitis media. A: the external ear; C: the internal ear; D: the Eustachian tube; and B: the middle ear, shown filled with pus which causes the ear drum to bulge outward.

Often, both of these organisms are involved. Pneumococci have been isolated from about 20% of cases. A particularly severe infection, with special danger of serious complications, is likely to be caused when the heavily capsulated pneumococci of Type III are present. Various other bacteria, such as *Hemophilus influenzae* and *Pseudomonas aeruginosa,* may be concerned in otitis media, particularly when the disease is of the subacute or chronic type, and in many such cases a mixture of three or four organisms is present.

When infection spreads to the mastoid region, the resulting *mastoiditis* is usually caused by a single kind of bacterium, representing, doubtless, the most invasive species present. Cultures from the infected mastoid tissues yield a pure growth of hemolytic streptococci in a large majority (75–80%) of cases, while pure pneumococci are likely to be found in a much smaller number (10–15%) and staphylococci only rarely (about 1%).

The running-ear discharge, which may contain virulent germs, ought to be carefully collected and destroyed.

Intracranial complications of otitis media or sinusitis; brain infections. Severe bacterial inflammation of the middle ear, mastoid, nose, or sinuses may lead to still further complicating infections within the cranium. Of course, a compound fracture of the skull may allow dangerous organisms to enter. Among the infectious processes likely to occur are *lateral sinus thrombosis, extradural abscess, brain abscess,* and *meningitis.*

Lateral sinus thrombosis. The lateral sinus is a venous channel which lies in close contact with the mastoid bone, so that infection may extend into it easily in cases of mastoiditis. As a result of inflammation of the infected cells lining the sinus, there is deposited upon its walls a collection of fibrin derived from the blood stream. This is a *thrombus.* The fibrin mass gradually gets larger, until it may stop up the channel. Almost always, the thrombus itself is infected with the streptococci, pneumococci, or staphylococci causing the original mastoiditis. From time to time, small pieces of the thrombus (infected emboli) break off and are carried around by the blood stream until they find lodgment in a lung capillary or in some other part of the body, where they may start up a new infectious process. The end result is often a fatal septicemia.

Extradural abscess. An abscess external to the dura mater (the tough membrane lining the skull cavities and enclosing the brain) may occur as a complication of otitis media or sinusitis. Such an infectious process may be merely a temporary stage leading to brain abscess or meningitis.

Brain abscess. An abscess in the brain substance may develop by direct extension of infection from the middle ear or sinuses, it may follow a fracture (especially a compound fracture) of the skull, or it may arise from lodgment in the brain of an infected embolus brought by the blood stream from some area of infection elsewhere in the body.

MENINGITIS

By *meningitis* is meant an inflammation of the meninges, i.e., the membranes which cover the spinal cord and brain. The strong outer membrane which lines the bony cavities of the skull and the spinal canal is called the *dura mater.* Just beneath this is another membrane, the *arachnoid.* The delicate inner membrane which immediately covers the brain and cord is called the *pia mater.* Between the pia mater and the arachnoid is the subarachnoid space which contains the *cerebrospinal fluid.* In meningitis, pus collects about the subarachnoid space.

Secondary meningitis. As indicated above, a meningitis may be one of the final complications of a severe ear or mastoid infec-

tion. In this so-called otogenous meningitis, the organism invading the meninges is likely to be a pneumococcus (especially Type III), or a hemolytic streptococcus, the "influenza bacillus," or a staphylococcus. On occasion, however, *Proteus* bacilli, colon bacilli, Friedländer's bacilli, diphtheroids, or other common varieties of bacteria are the guilty ones.

A meningitis following a compound fracture of the skull is usually due to staphylococci or streptococci. Sometimes staphylococci or other bacteria are accidentally introduced into the spinal canal in the course of making lumbar punctures, causing a fatal meningeal infection. It appears that almost any bacterium, whether ordinarily pathogenic or not, may produce an infection when introduced by any means into the subarachnoid space.

Primary meningitis. Illness characterized from the beginning by signs of meningitis may be caused by infection of the meninges with a variety of different bacteria and fungi. There are four kinds of bacteria, however, most commonly responsible for primary meningitis. These are the pneumococcus (*Diplococcus pneumoniae*), the hemolytic streptococcus (*Streptococcus hemolyticus*), the "influenza bacillus" (*Hemophilus influenzae*), and the meningococcus (*Neisseria meningitidis*). The latter is the cause of the serious and common communicable disease called *epidemic cerebrospinal meningitis.*

Influenzal meningitis. This disease requires special mention. In children's hospitals it is one of the more frequent forms of nonepidemic meningitis. Since it is caused by certain virulent strains of *Hemophilus influenzae* it is generally referred to under the unfortunate and misleading term "influenzal." We now understand, of course, that there is no direct relation to influenza. But this meningeal infection does make clear the very real pathogenic powers of some strains of the "influenza bacillus." Until the advent of the sulfonamide drugs and the therapeutic antiserum now used, this disease was almost always fatal in young children.

Recent investigations show that the majority of the cultures of *Hemophilus influenzae* isolated from cases of meningitis in children fall into Pittman's serological Type b. These strains are capsulated; and, as in the case of virulent pneumococci, the capsule-swelling phenomenon will occur in the presence of type-specific rabbit antiserum.

Diagnosis is usually made easily by direct smears from the cloudy spinal fluid. The organisms appear as faint-staining, Gram-negative

rods, varying greatly in length, from coccoid forms to long filaments. The marked pleomorphism is characteristic.

Whenever the bacilli are sufficiently numerous in the spinal fluid specimen, direct typing by capsule-swelling, using specific rabbit antisera, is attempted. Otherwise the typing is postponed until pure cultures have been secured from the spinal fluid and nasopharynx. These cultures are made by streaking warmed blood agar or chocolate agar plates. A useful trick is to inoculate portions of the same plates at the same time with a staphylococcus culture. The staphylococci will supply needed growth-accessory substances, and in the neighborhood of the staphylococcus colonies growth of *Hemophilus influenzae* will be enhanced (the satellite phenomenon, Fig. 36). The organisms are identified by checking their morphology and by showing that they require both V and X growth factors.

Typing of the bacillus at the earliest possible moment is of truly vital importance, for Alexander and others have recently shown that once the causative organism is identified as a Type b "influenza bacillus," a type-specific, therapeutic rabbit antiserum may be administered with lifesaving effect. Use of this serum, together with sulfonamides and symptomatic treatment, has brought about a dramatic reduction in mortality.

EPIDEMIC CEREBROSPINAL MENINGITIS; MENINGOCOCCAL INFECTIONS

Epidemic meningitis is a specific, communicable disease always caused by the Gram-negative diplococcus called *Neisseria meningitidis* or *N. intracellularis* (the *meningococcus*), first studied by Weichselbaum in 1887. Meningitis frequently occurs in epidemic form, among groups of persons living in crowded quarters, especially in military camps. In the United States, during World War II, a marked increase in the prevalence of this illness occurred among the civil population, as well as in military and naval establishments. At the same time, a striking reduction in the death rate of this formerly highly fatal infection was obtained, because of the therapeutic effectiveness of the newly introduced sulfa drugs and antibiotics.

Neisseria meningitidis. *Morphology and staining.* Meningococci are small coffee-bean-shaped Gram-negative diplococci not differing in morphology from other pathogenic and nonpathogenic species of

Neisseria. Virulent strains in infected spinal fluid, and in young cultures 4–6 hours old, are capsulated. In cultures, there is often great variation in size among the individual cocci, and some stain more deeply than others. This is due to the fact that the organisms die and disintegrate very quickly, and many swollen and irregular-shaped cells are likely to be present. In spinal fluid from meningitis patients the cocci are more regular in form, and occur almost entirely within the cyptoplasm of the pus cells—i.e., they are *intracellular.* In shape and grouping, and in their tendency to be intracellular in pus, they are indistinguishable from gonococci.

Physiological properties. Meningococci are strictly parasitic organisms, and are cultivated in the laboratory with considerable difficulty. They require a medium enriched with blood or other body fluid. They will grow only at body temperature, and cultures must be maintained at 37° C and transferred to new media frequently. The organisms are aerobic, but grow best when the oxygen tension is somewhat reduced, and there is an increased carbon-dioxide content in the atmosphere. They have no natural existence outside of the human body, and die rather quickly when exposed to light, moderate heat, or common disinfectants.

Mode of infection and spread of epidemic meningitis. It might well be asked how an infection localized in the subarachnoid space could become epidemic and spread by contact as meningococcic meningitis does. The answer is that the meningococci are present not only in the infected spinal fluid, but also in the *nasopharynx* of cases and of carriers. The germs are passed from person to person chiefly by droplet infection. It is easy to understand, therefore, why *overcrowding* plays so important a part in the spread of the disease.

A striking feature of meningitis epidemics is the small number of cases with typical meningeal involvement in proportion to the number of individuals exposed to the germs. Many persons become carriers of the organisms, rather than victims of a typical meningitis. There may be ten times as many carriers as there are cases of frank meningitis. These carriers, of course, act as important sources of infection for the more susceptible members of the group, and particularly for the newcomers. The organisms may remain in the nasopharynx of carriers for a month or more. In army camps, it has been noted that the majority of the cases have occurred among new recruits, and that the prevalence was especially high in large camps,

where there were a good many unseasoned troops and frequent changes in the camp population.

Meningococcal bacteremia and septicemia. Once virulent meningococci have reached the nasopharynx of a susceptible person, there is little doubt that the usual course of events is as follows:

The organisms set up a mild local inflammation, though this is probably not clinically recognizable unless there are accompanying virulent bacteria or viruses. Then the meningococci invade the blood stream, and soon the signs and symptoms of meningitis, together with more or less obvious evidence of a generalized infection, suddenly appear. The meningococci are present in the circulating blood, but also, with a predilection not explained, they have localized on the meninges. Severely ill patients show a rash, which is a further sign of a bacteremia, and which accounts for the name "spotted fever," sometimes applied to epidemic meningitis. The organisms may sometimes be recognized in direct smears from these skin lesions.

The clinical picture of meningococcal disease varies a great deal, according to whether the microorganisms are causing a generalized blood invasion primarily, with little or no localization on the meninges, or whether there is an infection confined almost entirely to the meninges, with only a transient or intermittent bacteremia. In any sizable epidemic among children or young adults, there will always occur a certain proportion of cases that would be better described as meningococcic septicemia, rather than typical meningitis. Some of these cases, moreover, especially in young children, are extraordinarily acute, overwhelming blood invasions, which progress so rapidly that the patient dies within five or six hours after the onset of symptoms. At autopsy, a massive hemorrhage into the adrenal glands is often, though not invariably, found. Sometimes, in an adult, death comes in this way with such unexpected suddenness that the case is one requiring investigation by the coroner. On the other hand, some patients have a low-grade, chronic meningococcus bacteremia that may persist for months, if untreated.

Laboratory diagnosis and detection of carriers. The bacteriological diagnosis of meningococcic infection depends upon finding meningococci in the spinal fluid, or blood. Nasopharyngeal cultures are also indicated. Sometimes microscopic preparations are made from the skin spots.

Spinal fluid is collected by inserting a sterile lumbar puncture needle into the spinal canal; the fluid is usually turbid and contains many pus cells. A smear made from the sediment after centrifuging the fluid will show the typical Gram-negative diplococci, most of them within the cytoplasm of the leukocytes. If free organisms are plentiful, they may be positively identified, and grouped at the same time, by performing capsule-swelling tests with specific rabbit antisera directly on the spinal fluid sediment. It may happen, however, that there are few or no recognizable organisms in the fluid, because the meningococci have autolyzed. In any case, cultures should be made by streaking the fluid over plates of fresh blood agar or chocolate agar previously warmed to body temperature. The plates should be incubated at once in a CO_2 jar. Often it is worth while to incubate the spinal-fluid specimen itself for about eighteen hours, and then make further microscopic studies and transfers.

Fig. 92. A West tube for making cultures from the nasopharynx. The cotton plug at the curved end of the glass tube is removed, and this end of the tube is passed into the mouth and back of the uvula. The swab is then pushed out, and rubbed over the nasopharynx, then withdrawn into the tube before removal from the mouth.

Blood cultures are made by drawing 10 cc of the patient's blood from an arm vein and adding it directly to a flask containing 100 cc of a warmed, infusion glucose broth, or brain medium.

Cultures from the nasopharynx are obtained by use of a special type of swab, made on a bent wire (Fig. 92). After this swab has been rubbed over the nasopharynx, it is streaked at once upon a warmed blood agar plate. This method of culturing from the nasopharynx is the routine method used for the *detection of carriers* of meningococcus.

The identification of the meningococci obtained in cultures, and their differentiation from the several varieties of harmless Gram-negative diplococci found normally in the throat, depends upon recognition of their somewhat characteristic colonies on blood agar, their failure to develop on simple culture medium or at room temperature, their capacity to ferment glucose and maltose but not

lactose or sucrose, absence of pigment, and their agglutination by a polyvalent or a group-specific antimeningococcus serum. The use of diagnostic antisera prepared by immunization of chickens is a recent development. It is necessary to identify the cocci definitely because rare cases of meningitis are caused by Gram-negative diplococci other than *N. meningitidis,* especially *N. flavescens.*

Serum treatment versus chemotherapy. Until recent years, the treatment of meningococcus meningitis with a polyvalent therapeutic horse antiserum was regarded as a valuable measure for reducing mortality. At present, however, serum is used only in exceptional cases. The use of sulfa drugs alone is apparently sufficient in the vast majority of instances. The death rate in this formerly highly fatal disease now averages only about 10–15%.

REFERENCES

Eye Infections

KORNS, R. F., SANDERS, M. and ALEXANDER, R. C.: "Epidemic Keratoconjunctivitis," *Am. J. Pub. Health,* June 1944, 34:567.

MAY, C. H.: *Manual of the Diseases of the Eye.* Baltimore: Wm. Wood & Co., 16th Ed., 1939.

MCKEE, S. H.: "Certain Virus Diseases of the Eye," *Can. Med. Assn. J.,* Mar. 1944, 50:261.

REID, R. D. and BRONSTEIN, L. H.: "Meningococci Conjunctivitis," *J.A.M.A.,* Mar. 11, 1944, 124:703.

THYGESON, P.: "Viruses and Virus Diseases of the Eye—II. Viruses of Ocular Importance," *Arch. Ophth.,* Mar.–Apr. 1943, 29:418, 635.

WEISS, C.: "Bacteriologic Observations on Infections of the Eye," *Arch. Ophth.,* July 1943, 30:110.

Ear Infections

BALLENGER, A. C.: *A Manual of Otology, Rhinology, and Laryngology.* Philadelphia: Lea & Febiger, 1940.

MARTIN, R. C., DANNA, D. and SKELLY, J. F.: "Suppurative Otitis Media and Its Complications," *Arch. Otol.,* May 1943, 37:727.

Meningitis

ALEXANDER, HATTIE E.: "Experimental Basis for Treatment of Hemophilus Influenzae Infections," *Am. J. Dis. Children,* Aug. 1943, 66:160; "Treatment of *Hemophilus influenzae* Infections and of Meningococcic and Pneumococcic Meningitis," *Ibid.,* Aug. 1943, 66:172.

BELSON, P. B. and WESTERMAN, E.: "Cerebrospinal Fever; Analysis of 3575 Case Reports, with Special Reference to Sulfonamide Therapy," *Brit. Med. J.,* Apr. 24, 1943, 1:497.

BIRDSONG, M., WADDELL, W. W., JR. and WHITEHEAD, B. W.: "Influenzal Meningitis," *Am. J. Dis. Children,* Mar. 1944, 67:194.

MARTLAND, H. S.: "Fulminating Meningococcic Infection with Bilateral Massive Adrenal Hemorrhage (Waterhouse-Friderichsen Syndrome)," *Arch. Path.,* Feb. 1944, 37:147.

PHAIR, J. J., SHOENBACH, E. B. and ROOT, C. M.: "Meningococcal Carrier Studies," *Am. J. Pub. Health,* Feb. 1944, 34:148.

THOMAS, L. and DINGLE, J. H.: "Investigations of Meningococcal Infection—I. Bacteriologic Aspects," *J. Clin. Invest.*, May 1943, 22:353.

SMITH, H. W., THOMAS, L., DINGLE, J. H. and FINLAND, M.: "Meningococcemia," *An. Int. Med.*, Jan. 1944, 20:12.

SARTWELL, P. E. and SMITH, W. M.: "Epidemiological Notes on Meningococcal Meningitis in the Army," *Am. J. Pub. Health,* Jan. 1944, 34:40.

REVIEW QUESTIONS—CHAPTER XXX

1. What organisms are normally found on the conjunctiva? What is lysozyme? What may happen when the secretions which normally bathe the eyeball dry up?
2. Describe the nature and the cause of: (*a*) sties, (*b*) ulcerations of the cornea, (*c*) infections of the lacrimal apparatus.
3. What is "pink-eye"? How does this infection spread?
4. Name another organism commonly causing acute catarrhal conjunctivitis.
5. Describe some other forms of subacute or chronic conjunctivitis, and name the causative organisms.
6. Describe gonorrheal ophthalmia in the newborn. How is this infection prevented?
7. Name three important virus infections of the eyes.
8. Describe the occurrence, manner of spread, and causative agents of trachoma and infectious keratoconjunctivitis.
9. Describe two infections of the external auditory canal.
10. What is *otitis media*? When may it occur? What organisms may cause it? What is *mastoiditis,* and what germs are usually responsible?
11. Describe lateral sinus thrombosis, extradural abscess, and brain abscess. What bacteria are likely to be responsible?
12. What is meant by *meningitis*? What are the meninges?
13. What organisms may cause a secondary meningitis, and under what circumstances is this infection likely to occur?
14. Name the four organisms most commonly responsible for a primary meningitis.
15. What is the importance of influenzal meningitis? Name and characterize the causative organism.
16. Outline the essential procedures for making a bacteriological diagnosis of influenzal meningitis. Why is it important to ascertain the serological type of the bacillus present?
17. What is the importance of epidemic cerebrospinal meningitis? Describe morphological and physiological properties of meningococci.
18. Describe the mode of infection, and the manner of spread, of epidemic meningitis. What clinical states, other than a frank meningitis, may occur as the result of infection with meningococci?

19. Outline methods of making a bacteriological diagnosis of epidemic meningitis. What method is used to detect meningococcus carriers?
20. What is the present-day opinion concerning the use and value of antimeningococcus serum versus chemotherapy in the treatment of meningitis?

CHAPTER XXXI

INFECTIONS OF THE GENITO-URINARY TRACT. THE VENEREAL DISEASES

A considerable number of microorganisms, of both aerobic and anaerobic types, are to be found normally upon the external genitals in both sexes. In the female an especially varied flora is always present, including aerobic diphtheroids, staphylococci, sarcinae, yeasts, colon bacilli and related organisms, and spirochetes, fusiform bacilli, and other anaerobes like those in the mouth. Protozoa of the genus *Trichomonas* are important members of this flora (Chapter XLII). The harmless acid-fast bacillus known as *Mycobacterium smegmatis* is usually found in the genital region.

The normal flora of the vagina is a limited one, probably because of the acidity of the normal vaginal secretions. A large aerobic Gram-positive bacillus, called *Döderlein's bacillus* (probably identical with *Lactobacillus acidophilus*), is often the most conspicuous organism seen in vaginal cultures. It is not always present, however. There may be a few other organisms, such as diphtheroids, and Gram-positive cocci. Many of these are *anaerobic.*

The bacteria on the external genitals are ordinarily harmless. Some of them, however—the fusiform bacilli and spirochetes, for example—are potentially pathogenic, and may be responsible for local ulcerations or other inflammations. Infection of the uterus after childbirth (puerperal fever) is undoubtedly caused, in some instances, by bacteria normally in the vulva and vagina.

The internal genito-urinary organs—bladder, ureters, kidneys, testes, uterus, and ovaries—are normally sterile.

Infections of the urinary tract. Acute or chronic infections, extending up the urethra to the bladder (*cystitis*) or involving the ureter and the pelvis of the kidney (*pyelitis*), are common. Young female children are especially liable to develop a urinary-tract infection, which may persist in chronic form for years. In adults, kidney stones, enlarged prostate glands, and other conditions which may

interfere mechanically with the urinary passages often lead to cystitis or pyelitis.

The organisms usually responsible for these infections are customarily thought of as falling into two groups: Gram-positive cocci or Gram-negative bacilli. The varieties of cocci most frequently found are *Staphylococci,* and the enterococci (*Streptococcus fecalis*). Occasionally, virulent hemolytic streptococci of Lancefield's Group A are present. Among the Gram-negative bacilli, the organism most commonly encountered is *Escherichia coli.* Various other intestinal bacteria, however, may be involved, especially members of the genus *Proteus* and, less commonly, organisms of the genera *Pseudomonas, Klebsiella,* or *Alkaligenes.* Cystitis sometimes occurs as a complication of gonorrhea, and then is due to *Neisseria gonorrhea* alone or in association with other organisms. Tuberculosis of the kidney or bladder is often complicated by a secondary infection with staphylococci, enterococci or colon bacilli, or with a mixture of these bacteria.

Puerperal fever. This disease, sometimes called childbed fever, is due to infection of the uterus after childbirth. The germs usually spread into the blood stream from the infected uterus, and a fatal septicemia often results.

The great majority of severe and fatal cases of puerperal fever are caused by *hemolytic streptococci* (rarely other organisms) *introduced into the uterus from without.* This type of puerperal infection was common before the introduction of aseptic methods into obstetrics. It was then the doctor, nurse, or midwife who innocently carried the deadly germs from patient to patient.

Oliver Wendell Holmes (1809–1894), in his celebrated essay "On the Contagiousness of Puerperal Fever" (1843), was among the first to call attention to the infectious nature of puerperal fever. About the same time, a young Hungarian physician named Semmelweis (1818–1865) began to teach the same idea. He observed how frequently puerperal fever occurred in patients attended by students who were accustomed to go directly from the autopsy room to the maternity ward, and he came to the conclusion that uterine infection is caused by matter introduced into the birth canal from outside sources, through the hands of the nurse, doctor, or other attendant. He succeeded in greatly reducing the amount of puerperal fever in his maternity clinics by the simple measure of requiring all attendants to wash and disinfect their hands before examining

a patient. Other doctors were strongly opposed, however, to the ideas of Semmelweis, and his methods were never widely adopted nor well known until long after the independent work of Lister (beginning 1864) had finally brought about the general use of antiseptic technique. Semmelweis struggled with such fanatical zeal to get his methods accepted that he finally lost his reason, and in 1865 was committed to an asylum. Curiously enough, at the time of this retirement, he was suffering from an infection of a wound on his hand, and in a short time he died of septicemia—the very disease for the relief of which he had already sacrificed so much.

Nowadays, infection of the uterus, after childbirth, with bacteria introduced *from outside* should never occur, and when it does, it is evidence of carelessness, or ignorance of modern aseptic technique, on the part of the midwife or other attendant. *Streptococcus hemolyticus* (Group A) is usually the causative germ. Fatal streptococcus infections following criminal attempts to produce abortion are common. Other cases of postabortal infection are due to *Clostridium perfringens,* the gas gangrene bacillus.

Puerperal fever is apparently caused, in some instances, not by germs introduced from outside sources, but by *organisms naturally present in the birth canal.* These bacteria are especially liable to be carried into the uterus in cases with long and difficult labor. They set up, usually, a mild infection, but sometimes cause a serious or fatal illness. In these cases, cultures from the infected uterus and blood often yield the same type of bacteria that are normally found on the surfaces of the vulva and vagina. Certain *anaerobic streptococci* are the predominant germs in many cases. *Bacterium melaninogenicum* is often present, accompanied by other anaerobes, in both the uterine and the blood cultures. The danger of uterine infection arising from these organisms normally present emphasizes the great importance of thorough disinfection of the external genitals before delivery.

Specific infections of the genital organs. Prevalence and importance of venereal diseases. There are three common infectious diseases which are usually localized, in their early stages, at least, on the genital organs. These are (1) *chancroid,* (2) *gonorrhea,* and (3) *syphilis.* These diseases are generally referred to as *venereal diseases,* i.e., diseases acquired by sexual contact, although by no means all cases arise in this way.

It would be difficult to overemphasize the importance of gon-

orrhea and syphilis. Together, they constitute the greatest of perils, not merely to the individual sufferer, but to the happiness of the family and the future of the race. There are no wholly reliable figures as to the prevalence of these diseases, because only a fraction of all the cases is ever discovered by public-health authorities, but there is no question that these infections are among the most widespread of human diseases. Recent estimates put at 3,200,000 the total number of persons who had syphilis in the United States in 1941. Among the entire white population, about 2% are thought to have syphilis, whereas the rate among Negroes is about 12%. Gonorrhea is still more prevalent. Both diseases are especially common among young people 20–30 years of age.

Three other, less prevalent, specific infections that primarily involve the genital region are: (4) *granuloma inguinale,* (5) *fusospirochetal infection,* and (6) *lymphogranuloma venereum.*

CHANCROID

Chancroid is a common infection of the external genitals, characterized by multiple, small, painful ulcerations. The lesion is called a *soft chancre.* It somewhat resembles the primary sore of syphilis, but never becomes hardened as in the case of the syphilitic chancre. Often the inguinal glands become involved, eventually forming pus-filled buboes. Chancroid spreads almost entirely by sexual contact. It has no serious consequences, and yields promptly to local treatment.

Hemophilus ducreyi. Chancroid is due to infection with a small Gram-negative, nonsporebearing, nonmotile bacillus, first described by Ducrey (1889), and generally known as the *Ducrey bacillus.* The organism is now formally named *Hemophilus ducreyi.* In smears from the ulcerating sore, the bacilli appear as short, oval rods, in pairs or chains. They are delicate, parasitic organisms, not resistant outside the body, but easily killed by dilute disinfectants or by unfavorable temperatures. As would be expected, they are difficult to cultivate in the laboratory, but they can be isolated on freshly made media enriched with blood.

Bacteriological diagnosis. Direct smears, cultures, and skin tests are used in the diagnosis of chancroid. Gram-stained *smears* made directly from the soft chancre, or from the pus aspirated from a bubo, reveal the typical organisms (Fig. 93).

Cultures from the same materials may be made by inoculating freshly made cystine-glucose-beef-infusion rabbit blood agar. Incubation is best carried out at 30° C, with care to maintain a moist atmosphere in the cultures. *Skin tests* are made by introducing intradermally 0.1 cc of a suspension in saline of heat-killed *H. ducreyi*. If the patient has chancroid infection, and has become sensitized to the organism, an inflammatory reaction appears at the site of injection, in about forty-eight hours.

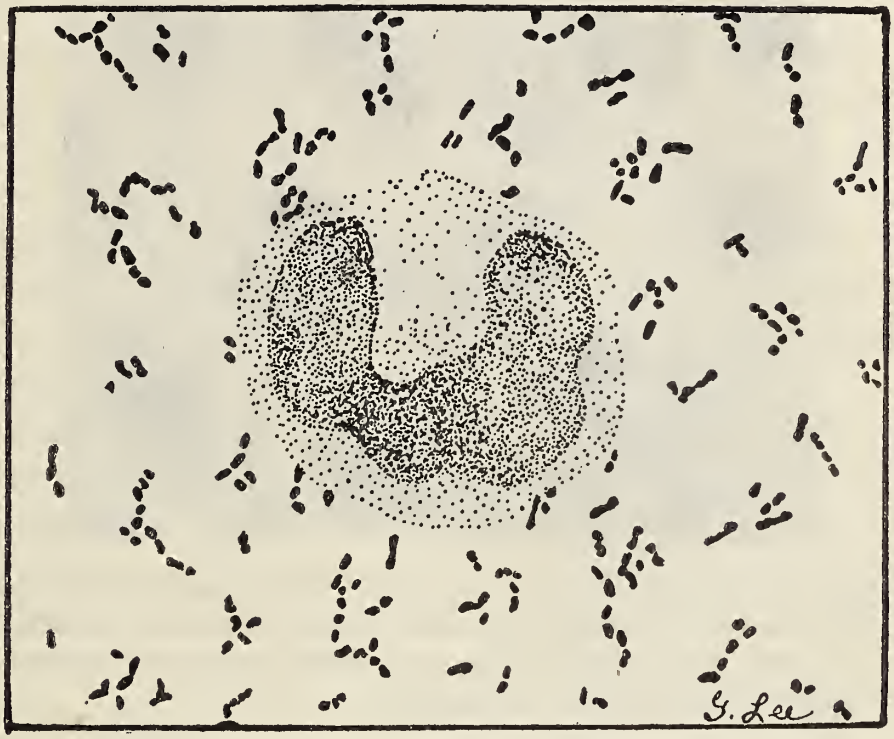


FIG. 93. *Hemophilus ducreyi* in the pus aspirated from a typical bubo in a patient suffering from chancroid infection. Drawing represents a single microscopic field.

With pure cultures of *H. ducreyi* Greenblatt, Sanderson and coworkers have reproduced typical chancroid disease in human volunteers, and also have demonstrated the remarkable prophylactic and therapeutic action of sulfonamide drugs in this disease.

GONORRHEA

Gonorrhea is a specific disease always caused by a particular species of Gram-negative diplococci, called *Neisseria gonorrheae* (the *gonococcus*). This organism was discovered by Neisser in 1879. Gonorrhea does not occur in any of the lower animals; it is strictly a human disease.

Neisseria gonorrheae. *Morphology and staining.* The gonococci are small, kidney-bean-shaped cocci, occurring in pairs, with the concave borders facing one another. They are indistinguishable, in appearance, from meningococci and other members of the genus *Neisseria.* Capsules have been demonstrated (with difficulty) by a few investigators, but they are not to be seen in ordinary preparations. In pus from acute cases of gonorrhea, the gonococci are chiefly intracellular—i.e., they occur *within the cytoplasm of the pus cells;*

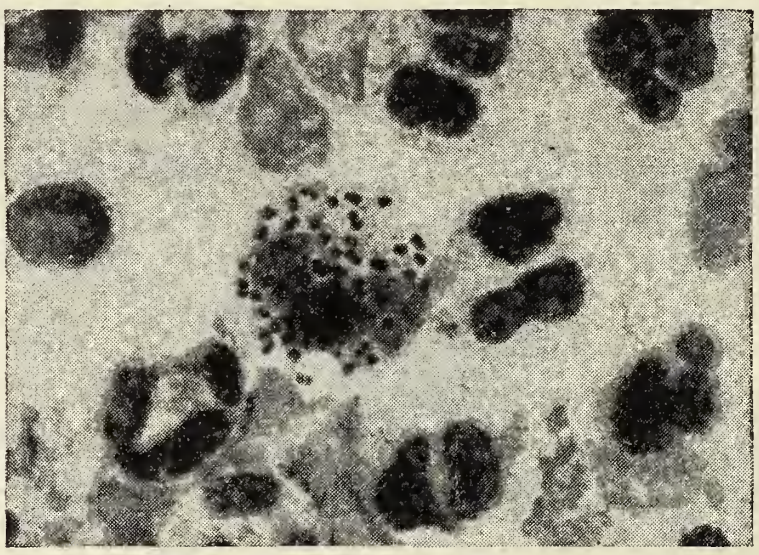


FIG. 94. *Neisseria gonorrheae* (gonococci) in a direct smear of the pus from a case of acute gonorrhea, showing the characteristic occurrence within the cytoplasm of a pus cell. The large dark bodies in the photograph are the nuclei of the pus cells (polymorphonuclear leukocytes).

often thirty or more pairs may be crowded into a single cell (Fig. 94). They may also be found in small numbers outside the cells. They color intensely with the usual stains, and are frankly Gram-negative.

Physiological properties. The gonococcus, like the meningococcus, is strictly parasitic, and is even more difficult to cultivate in the laboratory. Various special types of media, enriched with blood or other body fluids, are used. When a growth is obtained, it dies off quickly unless transplanted frequently to new media. Multiplication occurs only at body temperature, in the presence of abundant moisture, and is improved by incubation of the cultures in a CO_2 jar.

The difficulty in growing gonococci in the laboratory, and their failure to infect animals, make clear how very closely these organisms are adapted to growth on the *living human body.* They will survive only for brief periods outside of the body. This is the rea-

son why gonorrhea is almost always transmitted by direct bodily contact, and principally by sexual intercourse. Moderately high temperatures and weak disinfectants kill gonococci readily. It must be remembered, however, that *the germs may remain alive in pus on clothing or other objects for several hours,* or until the pus is completely dried.

Clinical forms of gonorrhea. In adults, gonorrhea begins as an acute inflammation of the urethra, accompanied by a discharge of pus containing gonococci in great numbers. The inflammation usually appears within a week after the infection has been acquired. If the disease could always be successfully treated at this stage, it might not be greatly feared. But the inflammation almost invariably spreads, extending into the deeper structures, including the organs of reproduction. It becomes *chronic,* instead of acute, and chronic gonorrhea is an obstinate, treacherous disease, resistant to treatment. In the chronic stage there may be little or no discharge. The germs are not so numerous, but they are producing deep-seated degenerative changes in vital internal organs.

The effects of chronic gonorrhea are serious, both for the individual and for society. Gonorrhea is the prime cause of sterility—the condition in which conception cannot occur—resulting in childless marriages. This condition is brought about by injury to the reproductive organs themselves, or by blocking of the passages through which the reproductive cells—the male sperm and the female ovum—must travel. In women, chronic gonorrhea is responsible for a long list of troubles, some requiring surgical treatment; a large proportion of the patients who come to the gynecological clinic are suffering from the effects of this disease. The gonococci may invade the blood stream, and give rise to many complications, such as a severe arthritis or endocarditis. The gonococci are, in fact, among the most virulent germs known.

The complete cure of gonorrhea, especially if effective treatment is delayed until the disease has become chronic, is difficult and uncertain. Many persons have been innocently infected by others who thought themselves cured. There is mounting evidence that the new chemotherapeutic agents now used may often bring about a disappearance of symptoms without a real cure, that is, without eliminating all the virulent gonococci.

In view of its great frequency, its tendency to become chronic, the serious effects of the chronic form on the individual and the

race, and the uncertainty of cure, gonorrhea must be regarded as fully as serious a disease as the more greatly feared syphilis.

At one time, gonorrheal infection of the eyes of infants (*ophthalmia neonatorum*) was common, but since the universal adoption of the Credé method of prophylaxis—the application of silver nitrate solution to the eyes of all newborn babies immediately after birth—this disease has become rare.

In young female children there may occur a troublesome inflammation of the vulva and vagina due to gonococci. This does not usually spread to the internal genital organs, and although the infection is resistant to treatment, it eventually disappears without resulting in any permanent harm, when the mucous membrane of the vagina becomes matured. The importance of this disease lies in the ease with which it may spread among the children in a hospital or institution. Once a case appears in a children's hospital ward, the most careful attention to the sterilization of the patient's towels and other linen, and the most *conscientious practice of hand disinfection by the nurses,* are required to prevent the spread of the infection to other children. In children's hospitals vaginal smears are made from every female child upon her entering the institution. These smears are examined by the bacteriologist for gonococci, and if any suspicious organisms are found, the child is put under the strictest isolation at once.

Bacteriological diagnosis. A variety of procedures is used to determine the presence of gonorrheal infection. These include: (1) *direct smears,* (2) *cultures,* and (3) *immunological tests* (complement-fixation tests) *with the patient's blood.*

Direct smears. In *acute cases,* when the pus is abundant, the laboratory diagnosis of gonorrhea usually is not difficult. Smears are prepared from the urethral discharge and stained by Gram's method.

These should be carefully prepared by *rolling* the swab gently over a clean slide to make a thin, even film. If Gram-negative diplococci of the typical biscuit-shaped appearance are found grouped within the pus cells, this is sufficient evidence for all practical purposes that the infection is due to gonococci.

In *chronic gonorrhea,* diagnosis by means of smears may be impossible. The germs are not abundant and there may be no pus, so that it may not be possible to find the gonococci in their typical intracellular arrangement. In the male, it is usually necessary to

massage the prostate and seminal vesicles to obtain secretion for examination. In the female, smears are made from the cervix and other limited areas most likely to harbor the organisms.

Cultures. Cultivation of the gonococci from the patient is a supplemental procedure of proven value in both acute and chronic cases, and not infrequently will permit a definite diagnosis of gonorrhea when direct smears are doubtful or negative. A variety of special media has been devised for this purpose. Among the best is *chocolate agar medium*—made of infusion agar (with Difco proteose peptone #3) plus 10% of fresh, citrated blood, and heated just enough to make the medium turn brown. The medium must be moist, and previously warmed to body temperature. It should be heavily inoculated with material obtained directly from the patient. It is best to incubate the cultures in an atmosphere of 5–10% CO_2, as in a candle jar.

After growth appears, the colonies of gonococci may be quickly found by use of the *oxidase reaction.* This consists in pouring over the medium a sterile 1% solution of tetramethyl-phenylenediamine hydrochloride (or para-amino-dimethylaniline monohydrochloride), which is then drained off immediately. By action of the enzyme oxidase, possessed by the organisms, gonococcus colonies quickly turn a pinkish-purple color, and finally become black. The organisms are not killed at once by the reagent, and transplants from the reacting colonies may be made successfully if done before the colonies turn black. The general character of these colonies, the morphology of the organisms found on microscopic examination, the cultural requirements of these cocci in subculture, and fermentation reactions (acid from glucose but not from maltose) will definitely identify the gonococci.

No entirely satisfactory method has yet been found to keep gonococci alive in pus or urine long enough so that reliable results from culturing can be expected when the specimen must be transported any considerable distance from the patient to the laboratory. Many investigators are working on this problem, however, and further improvements in cultural methods for gonorrhea diagnosis may be expected.

Complement-fixation test. As additional evidence of gonorrheal infection, or as a diagnostic procedure when smears and cultures fail, particularly in those cases in which the germs have set up arthritis or other complicating infections in the internal organs, a

blood test similar in principal to the Wassermann test used for the diagnosis of syphilis may be performed. This is a complement-fixation test in which the patient's blood is mixed with an antigen consisting of a suspension of several different strains of gonococci.

Immunity. It is doubtful whether recovery from gonorrhea leaves any effective immunity at all; in any case, it is of only short duration, for in a brief period a second attack may be acquired as readily as the first. The present practice of using chemotherapeutic agents promptly will undoubtedly reduce materially the opportunity for the development of even a transient resistance.

SYPHILIS

This specific germ disease is caused by a kind of spirochete, now called *Treponema pallidum.* The organism was first recognized as the cause of syphilis by Schaudinn and Hoffmann, in 1905. It is still known to many doctors by its older name *Spirocheta pallida.*

Like gonorrhea, syphilis occurs naturally only in human beings. It is possible, however, to produce the disease in apes and in rabbits by artificial inoculation of material from cases of human syphilis. The disease was at one time an acute, severe infection, but after centuries, during which it has occurred continually throughout the world, it has now become milder, and often runs a long, chronic course. The curious name "syphilis" has its origin in a famous poem written by Fracastoro in 1530, in which a character named *Syphilus* is described as having the symptoms of the disease.

Treponema pallidum. *Morphology and staining.* Certain general properties of spirochetes of the genus *Treponema* have been described in Chapter V. The *Treponema pallidum*—the *pale* treponema—is an extremely delicate filament 7–14 microns long, with 6–14 sharp, closely set, and regular spirals. The ends taper to a fine point (Figs. 19, 95, and 96).

Photographs made with the electron microscope show that the spirochete is enclosed by a delicate cell wall, which is stretched between adjoining cells when longitudinal division occurs, then remains as a fine terminal filament. Flagella, often in groups of four, are found along the sides, or near the ends, of the treponema.

The syphilis spirochete is difficult to stain, and in smears prepared in the ordinary manner, and colored with the usual bacterial stains, it will not be seen at all. The method most widely used to demon-

strate *Treponema pallidum* in dried smears is the nigrosine relief stain.

The true morphology and characteristic motility of the syphilis spirochetes can be observed, however, only when the organisms are studied in the living state under a *dark-field microscope*. Here they appear as silvery coiled threads, the nearer half of each coil brightly illuminated, like a chain of commas. Ordinarily, in preparations from chancres, they show only a slight, slow, and intermittent locomotion, in contrast to the active motility of *Borellia refringens* and other kinds of spirochetes that may be present. When they do move, their stately, unhurried gait is characteristic. Though often remaining virtually stationary in the field, they do exhibit marked rotary movement—twirling around on their own axis, like a corkscrew—and also movements of flexion, i.e., sharp bending or buckling of the body, and sometimes an accordion-like compression and expansion of the coils. Throughout all these movements, however, the primary spirals remain intact.

FIG. 95. The syphilis organism (*Treponema pallidum*) in a section of the liver from a syphilitic fetus. The tissue is literally swarming with the spirochetes. Note the typical form in the upper right hand corner.

Physiological properties. In 1911, Noguchi reported that he had succeeded in cultivating *Treponema pallidum* in pure culture and with these cultures had reproduced syphilitic lesions in animals. Other investigators, however, have not been able to confirm his results entirely, and there is considerable doubt whether the truly *virulent* syphilis spirochetes have ever been isolated in pure culture.

Because of this uncertainty and the many technical difficulties encountered in its study, there is still much to be learned about this most important organism. It is clear that the syphilis spirochetes are extremely delicate microbes, and closely adapted to a parasitic life on the human body. They are killed rapidly by cold; by moderate heat; by drying; by weak disinfectants; and even by soap and water. They cannot live in the presence of air. These are fortunate circumstances, for if it were otherwise, syphilis would be even more common than it now is.

The sensitiveness of the spirochetes to mercury, bismuth, and arsenicals (salvarsan) is the basis of the successful chemical treatment long used everywhere for syphilis. Nowadays, it seems likely that these chemicals will eventually be replaced by penicillin, or other antibiotics. The fact that in infected animal tissue they are destroyed within an hour, when exposed to a temperature as low as 41° C, furnishes rational ground for the employment of fever-therapy in some cases.

How syphilis spreads. On account of the lack of resistance of the germ outside of the body, syphilis is rarely acquired in any way other than by actual bodily contact with the lesions of the disease in another person, though not necessarily through sexual intercourse. The infection may be transmitted by kissing a person who has a syphilitic sore in the mouth or throat. From the description given above, it is not hard to imagine how the syphilis organisms bore into the tissues. The spirochetes have the power of invading the body through very minute breaks in the skin, and probably can penetrate the unbroken mucous membranes. It is not uncommon for nurses, dentists, or physicians to acquire the disease as the result of infecting a finger during the examination or care of a patient.

It is possible for the disease to be transmitted through the common use of towels or drinking cups, but the chance of infection in this way is small. There is practically no danger in handling specimens of blood from syphilitic patients.

Syphilis is often transmitted by a syphilitic mother to her children (congenital syphilis). The transmission is not by direct inheritance, and the spirochetes cannot have come directly from the father, but the mother herself must be infected, even though outward symptoms of syphilis may be entirely lacking. The organisms reach the placenta from the mother's blood and, by growing through the placenta, invade the fetus. The result may be the death of the baby —stillbirths are far more common in syphilitic families than in non-syphilitic families; the baby may be born with active signs of the disease; or the infant may show no evidence of the disease at birth, but symptoms corresponding to the late stages of syphilis may develop several years later. Not all children of mothers with latent syphilis are necessarily infected. If the mother is treated with salvarsan during pregnancy, the danger of infecting the growing baby is practically eliminated. Hence, a blood test for *every mother* early in pregnancy is very much worth while.

Clinical forms of syphilis. The first sign of syphilis is the appearance of a sore called a *chancre,* at the spot where the spirochetes have entered, two to five weeks before. The chancre is not always typical in appearance, and it may be hidden so that it is overlooked. This primary sore literally swarms with the syphilis spirochetes.

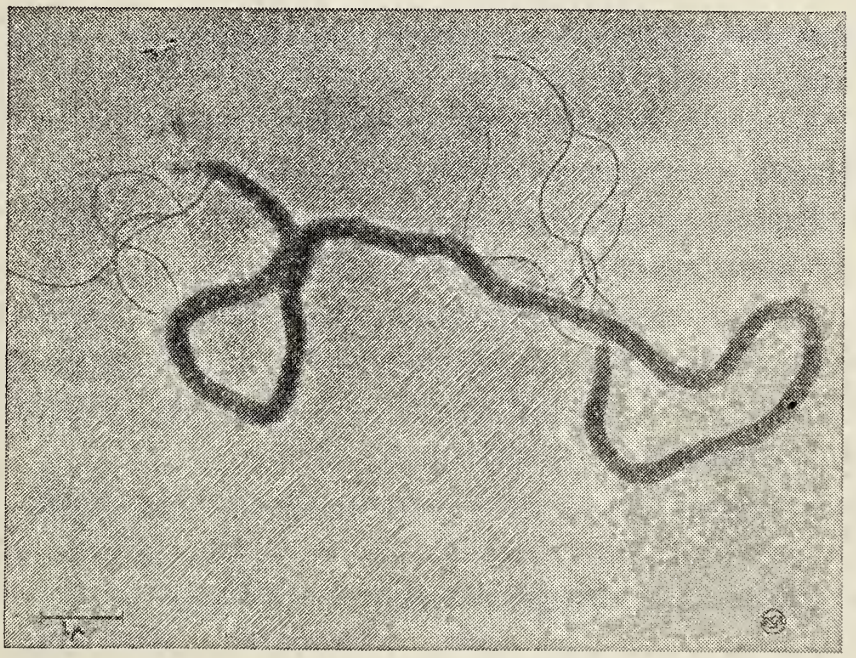


FIG. 96. *Treponema pallidum* (Kroo strain) photographed with the electron microscope. Note the tufts of flagella near the ends of the spirochete. (From Mudd, S., and Anderson, T. F., "Pathogenic Bacteria, Rickettsiae and Viruses as Shown by the Electron Microscope. I. Morphology," *J.A.M.A.,* 126:561, Oct. 28, 1944.)

Whether or not the proper treatment is given, the chancre soon disappears. (This makes possible the evil profits of the quack doctor.) However, serious trouble is just beginning, for the spirochetes are now invading the entire body. In about a month, or longer, the so-called secondary stage of the disease is established. This is characterized by a skin eruption, by sores in the mouth or throat, and by other evidences of an involvement of the entire body. At this secondary stage, the patient is a source of great danger to others, and syphilis has often been acquired innocently by contact with such persons.

After the acute symptoms of the secondary stage have passed, there follows a period of variable length, often months or years, in which the patient is practically without symptoms, though there may be relapses from time to time. The destructive effect of the

germs still present in the body becomes obvious at last, however, with the development of symptoms which may be of the most varied nature and may simulate many other diseases. The brain and spinal cord may become the seat of destructive lesions, resulting in locomotor ataxia (tabes dorsalis), general paresis, insanity, and death. During this third stage (late syphilis), the patient is not liable to transmit the infection to others.

The great variability in the clinical picture of syphilis, at all stages of the disease, is a well-recognized feature of the infection. For example, early syphilis may exist without characteristic symptoms; in females the disease is generally milder than in males; and its severity further varies with age and race. Good diagnostic laboratory aids are consequently of great importance.

Bacteriological and serological diagnosis. A laboratory diagnosis of syphilis may be made in two ways: (1) by microscopic examination of fluid from the chancre or (2) by serological tests with the patient's blood serum or spinal fluid.

Dark-field examination, and smears of chancre fluid. If typical spirochetes are found in the primary sore, this affords the earliest and surest proof that the patient has syphilis. Negative results are not entirely conclusive, however, because this dark-field examination of the chancre is difficult to carry out successfully. Only a trained person, aware of all the necessary minute details of technique, can be trusted to do it properly. In the well-made dark-field preparation of the serum squeezed out of a chancre, the spirochetes will be numerous, and readily recognizable by their characteristic morphology and motility, as described above. If a dark-field examination cannot be done, smears of the fluid may be made by the nigrosine method.

Blood (and spinal fluid) tests. After the primary stage, the laboratory diagnosis of syphilis is made by testing the blood (or, when involvement of the central nervous system is suspected, the spinal fluid) of the patient for the presence of the peculiar antibody formed as the result of syphilitic infection. Blood tests become positive two or three weeks after the appearance of the chancre. The best known of the various blood tests for syphilis is the *Wassermann test,* originally devised by Wassermann and Bruck (1906). This is a *complement-fixation test,* the principle of which has already been explained. The Wassermann test is occasionally positive in conditions other than syphilis, such as malaria and leprosy, and in

some circumstances may be negative in syphilitic individuals, but in general it gives reliable results and, when taken in connection with clinical signs and history, is of immense value as an aid to diagnosis and as a guide to treatment. The test is complicated, however, and subject to many errors. In all cases with doubtful clinical signs, repeated tests should be made before a positive diagnosis is accepted.

A number of other tests, simpler than the Wassermann, but equally reliable when properly performed, have been developed. Those most widely used in this country are the Kahn test and the Kline test. These are called "precipitation or flocculation tests," because a visible precipitate or clumps will be formed in positive cases when the blood serum of the syphilitic patient is mixed directly with the antigen preparation.

As the patient recovers following treatment, the Wassermann and precipitation tests become *negative*. This is in sharp contrast to the usual state of affairs. Ordinarily, the immunological tests we perform for diagnostic purposes on the patient's blood, in other infections, are most strongly positive at the time of recovery and shortly thereafter, indicating an abundant content of protective antibodies at that time. In the tests for syphilis, however, we detect an antibody not related to resistance, but associated in some way with the presence of spirochetes in the body. A positive Wassermann (or precipitation) test is, then, *a sign of syphilitic infection,* not an index of immunity, and naturally this sign disappears as the spirochetal infection is conquered.

Immunity. As indicated above, the course that syphilis takes in any individual is largely determined by the immune reactions of that person—by the character of the host-parasite relationship that is set up. In syphilis, these immunity factors are subtle and complex. Antibodies which will act directly upon the syphilis spirochetes may be demonstrated in the blood of syphilitic patients, but resistance to the infection seems to depend principally upon some obscure cellular change which makes the tissues unfavorable for the organisms. This change develops during the course of syphilis, and it is a well-established fact that as long as a person is obviously infected with the syphilis spirochete, *reinfection* with this germ is very rare. A full recovery from the disease, however, does not leave any effective immunity, for reinfection may then occur, and the individual appears to be as susceptible as ever.

GRANULOMA INGUINALE

Prevalence and clinical features. This infection (also called granuloma venereum) was formerly regarded as almost exclusively a tropical disease, but in recent years it has been shown to be endemic in the United States, and by no means uncommon, particularly among the colored race in the South. Beginning as a small, moist papule on or near the external genitalia, the infection gradually creeps into the surrounding mucous membranes and skin, and may eventually become a large superficial ulcerating lesion, involving a considerable area of the groin. The disease remains localized to the pubic region, and despite severe and extensive lesions there, the patient usually shows little or no signs of general illness. The causative agent of granuloma inguinale is presumably transmitted by sexual contact, but the disease is not highly contagious, and may possibly be acquired in some other way. Remarkable clinical improvement occurs, in most cases, following intravenous injections of tartar emetic, an antimony compound.

Etiology and diagnosis. Smears made from the ulcerating lesions reveal large mononuclear cells (macrophages) containing numerous round or oval bodies, called *Donovan bodies*. Laboratory diagnosis of the disease is now based upon this finding. The smears may be stained with Wright's stain or by other special methods. The intracellular Donovan bodies appear as deep pink, coccoid structures. Sometimes clusters of these bodies are seen lying within a vacuole in the cytoplasm (Fig. 97).

The nature of the Donovan bodies has been a matter of controversy. Recent investigations by Anderson and associates leave little doubt, however, that these bodies are capsulated bacteria-like microorganisms of peculiar nature, cultivable only in the yolk-sac cells or in the yolk of chick embryos. The name *Donovania granulomatis* is proposed. Suspensions of the microbes from cultures give specific skin reactions in persons with granuloma inguinale.

FUSOSPIROCHETAL INFECTION

The same combination of microorganisms responsible for the ulceration in the throat known as Vincent's angina (Fig. 80, p. 401) may cause similar ulcerating lesions of the external genitalia

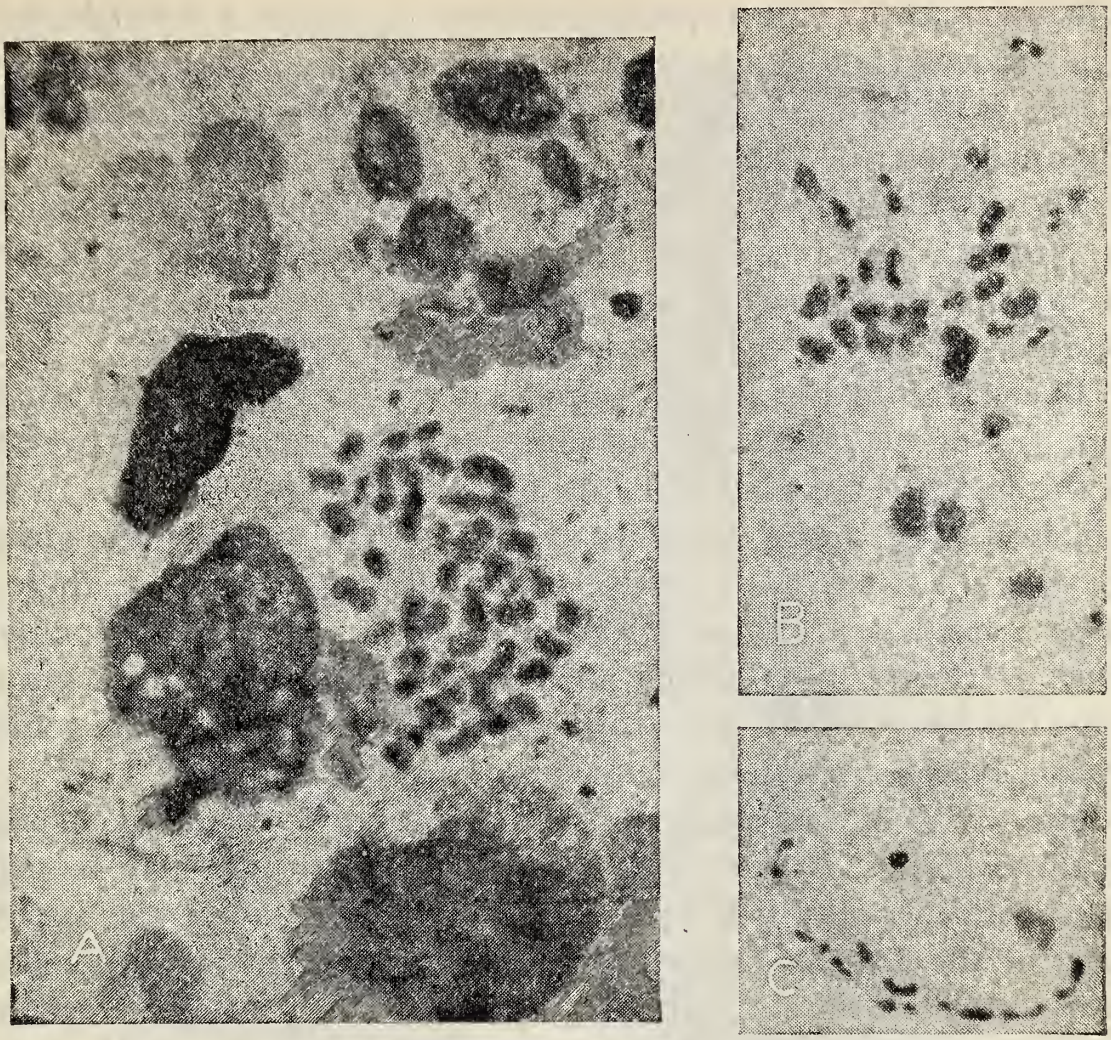


FIG. 97. A: *Donovan bodies* within the cytoplasm of a large mononuclear cell in a direct smear from the skin-ulceration in a case of granuloma inguinale. The large, roughly oval-shaped body at the lower left is the nucleus of this cell. B and C: Encapsulated and unencapsulated microorganisms in smears from the yolks of chick embryos inoculated with material from human lesions of granuloma inguinale. These are believed to be active, growing forms of the Donovan bodies. Smears stained by Wright's method; magnification 2000 times. (From Anderson, K., De Monbreun, W. A. and Goodpasture, E. W., "An Etiologic Consideration of *Donovania Granulomatis* Cultivated from Granuloma Inguinale [three cases] in Embryonic Yolk," *J. Exper. Med.*, 81:25, 1945.)

in either male or female. The bacteriological diagnosis is readily made by microscopic examination of direct smears from the lesions, stained by gentian violet or the nigrosine relief method.

LYMPHOGRANULOMA VENEREUM

Prevalence and clinical features. This is the disease known in the tropics as *climatic bubo,* and also called the sixth venereal disease. For a long time confused with other diseases of the genitalia,

it is now known to be a specific infection caused by a *filtrable virus.* The student should note particularly that *lymphogranuloma venereum* is a separate and distinct infection from the granuloma inguinale described above.

Though long known as a tropical disease, lymphogranuloma venereum has been recognized in recent years as an infection of wide occurrence in temperate regions as well. It is prevalent among the colored population in the southern United States.

There is probably a small initial sore, as in syphilis, but usually this cannot be found. In the male, there soon occurs a swelling of the inguinal lymphatic glands, forming the typical bubo. In the female it is the more deeply situated, intrapelvic lymphatic glands that are the usual site of active infection. In consequence, there is often considerable tissue-destruction and scarring, one common effect of which is to produce a stricture of the rectum. Unlike granuloma inguinale, which is a localized infection confined to the genital region, lymphogranuloma venereum may be generalized.

The virus of lymphogranuloma venereum. From the swollen inguinal glands, and other lesions, bacteria-free pus may be aspirated, filtrates of which have been shown to contain the specific virus of the disease. Inclusion bodies and elementary bodies have been described. Several animal species, including monkeys, are susceptible to experimental infection, and the virus is usually maintained in laboratories by serial transfer intracerebrally in white mice. Recently, cultures of the virus have been successfully made in the yolk sac of chick embryos. There is evidence that this virus, and also the psittacosis virus to which it is apparently related, form specific *toxins.*

Diagnosis; the Frei test. Frei, in 1925, found that an inflammatory (allergic) skin reaction is produced in lymphogranuloma-venereum patients when a minute amount of the diluted, heated pus from the swollen inguinal lymph gland of another patient is inoculated intradermally (Fig. 98). At the present time, the Frei test is widely used for diagnosis. The antigen now employed is a formalinized suspension of lymphogranuloma virus harvested by centrifugation of chicken-embryo yolk-sac cultures. This antigen is called *lygranum.* The test is performed in the same manner as the tuberculin test, and the results are interpreted similarly. A control test must be made with an antigen prepared from a noninfected yolk sac. The significance of the skin reactions obtained must be judged, of course, in the light of the full clinical history of the patient.

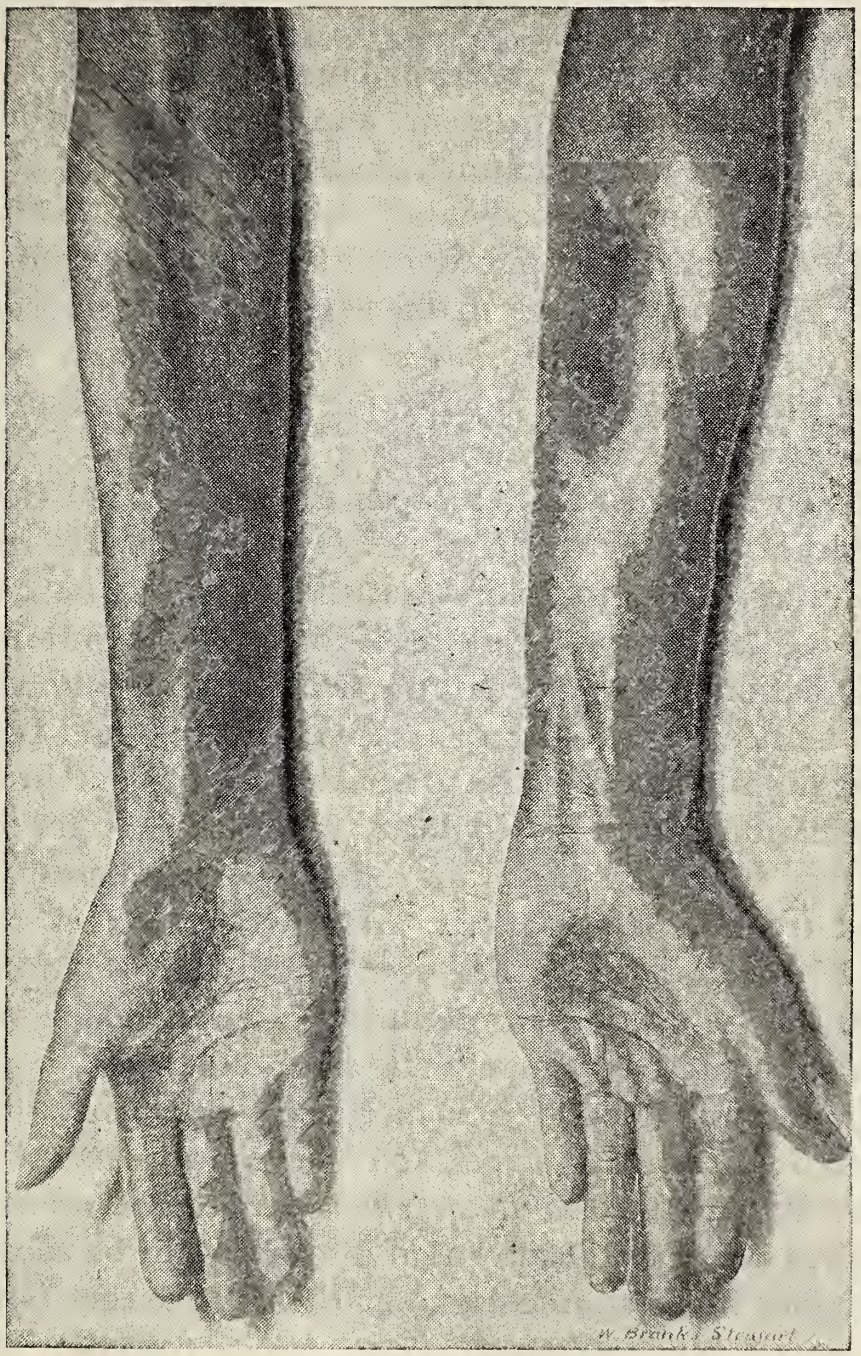


FIG. 98. Right, a positive Frei test (with each of two antigen preparations); left, the opposite arm showing no reactions from control injections. (Reproduced from a painting by W. Branks Stewart, Louisiana State University School of Medicine, New Orleans, La.; courtesy of Dr. Rigney D'Aunoy and Dr. Emmerich von Haam.)

PREVENTION OF VENEREAL DISEASES

Prevention of syphilis and gonorrhea is generally admitted to be a public-health matter of outstanding importance. It is partly a social and partly a medical problem, and obviously a difficult and complicated one. Efforts to improve social conditions in general,

and particularly measures to suppress commercialized prostitution, and promiscuity among adolescents and young adults, will eventually reduce the amount of venereal disease. The establishment of clinics for diagnosis and treatment, and of hospital beds for the isolation of cases in the communicable stages; discovery of cases by routine examination of food-handlers, employees in industrial plants, etc., and persons in public institutions of all kinds; reporting of all cases by physicians to the health authorities; and especially, *tracing cases to their sources* by investigations of trained nurses or social workers are among the principal measures which are now being tried in nearly every large community.

A great step forward will have been when every individual who has a suspicious sore or discharge, especially if he has knowingly exposed himself, comes to realize that the best thing he can do for himself, as well as for everyone else, is to submit at once to a thorough examination by a competent physician, and, if found diseased, to accept the necessary course of treatment. He should be willing, further, to supply the physician or health-department investigator with information about his contacts, in order that the chain of infection may be traced back, and other infected persons may be found and treated. Self-diagnosis, and particularly self-treatment, ought to be discouraged. It has been found that self-dosing with sulfa drugs may result only in producing cases of hidden gonorrhea —cases with symptoms temporarily suppressed, but not free of virulent gonococci. If every case of syphilis and gonorrhea could be discovered in its earliest stages, and *intensively treated by trained physicians,* the patients *would quickly be rendered noninfectious,* the disastrous effects of the advanced disease would be avoided, and these infections would soon cease to be the menace they now are.

REFERENCES

Urinary-Tract Infections

LEVINE, M. G.: "Methods of Urologic Bacteriology—I. Isolation and Identification of Cocci from Urine and Discharges," *Urol. and Cutan. Rev.,* 1942, 46:491. "II. Isolation and Identification of Gram-Negative Rods from Urine," *Ibid.,* Sept. 1943, 47:499.

Puerperal Fever

CONTI, E. A., O'LAUGHLIN, D. L., MCMEANS, J. W. and LIPMAN, G. S.: "A Study of the Bacteriology of the Cervix During Pregnancy, and Its Relation to Puerperal Morbidity," *Surg., Gyn. & Ob.,* Sept. 1941, 73:367.

DE KRUIF, P.: "Semmelweiss, Savior of Mothers," in *Men Against Death.* New York: Harcourt, Brace & Co., 1932.

Heaton, C. E.: "Control of Puerperal Infection in United States During Last Century," *Am. J. Ob. & Gyn.,* Oct. 1943, 46:479.

Salm, R.: "Occurrence and Significance of Clostridium Welchii in the Female Genital Tract," *J. Ob. & Gyn. Brit. Emp.,* Apr. 1944, 51:121.

Chancroid

Greenblatt, R. B., Sanderson, E. S., Mortara, F. and Kupperman, H. L.: "Experimental Prophylaxis of Chancroid Disease," *Am. J. Syph., Gonor. & Ven. Dis.,* Mar. 1943, 27:30.

Knott, L. W. and Others: "Differential Diagnosis of Lymphogranuloma Venereum and Chancroid by Laboratory and Skin Tests," *Am. J. Syph., Gonor. & Ven. Dis.,* Nov. 1943, 27:657.

Gonorrhea

Carpenter, C. M., Ackerman, H., Winchester, M. E. and Whittle, J.: "Correlation of In Vitro Sulfonamide Resistance of Gonococcus with Results of Sulfonamide Therapy," *Am. J. Pub. Health,* Mar. 1944, 34:250.

Goodale, W. T., Gould, R. G., Schwab, L. and Winter, V. C.: "Laboratory Identification of Sulfonamide-Resistant Gonococcic Infections," *J. A. M. A.,* 1943, 123:547.

Lankford, C. E.: "A Critical Study of the Laboratory Methods of Diagnosis of Gonorrhea in Women," *Tex. State J. Med.,* 1941, 37:553.

Mahoney, J. F., Van Slyke, C. J., Wolcott, R. R., Thayer, J. D. and Nimelman, A.: "Culture Studies in Chronic Gonorrhea of Women," *Am. J. Syph., Gonor. & Ven. Dis.,* 1942, 26:38.

Nelson, N. A.: "Present Status of Control of Gonorrhea," *Am. J. Pub. Health,* Feb. 1944, 34:159.

Thomas, R. B.: "The Gonococcus and Gonococcal Infections," *Am. J. Syph., Gonor. & Ven. Dis.,* 1942, 26:691.

Syphilis

Brunfield, W. A., Lade, J. H. and Feldman, L. L.: "Epidemology of Syphilis, Based upon Five Years' Experience in an Intensive Program in New York State," *Am. J. Pub. Health,* Aug. 1942, 32:793.

Cares, R.: "Dark-field Diagnosis of Penile Lesions; Differential Motility Characteristics of *Treponema pallidum,*" *J. Lab. & Clin. Med.,* Jan. 1944, 29:82.

Kampmeier, R. H.: *Essentials of Syphilology.* Philadelphia: J. B. Lippincott Co., 1943.

Rosebury, T.: "Text of the Motion Picture: A Lecture on Spirochetes," *J. Lab. & Clin. Med.,* Aug. 1942, 27:1470.

Vonderlehr, R. A. and Usilton, L. J.: "Syphilis Among Men of Draft Age in the United States," *J. A. M. A.,* Dec. 26, 1942, 120:1369.

Granuloma inguinale

Anderson, K., DeMonbreun, W. A. and Goodpasture, E. W.: "An Etiological Consideration of *Donovania granulomatis* Cultivated from Granuloma Inguinale (three cases) in Embryonic Yolk," *J. Exper. Med.,* 1945, 81:25.

Lymphogranuloma venereum

Blair, John E.: "Complement-Fixation Reaction with the Antigen of Lymphogranuloma Venereum (Lygranum)," *J. Immun.,* July 1944, 49:63.

Hersel, E. B., Stroud, G., III, and Others: "The Chick Embryo Antigen (Lygranum) Test for Lymphogranuloma Venereum," *Arch. Dermat. & Syphilol.,* 1943, 48:379.

Rake, A. and Jones, H. P.: "Studies on *Lymphogranuloma venereum*—II. Association of Specific Toxins with Agents of the Lymphogranuloma-psittacosis Group," *J. Exper. Med.,* May 1944, 79:463.

REVIEW QUESTIONS—CHAPTER XXXI

1. What organisms may be present on the external genitals?
2. Describe common infections of the urinary tract, and the organisms usually concerned.
3. How is a bacteriological diagnosis of cystitis or pyelitis made?
4. What is puerperal fever? What are the cause and origin of severe and fatal cases? May the infection be from the patient herself?
5. What did Holmes and Semmelweis contribute to early knowledge of the nature of puerperal fever?
6. Name the three common specific infections of the genital organs. Name three other, less prevalent, specific infections, primarily involving the genital region. Discuss the prevalence and importance of gonorrhea and syphilis.
7. Describe chancroid. Name and describe the causative organism, and outline practical methods of bacteriological diagnosis.
8. Name the organism causing gonorrhea. Describe the morphology and the staining of gonococci, and their appearance in smears of pus.
9. Describe the principal physiological properties of gonococci. What resistance do they have outside of the body?
10. Describe the clinical forms of gonorrhea in adults. What serious effects may the disease have on the individual, and on the race?
11. What form of gonorrheal infection was formerly common in infants? How is this now prevented?
12. What form of gonorrheal infection may occur in young female children? What precautions must be taken to prevent its spread?
13. How is a bacteriological diagnosis of gonorrhea made: (*a*) in acute cases, (*b*) in chronic cases? Discuss the value and the limitations of: (1) smears, (2) cultures, (3) serological tests. Describe the oxidase reaction.
14. Is there any immunity to gonorrhea?
15. Name the causative organism of syphilis. Who discovered the organism, and when? Who gave the disease its name?
16. Describe the morphology, motility, and staining of *Treponema pallidum*. What is its characteristic appearance and behavior in dark-field preparations from chancres?
17. Has the syphilis spirochete been cultivated? What is known of its resistance outside of the body? Within the tissues?
18. How may syphilis be acquired? What is congenital syphilis?
19. Describe briefly the three clinical stages of syphilis. Are they equally dangerous to others?
20. In what two ways may a laboratory diagnosis of syphilis be made? What is the value of microscopic examinations of chancre fluid?

21. What is the principle of the Wassermann test? Discuss its value and limitations. Name two other blood tests.
22. What can be said concerning immunity to syphilis?
23. Characterize the infection called granuloma inguinale. How is the laboratory diagnosis made? Name the probable causative agent.
24. What is meant by fusospirochetal infection of the genitalia? How is the bacteriological diagnosis made?
25. Describe the outstanding clinical features of lymphogranuloma venereum. What is the nature of the etiologic agent? Distinguish between granuloma inguinale and lymphogranuloma venereum.
26. Describe the properties of the virus of lymphogranuloma venereum.
27. Describe the materials and procedure used, and the proper interpretation of the Frei test.
28. Discuss the problem of the prevention of venereal diseases.

CHAPTER XXXII

INFECTIONS OF WOUNDS. TETANUS. GAS GANGRENE

Infection of surgical wounds. Before the introduction of antiseptic methods by Joseph Lister, whose revolutionary work we have already described in Chapter III, serious inflammation of operative wounds was regarded as a more or less inevitable feature of surgical practice. In these days of aseptic methods, however, infection of a surgical wound from without can result only from carelessness. The elaborate preparations for a modern operation, and the strict aseptic technique practiced in the operating room, in which the attending nurses play so prominent a part, are designed to prevent any possible chance for contamination of the wound. Nevertheless, surgical wounds may occasionally become infected because of some break in technique. When this happens, the organisms most likely to be introduced are staphylococci or streptococci. The character and severity of the infection will, of course, vary with the nature and virulence of the organisms.

One form of operative-wound infection occurs rather frequently. This is a mild inflammation with pus formation about the stitches which close the wounds—the so-called "stitch abscess." This infection is caused by staphylococci of low virulence, belonging to the species *Staphylococcus albus,* which normally inhabit the skin.

Of course, the surgeon must frequently operate in areas already infected. He is at pains to arrange for the adequate drainage of such wounds, in order that the accumulated exudate may escape. Usually, when free drainage from a localized focus of infection has been provided for, the defensive mechanisms of the body are enabled to dispose of the remaining bacteria and bring about healing.

The nurse will notice that the pus discharged from some infected wounds, especially deep wounds of long standing, imparts a greenish color to the dressings. This is due to infection with *Pseudomonas aeurginosa* (*Bacillus pyocyaneous*), the "bacillus of blue-green pus."

Infection of accidental wounds. It is natural, when we talk about accidental wounds, to think first of wounds of warfare, made by bullets, shell fragments, or other missiles. In the same general class of wounds, however, are all those suffered at home, and in the street or shop, as the result of *trauma,* a blow of some kind—an injury which cuts, tears, or crushes the skin and other tissues.

Types of traumatic wounds. Traumatic wounds may, of course, be either slight or extensive, and they differ in their tendency to become infected, according to the type of injury. *Abrasions* of the skin, such as those so common in children who, in the midst of play, fall and scuff off the skin of legs or hands against unyielding pavement, are easily infected. But also, such wounds are easily cleaned and disinfected, and a serious, complicating infection is not likely. *Incised wounds,* made with a sharp instrument, such as a knife or a razor, or by bits of glass, are less liable to infection than other types of traumatic wounds. They tend to bleed profusely, thus washing out dirt and germs, and since the tissues are cut cleanly, without much crushing, healing is generally prompt.

Lacerated wounds. On the other hand, wounds in which the tissues are bruised and torn—lacerated wounds—are certain to be heavily contaminated by bacteria, and, unless they are properly cared for, serious and perhaps fatal infection may follow. We have only to recall the hundreds of persons injured on the streets every day by automobiles, or at home by falls, to say nothing of the shell wounds and other serious casualties of warfare, to realize that lacerated wounds are common. Often these wounds are deep, and bacteria on the skin or clothing, and in dirt and other foreign matter, are carried into the depths of the injured flesh. In those wounds, conditions are favorable for the growth of pathogenic bacteria of *both aerobic and anaerobic types,* and bacteria which are ordinarily saprophytic may be able to develop there also, because of the presence of dead tissue. The most serious infection to which such wounds are liable is some form of *gas gangrene.*

Puncture wounds or stabs. Bullet wounds, or those suffered when a nail or a pitchfork is stepped upon, are dangerous for a special reason. These wounds usually bleed very little, and since they have only a small opening at the skin surface, they are not easily cleaned or disinfected. In the depths of such wounds, anaerobic bacteria are given an opportunity to multiply, and it is this type of wound that is especially liable to lead to *tetanus.*

Bacteria in infected wounds. Conspicuous among the many kinds of *aerobic* bacteria likely to be present in wounds are *staphylococci* and *streptococci*. There is always the possibility that these organisms, especially the streptococci, may be so virulent as to cause a severe local inflammation or a fatal septicemia.

The greatest danger in connection with infection of traumatic wounds, however, lies not in the presence of these aerobic bacteria, but in the almost unavoidable contamination of the wound with the pathogenic *anaerobic sporebearing bacilli* which cause *tetanus* and *gas gangrene*.

TETANUS

This disease has been known for centuries. It is characterized by strong and continuous contractions of the muscles (tetanus), beginning often in the neighborhood of an infected wound, usually involving all the muscles eventually and causing death. The frequency with which the muscles of the jaw are involved early in the disease has given rise to the popular term *lockjaw*.

Clostridium tetani. *Morphology and staining.* The tetanus organism is a slender, Gram-positive bacillus. Young forms are motile. It develops round spores, which are larger in diameter than the rod and appear at the very end, so that the bacterium looks like a microscopic drumstrick (Fig. 99).

Occurrence. The tetanus bacillus is a common inhabitant of the intestine of horses and other herbivorous animals, and also it is frequently present in the human intestine. It is remarkable that this very dangerous organism can exist there without harm. It forms spores when it leaves the body in the feces and is deposited upon the soil. The tetanus spores are extremely resistant. If protected from sunlight or other injurious influences, they may remain alive for many years. These resistant spores become widely distributed in the soil and on dirty objects everywhere.

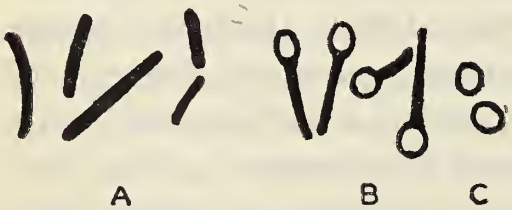


FIG. 99. *Clostridium tetani*. A: vegetative forms; B: the characteristic drumstick-shaped sporulating forms; C: free spores.

Physiological properties. Tetanus toxin. The tetanus bacillus is a *strict anaerobe*. Surface growth can be secured in the laboratory

only by use of an efficient anaerobic jar, or some other good method for removing contact with atmospheric oxygen. It will grow, however, in cooked-meat medium and in plain liquid or semisolid media containing thioglycollate or cysteine hydrochloride. Growth is abundant on enriched media, such as blood agar, at body temperature. Agar surface colonies are rhizoid—that is, they have filamentous, root-like outgrowths. This results from the habit of forming long, curling chains. The organism is readily distinguishable from other principal members of the *Clostridium* genus by its distinctive sporulating form and colony, and by its failure to produce fermentation of carbohydrates or other common biochemical changes.

The outstanding property of the tetanus bacillus is its capacity to produce a powerful, specific *exotoxin.* The toxin has a peculiar affinity for nervous tissue, and especially for the motor nerve centers. When the poison reaches these centers, tetanic convulsions of the muscles follow.

The tetanus toxin is one of the most powerful poisons known. The filtrate of a broth culture may contain enough toxin in one cubic centimeter to kill literally millions of mice. Human beings are even more susceptible to this toxin than mice. It is easy to understand, therefore, how slight a development of the tetanus organisms in a wound may be sufficient to cause a fatal toxemia.

Tetanus in man. *Special features of this infection.* Despite the almost universal distribution of tetanus spores, and the frequent occurrence of wounds which must be contaminated with them, tetanus is not a common disease. This is explained by the fact that the tetanus spores cannot germinate and multiply to produce toxin unless conditions in the wound suit their requirements. They are saprophytes, and not adapted to invasion of healthy tissue, hence the presence of some dead flesh, which may give them a start, is probably essential. The presence of other organisms in the wound favors the development of the tetanus germs. Further, the tetanus spores will not germinate and multiply unless carried deep into the tissues, where there is little atmospheric oxygen, or unless accompanied by aerobic organisms that use up the oxygen. Hence badly lacerated wounds in which splinters of wood and bits of glass are embedded, gunshot wounds, and wounds made by blank cartridges in which particles of paper or cotton-wadding bearing tetanus spores are driven into the flesh, and other types of *penetrating wounds,* are

especially liable to furnish the proper conditions for growth of tetanus bacilli.

Rarely, tetanus has occurred following contamination of a smallpox vaccination sore, or other superficial wound or ulceration, and after surgical operations upon the intestine. The use of suture material (usually catgut) contaminated with tetanus spores probably explains most of these rare cases of postoperative tetanus. In infants, fatal tetanus may follow if the germs enter the cut surface of the umbilical cord.

In any case, the tetanus bacilli multiply in the wound to a limited extent only; they do not invade the deeper tissues. The disease is caused entirely by absorption of the powerful tetanus toxin in the blood, and eventually into the motor nerves and muscles. The incubation period, in the usual cases of severe generalized tetanus, is about seven days. The tonic contractions of the muscles, which often begin in the region of the wound and early affect the muscles of the jaw, finally extend to the whole body, causing paralysis of respiration, and death.

Bacteriological diagnosis. Ordinarily the laboratory is of little help in arriving at a diagnosis of tetanus. Sometimes swabbings from the depths of the wound, or pieces of tissue excised from the wound area, may be cultured successfully for tetanus bacilli by inoculation into cooked-meat medium. The presence of tetanus toxin in the blood of the patient might be demonstrated by animal inoculations, but this is rarely attempted. Fortunately, the clinical features of tetanus are so clear-cut that they usually permit early diagnosis without laboratory aid.

Tetanus antitoxin. *Production and standardization.* Antitoxin for tetanus is prepared, like diphtheria antitoxin, by immunization of horses.

The methods used for standardizing this antitoxin are similar to those employed for diphtheria antitoxin. In the laboratories of the National Institute of Health, in Washington, a standard tetanus antitoxin and an official test dose of tetanus toxin are carefully preserved. A *unit of tetanus antitoxin,* according to the methods used in the United States, is *ten times the least amount of serum necessary to save for ninety-six hours the life of guinea pigs weighing 350 grams when the animals are inoculated subcutaneously with the official test dose of tetanus toxin.*

Therapeutic use. Once the symptoms of tetanus have fully developed, there is little hope of recovery, because tetanus antitoxin cannot neutralize toxin which has already combined with the tissues. Nevertheless, the life of the patient is sometimes saved by the intravenous injection of antitoxin—which serves at least to protect the body from further damage, and by the use of a combination of drugs which reduce the muscular spasms.

Prophylactic use. The principal value of tetanus antitoxin lies in its prophylactic effect. It has long been the general practice, everywhere, to administer a prophylactic dose of tetanus antitoxin at the earliest possible moment to every individual who has suffered a wound which might lead to tetanus. The indiscriminate use of tetanus antitoxin in this way has never been desirable, however, and fortunately the practice is now rapidly falling into disuse, as more and more persons are being *actively immunized* against tetanus.

To the injured individual *not* actively protected (by *two* or more previous injections of tetanus toxoid *at least three months before* the wound is suffered), tetanus antitoxin must be given prophylactically whenever there is a reasonable probability that tetanus will develop. It must be remembered that the passive immunity conferred by a single prophylactic injection of about 1,500 units of tetanus antitoxin wears off in about two weeks, and that when the antitoxin has all left the body, the individual is probably as susceptible to tetanus as a person who has never been inoculated at all. Hence, if tetanus bacilli are still present in the wound, they may yet give rise to the disease. *A second dose* of the antitoxin, about a week later, should be given if the wound is not healing properly. Also, in case it should be necessary to operate, later, in the area of the original wound, another dose of the antitoxin should be given. The tetanus spores originally introduced may remain alive (though inactive) for weeks, ready to germinate if the tissues are again devitalized by surgical procedures.

Prevention of tetanus by active immunization with toxoid. Active immunization against tetanus is a procedure now of recognized worth. The routine vaccination, with tetanus toxoid, of all men and women in the armed forces of the United States and of other nations, during World War II, resulted in the almost complete disappearance of tetanus as a complication of war wounds. Stimulated, in part, by this war experience, active immunization

of children and of the population generally is now widely practiced. All children (at the age of about 11 months) should be given tetanus toxoid, along with the injection of diphtheria toxoid.

The usual scheme followed in tetanus immunization is to give a series of two, or better, three subcutaneous injections of alum-precipitated or plain toxoid at intervals of from three to six weeks, followed by a "boosting" dose several months (usually a year) later. Development of the desired tetanus antitoxin in the vaccinated individual is slow, and, as in the case of diphtheria immunization, an effective immunity is not ordinarily established until about four or five months after completion of the first series of injections. If at that time, or within the next few months, a reinjection of a small dose of the toxoid is given, more antitoxin is rapidly produced, and the concentration of this protective antibody in the blood goes up and stays up for a relatively long period.

Combined vaccines, consisting of mixtures of tetanus and diphtheria toxoids, or of these toxoids with whooping-cough vaccine or typhoid vaccine, are under trial.

The fact that a person who has once received the primary immunizing injections, and who has had time to develop some tetanus antitoxin, responds so promptly to the secondary stimulus afforded by reinjection of a "booster" dose, makes possible the use of tetanus toxoid instead of a tetanus antiserum inoculation at the time of a traumatic injury. In the previously vaccinated individual, an injection of plain toxoid calls forth an almost immediate outpouring of his own tetanus antitoxin into the blood stream in a strength sufficient to protect against any ordinary risk of tetanus.

GAS GANGRENE

Aside from tetanus, other serious forms of wound infection may develop, following severe traumatic injury, when the wounds are not promptly cleaned or properly dressed. War wounds are naturally more liable to these complications than the accidental injuries of civil life, and there is always an increase in the prevalence of such cases after periods of intense fighting, when there was inevitable delay in collection of the wounded. These infections may take various clinical forms, but the more serious are all generally included under the term *gas gangrene*.

In typical cases, there is an acute, rapidly spreading, destructive

infection, involving especially the muscles. The tissues about the wound become greatly swollen, discolored, and infiltrated with gas; often they are crepitant, i.e., one can hear (and feel) the movement of fluid and gas within them. A thin, frothy, brownish-bloody fluid, which often has a nauseating odor, and may contain obvious bubbles of gas, exudes from the wound. The pressure of the gas in the tissues is so great that it actually tears them apart. The patient is at the same time poisoned by toxins secreted by the germs. The gangrene extends rapidly and is frequently fatal, even if attempts are made to stop it by amputation of the wounded limb.

Gas gangrene bacilli. The organisms responsible for this frightful condition are anaerobic sporebearing bacilli of several species. Like the tetanus bacillus, they are primarily saprophytic and are frequent inhabitants of the intestine. Their spores are carried into the wounded flesh with soil, and their development depends, as in tetanus, upon favorable conditions within the wound. Wounds with imperfect drainage, having extensive areas of dead or devitalized tissue, with extravasation of blood, are especially liable to a serious gangrenous infection. When the muscles are actually invaded and begin to be destroyed, gas gangrene has technically set in.

The three principal species of gas-gangrene bacilli are *Clostridium perfringens* (*Cl. welchii*) *Clostridium novyi* (*Cl. oedematiens*), and *Clostridium septicum* (*Cl. oedematis-maligni,* the *Vibrion septique* originally described by Pasteur). These species produce exotoxins and, as the organisms multiply in the wound, these toxins kill or devitalize the local tissues, thus enabling the bacilli to invade farther. *Clostridium bifermentans* (*Cl. sordelli*) is another toxigenic species that occurs less commonly. Of importance, also, are still other varieties, especially *Clostridium sporogenes* and *Clostridium histolyticum,* and to a lesser degree, *Clostridium fallax* and *Clostridium tertium.* Some of these last-mentioned species are toxic although apparently they are not themselves capable of causing gas gangrene. They contribute importantly to the pathological picture, however, by their intense proteolytic action.

Often, two or more of the principal species mentioned are present in the same infected wound, together with aerobic bacteria. *Clostridium perfringens,* however, is the predominating organism in the majority of typical cases of gas gangrene.

Clostridium perfringens. This bacillus is better known as *Clostridium welchii.* The name has reference to Dr. Welch, who, with

Nuttall, first described the organism in 1892. It is found almost constantly in the intestinal contents of animals and men, and it is frequently present in the stools of infants. Like those of the tetanus organism, its spores become widely distributed in the soil.

Cl. perfringens has a number of distinctive properties. Morphologically it differs from other clostridia in being a notably short, thick rod; moreover, it is nonmotile, and develops a definite capsule (Fig. 100). Spores are rarely seen in laboratory cultures. It grows readily in ordinary media, and is less exacting than the tetanus bacillus in regard to anaerobic conditions. On blood agar, its colonies are relatively large and smooth, and they are surrounded by a peculiar double zone of hemolysis.

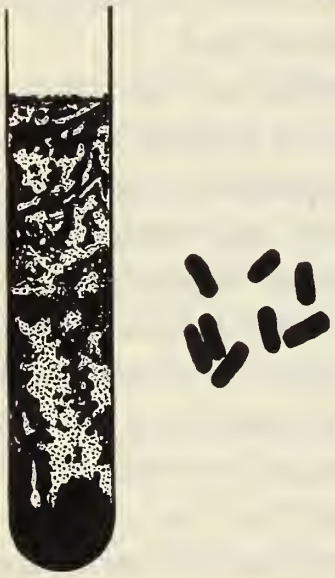


Fig 100. *Clostridium perfringens* (*Cl. welchii*). The sketch at the left illustrates the characteristic "stormy fermentation" produced by growth of this organism in a tube of milk medium. The milk is first clotted, then the clot (shown in black) is disrupted by the pressure of the gas formed by the organism.

A further outstanding property is its extremely vigorous fermentation of sugars, with the production of very large amounts of gas. Hence, it is commonly called the *"gas bacillus."* The rapid fermentation of the lactose in milk gives a characteristic appearance to cultures in this medium, the so-called *stormy fermentation of milk* (Fig. 100).

The disease-producing power of *Clostridium perfringens* depends in part upon its active gas-formation, and in part upon the poisons it develops during growth. In wounds, the organisms ferment the muscle sugar, causing the formation of gas bubbles which by their pressure disrupt the tissues, and carry the infection farther into the body. The organisms become numerous in the infected area, but usually do not invade the blood stream except in the few hours before death.

Clostridium perfringens is often found associated with pathological conditions other than gas gangrene. It is one of the organisms which may cause *diarrhea in infants.* It is sometimes the cause of fatal blood-poisoning, especially in the cases of *septicemia which follow a criminal abortion.* It is also regarded as one of the most important bacteria associated with *appendicitis.*

Bacteriological diagnosis of gas gangrene. Early diagnosis of anaerobic infection in wounds is important, and while various special procedures for the rapid identification of gas gangrene bacilli have been proposed, a straightforward bacteriological study of the wound exudate or of excised infected tissue is advisable. A direct smear from the wound may show numerous Gram-positive, stubby, nonmotile bacilli, so that a tentative diagnosis of *Cl. perfringens* infection may be justified. In any case, swabbings of the wound exudate or, better, fragments of infected muscle tissue, should be inoculated into tubes of cooked-meat medium and milk medium. These media should be sealed from the air by covering them with sterile vaseline. Further, dilutions of the material from the wound, made in thioglycollate broth, should be streaked on blood agar plates, which are then incubated in an anaerobic jar.

In such cultures, the growth of *Cl. perfringens* can be recognized within twenty-four hours. The unmistakable stormy fermentation of milk may appear within as short a time as five or six hours, and is in itself strong indication of presence of the gas bacillus. If microscopic examination of the milk culture shows a short, Gram-positive, capsulated, nonmotile bacillus, no further identification is needed.

Recognition of the other species of gas-gangrene bacilli will ordinarily require their isolation in pure culture, and the testing of their reactions in various differential media.

Prevention of gas gangrene and other serious wound infections. It goes without saying that the early and proper surgical cleaning (débridement) of any extensive traumatic wound is a fundamental measure of vital importance in preventing tetanus, gas gangrene, or other serious infections. Débridement consists in the removal of foreign bodies, and the cutting away of all dead and devitalized tissue, so that the injured flesh is converted, so far as may be possible, into an open, clean wound in which anaerobic organisms are not likely to thrive.

Once this surgical treatment is accomplished, principal reliance for control of infection is now placed upon the newer chemotherapeutic agents, especially penicillin.

REFERENCES

Tetanus

COOKE, J. V. and JANES, F. G.: "The Duration of Passive Tetanus Immunity and Its Effect on Active Immunization with Toxoid," *J. A. M. A.*, Apr. 10, 1943, 121:1201.

FRASER, D. J. and OTHERS: "Tetanus Toxoid and Its Use for Active Immunization," *Am. J. Pub. Health,* Sept. 1943, 33:1107.

MILLER, J. J., JR. and HUMBER, J. B.: "Observations on Tetanus Immunization; Dosage of Alum-Precipitated Toxoid and Use of Fluid Toxoid After Trauma," *J. Pediat.,* Nov. 1943, 23:516.

MUELLER, J. H., SEIDMAN, L. R. and MILLER, P. A.: "Antitoxin Response in Man to Tetanus Toxoids," *J. Clin. Invest.,* Mar. 1943, 22:325.

STEWART, S. E.: "Active Immunization of Human Beings with Combined *Clostridium Perfringens* and Tetanus Toxoids," *War Med.,* May 1943, 3:508.

Gas gangrene

ALTEMEIER, W. A.: "Rapid Identification of the *Clostridium welchii* in Accidental Wounds," *Surg., Gyn. & Ob.,* Apr. 1944, 78:411.

PETRIE, G. F. and STEABBEN, D.: "Specific Identifications of the Chief Pathogenic Clostridia of Gas Gangrene," *Brit. Med. J.,* Mar. 27, 1943, 1:377. 1943, 1:377.

REED, G. G. and ORR, J. H.: "Gas Gangrene," *Am. J. Med. Sci.,* Sept. 1943, 206:379.

REVIEW QUESTIONS—CHAPTER XXXII

1. Under what circumstances are surgical wounds likely to become infected? What is the cause of "stitch abscess"? Why does the surgeon leave drains in infected wounds?
2. Name and describe the "bacillus of blue-green pus."
3. How do traumatic wounds become infected? Name four types of traumatic wounds, and explain the likelihood of infection in each case.
4. What two aerobic organisms are likely to be present in infected wounds, and what is their importance? Explain how the germs of tetanus and gas gangrene may get into wounds.
5. Describe briefly: (*a*) the morphology and staining, (*b*) the important physiological properties, of *Clostridium tetani.* What is the one outstanding property of the tetanus bacillus which accounts for the disease it causes?
6. Describe the types of wounds which are likely to lead to tetanus in man, and explain why special circumstances are necessary to permit the development of the organism.
7. Can a diagnosis of tetanus be made in the bacteriological laboratory?
8. How is tetanus antitoxin produced and standardized? What value has this antitoxin in the treatment of tetanus?
9. Discuss the use and value of tetanus antitoxin for the prevention of tetanus.
10. Discuss the use and value of plain and alum-precipitated tetanus toxoids in the prevention of tetanus. Mention some advantages over the older practice of giving antitetanus serum.
11. Describe the infection called gas gangrene. Name the three most important organisms associated with this disease. Name five other bacteria

of lesser importance, and explain their part in the infection. What species is usually predominant?

12. Briefly describe the properties of *Clostridium perfringens*. Why is it called the "gas bacillus"? Explain how this organism brings about gas gangrene. What other pathological conditions may it cause?
13. Outline practical procedures for the laboratory diagnosis of gas gangrene. What special properties of *Cl. perfringens* permit its ready identification?
14. What is meant by débridement of a wound? Mention other measures which may help to prevent or control gas gangrene.

CHAPTER XXXIII

INTESTINAL INFECTIONS. FOOD POISONING

The intestinal tract, some thirty feet long, may be thought of as a tube which is within the body, and yet is really outside of the body proper. It contains continuously a remarkably varied and abundant flora of microorganisms. In fact, as a culture medium and incubator combined, the intestine has no rival in nature. In the colon (large intestine) especially a tremendous number of organisms are to be found at all times, and the bulk of the intestinal discharges (feces) consists of nothing more than the bodies of bacteria, the majority of which are dead. Obviously, the food we swallow does not contain anything like this number of bacteria; there must be a rapid multiplication of the organisms within the intestine.

Normal intestinal flora in infancy and in adult life. The intestinal canal of the new-born baby is sterile, but within a few hours after birth, bacteria gain entrance through the mouth and anus from objects in the immediate surroundings. During the first two or three days of life, the feces of the infant will contain the various bacteria that have accidentally found their way into the intestine, but by the fourth or fifth day, when the infant has begun to take milk regularly, these organisms largely disappear, and a peculiar bacterial flora characteristic of the infant intestine becomes established. The striking thing about this flora is its remarkable simplicity and uniformity, particularly in the case of infants who are breast-fed. In most cases, the feces of the breast-fed infant contain not more than three or four kinds of organisms, and as much as 99% of all the bacteria may be of a single kind—the anaerobic bacillus, called *Lactobacillus bifidus*. Smears made directly from the breast-fed infant's feces show in a striking way the great preponderance of this organism (Fig. 101). The few other bacteria most likely to be present include *Streptococcus fecalis, Lactobacillus acidophilus,* and *Escherichia coli* and related Gram-negative bacilli. The latter, how-

ever, are always in small numbers as compared with their abundance in the adult intestine.

In the case of bottle-fed infants, the intestinal flora is likely to be more varied, with more *Escherichia coli* and other organisms

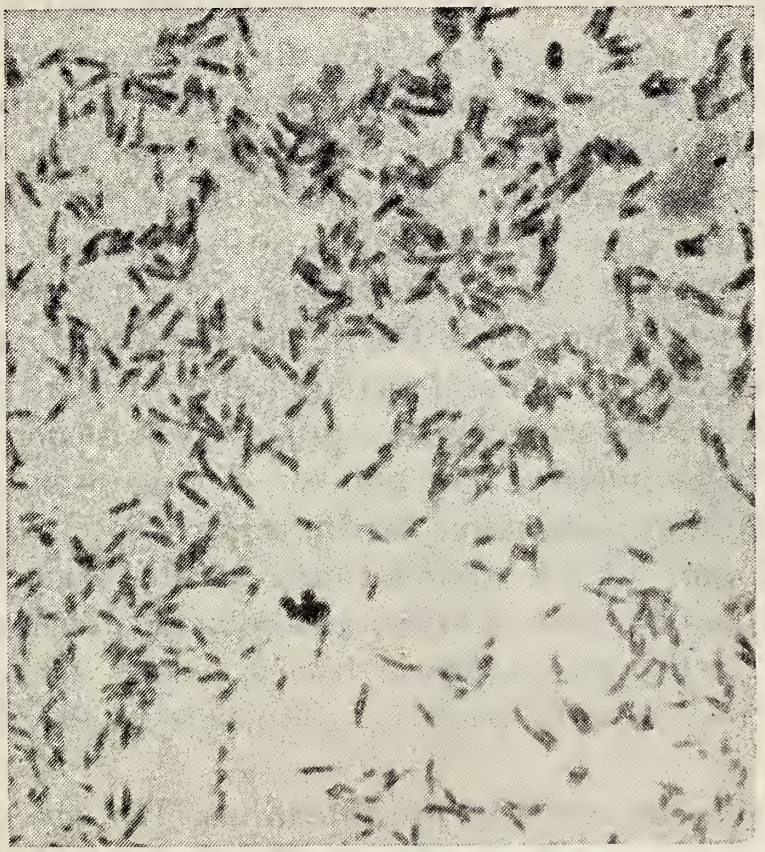


FIG. 101. Direct smear from the feces of a five-day-old breast-fed infant. Note that the bacteria are almost all of a single kind. They belong to the species *Lactobacillus bifidus*. Note forked (bifid) form near upper left corner of this photograph.

characteristic of the adult intestine. However, if the infant is fed soured (*acid*) milk, almost the same type of intestinal flora may develop as in the breast-fed child.

The *Lactobacillus bifidus* is so called because in broth cultures from the feces it shows characteristic forked (bifid) ends. This organism forms considerable acid from the lactose in milk, and its presence in large numbers in the infant intestine tends to inhibit the development of the proteolytic type of bacteria, which are, in general, sensitive to acid and unable to multiply in an acid environment. Thus, so long as the infant is receiving an exclusive milk diet and this acid-producing flora predominates, protein-splitting organisms are kept out, the feces do not have a putrefactive character as

in the adult, and the possibility of the absorption from the intestine of toxic substances formed there by proteolytic bacteria is avoided.

As the individual grows older, and milk is no longer the sole or the principal article of diet, the intestinal flora becomes more and more complex, until, in the adult, organisms of many different kinds become abundant. *Escherichia coli* and related Gram-negative bacilli—the group of organisms generally referred to as the *colon bacilli*—come to be the predominant bacteria in the lower intestine, and they constitute about 75% of all the living bacteria in the feces.

Bacteria are not uniformly distributed throughout the length of the intestine, but there are differences in the number and kinds of organisms at different levels. The empty stomach is usually sterile, and there are few organisms in the duodenum and upper jejunum. This is probably due to the acid secretion of the stomach. The lower levels of the small intestines become progressively richer in bacteria, and in the large intestine the number of organisms reaches the maximum. Here, besides colon bacilli, staphylococci, sarcinae, and yeasts, are found aerobic sporeforming bacilli, such as *Bacillus mesentericus* and *Bacillus subtilis;* anaerobic sporebearers, such as *Clostridium perfringens;* aciduric bacteria, such as *Lactobacillus acidophilus;* thermophilic bacteria; spirochetes; and various other types.

Importance of bacteria in the intestines. The masses of organisms in the intestines under normal conditions do no harm. Indeed, recent studies indicate that the intestinal bacteria contribute definitely to the general well-being of both animals and man, by synthesizing a number of the growth-factors (vitamins) essential for good nutrition. Vitamin K and various elements of the vitamin-B complex are formed in significant amounts. Nutritionists have found that a useful way to produce a vitamin deficiency in a rat is to feed it a sulfonamide compound. The drug presumably inhibits the growth of some of the bacteria in the intestine, and thus reduces the synthesis of certain needed vitamins there.

The colon-typhoid-dysentery group of bacilli. Despite the great complexity of the bacterial flora in the intestine, only a few kinds of organisms need be considered by bacteriologists in connection with common infections of the intestinal tract. We have already mentioned that a large proportion of the living bacteria in the normal intestine belong to the species called *Escherichia coli,* or to one of the related varieties constituting the colon group. The colon

TABLE XX. Genera of Intestinal Bacilli

GENUS	MOTILITY	FERMENTATION * OF			RUSSELL'S DOUBLE-SUGAR		HYDROLYSIS OF UREA	PATHOGENICITY
		GLUCOSE	LACTOSE	SUCROSE	SLANT	BUTT		
Escherichia	+	ag	ag (rapid) [2]	ag [1]	a	ag	—	Slight—opportunists only (urinary tract infections, etc.)
Aerobacter	— [1]	ag	ag (rapid) [2]	ag	a	ag	—	Doubtful—(gastroenteritis?)
Alkaligenes	+	—	—	—	alk.	no change	—	Slight—opportunists only (urinary tract infections)
Proteus	+	ag	—	ag [1]	no change	ag	+	Considerable—(gastroenteritis in children, cystitis, etc.)
Eberthella [4]	+	a	—	—	no change	a	—	Specific cause of typhoid fever in man
Salmonella [4]	+	ag	—	—	no change	ag	—	Cause of animal infections; and of paratyphoid fever and food infection in man
Shigella [4]	—	a	— [3]	—	no change [3]	a [5]	—	Cause of bacillary dysentery

* a = acid; ag = acid and gas.

[1] Strains varying in this respect are especially common.

[2] So-called paracolon bacilli ferment lactose *slowly*.

[3] *Shigella sonnei* and *Shigella dispar* cause *slow* acid formation from lactose.

[4] Special emphasis is placed upon antigenic structure, that is, upon the results of agglutination tests with group-specific and type-specific rabbit antiserums, in defining the limits of these genera.

[5] The so-called "Newcastle bacillus" may form a small bubble of gas.

bacilli are all Gram-negative rods, without spores, and the different varieties are morphologically indistinguishable from one another. Moreover, the bacilli that cause the common acute infections of the intestinal tract—typhoid fever, dysentery, and food infection—are *also* Gram-negative, nonsporebearing rods, of almost identical appearance, differing only in comparatively slight degree from the colon bacilli of the normal intestine. Thus, all these organisms, pathogenic and nonpathogenic, may be regarded as forming a single group of closely interrelated species—the *colon-typhoid-dysentery-group.*

Characteristics of the genera of intestinal bacilli. At present, seven genera are usually listed: *Escherichia, Aerobacter, Alkaligenes,* and *Proteus,* containing the nonpathogenic or occasionally pathogenic ("opportunist") varieties; and *Eberthella, Salmonella,* and *Shigella,* which include the frankly disease-producing bacteria causing typhoid and paratyphoid fevers, the acute enteritis associated with food infection, and bacillary dysentery (Table XX).

As this table indicates, there is a curious relation between the *lack of capacity to produce a rapid fermentation of lactose* (with acid and gas) and pathogenicity. Those varieties (typical *Escherichia* and *Aerobacter*) that regularly ferment lactose actively (forming acid and gas within 24 hours at 37° C) are *not* pathogenic in the intestinal tract. But the assumption of a parasitic habit and the development of disease-producing powers, on the part of the intestinal bacilli, seems to be associated almost invariably with a partial or total loss of ability to split lactose. The lactose-fermentation reactions are therefore of practical value in identifying and in classifying the intestinal bacilli. The fact that lactose is fermented *rapidly* only by the nonpathogenic coliform bacilli, and not by any of the species causing enteric infections, is the basis for the practical methods used to isolate these pathogens for the purpose of diagnosis.

Coliform bacteria (*genera Escherichia and Aerobacter*). In this group are included all those lactose-fermenting bacilli that are used as the index of pollution in the bacteriological analysis of water (p. 278). The coliform bacilli actually comprise a group of closely intergrading forms, many of which are intermediate in their properties and, indeed, defy precise classification. Included are not only typical strains of *Aerobacter aerogenes* and of *Escherichie coli,* but many variants of these species. Some of the variants are capsulated

(mucoid) forms, and these are closely similar to the heavily capsulated bacilli classified as *Klebsiella.*

Escherichia coli is important not only in connection with water analysis, but medically also, as a cause of several common forms of human infection. Although it lives quite harmlessly in the human intestine, it is not without some capacity to grow elsewhere in the body, and is a typical "opportunist." It is one of the bacteria most commonly causing inflammation of the urinary tract (*cystitis, pyelitis*) and of the gall bladder (*cholecystitis*). It is also abundant in the infected tissues in many cases of appendicitis and peritonitis.

Paracolon bacilli. These constitute a group of atypical coliforms, isolated from the human intestine, that are characterized by consistently *delayed* fermentation of lactose. Most strains would be classified, at present, in the genus *Aerobacter.* Recently, attention has been called to the fact that these organisms are encountered with special frequency in cultures from fecal specimens in cases of mild gastroenteritis, and it is now thought that some strains may have a definite pathogenicity.

Proteus. Members of this genus are noteworthy for their proteolytic activity. Also, they stand apart from other organisms of the intestinal group by their capacity to hydrolyse urea within forty-eight hours. The motile strains develop a characteristic spreading growth—forming a continuous veil or film—on the surface of moist agar. Typical strains of the commonest species (*Proteus vulgaris*) attack sucrose as well as glucose with resultant acid and gas formation.

Like the coliform bacteria, the *Proteus* bacilli ordinarily do no harm in the intestine. Certain strains, however, particularly of the species *Proteus morgani,* have been suspected of causing some cases of gastroenteritis, especially in children. *Proteus ammoniae* has been implicated in cases of severe cystitis and other forms of urinary-tract infection. *Proteus* bacilli have been isolated, also, from a variety of other pathological conditions, such as gangrenous wounds, appendicitis, peritonitis, and otitis media.

Alkaligenes fecalis. This is a common inhabitant of the intestine, which is also found occasionally in association with pathological states in man, especially in infections of the urinary tract. It is conspicuous among the intestinal bacilli, because it fails to ferment any carbohydrates, and gives a strongly alkaline reaction to cultures in litmus milk. It is also the only species among the common Gram-negative enteric bacilli that regularly

shows conspicuous fatty inclusions, when stained by Burdon's Sudan Black-B-safranin fat stain.

Common nonspecific infections of the intestinal tract. *Peritonitis.* The delicate membrane which lines the abdominal cavity and envelops the intestine and other organs there—the peritoneum—is sometimes inflamed as the result of a primary infection with *Pneumococcus* or other organisms, particularly in children. In most cases, however, peritonitis is secondary to some lesion in the stomach or intestines, and follows perforation of a gastric or duodenal ulcer, intestinal obstruction caused by intersusception or hernia, rupture of an inflamed appendix, intestinal operations, or abortions. Though many types of bacteria may be present, the peritoneal exudate usually contains a predominance of *Streptococcus hemolyticus* or *Escherichia coli.*

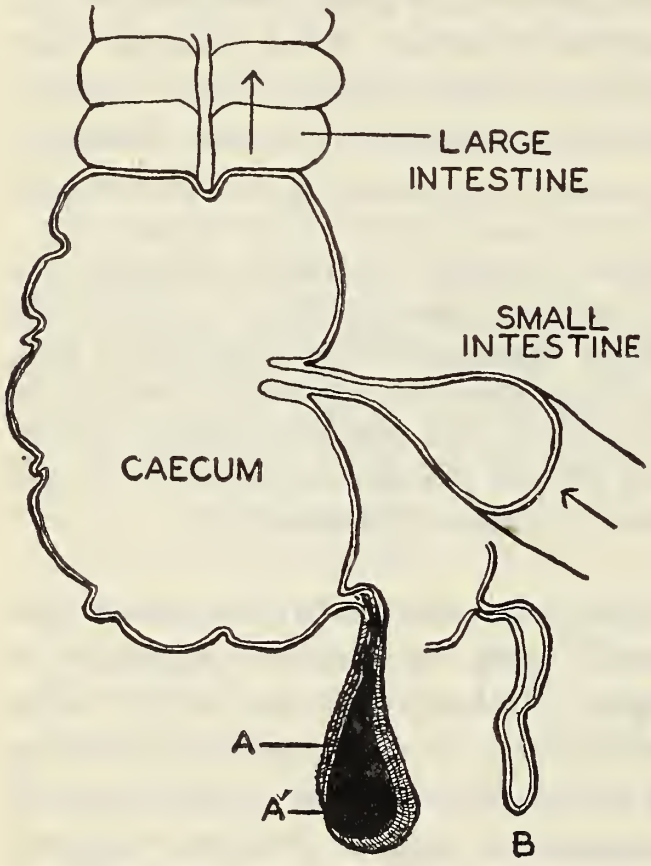


FIG. 102. Diagram of a portion of the intestine at the junction of the small and large intestine, cut open to show the position of the appendix. The arrows indicate the direction in which the food passes through. B: the normal appendix. A: the condition in acute appendicitis. The appendix is swollen and filled with pus; perforation is about to occur at A'.

Cholecystitis. Inflammation of the gall bladder is commonly caused by coliform bacilli, sometimes associated with streptococci. The presence of gallstones probably greatly increases the liability to gall bladder infections, by causing irritation, and by obstructing the free flow of bile, and thus making more likely the passage of bacteria up the bile ducts to the gall bladder from the intestine. Doubtless, the gall bladder is at times infected by organisms brought to it in the blood, rather than directly from the intestine.

Appendicitis. The appendix in man is a small tube about four inches long, and as large around as the little finger, attached as a

sort of worm-like appendage to the large intestine, just below the junction with the small intestine (Fig. 102).

In human beings, the appendix is a useless organ—an evolutionary remnant—but in certain herbivorous animals, rabbits for example, a corresponding structure, much larger, serves as a pouch in which partial digestion of the food takes place.

Both acute and chronic appendicitis are common. Probably the thin, inner epithelium of the appendix is easily injured, especially when the walls are under tension as a result of obstruction of the lumen, or because of the presence of a hard fecal mass (stercolith). Invasion of the weakened tissues by some of the bacteria that happen to be present is then to be expected. In the acute cases, a rapidly developing inflammation, with formation of pus, takes place. Usually the only adequate treatment is the immediate surgical removal of the inflamed organ. If this is done before the wall of the appendix becomes gangrenous and perforation occurs, an immediate cure is effected; otherwise, a localized peritoneal inflammation or a fatal generalized peritonitis may develop.

A mixture of many different organisms of both aerobic and anaerobic types may be found in cases of acute appendicitis. Among the aerobic bacteria likely to be present are *Escherichia coli, Proteus vulgaris,* staphylococci, and streptococci. Anaerobic organisms are usually abundant, especially when the infection is of the severe, gangrenous character. They include *Clostridium perfringens,* and other Gram-positive, anaerobic bacilli of this genus, *Bacterium melaninogenicum* and *fusiform bacilli.*

BACTERIAL FOOD POISONING AND FOOD INFECTION

Prevalance and importance. Cases of the illness popularly called "ptomaine poisoning," characterized by the sudden development of nausea, vomiting, abdominal pain, and diarrhea shortly after a meal, are very common. The symptoms are as familiar to most persons as those of a common cold. Few individuals escape an occasional mild attack, and spectacular outbreaks involving large groups of people are frequently reported in the newspapers. In wartime, when hundreds of persons in military establishments and industrial plants are fed regularly from a central kitchen, and when maintenance of high standards of sanitation in home-front eating places

is difficult, the hazard is increased. Some costly outbreaks among the armed forces and civilian war workers were reported during World War II.

Causes of acute illness from food. These cases of diarrheal disease following the eating of a certain meal are due to the presence, in the food, of poisons (toxins) already formed there by certain particular kinds of bacteria (in which case the patient suffers from bacterial *food poisoning*); or the cases are due to the presence of certain bacteria capable of setting up an almost immediate, acute, infectious diarrhea (in which instance the condition is properly described as a *food infection*). *Ptomaines have nothing whatever to do with the disease,* and the expression "ptomaine poisoning" ought to be abandoned.

There are four principal kinds of this acute foodborne illness: (1) *staphylococcus food poisoning,* (2) *Salmonella food infection,* (3) *streptococcus food infection,* and (4) *botulism.* In addition, some outbreaks of food infection are attributed to *paracolon bacilli.* Probably most common in the United States are cases arising from foods contaminated with enterotoxin-forming *staphylococci.* Next in frequency are the *Salmonella* infections and those due to paracolon organisms. Only a few instances of intestinal disease from streptococcus-contaminated foods have been described; and fortunately, the highly dangerous food poisoning called botulism is also rare.

Staphylococcus food poisoning. When we remember that staphylococci are always to be found on the human skin it is perhaps not surprising that food-poisoning cases due to these organisms occur so frequently. The kinds of food most often involved are cream puffs and similar pastry, though outbreaks have been traced to prepared delicacies such as hollandaise sauce, soups, and sandwiches, and to other foods that are much handled, like ham and cheese. The clinical features of staphylococcus food poisoning are characteristic. Symptoms begin within 1–6 hours after eating of the contaminated food, and usually within 2–3 hours. Dizziness, nausea, abdominal cramps, vomiting, diarrhea, and acute prostration come on rapidly. The patient is often severely dehydrated. The acute symptoms usually last for only a few hours, however, whereupon rapid recovery occurs.

Bacteriological examination of the incriminated food usually reveals unmistakable evidence of gross contamination with staphylo-

cocci. Counts of the number present in a custard-filling, for example, may reveal hundreds of millions of cocci per gram.

When isolated and studied in pure culture, the majority of strains of food-poisoning staphylococci are hemolytic, and will liquefy gelatin, ferment mannite, and give a positive coagulase test. But there are exceptions, and the food-poisoning cocci do not fall into any clearly defined group according to the usual cultural, biochemical, or serological tests. It is their capacity, while multiplying in the food, to form the special poison—the *staphylococcus enterotoxin*—to which the human intestine is so sensitive, that gives the food-poisoning strains their significance.

An important feature of staphylococcus food poisoning is the necessity for multiplication of the cocci within the food during the interval between its original preparation (when the staphylococci were introduced) and the time it is consumed. Obviously, refrigeration during this time would prevent or delay growth of the organisms. Avoidance of the *original* contamination of foods with enterotoxin-forming staphylococci is largely, of course, a matter of strict *personal cleanliness* on the part of cooks and bakers. The rebaking of cream-filled pastries, to destroy organisms introduced in their preparation, is a helpful step now practiced by some bakeries.

Salmonella food infection. This form of foodborne intestinal disease is characterized clinically by a delayed onset, with symptoms appearing not earlier than 7, and usually 12–72, hours after eating, by a relatively slow evolution, by high fever, and by a gradual recovery, which is often not complete for a week or more. Otherwise, the symptoms are essentially the same as in staphylococcus food poisoning. Rarely, the patient dies.

The principal species of *Salmonella* concerned are *Salmonella typhimurium* (*Sal. aertrycke*), *Salmonella enteritidis,* and *Salmonella choleraesuis.* These organisms sometimes cause infections in cattle and swine, and cases of food infection in man have been traced to the eating of imperfectly cooked meat derived from these sick animals. *More commonly, however, the source of the contamination of the food is a human carrier of the germ in the person of the cook.*

In most outbreaks, the food concerned is meat, milk, fish, or other animal protein, and rarely vegetables or fruits. Often the food is of the kind which has been made up into pies, stews, jellies, salads, puddings, sausages, or similar products, and has therefore been handled to an unusual extent. Also, it often happens that the food

responsible for the illness is a large dish, prepared to serve a number of guests at a dinner or picnic, and usually such a dish has not been thoroughly cooked. The temperatures reached in ordinary cooking are not as high as is generally believed, and it is easy to see how the center of a large meat pie, for example, might not be heated sufficiently to kill the bacteria present. Now, if the incompletely cooked food is allowed to stand outside the refrigerator for some time before it is eaten, the organisms will multiply to a great extent, and become so numerous that they readily set up an acute intestinal inflammation in nearly every person who partakes of the food. Very dangerous food, heavily contaminated with *Salmonella enteritidis*, for example, may still have an entirely normal appearance and odor.

A bacteriological diagnosis of *Salmonella* food infection is not often possible, since laboratory studies are usually not begun until after the suspected food has been discarded, and often fecal specimens from the victims are not obtained until after the acute symptoms have subsided. Whenever stool cultures *are* made at the height of the illness, the offending strain of *Salmonella* may often be found to be present in practically pure culture. Sometimes the same type of *Salmonella* can be isolated also from the particular food which circumstantial evidence indicates is the source of the infection, thus completing the proof of its guilt.

For isolation of *Salmonella* from the ground-up food, or from feces, the medium known as S-S agar and other special differential and selective plating media devised for the intestinal bacilli are used, as described in Chapter XXXIV.

Prevention of *Salmonella* food infection requires cleanliness and care during the whole process of securing and preparing food for consumption. A thorough system of meat inspection, sanitary control of bakeries, slaughter houses, and establishments making sausages and similar products, and of public eating places of all kinds are valuable measures. Foods should be as fresh as possible and continuously refrigerated until they are to be consumed. Then the safest meal will be one that is *thoroughly cooked.*

Paracolon bacilli food infection. All that is said above about *Salmonella* food infection applies also to those cases of acute gastro-enteritis caused by eating of food contaminated with virulent strains of paracolon bacilli. The peculiar organisms concerned here closely resemble the *Salmonella,*

but usually form indol and bring about a very slow fermentation of lactose.

Streptococcus food infection. A few outbreaks of relatively mild and transient nausea and diarrhea have been traced to the eating of foods (e.g., cocoanut-cream pie, turkey dressing, beef croquettes) heavily contaminated with alpha type (viridans) streptococci. Also, an episode has been reported in which a number of persons exhibited marked gastrointestinal symptoms after eating a ham prepared by an individual who was in the preeruptive stage of scarlet fever. In this instance, a hemolytic streptococcus of Lancefield's group A was recovered from the food, as well as from the human carrier.

Symptoms of streptococcus foodborne illness appear within 5–18 hours —somewhat later than in the case of staphylococcus food poisoning. It is thought that the streptococci bring about an actual intestinal infection of brief duration; they apparently do not form a filtrable enterotoxin, though the feeding of whole cultures to human volunteers has resulted in characteristic symptoms. Blood agar plate cultures of the incriminated food will usually reveal great numbers of streptococci of green-colony type.

It is evident that persons having head colds, sore throats, or other forms of upper respiratory tract infection ought not to participate in the preparation of food for consumption by others.

Botulism. This interesting (and fortunately rare) disease is due to the *exotoxin* formed by the prior growth of *Clostridium botulinum* in some kind of prepared or preserved food. The name "botulism" is from the Latin *botulus,* a sausage, and was originally applied to this disease because numerous cases, particularly in Germany, were caused by eating contaminated sausages. In the United States, most cases have been traced to *canned vegetables,* especially string beans, corn, or spinach. Meyer has reported that in this country and Canada, between 1899 and 1941, there occurred 359 outbreaks of botulism, with 1024 cases, and 669 deaths, a case fatality rate of 65%!

Clostridium botulinum was first isolated by van Ermengem, in 1896, from a piece of raw ham, and from the organs of persons who had died of botulism after eating part of this ham. It is an anaerobic, Gram-positive, saprophytic, sporebearing bacillus, native to the soil. Five types (A, B, C, D, E) have been recognized within this species, based upon the specific nature of their exotoxins.

Botulism is essentially an intoxication, not an infection. The spores of the bacillus are widely distributed and are likely to get upon all kinds of foods, but the eating of fresh foods never causes

botulism. All human cases can be traced to foods which have been *preserved* in some way, usually by canning, but *insufficiently processed,* so that sterilization is not complete and some resistant spores of *Cl. botulinum* survive. These spores then eventually germinate, and the newly germinated bacilli—since they are anaerobic and saprophytic—find conditions in the sealed can suitable for multiplication. In time, there accumulates in the food an extraordinarily powerful soluble toxin.

Several cases are known in which deaths from botulism have followed the mere tasting of such food. The botulinus exotoxin has been found to be much more deadly than the strongest tetanus toxin. It can pass through the walls of the stomach and intestine unchanged, differing in this respect from the toxins of the tetanus and diphtheria bacilli, which are harmless when taken by mouth. Cases of human botulism are caused principally by the exotoxins of Type A and Type B *Cl. botulinum.*

The symptoms of botulism often develop within less than twenty-four hours after eating the poisonous food, but sometimes, when less toxin has been absorbed, they are delayed for several days. In contrast to the more familiar types of bacterial food poisoning, there are usually no signs of acute gastrointestinal irritation; there is no diarrhea, but instead the patient is constipated. The very distressing symptoms are due to the injurious effect of the botulinus toxin on the nerves. Paralysis of the eye muscles brings on early disturbances of vision, and soon the paralysis affects the pharynx; the throat becomes clogged with a thick, glairy mucus; and, although very thirsty, the patient cannot swallow, and often cannot talk. There is marked general muscular weakness. Usually the patient feels no pain and the mind remains clear. Death is caused primarily by paralysis of the respiratory center.

Diagnosis is ordinarily made on clinical grounds and history, but if remnants of the responsible food can be found, it may be shown, by injection into mice or other laboratory animals, to contain botulinus toxin. The presence of the toxin in the blood serum or vomitus of the patient, or in material obtained at autopsy from the intestinal tract, may be demonstrated by the same means.

Although specific *antitoxins* for the toxin of each of the types of *Clostridium botulinum* are available, it is seldom possible to use them effectively in the treatment of a case of human botulism.

The toxins of this bacillus are responsible for various forms of

poisoning in animals, such as limberneck of chickens, forage poisoning in horses, duck sickness, and paralytic disease in cattle.

In recent years, botulism in human beings due to commercially canned food is almost unknown. On the other hand, the disease still is occasionally caused by *home-canned* string beans, corn, or other foods. This emphasizes the importance of selecting for home canning only clean, fresh food, and of using the utmost care and thoroughness in the canning process. Thorough cooking of all canned food (boiling for five minutes) just before eating is the best safeguard against botulism, since the toxins (in contrast to the resistant spores) are destroyed by heat.

REFERENCES

Anonymous: "Biosynthesis of Thiamin in Man," *Nutrition Reviews,* Apr. 1944, 2:109; "Synthesis of Vitamins in the Digestive Tract," *Ibid.,* May 1943, 1:220; "Vitamin Synthesis by Intestinal Bacteria," *Ibid.,* Apr. 1943, 1:175.

Borman, E. K., Stuart, C. A. and Wheeler, K. M.: "Taxonomy of the Family Enterobacteriaceae," *J. Bact.,* Sept. 1944, 48:351.

Bowen, W. H.: "Etiology of Appendictis," *Brit. J. Surg.,* Oct. 1943, 31:121.

Parr, L. W.: "Coliform Bacteria," *Bact. Rev.,* 1939–40, 3–4:1.

Stuart, C. A.: "Sanitary Significance of the Coliform Bacteria in Water," *New Eng. Waterworks J.,* 1941, 55:355.

Food Infections and Intoxications

Breese, B. B., Stanbury, J. B. and Upham, H. C.: "Food Poisoning at a Naval Training Station," *U. S. Navy Med. Bull.,* May 1944, 42:1205.

Dack, G. M.: *Food Poisoning.* Chicago: Univ. of Chicago Press, 1943.

Dolman, C. E.: "Bacterial Food Poisoning," *Can. J. Pub. Health,* Mar. and May 1943, 34:97 and 205.

Geiger, J. C.: "An Outbreak of Botulism," *J. A. M. A.,* July 5, 1941, 117:22.

Getting, V. A. and Rubenstein, A. D.: "Staphylococcus and Streptococcus Carriers," *Am. J. Pub. Health,* Aug. 1944, 34:833.

Rubenstein, A. O., Feemster, R. F. and Smith, H. M.: "Salmonellosis as a Public Health Problem in Wartime," *Am. J. Pub. Health,* Aug. 1944, 34:841.

Stuart, C. A., and Rustigian, R.: "Further Studies on One Type of Paracolon Organism," *Am. J. Pub. Health,* 1943, 33:1323.

REVIEW QUESTIONS—CHAPTER XXXIII

1. How do the feces come to contain so many bacteria? Describe the normal bacterial flora of the infant intestine. How does *Lactobacillus bifidus* get its name? Of what significance is the fact that this organism predominates in the infant intestine?
2. Describe the distribution of bacteria in the adult intestine, and name some of the principal kinds usually present.
3. What importance have the bacteria in the normal intestine for the health of the individual?

4. What are the principal genera constituting the colon-typhoid-dysentery group of bacilli? Why are they classed together as one group? What fermentation reaction distinguishes the species that are nonpathogenic in the intestinal tract from other members of the group?
5. Mention some of the principal properties and the medical importance of each of the following: coliform bacteria, paracolon bacilli, members of the genera *Proteus, Alkaligenes fecalis.*
6. Name three common nonspecific infections of the intestinal tract, and explain how these infections may occur. What organisms are most commonly concerned in each?
7. What is the real nature of the illnesses popularly referred to as "ptomaine poisoning"? What is their importance? Name the four principal kinds of acute foodborne disease. What additional kind of organism is thought to be responsible for some outbreaks?
8. Describe the symptoms, diagnosis, cause and prevention of staphylococcus food poisoning.
9. Describe the clinical features, causative factors, diagnosis and prevention of *Salmonella* food infection. Explain clearly differences between this disease and staphylococcus food poisoning. Name the three principal species of food-poisoning *Salmonella.*
10. Discuss streptococcus food infection.
11. What is the disease called botulism? What does the name mean? Name and describe the bacterium responsible for this disease.
12. Explain why botulism is always due to the eating of a food which has been preserved in some way. Does botulism occur in animals? What procedures would prevent botulism?

CHAPTER XXXIV

TYPHOID AND PARATYPHOID FEVERS. DYSENTERY. CHOLERA

TYPHOID FEVER

In earlier times, typhoid fever was one of the most prevalent, and most deadly, of human diseases. During the past century, however, and particularly in the last forty years, there has been a marked reduction in the incidence of this disease, and consequently in the proportion of deaths attributed to it. One of the most striking features in the history of public health is the decline of typhoid fever, since 1900, from its former position as one of the principal causes of death to its present relatively insignificant place in the mortality tables. An average of only about 5 typhoid cases per 100,000 population was recorded in the United States for the years 1941–1943, inclusive. There are now few cases indeed in the large cities. In smaller towns and rural regions, however, the disease still occurs in sporadic cases and in the form of small-scale epidemics. Persistence of typhoid fever there is due to the fact that sanitation in such small communities is backward; in particular, the facilities for safe disposal of sewage and for the control of the purity of the milk and water supply are inadequate.

How successful the control of typhoid fever can be, when good sanitary practices are supplemented by active immunization of the susceptible population, is strikingly illustrated by the almost complete elimination of this disease as a health problem among American troops during World War II.

Eberthella typhosa. The germ of typhoid fever is not naturally pathogenic for animals, and typhoid fever cannot be produced in the usual laboratory animals by the inoculation of the bacilli. However, it has been possible to cause a disease similar to human typhoid in chimpanzees, by feeding them the organisms.

Morphology and staining. Like the other members of the colon-

typhoid-dysentery group, the typhoid bacillus is a small, Gram-negative non-sporebearing bacillus. There is nothing to distinguish it, in stained smears under the microscope, from the normal inhabitants of the intestine (colon bacilli) on the one hand, or from other pathogenic members of the group (paratyphoid and dysentery organisms) on the other. It is actively motile.

Physiological properties and biochemical activities. The typhoid bacillus grows readily on simple culture media and at room temperature, as well as at body temperature. It forms acid *but no gas* from glucose, mannitol, xylose, and a few other carbohydrates, and produces H_2S (Tables XX and XXI). In common with the paratyphoid and dysentery bacilli, it *does not ferment lactose,* and on this basis it can be differentiated from the colon bacilli always present in feces.

Typhoid bacilli are killed by heat and by chemical disinfectants about as readily as most other non-sporebearing organisms. If present in milk, they would be destroyed by the process of pasteurization. They are able to survive outside of the body in some circumstances, however, much longer than other germs, and this accounts for the fact that typhoid has often been transmitted through contaminated drinking water, milk, or other food. In feces, deposited in a privy vault or on the ground, typhoid bacilli tend to die out rapidly, but a few might still survive after twenty-four hours or more, and if it were wintertime, the germs might remain alive in the cold or frozen fecal mass for many weeks. They are said to survive in sewage-polluted water for about a week. In oysters or other shellfish, they may live for a month. In milk or milk products, such as ice cream or cheese, they are able not only to survive, but to multiply. Nevertheless, typhoid germs have no natural or continued existence outside the human body. It merely happens that they have a somewhat greater resistance to environmental conditions than most of the organisms causing communicable diseases, and therefore are sometimes carried from person to person by such remote means as contaminated water or food.

Typhoid bacilli in the body during the disease. In order to bring about typhoid fever, the typhoid bacillus must reach the intestine through the mouth. Following entrance of the germ, there is always an incubation period of from seven to fourteen days, averaging about ten days, before symptoms appear. During this time, the organisms penetrate the wall of the upper intestine, and

Table XXI. Typical Reactions of the Principal Species of Enteric Pathogens

Organism	Motility	Fermentation † of: Glucose	Lactose	Mannitol	Xylose	Rhamnose	Inositol	Litmus milk	Indol formation	H_2S production	Disease
Eberthella typhosa	+	a	—	a	a	—	—	sl. acid → neutral	--	+	Typhoid Fever
Salmonella paratyphi * (para A)	+	ag	—	ag	—	ag	—	sl. acid → alk.	—	--	Paratyphoid Fever
Salmonella schottmülleri * (para B)	+	ag	—	ag	ag	ag	ag	sl. acid → alk.	—	++	Paratyphoid Fever
Salmonella typhimurium (Sal. aertrycke)	+	ag	—	ag	ag	ag	ag	sl. acid → alk.	—	++	Food Infection
Salmonella enteritidis	+	ag	—	ag	ag	ag	—	sl. acid → alk.	—	++	Food Infection
Salmonella choleraesuis	+	ag	—	ag	ag	ag	—	sl. acid → alk.	—	+ —	Food Infection
Shigella dysenteriae (Shiga)	—	a	—	—	—	—	—	sl. acid → alk.	—	—	Bacillary Dysentery
Shigella paradysenteriae (Flexner, etc.)	—	a	—	a	—	—	—	sl. acid → alk.	+ —	—	Bacillary Dysentery
Shigella sonnei (Sonne)	—	a	a (late)	a	—	a	—	acid; may coag.	—	—	Bacillary Dysentery
Shigella ambigua (Schmitz)	—	a	—	—	—	a	—	sl. acid, no coag.	+	--	Bacillary Dysentery

† a = acid; ag = acid and gas.

* These two species of *Salmonella* are the only ones that fail to form acid from tartrate medium. An alkaline reaction on tartrate medium is the one cultural reaction that differentiates *Sal. schottmülleri* from *Sal. typhimurium*.

cause an inflammation, especially in patches of lymphoid tissue (Peyer's patches) in the intestinal wall. They probably pass into the mesenteric lymph glands very early and thus reach the lymph channels, then the blood stream, and are carried all over the body. They may localize in various internal organs, but especially often in the spleen, bone marrow, and gall bladder. At the time symptoms begin, there is nearly always a *bacteremia,* that is, the typhoid bacilli are present in the circulating blood. In its early stages, typhoid fever is a generalized infection, not confined to the intestinal tract. One evidence of this is the occasional appearance, during the first few days of illness, of characteristic "rose spots," which are really little colonies of typhoid bacilli in the skin. Serious complications may arise as the result of growth of the germs in parts of the body other than the intestine. In the later stages of the illness, however, the infection is most active in the intestinal tract.

The accompanying chart (Fig. 103), which is based on the findings in many different cases of typhoid fever, averaged together, gives a good idea of the usual course of events during the disease. The typhoid bacilli begin to disappear from the blood during the first week of the illness, and especially rapidly after the second week, so that they can be found in the blood stream only rarely after about the middle of the third week. In individual cases, of course, there may be variations from this general trend. In favorable instances, the bacilli may all disappear from the blood within the first few days of the illness, whereas in fatal cases the bacteremia may persist until death.

The disappearance of the organisms from the blood is clearly associated with the development of specific antibodies against the germs. We learn from the chart that agglutinins for typhoid bacilli can be demonstrated in the serum of most patients toward the later part of the first week of illness, and in a still higher proportion of cases later in the disease. These antibodies, in any particular case, reach their maximum about the time of recovery; then they gradually decrease in amount, but can still be detected in the patient's serum weeks or months later.

Even before symptoms begin, that is, during the last days of the incubation period, typhoid bacilli may be found in the feces, and they are eliminated in these excreta, and in many cases in the urine also, throughout the illness, and often in the days of convalescence, as well. They become most abundant in the feces during the second

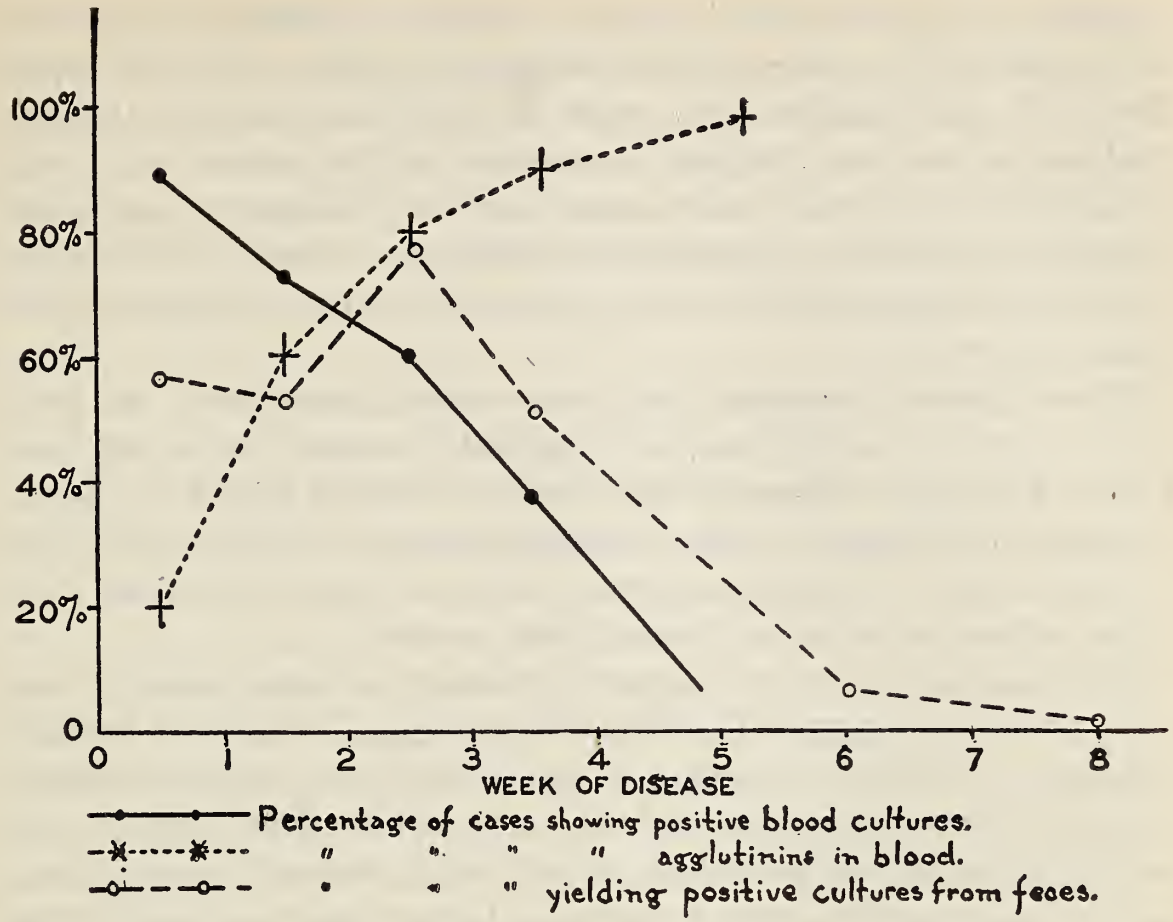


FIG. 103. Chart showing the course of events in typhoid fever. (Based on Topley & Wilson's *Principles of Bacteriology and Immunity*. Baltimore: William Wood & Co., 1936, 2nd Ed.)

and third week of the disease. This probably means that there is a secondary localization of the infection in the intestine after the bacilli have largely disappeared from the circulating blood and other places in the body. Sometimes the inflammation in the intestinal wall is so severe, as a result of invasion of the damaged mucous membrane by other bacteria besides the typhoid bacilli, that the wall is actually *perforated,* causing a fatal peritonitis. In most cases, however, the infection in the intestine gradually subsides, and the intestinal wall eventually returns to its normal state.

It is most important to realize that recovery from the illness does not mean that all the germs have disappeared from the excreta. A striking feature of typhoid infection is the tendency of the bacilli to persist in the body, and to appear in the feces or urine, at intervals, for long periods after recovery. In approximately 5% of cases the germs may still be found in the excreta for as long as three months after recovery (such an individual is called a *convalescent carrier*), and in a smaller percentage of cases the recovered patient

becomes a *chronic carrier* for years thereafter perhaps for the rest of his life. In the case of chronic carriers, the germs most often persist in the gall bladder, and reach the intestinal contents through the bile; or they may localize in some part of the urinary tract, and be passed in the urine. Such carriers are often discovered only after they have innocently transmitted the disease to others, and the presence of the organisms in their excreta has been demonstrated by laboratory tests.

How typhoid spreads. We have already mentioned the principal ways in which the bacilli of typhoid fever may be carried from person to person. Whatever the mode of transfer may be, *it is the intestinal discharges or urine of typhoid cases or carriers which are the real sources of typhoid germs,* and new cases arise when these germs reach the mouths of susceptible persons.

The infection may be acquired through personal contact with cases or carriers by way of contaminated fingers. Transfer of typhoid infection in this way is always an important factor in any outbreak of the disease. Vegetables, salads, and similar foods may be the vehicle by which the germs are carried, when they are contaminated by the fingers of the cook. Oysters, and other shellfish, which have been grown or fattened in sewage-polluted waters may harbor typhoid bacilli, and infection may follow when these are eaten in the raw state. At the present time, the most common source of typhoid outbreaks is contaminated (raw) *milk* or milk products. When milkborne epidemics are investigated, the trail usually leads to a single dairy, and to a single individual milker or other dairy worker who has a mild case of typhoid, or who is a carrier. Finally, typhoid fever has often been caused by a contaminated public *water* supply. Waterborne epidemics are now uncommon in cities, because of the almost universal use of artificial water-purification methods. Small outbreaks in country districts are frequently traced to contaminated well water.

As in the case of many other infectious diseases, mild cases of typhoid fever, not showing the usual signs of the typical disease, are common, and these often go unrecognized, neither the patient nor anyone else being aware that he is infected with typhoid bacilli. In young children, especially, such cases are often overlooked. It is these mild, unrecognized cases, and the healthy carriers of the organisms, which constitute the greatest danger to the public health.

The importance of *carriers* in the spread of typhoid fever can

scarcely be overstated. Chronic carriers, who harbor the germs for years, have often been responsible for outbreaks of the disease. A famous case is that of "Typhoid Mary." This woman served as cook for several families in New York State. Within ten years' time, seven outbreaks of typhoid fever occurred which could be clearly traced to her. She refused to cooperate with health authorities, and would not give up her occupation as cook, or submit to an operation for removal of the gall bladder, which in all probability would have freed her of the germs. She was forcibly detained in a New York hospital for three years, and finally released on her promise not to engage in cooking. A few years later she turned up again as the cook in an institution where an outbreak of typhoid had occurred. Her complete history has never been learned, but she is known to have been responsible for at least fifty-one typhoid cases. She spent her declining years as a ward of the State of New York on North Brother Island, and died there in November 1938.

Bacteriological diagnosis. A laboratory diagnosis of typhoid fever may be made by: (1) demonstrating the presence of *Eberthella typhosa* in (*a*) the blood or (*b*) the feces or urine of the patient or (2) by demonstrating the presence, in significant concentration, of specific antibodies (agglutinins) for the bacillus in the patient's blood. This latter test is called the *Widal test,* after the man who first developed the method. The routine procedures for the diagnosis of a suspected case of typhoid fever include: (1) *blood cultures,* (2) *feces* (*or urine*) *cultures,* and (3) *agglutination* (*Widal*) *tests.*

Blood cultures are made by inoculating 5 cc or more of the *patient's blood* directly into a liquid medium. The blood-broth mixture is then incubated for twenty-four hours. If this culture is made from a typhoid patient during the early course of the disease, it will usually yield a pure growth of *Eberthella typhosa.* Identity of the organism in the blood culture is determined by microscopic examination, inoculation into various media for fermentation, and other tests (Table XXI), and finally by agglutination tests in known antityphoid serum.

Feces cultures. These are made by streaking a portion of the diluted feces on plates of two or more of the specially prepared agar media listed in Table XXII. All these media are available in dehydrated form. Most favorable for isolation of typhoid bacilli is *MacConkey's bile-salt medium,* or *bismuth-sulphite agar* (Wilson and Blair), but since growth may fail on any one medium, yet

appear on another, it is advisable to inoculate, also, additional plates of SS agar or desoxycholate agar. Moreover, better results are obtained in attempts to recover the typhoid bacilli if plating is preceded by a period of growth of the fecal specimen in a selective enriching broth. Media most widely used for this purpose are Leifson's *selenite-F broth,* or *tetrathionate broth.* These media have the effect of suppressing the growth of *Escherichia coli,* which is always present in abundance in fecal specimens (even those taken during the height of typhoid fever), while at the same time permitting the free multiplication of typhoid and paratyphoid bacilli. A bit of the feces is emulsified in one of these broths, and the cultures are incubated for not more than 12–18 hours at 37° C. Then the plates are streaked.

As will be seen from Table XXII, colonies of *Eberthella typhosa* may be recognized more or less easily on these differential plating media, after incubation overnight. Suspicious colonies are then carefully fished (avoiding *coli* colonies), and inoculations are made into Russell's double-sugar agar butt-slants and into fermentation tubes of glucose broth. The latter medium is used as a check upon the ability of the organism to form gas—a question not always clearly decided from the cultures in double-sugar agar. Next day, those cultures in Russell's medium that show an acid butt and unchanged slant, without gas, are selected for further study, since this is the way a pure culture of the typhoid bacillus would appear (Table XX). Identifying procedures are then carried out, including microscopic observations to check morphology, staining, and motility, fermentation tests with the significant sugars, and agglutination tests with known antityphoid serum.

Agglutination (Widal) tests. Such tests are made by mixing a known culture of *Eberthella typhosa* with the *patient's serum* and observing the preparation to see if the bacilli are clumped together (agglutinated). It should be noted that this serum will ordinarily be sterile and, in any case, we are *not looking for typhoid bacilli* in it, but are going to test it for the presence of specific typhoid *antibodies.*

A series of graded dilutions of the patient's serum is made from 1:20 through 1:1280, and each of these dilutions is set up separately with a standardized typhoid suspension in hanging drops or in test tubes. The important point is not the mere presence or absence of agglutinins for typhoid bacilli in the serum, but the concentration

TABLE XXII. Growth Characteristics of Intestinal Bacilli on Differential and Selective Media

MEDIUM	USUAL APPEARANCE OF COLONIES ON ORIGINAL PLATES
Eosin-methylene blue (EMB) *agar* (lactose 0.5%, sucrose 0.5%, dipotassium phosphate 0.2%, eosin and methylene blue)	*Escherichia*—large, opaque, with dark centers and metallic sheen. *Aerobacter and Klebsiella*—large, opaque, central dark spot, but no sheen. *Proteus, Eberthella typhosa, Salmonella, Shigella,* and *Alkaligenes fecalis*—colorless or bluish gray, translucent; *Eberthella* and *Shigella* colonies relatively small; *Proteus* colonies may be of spreading type.
MacConkey's bile-salt agar[1] (lactose 1%, bile salts 0.15%, neutral red as indicator)	*Escherichia, Aerobacter,* and *Klebsiella*—large, opaque, deep pink or red. *Proteus, Eberthella typhosa, Salmonella, Shigella,* and *Alkaligenes fecalis*—colorless or grayish, translucent; *Eberthella* and *Shigella* colonies relatively small; *Proteus* colonies may be of spreading type; *Alkaligenes fecalis* colonies may be surrounded by light-yellow zone, due to alteration of neutral red by alkali.
Bismuth-sulfite agar[1] (Wilson & Blair) (mixture of bismuth-sulphite, phosphate and glucose 20%, and an iron citrate-brilliant green mixture 4.5%, in nutrient agar)	*Escherichia, Aerobacter,* and *Klebsiella*—growth is largely inhibited; occasional colonies are large, opaque, may be colorless or gray-black. *Proteus*—growth may be inhibited; occasional colonies colorless, translucent, may be spreading. *Eberthella typhosa*—flat, black, surrounded by dark halo with metallic sheen. *Salmonella*—relatively large, convex, gray to black. *Shigella* and *Alkaligenes fecalis*—growth inhibited.
Desoxycholate-citrate agar[2] (Leifson) (lactose 1%, sodium citrate 2.0%. sodium desoxycholate 0.5%, ferric citrate 0.1%, neutral red as indicator)	*Escherichia, Aerobacter, Klebsiella*—growth inhibited; occasional colonies large, opaque, pink to red. *Proteus*—may be inhibited; colonies colorless, non-spreading. *Eberthella typhosa* and *Salmonella paratyphi*—translucent, colorless. *Salmonella*—relatively large, opaque, colorless; some strains are inhibited. *Shigella*—translucent or opaque; Shiga, Sonne, *dispar,* and *alkalescens* types are often inhibited. *Alkaligenes fecalis*—growth inhibited.
Salmonella-Shigella (SS) *agar*[2] (lactose 1%, bile salts 0.85%, sodium citrate 0.85%, sodium thiosulfate 0.85%, ferric citrate 0.1%, brilliant green, neutral red as indicator)	*Escherichia, Aerobacter, Klebsiella,* and *Proteus*—growth inhibited; occasional colonies large, pink to red, except *Proteus* colonies, which are colorless with brownish center, non-spreading. *Eberthella typhosa*—may be inhibited; colonies are translucent, colorless or bluish, slightly granular. *Salmonella*—relatively large, translucent or opaque, colorless with brown center, except *Sal. paratyphi,* which forms translucent colonies without the brown center. *Shigella*—translucent or opaque, colorless; Shiga and Sonne types often inhibited. *Alkaligenes fecalis*—growth inhibited; occasional colonies colorless.

[1] Generally regarded as superior for the isolation of *Eberthella typhosa.*

[2] Generally regarded as superior for the isolation of the common varieties of *Salmonella* and *Shigella,* except *Sh. sonnei,* which is likely to grow out better on EMB agar.

Note. Slow-lactose-fermenting (paracolon) organisms give colonies that are colorless in the first 24 hours, then slowly turn pink or red.

of these antibodies; i.e., the *titer* of agglutinins. It is not uncommon for the serum of persons not infected with typhoid germs to agglutinate the bacilli in dilutions of 1:20 or 1:40, but in frank cases of typhoid fever the titer is at least 1:100 and usually considerably higher. Only rarely, however, does the titer exceed 1:320.

In diagnostic laboratories, tests parallel to those with typhoid bacilli are set up routinely with the paratyphoid bacilli (*Salmonella paratyphi* and *schottmülleri*) whenever a serum from a patient suspected of having typhoid fever is submitted for examination. In this way, cases of paratyphoid fever may be discovered.

Interpretation of laboratory findings. All these methods of diagnosis—blood cultures, feces or urine cultures, and Widal tests—are tried in any case of suspected typhoid fever, and repeated until a positive diagnosis is made, but it will be clear, from the outline of the usual course of the infection given above, that not all the methods are equally valuable as diagnostic tests at all stages of the disease. A single negative result with any of these methods does not rule out typhoid infection. Technical difficulties sometimes account for failure to get a positive result. On the other hand, it must be remembered that the finding of *Eberthella typhosa* in the feces or urine does not in itself prove that the patient has typhoid fever, for he may be a *carrier,* and a positive response to a Widal test may be given by *immune* as well as infected individuals. It is often necessary to repeat the various tests several times, and they must always be interpreted in the light of the *patient's clinical condition and previous history,* before a diagnosis of typhoid fever can be made with certainty.

Immunity. Following recovery from an attack of typhoid fever, an individual has a high and lasting immunity to this infection. The resistance is not absolute, but it is so strong that second attacks of typhoid are rare. All the immune mechanisms—the inflammatory and phagocytic cells, as well as bacteriolytic and other antibodies—participate in the defensive reactions during a typhoid attack. After recovery, resistance seems to reside chiefly in the tissues themselves, and persists long after antibodies in the circulating blood are reduced to a very low titer. A person who has recovered from typhoid fever is *not* resistant to paratyphoid fever or dysentery.

A high degree of immunity to both typhoid and paratyphoid infection may be obtained by active immunization with a vaccine

consisting of a suspension of killed typhoid and paratyphoid bacilli, as described below.

Serum treatment for typhoid fever has been tried on a limited scale, but is not yet beyond the experimental stage.

Prevention. From what has been said about the spread of typhoid fever, it will be clear that prevention of this disease depends *in part* upon certain public sanitary measures, for which municipal and state health authorities are primarily responsible, namely: (1) an efficient sewage-disposal system, (2) a pure water supply—in cities this usually requires artificial purification of the water, (3) a pure milk supply, which involves sanitary control of dairies and of the marketing of milk, and pasteurization, (4) sanitary control over the preparation and marketing of foods, especially milk products and shellfish, (5) exclusion of known typhoid carriers from occupations in which they handle foods for public consumption, and detection and control of all carriers, so far as this is possible, and (6) the extermination of flies.

These public measures have been remarkably successful, since typhoid fever is one of the few diseases which is directly affected by sanitary conditions and is always greatly reduced in frequency when these conditions improve. But typhoid must continue to spread by personal contact unless individual cases of the disease are promptly diagnosed and carefully *isolated.* The responsibility, here, lies with the physician and the nurse. The nurse, especially, must know how to protect herself, the members of the patient's family, and the entire community from the spread of the disease.

If the patient remains at home, the nurse should avoid intimate contact with other members of the household, and, in particular, should have nothing to do with the preparation of their food. Flies must be kept out of the sickroom. Concurrent disinfection of the patient's dishes, linens, etc., and all the other necessary procedures in caring for an isolated patient must be carried out most conscientiously. Special effort must be made to disinfect promptly and thoroughly all the feces and urine.

It is important to remember that in intestinal infections the greatest danger lies in the possibility of conveying the germs to the mouth through the contaminated hands. It is therefore essential that the nurse and other attendants disinfect their hands immediately after every contact with the patient. These precautions must be

continued not only throughout the illness, but into the days of convalescence as well, *until repeated laboratory examinations show that the patient is no longer excreting typhoid germs.*

Prophylactic vaccination. Finally, a most important measure for the prevention of typhoid fever is *prophylactic active immunization.* Now most widely used in the United States is a triple vaccine, the so-called T.A.B. vaccine, manufactured according to the techniques developed at the Army Medical School in Washington, D. C., and standardized to contain one billion heat-killed typhoid bacilli per cubic centimeter, 250,000,000 paratyphoid A bacilli, and 250,000,000 paratyphoid B bacilli. These bacteria are suspended in buffered salt solution, containing about 0.3% tricresol or other preservative. They have been killed by heating the suspension at 56° C for an hour.

The strains used for the vaccine are especially selected. The typhoid-bacillus culture is known as No. 58; it is a smooth strain, virulent for mice (when inoculated along with mucin), and it contains all the antigenic elements (including the so-called Vi antigen) known to occur in freshly isolated bacilli. Similarly the cultures of *Salmonella paratyphi* and *Salmonella schottmülleri* are typical, highly virulent strains.

The usual routine is to give three injections of the T.A.B. vaccine subcutaneously, at weekly intervals, in the amount of 0.5 cc, 1.0 cc, and 1.0 cc. Some degree of protective effect from this primary series of injections probably persists for at least three years; but for all persons exposed to special risk of typhoid infection, an annual booster dose of the vaccine, which will strengthen and prolong resistance, is advisable. In the United States Army, annual revaccination is done by injecting 0.5 cc of the T.A.B. vaccine subcutaneously, while in the Navy the annual booster dose consists of 0.1 cc of the vaccine inoculated intradermally.

It must be remembered that, despite the proved effectiveness of prophylactic vaccination, the vaccinated individual is never more than *relatively* resistant, and may still contract typhoid fever if exposed to a particularly heavy dose of virulent germs. Whether a person is vaccinated or not, the same care must be exercised to avoid infection.

PARATYPHOID FEVER

This infection is similar, in every way, to typhoid fever. In general, however, it tends to be less severe. The causative organisms

are *Salmonella paratyphi* (para A) or *Salmonella schottmülleri* (para B). The mode of infection, methods of laboratory diagnosis, and prevention are essentially the same as in typhoid fever.

BACILLARY DYSENTERY

The term *dysentery* is really a clinical expression meaning simply an illness characterized by diarrhea. One type of dysentery is due to an ameba—*Endameba histolytica*—and is called *amebic dysentery*. This disease is discussed in Chapter XLII. The type of dysentery with which we are concerned here is *bacillary dysentery,* caused by bacteria of the genus *Shigella.*

Prevalence and importance. Bacillary dysentery is one of the commonest illnesses of man. Whenever and wherever ordinary sanitary precautions are relaxed the disease appears, in epidemic form. While the incidence of typhoid and paratyphoid fever has continued to decline in recent years, cases of bacillary dysentery have been occurring as frequently as ever. As in all past wars, dysentery was a major cause of sickness and loss of fighting effectiveness among troops of all nations in World War II. Soldiers sent to tropical countries were especially liable to contract the disease; in these places hardly anyone escapes dysenteric infection. Even in peacetime, dysentery remains an ever-present threat, the world over. The mortality rate in adults is generally low (perhaps about 2%), but dysentery is one of the chief causes of death in infants and young children.

Varieties of dysentery bacilli. The dysentery germs are distinguishable from all the other intestinal bacilli by one conspicuous property—they are *not motile.* There are four main varieties of these organisms: *Shigella dysenteriae,* first isolated by Shiga, in 1898, during an epidemic of dysentery in Japan; *Shigella paradysenteriae,* of which the *Flexner* subtype, first obtained by Flexner, in 1900, from cases of the disease in the Philippine Islands, is the most important; *Shigella sonnei,* first described by Duval, but now generally referred to as the Sonne type; and *Shigella ambigua,* the Schmitz bacillus (Table XXI).

The Shiga type is a distinct species, set apart from all other dysentery bacilli by its failure to bring about any of the usual biochemical changes (other than acid-formation from glucose) and by its capacity to form a

powerful *exotoxin*. Infections with *Shigella dysenteriae* are likely to be especially severe because of this toxin.

Outbreaks due to the Shiga bacillus occur most frequently in Asiatic countries; this species has been encountered only very rarely in the United States. In this country and in England, the Flexner and Sonne types of dysentery bacilli are responsible for the great majority of cases.

Features of dysentery in man. Like typhoid, bacillary dysentery is a human disease, and the germs do not naturally infect animals. *Acute* dysentery differs from typhoid in having a short incubation period, rarely of more than forty-eight hours, and in being strictly an intestinal infection. In dysentery, the organisms are not found in the patient's blood, and they do not invade the internal organs to any great extent. They may cause an intense inflammation of the walls of the intestine, often resulting in permanent damage. Cases vary a great deal in severity; some patients will have the "bloody diarrhea" so often mentioned in classic descriptions of the disease, but the majority will have a simple diarrhea without conspicuous blood in the stools.

Carriers of dysentery bacilli are numerous. Also, cases of *chronic infection* with members of the genus *Shigella* are common; these may assume various clinical forms.

Bacteriological diagnosis. The only satisfactory method of laboratory diagnosis is to cultivate the bacilli from the patient. In the early stages of acute bacillary dysentery, isolation of the causative organisms from the feces is usually accomplished without difficulty by using the same special media and methods employed for *Salmonella*.

An ordinary stool specimen may be used for the cultures, but when the making of a number of cultures from different persons is desired—for example, in the study of a dysentery outbreak in a camp or institution—the substitution of a *rectal swab specimen* has been found a very helpful procedure. A cotton swab, tightly wound on the usual wooden applicator, is moistened, then placed inside a short length of soft rubber tubing which has the distal end cut on a bevel and lubricated. The beveled end is easily inserted past the sphincter, then withdrawn slightly to expose the swab. The specimen is taken by rotating the applicator, then drawing it back into the rubber tube. Immediately upon removal from the patient, the swab may be streaked directly over the surface of SS agar, and one or two of the other special media listed in Table XXII.

Final identification of the organisms fished from *Shigella*-like colonies on the original plates will depend upon tests for staining and motility, fermentation reactions, indol tests, and agglutination in known specific antiserums.

The spread of dysentery; prevention. Typical, slowly developing epidemics of dysentery are especially liable to occur among the inmates of prisons, asylums, and other institutions, where men and women are crowded together under poor hygienic conditions. *Carriers* of one or the other variety of dysentery bacilli are often responsible for outbreaks. It must be remembered that the infection may persist long after clinical recovery, often for a period three or four times as long as the total duration of symptoms. The infection spreads largely by *personal contact,* and through the agency of *flies,* but also may be transmitted through contaminated food or water.

Prevention demands good sanitary facilities and practices. The element of personal cleanliness is most important, especially among the personnel of public institutions, boarding houses, hospitals and the like.

Active immunization against bacillary dysentery is not often attempted. There is a multiplicity of antigenically different strains included among the dysentery bacilli; this obviously adds to the difficulty of preparing an effective vaccine. Moreover, killed dysentery organisms contain so much endotoxin that they cannot be safely injected into human beings, unless their toxicity is in some way reduced; altogether satisfactory methods of accomplishing this have not been found.

Summer diarrhea. An important form of dysentery is the so-called *summer diarrhea,* or *infantile diarrhea,* which may become prevalent in the hot months of the year, especially among bottle-fed infants in homes where there are poor hygienic conditions. Explosive outbreaks of epidemic diarrhea in the newborn infants occur occasionally in first-class, modern maternity hospitals.

When the feces of infants suffering from diarrhea are examined, bacilli of the same varieties as those associated with dysentery in adults (*Shigella*) are sometimes found, but in many instances there are organisms of other kinds, such as varieties of *Salmonella, Proteus morgani,* or *Clostridium perfringens.* The exact etiologic agent in the hospital epidemics among the newborn babies has not been determined.

These infectious diarrheas in bottle-fed infants can be prevented

only by extreme care in the preparation of their food. All water and milk that they receive should be boiled, and the bottles and nipples should be sterilized. If an infant can be breast-fed during its first summer, most of the danger will be avoided.

CHOLERA

Cholera is caused by infection of the intestine with a spirillum discovered by Koch in 1884 and named *Vibrio cholerae* (or *Vibrio comma*). The incubation period is commonly only a few hours—at most, five or six days. Onset of the disease is marked by a profuse diarrhea, persistent vomiting, and cramps, and often there is early and profound prostration. The stools become watery, containing only cellular debris and shreds of mucus—so-called rice-water stools. Soon the patient is extremely dehydrated from continual loss of fluid; secretion of urine is diminished; and acidosis and uremia develop. In many outbreaks, most of the patients die, though mild cases also occur.

The real home of cholera is India, especially along the delta of the Ganges River, where it has been endemic for centuries. From this focus, the disease has swept many times in severe epidemics over most of the rest of the world, and numerous cases have occurred in North America. Since about 1875, however, cholera has been kept out of the United States and England, and, with the development of Western civilization, it has been gradually restricted to the warmer Asiatic countries. In parts of India, Iraq, and southern China, it is still a great scourge.

There is little likelihood that cholera will ever again get a foothold in this country. It is necessary, however, for authorities of the United States Public Health Service to maintain a constant watch at ocean ports to prevent the admission of immigrants who are carriers of the spirilla. To this vigilance we owe our present freedom from the disease.

Vibrio cholerae. Cholera spirilla appear most typically as short spirals in the shape of a comma; for this reason, the organism is often referred to as the "comma bacillus." In smears from "nests" of the organisms in the feces, they appear lined up, with each "comma" headed in the same direction, like fish in a stream. In cultures, longer and more irregular, curved forms are seen. The spirilla are actively motile, by virtue of a single, long terminal flagellum, and they are Gram-negative (Fig. 104).

They will grow in the laboratory upon ordinary media, but they are sensitive to even a trace of acid, and multiply best in highly alkaline media of pH 8.0 to 9.0. They liquefy gelatin and coagulated blood serum. In broth they form indol, and they also reduce nitrates to nitrites. When a broth culture is made strongly acid by the addition of hydrochloric or other mineral acid, a deep-red color appears, owing to the presence of nitroso-indol. This is the so-called "cholera-red test." The organisms do not produce an exotoxin, but they contain considerable endotoxin.

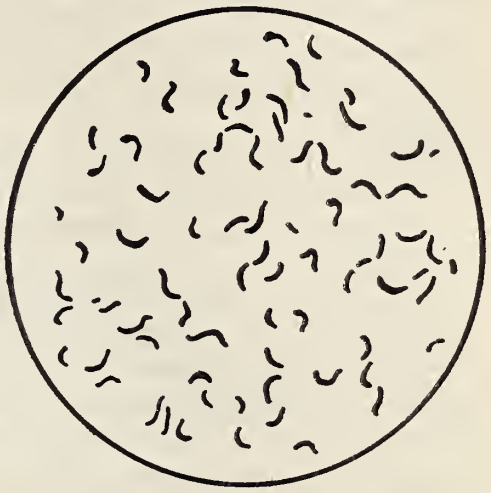


FIG. 104. The spirillum of cholera (*Vibrio cholerae*).

Bacteriological diagnosis. A provisional diagnosis of cholera may sometimes be made by a simple microscopical examination of the mucous flakes in the watery feces. A definite diagnosis is reached only by isolating the spirilla from the feces, using alkaline media, and by conducting agglutination tests and other identifying procedures on the pure cultures obtained.

Carriers are detected by feces examination and tests for cholera agglutinins in the blood.

The spread of cholera; prevention. Cholera spreads by the same means as other intestinal infections discussed above, and measures to prevent cholera are similar to those for typhoid. In most countries, stringent regulations are enforced by health authorities at seaports to prevent the introduction of the disease by immigrants from places where cholera is endemic.

Recovery from cholera confers a lasting immunity. Several forms of cholera vaccines have been used with good effect for prophylactic immunization in India and China. The immunity produced by this means is thought to be of short duration, a year or less. The United States Navy recommends a vaccine, similar to the T.A.B. vaccine, consisting of a suspension of heat-killed cholera organisms.

REFERENCES

Typhoid Fever

ALMON, L.: "Significance of the Vi Antigen," *Bact. Rev.,* 1940, 4:43.

CALLENDER, G. R. and LUIPPOLD, G. F.: "The Effectiveness of Typhoid Vaccine Prepared by the U. S. Army," *J. A. M. A.,* 1943, 123:319.

SCHLESINGER, E. R.: "Use of Modern Laboratory Aids in the Investigation of a Typhoid Outbreak," *Am. J. Pub. Health,* Oct. 1943, 33:1257.

Dysentery

HARDY, A. V. and WATT, J.: "The Acute Diarrheal Diseases," *J. A. M. A.,* Apr. 22, 1944, 124:1173; "Newer Procedures in Laboratory Diagnosis and Therapy in the Control of Bacillary Dysentery," *Am. J. Pub. Health,* May 1944, 34:503.

CALLENDER, G. R.: "Diarrheal Diseases," *Am. J. Trop. Med.,* Jan. 1944, 24:7.

HORMAECHE, E., SURRACO, N. L., PELUFFO, C. A. and ALEPPO, P. L.: "Causes of Infantile Summer Diarrhea," *Am. J. Dis. of Children,* 1943, 66:539.

KUHNS, O. M. and ANDERSON, T. G.: "A Fly-borne Bacillary Dysentery Epidemic in a Large Military Organization," *Am. J. Pub. Health,* July 1944, 34:750.

LEMBCKE, P. A., QUINLIVARR, J. J. and ORCHARD, N. G.: "Epidemic Diarrhea of the Newborn: A Report of Two Outbreaks," *Am. J. Pub. Health,* Oct. 1943, 33:1263.

LITTMAN, M. L.: "Rapid Identification of Enteric Pathogenic Bacteria," *War Med.,* July 1943, 4:31.

NETER, E.: "The Genus *Shigella*," *Bact. Rev.,* 1942, 6:1.

WEIL, A. J.: "Progress in the Study of Bacillary Dysentery," *J. Immun.,* 1943, 46:13.

REVIEW QUESTIONS—CHAPTER XXXIV

1. Discuss the prevalence of typhoid fever. What are some of the factors which have caused it to be less common?
2. Name and describe the morphological and physiological properties and biochemical activities of the germ of typhoid fever.
3. Trace the typhoid bacilli through the body during the course of typhoid fever. When are the germs to be found in the blood? In the feces? When do specific antibodies appear in the blood?
4. Explain how the germs of typhoid may be carried from person to person. What is the importance of personal contact? Of carriers?
5. In what two ways may a laboratory diagnosis of typhoid fever be made? What are the three routine diagnostic procedures?
6. How is a diagnosis made by blood cultures? At what time during the infection are they most likely to reveal the presence of *Eberthella typhosa*?
7. Outline the method of examining feces for typhoid bacilli.
8. Explain in detail the method of performing a Widal test. In this test, what are you looking for? In a positive test, what is agglutinated? What causes this agglutination? Discuss interpretation of the results.

9. Discuss immunity to typhoid infection. How may immunity be acquired?
10. Discuss the prevention of typhoid fever. Explain the importance of: (*a*) sanitation and the work of public-health authorities, (*b*) care of the isolated case, and (*c*) prophylactic vaccination. Describe the present U. S. Army vaccine, and the usual immunization procedures.
11. What is paratyphoid fever?
12. What is "dysentery"? What type of dysentery is caused by a protozoan? Name and describe the principal varieties of the dysentery bacilli.
13. Outline the chief clinical features of bacillary dysentery in man and the methods used for the bacteriological diagnosis.
14. Discuss the prevention of bacillary dysentery.
15. Discuss the nature, cause, and prevention of infantile summer diarrhea.
16. Where does cholera occur? What are the outstanding features of the illness? How is it kept out of the United States?
17. Name and describe the causative organism of cholera. Outline methods used in laboratory diagnosis.
18. Discuss the mode of spread and prevention of cholera.

CHAPTER XXXV

BACTERIAL INFECTIONS ACQUIRED FROM ANIMALS

In this chapter we shall describe a group of infectious diseases that have a number of features in common. All of them affect animals primarily, though they are often transmitted to man, and in each case there exists among certain animal species a reservoir of the infection which constitutes a perpetual menace to the health of human beings. In the spread of several of these diseases, insects and arthropods play an important part. The germs concerned are all highly invasive, and virulent, causing severe, prolonged, and often fatal illness in man. Most of them remain dangerous for the bacteriologist, even after prolonged laboratory cultivation.

BRUCELLOSIS (UNDULANT FEVER)

In a previous chapter we mentioned that the *Brucella* organisms cause one of the most prevalent and dangerous of the milkborne diseases—*undulant fever,* or *brucellosis.*

Brucella. The causative organisms of brucellosis are small, somewhat pleomorphic, Gram-negative, non-sporebearing, nonmotile bacilli; no capsules have been observed. Laboratory strains may be cultivated on plain media, but, for primary isolation from the body, richer media—such as liver infusion or serum agar—are necessary. Growth is generally slow and sparse. Isolation of *Brucella abortus* from animals or human patients is greatly aided if the cultures are incubated in an atmosphere containing 5–10% CO_2. No signs of fermentation appear in cultures in the usual sugar media. Indol is not formed. No exotoxin has been demonstrated.

Three "species" are recognized: (1) *Brucella melitensis,* originally isolated by Bruce in 1886, and shown to be the causative agent of typical Malta fever, which is contracted by drinking *goat's* milk containing these organisms; (2) *Brucella abortus,* originally identi-

fied by Bang in 1897 as the causative agent of contagious abortion (Bang's disease) in cattle; and (3) *Brucella suis,* isolated by Traum from aborting swine, in 1914. These organisms are virtually alike in morphology, but they do show certain physiological differences which are used in differentiating them (Table XXIII). All three varieties are capable of causing undulant fever in man.

Table XXIII. Differential Properties of Brucellosis Organisms

Species	CO_2 required for primary growth	H_2S production	Growth in presence of	
			Basic fuchsin 1:25,000	Thionin 1:50,000
Brucella melitensis	—	— or ±	good	good
Brucella abortus	+	+ (continues through 4 days' incubation)	good	poor or none
Brucella suis	—	++ (continues through 6–10 days' incubation)	poor or none	good

Sources of infection. Human beings are infected secondarily from animals; brucellosis probably never spreads directly from man to man. The germs may gain entrance either through the skin or through the mucous membrane of the gastrointestinal tract. Persons acquire the infection by drinking *raw milk* from infected cows or goats (or possibly through butter or cheese made from contaminated milk), by direct contact with infected living animals, or by handling infected carcasses or meat. Many bacteriologists have been infected while working with *Brucella* in the laboratory. Hence, sporadic cases occur most commonly among farmers and their families, butchers, slaughterhouse workers, veterinarians, and laboratory personnel, while localized epidemics, usually involving a small group of individuals, occur among the consumers of the same contaminated raw-milk supply. Multiple cases are especially likely to appear among

persons using a milk contaminated with *suis* strains, for these are generally of relatively high virulence. *Brucella melitensis* has been isolated from human cases of brucellosis in places in the United States where goat-raising is a prominent activity, as in some parts of Texas and California, but in the great swine- and cattle-raising states of the Middle West, and the country generally, most cases are due to infection with the *suis* or *abortus* varieties.

Brucellosis in man. Virulent strains of *Brucella* produce in human beings a specific disease, characterized by periods of high fever, chills, sweating, rheumatic pains, and other signs and symptoms of a generalized infection, alternating with periods in which the fever and other symptoms temporarily disappear. Some cases are so mild that the patient is never confined to bed. Eventual recovery is the rule, but often the illness is both severe and prolonged.

The onset of symptoms is usually gradual, after an incubation period commonly of from two or three weeks, but varying all the way from one week to four months. The fever may follow the typical undulating course for weeks and months at a time. Often each bout of fever lasts about 7–10 days, and the total duration of the acute illness commonly extends over 6–8 weeks. In the later stages of the acute disease, the organisms may localize at various sites within the body, as in the joints, brain, or meninges.

In certain individuals, the illness becomes *chronic;* symptoms disappear, only to reappear from time to time, months or even years later, in a variety of clinical forms. Brucellosis is somewhat like syphilis and tuberculosis in its tendency to become latent, and to assume various clinical disguises in its chronic form.

Bacteriological diagnosis. The clinical picture of brucellosis in man, especially in mild and chronic cases, is not sufficiently distinctive to permit a definite diagnosis without the aid of the laboratory. Four types of diagnostic tests may be performed: (1) *blood cultures,* (2) *agglutination tests with the patient's blood serum,* (3) *opsono-cytophagic tests* with the patient's blood, and (4) an intradermal *skin test* with an antigen made from *Brucella* organisms.

Blood cultures. These may be made in the usual way, by introducing 5–10 cc of the patient's blood directly into three or four flasks of liver infusion broth or Bacto-tryptose broth. Always some of the cultures are placed in a chamber with 10% CO_2, while others are incubated in the ordinary way. Cultures may be made, also, from joint fluid, bile, lymph nodes, or other tissues. It is important that

blood and all other materials be cultured as soon as possible after collection.

The *Brucella* grow out slowly, and cultures must be watched for two weeks or more. The bacteria that finally appear may be identified by their morphological and cultural properties, and by their agglutination in specific antiserum. The most dependable procedure for identifying the species of Brucella is inoculation upon the differential dye media of Huddleson (Table XXIII).

Agglutination tests. Such tests with the blood serum of the patient and *Brucella abortus* as the antigen are relied on for diagnosis in most cases. The general method is the same as in the Widal test for typhoid fever.

Opsono-cytophagic tests. These are done to determine the relative opsonic activity of the patient's leukocytes toward *Brucella.* By a recently recommended method a standardized, formalin-killed suspension of *Brucella abortus* in a sodium citrate solution is mixed with an equal volume of the patient's freshly drawn blood (final concentration of citrate 1%). The mixture is incubated at 37° C for 30 minutes; then smears are prepared and stained with a special stain. The stained smears are then examined microscopically, and a count is made of the number of bacteria found phagocytized (within the cytoplasm) of 25 polymorphonuclear leukocytes. The figures thus obtained may be used to express the avidity with which the patient's leukocytes take up the *Brucella* organisms.

Skin tests. Such tests for brucellosis are performed by injecting intradermally 0.1 cc either of a heat-killed suspension of *Br. abortus* or of the material called *brucellergen,* a preparation of nucleoprotein from *Brucella,* originally made by Huddleson. Intense local inflammation and rather alarming general symptoms not infrequently follow the injection in persons sensitized by previous infection with *Brucella.* As in the tuberculin test, the local reaction is delayed; it is usually read forty-eight hours after the injection.

Significance of the diagnostic tests. Actual isolation of a strain of *Brucella* from the blood or other tissues of a patient is, of course, the best evidence that the individual has brucellosis. Cultures are not infrequently negative, however, even in the presence of very suggestive symptoms. On the other hand, a blood culture may be positive during a period when clinical signs of the disease are largely absent. Repeated cultures must be made, and negative findings will not exclude a diagnosis of undulant fever.

Agglutination tests are the most useful means of reaching a definite diagnosis. A titer of 1:160 or more is significant in clinically suggestive cases. The patient's serum will not ordinarily agglutinate the bacilli in high titer until some time in the third or fourth week of illness; then the titer often reaches 1:1280. Blood for agglutination tests must be obtained from the patient *before a skin test is done,* since the injection of brucellergen or other skin-test antigen may in itself stimulate the formation of *Brucella* agglutinins.

The diagnostic value of phagocytic tests on the patient's blood is problematical.

A negative skin test may ordinarily be regarded as clear evidence that the patient has never been infected with any variety of *Brucella.* On the other hand, a positive test—like a positive Frei test or tuberculin test—signifies only that infection has occurred at some previous time, and not necessarily that the patient has active brucellosis at the moment.

Specific therapy. Antiserums prepared by immunizing goats, cattle, or horses against all three varieties of *Brucella* have been used successfully in the treatment of acute brucellosis in man. In subacute and chronic cases clinical improvement has often followed the judicious use of vaccines made up of killed suspensions of *Brucella abortus* or *Brucella suis.*

Prevention. The control of brucellosis is a complicated and difficult problem. Detection of *Brucella* infection among dairy cattle or other domestic animals, by agglutination tests with their blood, and destruction or isolation of infected animals are valuable measures. For the protection of the general public, *all milk* for direct consumption, or for use in making other dairy products, *should be pasteurized.* No practicable method has yet been devised to prevent those cases that arise from direct contact with infected animals.

PLAGUE

Plague has been one of the most destructive pestilences ever suffered by mankind. Great epidemics have swept over most of the world, repeatedly. Celebrated accounts of the "Black Death" (as plague was called in the Middle Ages) are given by Boccaccio, by De Foe in his vividly imaginative *Journal of the Plague Year,* and by Pepys in his *Diary.* The last extensive epidemic began in Hong Kong in 1894, and spread widely through most of the world. It was

during this epidemic in China that the germ of plague, *Pasteurella pestis,* was discovered independently by Kitasato and Yersin. The disease is still common, and terribly destructive to human life, in such countries as India, Burma, Manchuria, parts of China, and in Africa. Small epidemics and sporadic cases continue to occur in Russia, southern Europe, the Middle East, South America, and Hawaii.

Sources of infection; the animal reservoir of plague. Plague is primarily a disease of *rats* and other rodents, wild and domestic. Rats are great travelers, and almost all ships are infested with them. Ships harboring plague-infected rats have carried the disease to ports throughout the world. In the United States, plague first appeared in San Francisco, in 1900, and it subsequently occurred in New Orleans, Texas, and Florida. In California there have been several hundred cases, but never an extensive epidemic, owing to the energetic work of health officers. Since about 1924, there has been a very little plague in this country, but it must still be regarded as a menace because the ground squirrels and other wild rodents in the western states have become infected, and therefore a great and uncontrollable animal reservoir of plague exists there. The possibility that occasional human cases of plague may arise from contact with these infected wild rodents must be reckoned with, and still more important is the danger that an epidemic (epizoötic) among the ground squirrels may be communicated to domestic rats in the towns and cities, with consequent threat to the human population. A similar situation exists in various other parts of the world where animal reservoirs of plague-infection have become established. Plague in wild rodents is called *sylvatic plague.*

The causative organisms of plague are carried from animal to animal, and from rat (or other rodent) to man, through the bite of *fleas.* Among the several varieties of fleas which may act as vector of the plague bacilli from rodent to man, the oriental rat flea *Xenopsylla cheopis* is the most important. When fleas bite an infected rodent, they swallow some of the plague bacilli; then, in some of the fleas, these organisms multiply to such an extent that they block the esophagus and proventriculus with a solid mass. Now, when such a flea attempts to get a blood meal from a human, this mass of plague organisms is regurgitated, and deposited upon the skin. The bacilli are able to invade the body easily through the tiny wound

made by the fleabite, or the organisms may be rubbed in by the clothing, or by scratching the fleabite.

Human plague is obviously most likely to occur among those persons who live or work in rat-infested buildings, or who handle dead rats or other rodents that might be infected. Large plague epidemics arise almost always as a consequence of an epizoötic of the disease among domestic rats. *Xenopsylla cheopis,* though primarily a rodent flea, leaves the dead or dying plague-infected rat and readily attacks man.

Clinical forms of plague in man. The most characteristic form of plague in human beings is so-called *bubonic plague.* The germs, which have entered by means of a fleabite, localize in the nearest lymph glands, which swell and later soften and discharge pus. The femoral glands in the groin are most often involved, since fleas often bite the legs. When these glands swell, they are called "buboes"—hence the name bubonic plague. The bacilli spread to other glands and to the blood stream, and in the majority of cases a fatal septicemia results. Sometimes a very acute, fatal blood infection (*septicemic plague*) develops without primary localization in the glands. These forms of plague probably never spread directly from man to man.

A third form of the disease, however, is directly communicable from one person to another, and doubtless cases of this kind always occur in extensive human epidemics. This is *pneumonic plague,* in which the lungs are primarily involved, then later the blood, with death resulting in practically every instance. This infection spreads rapidly by personal contact in the same manner as other respiratory diseases. Sometimes pneumonic plague develops in a case which was originally bubonic; the individual then becomes, in the few days of life that may remain to him, a source of infection for plague of the pneumonic type.

Patients with bubonic plague sometimes recover, and, when they do, they possess a lasting immunity to the disease.

Pasteurella pestis. The organisms of plague are small Gram-negative nonmotile bacilli, usually oval in outline, and staining only at the poles (Fig. 105). In smears from the body, they show a narrow capsule. They grow moderately well on ordinary culture media at an optimum temperature of 30°–35° C. In cultures, they have a marked tendency to develop involution forms, of bizarre shapes, particularly when grown on agar containing 3% sodium

chloride. They have no spores, and are easily killed by heat and the usual chemical disinfectants. In laboratory cultures, however, they survive in virulent form for many years.

The organisms do not form an exotoxin, and, like the germs of anthrax, glanders, and tularemia, they owe their disease-producing power solely to their capacity to invade the tissues and multiply there very rapidly. They are highly pathogenic for laboratory animals. Plague bacilli belong to the group of germs called the hemorrhagic septicemia group, and closely related species are responsible for the common disease called *hemorrhagic septicemia* in cattle, swine, sheep, guinea pigs, and other animals.

FIG. 105. *Pasteurella pestis,* the cause of plague.

Bacteriological diagnosis. Prompt laboratory diagnosis of any case of human plague is of the greatest importance. This dangerous task is best done by an expert familiar with the germ. It goes without saying that the utmost precaution must be taken by physician, nurse, and bacteriologist to avoid infection. The plague bacilli may be demonstrated in smears and in cultures on blood agar plates from the bubo, or (in pneumonic cases) from the sputum. Sometimes blood cultures will reveal the organisms. Their identity may be established by agglutination tests with known diagnostic antiserum. Final proof that one is dealing with *Pasteurella pestis* may be obtained by inoculating laboratory animals. The germs are so highly invasive that they will enter and infect a guinea pig if material containing them is merely rubbed on the shaven skin; and a pure culture can be recovered from the organs of the animal.

Specific prophylaxis and therapy. Active immunization against plague is an important measure for the protection of members of the armed forces who are assigned to areas where plague is endemic, and for laboratory personnel and other workers handling infected materials. A vaccine made from formalin-killed, virulent plague bacilli has been used by the U. S. Army and Navy. Vaccines composed of living *avirulent* strains have been tried in Africa and elsewhere. These are probably superior in immunizing power, but they may not be safe. Although antiserums for the treatment of bubonic plague have been used widely, their effectiveness is doubtful. Promising results in treating experimental plague infections with sulfonamides and with some of the newer antibiotics are reported.

Prevention. There is little danger that plague will ever reach serious epidemic proportions in the United States so long as the present precautions are continued. Health officers in the Pacific states, and elsewhere where plague has appeared, maintain a constant vigilance to detect the possible outbreak of plague among the rat population, and they are prepared to take vigorous steps to stamp out the focus of infection before the disease spreads to man. Continual warfare is waged against rats and other rodents. At ocean ports, ships are quarantined and fumigated routinely, and efforts are made to prevent rats from getting from ship to shore.

The extermination of rats everywhere is worth while, not only because they are a possible source of plague, typhus, and other human diseases, but because they cause a tremendous economic loss. As in the control of any other living pest, the most effective measures are those which help to prevent the *breeding* of the animals. The best means of accomplishing this is to make all houses, stores, granaries, ship, etc., *rat-proof,* i.e., contruct them in such a way that rats cannot find safe harborage and cannot readily secure food.

TULAREMIA

This disease occurs naturally in birds (such as quail and ducks), wild rabbits, ground squirrels, and other animals, and from this reservoir of infection it may be transmitted secondarily to man. It is spread from animal to animal by blood-sucking insects, especially ticks. Man is sometimes infected by the bite of an infected insect or arthropod, but most commonly through the contamination of his hands or conjunctiva by contact with the germ-laden organs and body fluids of infected rabbits or other animals.

In 1911, McCoy first described the infection in animals as a "plague-like disease of rodents," and in the following year McCoy and Chapin isolated the causative organism. They called the germ *Bacterium tularense*—from Tulare, the county in California where they were working. The first human case of *tularense* infection to be proved bacteriologically was diagnosed by Wherry and Lamb in Cincinnati, in 1914. In 1920, Francis proved that the "deer-fly fever" of Utah was a form of the same infection, and it was he who suggested naming the disease *tularemia,* a logical name since the organism was found in the blood.

Human tularemia has since been reported in almost every state in this country, and it has been recognized also in various Asiatic and European countries.

Sources of infection. There are many ways in which human beings may be infected with tularemia. Deer flies, horse flies, ticks (*Dermacentor andersoni,* the wood tick of western states, and *Dermacentor variabilis,* the dog tick of eastern and southern states), and other biting insects may carry the germs to human beings from the numerous forms of wild life that are infected. Infection has followed the bite or scratch of various kinds of animals, including the domestic cat. (Presumably, in these instances the mouth parts of the biting animal were contaminated by feeding on infected material.) Cases have been traced to the eating of *partially* cooked rabbit meat, and to the drinking of water from a brook contaminated by infected animals. The highly invasive germs have often infected bacteriologists and other laboratory workers. By far the largest percentage of cases in the United States, however, has resulted from infection of the hands or eyes while dressing infected wild rabbits. Thus hunters, market men, and housewives have often been victims, and cases are likely to be most numerous during the rabbit-hunting season.

Tularemia in man. The incubation period is commonly about 2–5 days, but may be as long as 9 days. Onset of symptoms is usually sudden, with headache, vomiting, body pains, and chills and fever. In typical cases, an ulcerating sore develops on the finger or at another point where the germs originally penetrated the skin, and the associated lymph glands become markedly swollen and painful. In another common and severe form of the disease, the infection begins in the eye, and involves, primarily, the regional lymph glands (so-called *oculoglandular tularemia*). The infection becomes generalized early; there is at least a transient bacteremia; and there may be localization of the germs in the lungs, pleura, meninges, or elsewhere. In some cases of pulmonary tularemia the lungs are involved primarily, without any skin sore or glandular enlargement. The acute illness often persists for two or three weeks, or more. Recovery is usually slow, and relapses months or even years later may occur. About 6% of cases are fatal. Encouraging results have been obtained recently by treatment with streptomycin.

Bacterium tularense. The causative organisms of tularemia are tiny, Gram-negative, nonmotile bacilli, having at times a coccoid

form, and at other times appearing as rods less than one micron long. Capsules probably develop in the tissues. Although usually classified with the plague bacilli in the genus *Pasteurella* (*Pasteurella tularensis*), the tularemia organisms differ from the germs of plague, especially in their cultural requirements. They will not grow on ordinary media, but demand enriched media *containing cystine.* Most widely used is a glucose-blood-cystine agar medium. Only recently have methods been developed for cultivating these fastidious bacteria in liquid media. No toxins have been found. The organisms have no spores, and are not especially resistant to heat.

Bacteriological diagnosis. The tularemia bacilli may sometimes be found microscopically in discharges from ulcerating lesions, and may be *cultured* from this material or (rarely) from the blood. Cultures, or the patient's blood, may be used to reproduce the infection in guinea pigs. These procedures are dangerous for the bacteriologists, however, and are not performed routinely. Diagnosis is usually made by *agglutination tests,* using the patient's blood serum and a standard suspension of killed *Bacterium tularense.* After the second week of the disease (and for months or years after recovery), the blood of most patients will agglutinate tularemia bacilli in high dilution (1:500 or more). The patient's blood should be tested at the same time with *Brucella* organisms, and with plague bacilli, since brucellosis, plague, and tularemia may be confused with one another clinically.

Prevention. To avoid danger of tularemia, hunters should beware of rabbits, other animals, and birds which appear ill, or which have been too easily caught. In dressing these animals, great care must be taken not to cut the skin of the hands. It would be advisable to wear rubber gloves. *Thoroughly cooked* rabbit meat, even from an infected animal, is harmless for food.

ANTHRAX

Anthrax is primarily a disease of cattle, sheep, horses, and mules, from which human beings are infected secondarily. It is caused by *Bacillus anthracis,* a Gram-positive, aerobic, sporebearing bacillus. As we have explained in Chapter III, anthrax was one of the first diseases to be studied by the newer bacteriological methods worked out by Koch and Pasteur (1877), and the first human infection for

which a particular germ was proved to be responsible. The Latin word *anthrax* comes from a Greek word meaning a *coal,* and refers to the characteristic dark, tarry color of the blood of animals which have died from the disease.

In animals, anthrax usually takes the form of a septicemia, and death often occurs within a few days. The bacilli remain almost entirely within the blood vessels, but become so numerous there that the capillaries are literally choked with them. When an animal dies, the anthrax organisms escape to the ground with blood and excretions from the body. In the presence of oxygen outside of the body, the bacilli are converted to the spore form. These *anthrax spores* are extremely resistant. The still-viable spores become widely distributed on the skin and hair of other animals in the same pasture; in the food; and in all surrounding objects. The fully virulent spores may remain alive for years upon the surface of the ground, particularly in moist soil. When cattle, sheep, or other animals graze upon such land, or eat contaminated fodder, they may swallow these spores, or the germs may enter through breaks in the mucous membrane of the mouth.

Sources of human infection. Human anthrax is always contracted either directly from animals, or from animal products containing the spores. In the United States, anthrax is an industrial hazard of considerable importance. The raw materials—wool, hides, horsehair, etc.—used in a variety of manufacturing processes are largely imported from countries where anthrax is prevalent, and often these reach the workbench still contaminated with viable anthrax spores. During World War II, a decided increase in the number of cases of human anthrax among workers in these industries occurred, as the demands of war brought about relaxation of anthrax-preventive measures abroad and the use of inferior (and more heavily contaminated) grades of wool and hides. In England, a central disinfecting station for imported wool and hair operates efficiently to protect industrial workers, but there is no such station in this country, and the use of really effective disinfecting methods by the individual manufacturer is difficult and expensive.

Anthrax in man. The commonest form of anthrax in human beings is a localized skin infection, which follows when anthrax spores get into an abrasion on the hands, face, or other exposed part. This is an occupational disease of workers in tanneries and other industries handling animal products from cattle, sheep, or

horses. Also, it is the clinical form of anthrax usually seen in farmers, butchers, and other persons infected more directly from animals. After an incubation period of 1–3 days, the organisms produce in the skin a characteristic boil-like lesion, known as a *malignant pustule.* The infection usually remains localized, and recovery is the rule. When death occurs, there is a terminal septicemia. In workers who handle wool, a pulmonary anthrax, or *woolsorter's disease,* sometimes occurs. This severe, and often fatal, infection is contracted when a large amount of dust containing anthrax spores is inhaled. Rarely, there may occur an *intestinal* form of *anthrax,* caused by eating food contaminated with the spores.

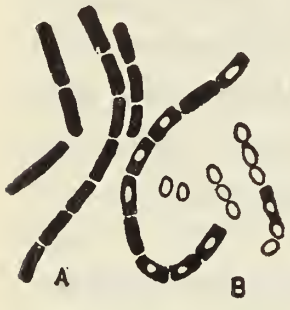


FIG. 106. *Bacillus anthracis,* the cause of anthrax. A: vegetative forms in the characteristic chain arrangement; B: sporulating forms and free spores.

Bacillus anthracis. The anthrax organisms are relatively large Gram-positive, aerobic, sporebearing bacilli. *Capsules* about the organisms may be seen in smears from infected tissues. These are best brought out by staining with a polychrome methylene blue. In cultures, the bacilli form long chains; the oval, centrally placed spores develop while the organisms remain in chain formation (Fig. 106). They grow readily on infusion culture media. In both morphological and cultural characteristics they resemble the large-celled species of the nonpathogenic, saprophytic, sporeforming bacilli common in the dust and soil, especially *Bacillus cereus.* Like *B. cereus* and *B. mycoides,* the anthrax bacilli regularly contain large central droplets of fat, revealed by the Sudan Black B-safranin fat stain.

Bacillus anthracis is distinguished from these related saprophytic organisms principally by its *lack of motility,* presence of a *capsule,* and *high pathogenicity for laboratory animals.*

Bacteriological diagnosis. When a cow or steer dies of what is presumed to be anthrax, it is customary to cut off an ear, place it in a jar, and send the specimen to the diagnostic laboratory. The bacteriologist prepares smears from the blood that has escaped into the jar, and stains them with Gram's stain and with polychrome methylene blue. Microscopic examination usually reveals an abundance of large, Gram-positive bacilli, occurring characteristically as long chains of square-ended rods, surrounded by definite, pinkish-colored capsules. Such a finding permits a presumptive diagnosis of

anthrax. Similarly, the anthrax bacilli may be found in direct smears from the skin pustules or sputum in cases of human anthrax.

The capsules may not be clearly seen, however, in some cases, and nonpathogenic bacilli of similar appearance may be present to confuse the picture. Hence it is best in all cases to confirm the diagnosis by cultures and pathogenicity tests. The original material should be streaked on blood agar plates. The large, easily recognizable, nonhemolytic colonies of *B. anthracis,* appearing after overnight incubation, should be fished to agar slants, plain broth, and other media, including methylene blue broth, litmus milk, and salicin fermentation medium. It will be found that although its capsule is largely or wholly lost in these cultures, the anthrax bacillus may be differentiated from *B. cereus*—the closely similar organism most liable to be confused with it—by its failure to multiply at 45° C, or in the presence of penicillin, its inability to ferment salicin, and its relatively slow and feeble power to hemolyze blood, reduce methylene blue, and peptonize milk.

The final step to prove the presence of *B. anthracis* in any material is to inoculate a mouse or guinea pig. Most strains of *B. cereus* are strongly hemolytic and broth cultures may kill mice when inoculated in relatively large doses (e.g., 1.0 cc), but virulent anthrax bacilli will cause a fatal septicemia in very small doses (e.g., 0.2 cc of a thin suspension in saline). At autopsy, smears from the spleen of the anthrax-inoculated animals will show bacilli with typical capsules. Animals killed by injection of *B. cereus* may show many bacilli in spleen smears also, but these organisms will not be capsulated.

Prevention. *General measures.* To protect industrial workers from anthrax infection, all hides, hair, and wool, which might possibly be contaminated with anthrax spores ought to be thoroughly disinfected before reaching the workbench. Failing this, special hygienic precautions must be enforced in the workshops. Final manufactured products, like shaving brushes, should be sterilized.

To reduce anthrax in rural areas it is now the practice to exercise great care, when an animal dies of anthrax, to prevent contamination of the ground. The carcass is not opened, and every effort is made to avoid spilling blood. The body is completely burned, or buried deep and covered with quicklime.

Prophylactic vaccination of animals. In regions where anthrax is continually occurring among cattle and sheep because the pasture

land is contaminated with spores, the only successful method of controlling the disease is to vaccinate all the animals periodically. A method still widely employed, though many modifications have been suggested, is that introduced by Pasteur in 1881. Two vaccines are used: (1) a subculture in broth from a strain of anthrax bacilli incubated at 42°–43° C for from fifteen to twenty days, so that it no longer forms spores, and has lost its virulence for guinea pigs but will still kill mice; and (2) a subculture from a strain kept at the same temperature for only from ten to twelve days. This second vaccine is much more virulent than the first; it is fatal for guinea pigs, but not for rabbits. Cattle and sheep are given an inoculation of vaccine (1), then, about twelve days later, a smaller dose of vaccine (2). The protection conferred by this process of active immunization lasts for only about a year, and to keep animals permanently immune the vaccination must be repeated annually. Wholesale vaccination of herds of cattle and sheep has been carried out in many countries, with good results.

Vaccines made from heated anthrax *spores* now have a wide use.

Serum treatment of human anthrax infection. Immune serums, prepared by immunizing sheep or horses against anthrax bacilli, have proved of value in the treatment of human anthrax, particularly in those cases in which the organisms appear in the blood stream and there is grave danger of fatal septicemia. Sulfonamide drugs and penicillin also have therapeutic value.

GLANDERS

Glanders is an ancient disease of horses which is occasionally transmitted to man. It is caused by a small Gram-negative bacillus, first isolated by Loeffler and Schutz in 1882, and now known as *Malleomyces mallei.*

In horses. The disease may appear in an obvious clinical form, with a persistent nasal discharge, enlargement and induration of the submaxillary lymph glands, and pustules and ulcers ("farcy buds") on the skin of the legs or elsewhere; or it may appear in a chronic, subclinical, or latent form. Cases of the chronic infection are probably most common. The animals with this type usually have a tuberculosis-like lung infection. The disease spreads among horses and mules by direct contact.

In man. Glanders is not common in man, but when it does occur it is almost invariably fatal. It attacks chiefly those persons whose work brings them in close and continual contact with horses. The organisms invade the body through some break in the skin, or by way of the nasal mucous membrane, following direct contact with a germ-laden discharge from an infected animal. The acute form of glanders in man is characterized by fever, nasal discharge, and a nodular skin eruption, and may be terminated by death within a week or two. Numerous cases of glanders have occurred among laboratory workers, from handling cultures. There are few bacteria so dangerous for the bacteriologist.

Malleomyces mallei. This organism, the specific cause of glanders, is a slender, Gram-negative, nonmotile rod. It grows rather poorly on laboratory media when first isolated. Its most characteristic growth is developed on potato media, where it forms a brownish, honey-like mass. It is easily killed by heat and the ordinary chemical germicides.

Bacteriological diagnosis. Diagnosis of human glanders may be made by microscopic examination of the discharge from fresh lesions, and by cultures made on infusion agar and potato media. Guinea pigs may be inoculated intraperitoneally to elicit the so-called *Straus reaction,* an intense swelling and congestion of the testicles.

Chronic or latent glanders in horses is diagnosed by the *mallein* test, which is in every respect comparable to the tuberculin test for tuberculosis. Use of the mallein test, and subsequent slaughter of all positive reactors has resulted in almost complete eradication of glanders among horses in the United States and Canada, and this accounts for the rarity of human cases. *Complement-fixation tests,* using suspensions of *Malleomyces mallei* as antigen, are successful in the diagnosis of glanders in either hore or human beings.

LEPTOSPIROSIS (WEIL'S DISEASE)

Leptospirosis, or Weil's disease (also called spirochetal jaundice, or simply *infectious jaundice*), is an acute disease in man caused by the spirochete *Leptospira icterohemorrhagiae,* or by the related species *L. canicola.* It is acquired by contact with soil, water, food, or other materials contaminated with the urine of infected wild rats

and mice, or dogs. These animals constitute a natural reservoir of the casusative spirochetes.

Weil's disease occurs all over the world; it is especially prevalent in Japan, and is common also in Holland and in parts of Africa, the Middle East, and South America. In the United States, the reported cases are few, but the disease has attracted increasing attention in recent years, and the impression now is that the infection is actually more common than has been thought. Many of the milder cases fail to show any evident jaundice, and so remain undiagnosed.

Sources of infection; clinical features. Most patients give a clear history of exposure to wet places polluted by the urine of rats. Thus, the disease is encountered most commonly among miners in wet coal mines, in sewer workers, in fish-curers, and in workers along canals and in rice and sugar-cane fields. The infection has occurred following bathing in stagnant pools, and after accidental immersion in ditches and canals containing rat-polluted water. Infection in man probably occurs through rubbing of moist soil contaminated with the spirochetes into the skin, nose, or eyes, or as a consequence of swallowing polluted water or food. Occasional cases in the United States have been traced to contact with infected dogs, rather than rats.

Leptospirosis is a severe disease, clinically resembling yellow fever. It is characterized by an abrupt onset, after an incubation period of about 5–10 days with chills, headache, vomiting, and body aches. A high fever develops, there are catarrhal symptoms in the throat and eyes, and in about 50% of cases there is a marked jaundice. Most patients are prostrated for a period of about two weeks. The mortality rate may be as high as 18%.

In the early stages, the leptospira are present in the circulating blood, but later they disappear from the blood, while they remain in the enlarged liver and other internal organs, and are excreted in the urine.

Leptospira icterohemorrhagiae. This organism was first described by Inado, Ido, and their collaborators in Japan in 1916. It is readily distinguishable from other pathogenic spirochetes by its characteristic morphology. It is an extremely slender, coiled thread, only 0.07–0.15μ wide, and varying in length from about 5 to 12μ (Fig. 19). In fresh dark-field preparations these spirochetes are actively motile, showing flexing movements and coarse undulations,

and a vigorous rotary motion which makes the hooked ends appear like slits or buttonholes.

The leptospira are microaerophilic, and have been cultivated in semisolid agar medium enriched with blood or serum, where they grow just beneath the surface.

Bacteriological diagnosis. During the first six days of the illness, a diagnosis may sometimes be made by finding the *Leptospira* microscopically in smears or dark-field preparations from the patient's blood. A more reliable procedure is to inject about 5 cc of the patient's blood intraperitoneally into a guinea pig. In positive cases, the animal develops jaundice and dies in about 10 days; the blood and tissues will contain many of the spirochetes.

In nearly all cases, the spirochetes are present in the patient's urine at least by the twentieth day; their presence may be demonstrated by microscopic study, or by animal inoculation of the sedimented fluid.

Most convenient and reliable of all diagnostic procedures are *agglutination tests,* using the patient's blood serum and a standardized suspension of living or formalinized *Leptospira icterohemorrhagiae*. Within about a week after onset of the illness, antibodies are usually present in sufficient amount to give an agglutination titer of 1:500, or thereabouts, and the titer rapidly increases, often reaching 1:20,000, or more, at the height of the disease.

RELAPSING FEVER

Relapsing fever is a severe infectious disease characterized by an initial febrile period which lasts a few days, begins and ends abruptly, and is usually followed, after a week or two, by a similar period of fever. There may be several such relapses, each tending to be less severe than the previous ones. The infection is caused by *spirochetes* of the genus *Borrelia* (Fig. 107). Several varieties of these spirochetes, differing serologically, but otherwise closely similar, are found in association with the disease in different geographical areas. The causative organisms are transmitted from man to man by body *lice* in some regions, and in other places by *ticks*. A reservoir of the tick-borne relapsing-fever infection exists in wild rodents.

The epidemic, louse-borne disease. The *epidemic form* of this spirochetal infection (so-called relapsing fever of the European

type) is carried from person to person by body lice. It occurs principally in Europe, in North and West Africa, and in India and China. Occasional cases have been observed in the United States. Like epidemic typhus (also a louse-borne infection), relapsing fever has often been a scourge of armies, especially in the battlefields of eastern Europe.

When the louse (*Pediculus humanus*) feeds upon a person in the febrile stage of relapsing fever—i.e., when the causative spirochetes are in the circulating blood—the organisms are taken into the alimentary tract, where they remain for a day, then disappear. The louse is then unable to transmit the infection at once. But it becomes infective about a week later, when the spirochetes reappear in large numbers in the blood and the internal organs. This heavy infection does not appear to injure the louse, which remains capable of transmitting relapsing fever to man for the next two or three weeks. Since the spirochetes are not present in the salivary glands (or feces) of infected lice, the infection of human being is not the direct result of a bite. Instead, inoculation occurs when infected lice are crushed by the fingers or clothing and the spirochetes are rubbed into the inflamed or abraded skin by scratching.

The illness begins abruptly after an incubation period of about eight days, and varies greatly in severity in different individuals and in different outbreaks. Typically, patients have a high fever, intense headache, pains in back and limbs, and other symptoms of a severe generalized infection. The first attack usually lasts about four days; usually there are three or four relapses.

The endemic, tick-borne disease. An *endemic* type of relapsing fever is transmitted from animal to animal, and from animal to man, by ticks of the genus *Ornithodorus*. The tick-borne infection is especially prevalent in central Africa—hence, it is often referred to as African tick fever. The species of tick concerned is called *Ornithodorus moubata*.

In other parts of the world, other species of ticks carry the relapsing-fever spirochetes. Many cases of tick-borne disease have been reported from Spain, various parts of Central Asia, Mexico, and Central and South America. In the United States, relapsing fever has occurred in several of our western states, and with special frequency in Texas and California. In Texas, the tick vector belongs to the species *Ornithodorus turicata,* while in states farther west the ticks named *O. hermsi* and *O. parkeri* have been implicated. Human

cases of this endemic form of relapsing fever in the United States have been confined largely to residents or travelers in rural and sparsely settled areas, and to investigators who have exposed themselves during studies of the disease. In Texas, infected ticks are found principally in caves.

Exactly how ticks transmit the relapsing-fever infection to human beings is not certain. The spirochetes may be introduced into the human skin directly by the tick bite, or they may be carried into the wound from the coxal fluid of the tick. Wild rodents, and other

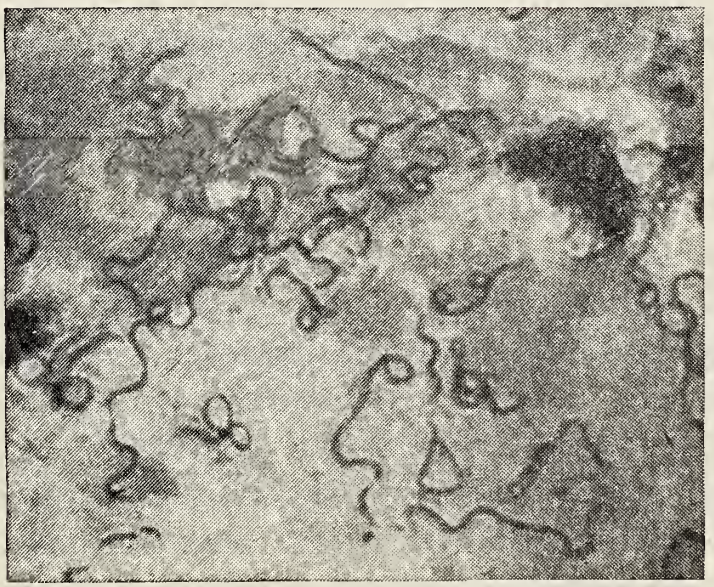


FIG. 107. A species of relapsing-fever spirochete (*Borrelia duttoni*) in a thick smear from the blood of an experimentally infected white rat. (Photo by C. G. Breckenridge, from preparation of Dr. W. M. Fisher, Baylor University College of Medicine, Houston, Texas.)

animals, are natural hosts of the tick-borne relapsing-fever spirochetes, but *ticks themselves constitute a reservoir* of the disease, for once infected, they remain capable of transmitting the disease to other animals or to man for years afterwards. Moreover, the spirochetes may be passed through the eggs to successive generations of ticks.

Endemic relapsing fever is similar clinically to the louse-borne type, except that the febrile periods tend to be of shorter duration, though the symptoms are more intense and more often involve the central nervous system, and there are a greater number of relapses. The mortality rate averages only about 5%, whereas the rate in some outbreaks of the louse-borne disease has been reported as high as 50%.

Relapsing-fever spirochetes. The species of spirochete responsible for the epidemic, louse-borne type of relapsing fever is *Borrelia recurrentis.* This was the first pathogenic microbe ever to be recognized in human blood, when Obermeier observed it as early as 1868. The spirochete of the African tick-borne fever is called *Borrelia duttoni.* Various other names have been applied to the relapsing-fever spirochetes encountered in other parts of the world, but it is probable that all those causing the tick-borne disease should be regarded as varieties of the single species, *Bor. duttoni.*

All the relapsing-fever organisms are morphologically identical. They are of variable size, averaging about 15μ in length, and 0.2–0.4μ in width; the ends are pointed (Fig. 107). There are from three to six loosely wound spirals, and the spirochetes have the marked flexibility characteristic of the *Borrelia* group. In fresh blood, they show an active corkscrew-like motility.

Growth has been obtained by inoculating infected blood beneath the chorio-allantoic membrane of the chick embryo.

Bacteriological diagnosis. Relapsing fever may be confused clinically with malaria, dengue, yellow fever, typhus fever and some other infections, and the diagnosis can be definitely established only by demonstrating the presence of the causative spirochetes in the blood or cerebrospinal fluid. During the fever periods, the organisms can frequently be found by looking at a drop of the patient's blood under a dark-field microscope, or by examining a stained blood smear.

If the spirochetes cannot be detected microscopically, some of the patient's blood may be inoculated into white mice. In positive cases, the injected mice will show the *Borrelia* in their own blood stream within forty-eight hours.

REFERENCES

HOLMES, W. H.: *Bacillary and Rickettsial Infections.* New York: The Macmillan Co., 1940.

HULL, T. G.: *Diseases Transmitted from Animals to Man.* Springfield, Ill.: Chas. C Thomas, 2nd Ed., 1941.

MANSON-BAHR, P.: *Synopsis of Tropical Medicine.* Baltimore: Williams & Wilkins Co., 1943.

Brucellosis

CARPENTER, C. M.: "Brucellosis," *Med. Clinics of N. Am.* (*N. Y. Number*), May 1943, 27:698.

HARRIS, H. J.: *Brucellosis.* New York: P. B. Hoeber, Inc., 1941; also, *Bull. N. Y. Acad. Med.,* Sept. 1943, 19:631.

HUDDLESON, I. F.: *Brucellosis in Man and Animals.* New York: Commonwealth Fund, 1939.

JORDAN, A. F., BORTS, I. H., HARRIS, D. M. and JENNINGS, J. R.: "Brucellosis: Consideration of its Epidemiology, Diagnosis, and Control," *Am. J. Pub. Health,* July 1943, 33:773.

WISE, B.: "Acute Brucellosis," *Arch. Int. Med.,* Sept. 1943, 72:346.

Plague

DOUGLAS, J. R. and WHEELER, C. M.: "Sylvatic Plague Studies—II. The Fate of *Pasteurella pestis* in the Flea," *J. Infect. Dis.,* Jan.–Feb. 1943, 72:18.

EVANS, F. C., WHEELER, C. M. and DOUGLAS, J. R.: "Sylvatic Plague Studies—III. An Epizootic of Plague among Ground Squirrels (*Citellus Beecheyi*) in Kern County, Cal.," *J. Infect. Dis.,* Jan.–Feb. 1943, 72:68; and "Plague Infection Reported in the United States during 1943," *Pub. Health Rep.,* July 14, 1944, 59:911.

FRANCIS, E.: "Twenty-year Survival of Virulent Plague Cultures without Transfer," *Pub. Health Rep.,* Sept. 10, 1943, 58:1379.

MEYER, K. F.: "The Known and Unknown in Plague," *Am. J. Trop. Med.,* Jan. 1942, 22:9; "Plague," *Med. Clinics of N. Am.* (*N. Y. Number*), May 1943, 27:745.

Tularemia

DAVID, J. K., JR. and OWENS, J. N., JR.: "Tularemia meningitis," *Am. J. Dis. Children,* Jan. 1944, 67:44.

FRANCIS, E.: "Oculoglandular Tularemia," *Arch. Ophth.,* Oct. 1942, 28:711.

GREEN, R. G.: "Virulence of Tularemia as Related to Animals and Arthropod Hosts," *Am. J. Hyg.,* Nov. 1943, 38:282.

KENNEDY, J. A.: "Pulmonary Tularemia: Discussion of Disease as Clinical Entity with Report of 3 Cases," *J.A.M.A.,* Mar. 7, 1942, 118:781.

LARSON, C. L.: "Growth of *Pasteurella Tularensis* in the Yolk Sac of Developing Chick Embryo," *Pub. Health Rep.,* May 25, 1945, 60:587.

Anthrax

"Anthrax in Philadelphia," *Am. J. Pub. Health* (*supp.*), Mar. 1942, 32:114; "Check Anthrax—a Warning and a Plea," *Am. J. Pub. Health,* July 1943, 33:854.

LEBOWICH, R. J., MCKILLIP, B. G. and CONBOY, J. R.: "Cutaneous Anthrax. A Pathogenic Study with Clinical Correlation," *Am. J. Clin. Path.,* Oct. 1943, 13:505.

STEIN, C. D.: "Differentiation of *Bacillus Anthracis* from Nonpathogenic Aerobic Sporeforming Bacilli," *Am. J. Vet. Research,* Jan. 1944, 5:38.

TABERSHAW, J. R. and MORRIS, G. E.: "Epidemiological Aspects of Anthrax in Massachusetts," *New Eng. J. Med.,* Jan. 21, 1943, 228:92.

Glanders

HEROLD, A. A. and ERICKSON, C. B.: "Human Glanders: Case Report," *So. Med. J.,* Sept. 1938, 31:1022.

MCGILVRAY, C. D.: "Transmission of Glanders from Horse to Man," *J. Am. Vet. Med. Assn.,* May 1944, 104:255.

Leptospirosis

BRUNO, F. E., WILEN, C. J. W. and SNAVELY, J. R.: "Spirochetal Jaundice," *J.A.M.A.,* Oct. 30, 1943, 123:519.

LARSON, C. L.: "Leptospirosis in Rats (*R. Norvegicus*) In and About Washington, D. C.: An Evaluation of the Methods Used for Diagnosis," *Pub. Health Rep.,* June 18, 1943, 58:949.

LEWIS, M.: "Incidence of *Leptospira Icterohemorrhagiae* in Trapped Rats in Philadelphia," *Amer. J. Trop. Med.,* 1942, 22:571.

Loreny, G. Jr.: "Studies in Weil's Disease," *U. S. Naval Med. Bull.,* Mar. 1944, 42:560.

Packchanian, A. and Tom, N.: "Persistence of Leptospira Antibodies in the Circulating Blood of Patients Recovered One to Over Twenty Years from Weil's Disease," *J. Immun.,* May 1943, 46:263.

Relapsing Fever

Davis, G. E.: "Relapsing Fever: The Tick *Ornithodoros Turicata* as a Spirochetal Reservoir," *Pub. Health Rep.,* May 28, 1943, 58:839.

Moursund, W. H. and Others: *Relapsing Fever in the Americas.* Washington, D. C.: Amer. Assoc. for Advancement of Sci., 1942.

Stein, G. J.: "The Serological Diagnosis of Relapsing Fever," *J. Exper. Med.,* Jan. 1944, 79:115.

Strong, R. P.: "Relapsing Fever," *Med. Clinics of N. Am. (N. Y. Number),* May 1943, 27:734.

REVIEW QUESTIONS—CHAPTER XXXV

1. Name and describe the three varieties of bacteria that may be responsible for brucellosis in man. By what means may they be differentiated?
2. Describe how human beings may be infected with *Brucella.*
3. What is the usual course of brucellosis in man?
4. Outline practical methods used in making a bacteriological diagnosis of human brucellosis. Discuss the practical significance of the several diagnostic tests.
5. Are antiserums or vaccines used in the treatment of brucellosis?
6. What are the principal measures that may help to prevent brucellosis?
7. Discuss the prevalence and importance of plague. What is the origin of human plague? Define *sylvatic plague.* How is the infection transmitted?
8. What are the principal forms of plague in man? How are human beings infected?
9. How may a laboratory diagnosis of plague be made? Name and describe the causative organism.
10. Discuss specific prophylaxis and therapy and the prevention of plague.
11. Describe briefly the nature of tularemia, its occurrence in man, sources of infection, bacteriological diagnosis, and prevention. Name and describe the causative organism.
12. What is the cause of anthrax? By whom was this organism first studied, and when? What does the word "anthrax" mean?
13. Describe anthrax in animals. How do animals become infected?
14. What are the three principal forms of anthrax infection in man? How does infection occur? What is its importance as an occupational disease?
15. Name and describe the anthrax bacillus. How may a bacteriological diagnosis of human anthrax be made?

16. Discuss prevention of anthrax in animals; in man. Describe a widely used form of anthrax vaccine. Who developed this vaccine and when?
17. Is a specific treatment available for human anthrax?
18. Discuss briefly the nature of glanders in animals, its occurrence in man, and methods of bacteriological diagnosis. Name and describe the causative organism.
19. Describe the prevalence and importance of leptospirosis, the modes of infection, and the clinical features in man.
20. Name and describe the causative organism of leptospirosis, and outline procedures used for diagnosis.
21. Outline the principal features of relapsing-fever infection.
22. Describe the occurrence, mode of transmission, and clinical features of: (a) the epidemic, louse-borne disease, (b) the endemic, tick-borne relapsing fever.
23. Name and describe the relapsing-fever spirochetes, and outline methods of bacteriological diagnosis.

CHAPTER XXXVI

TUBERCULOSIS AND LEPROSY

TUBERCULOSIS

Prevalence and importance. Tuberculosis, in various clinical forms, has been for centuries one of the most common and most fatal of human diseases. It has attacked persons of all ages and all races, the world over. Among the crowded populations of cities it has been a veritable plague. At the present time, its wide prevalence and chronic nature, with the consequent great loss of time and money on the part of its victims, and its close relationship to general health conditions within a community, combine to make it a disease of outstanding importance and of more than ordinary significance for the well-being of the people as a whole. Tuberculosis attacks individuals in so many families, and the measures which help to prevent it are so fundamental for the promotion of health in general, that efforts to control it have a very prominent place in the public-health program of every up-to-date community.

In olden times, tuberculosis was not only exceedingly common, but it was also a severe, acute disease, often rapidly fatal. It still may have this character when it occurs in infants, or in individuals of a savage race not often exposed to the infection. But for the past hundred years or more the disease has steadily decreased in severity in all the more highly civilized countries of the world. This change may be due, in part, to a gradual rise in the average level of resistance to tuberculosis, but probably the general sanitary and hygienic reform which has taken place during this time has had the greatest influence. The decline has been especially marked during the past fifty years. In 1900, the death rate from tuberculosis per 100,000 population in the United States was 201.9, while in 1942 it was only 43.1. The rate for 1943 (approximately 41.9) was still lower; the present mortality is therefore about 1/5 that of 1900.

Nevertheless, tuberculosis is still one of the most prevalent of all

infections and, although the disease tends now to be less severe, it remains one of the major causes of death. Among the infectious diseases, only pneumonia is now responsible for more fatalities. In the United States, between 1939 and 1941, tuberculosis (mostly of the pulmonary type) was responsible for 4.3% of deaths from all causes. The over-all death rate was considerably higher for males than for females, and nearly 3½ times as great among colored as among white persons. From early adulthood to age 35, tuberculosis remains the leading cause of death.

The prevalence of active tuberculous infection is always increased by factors which lower the economic status and the living standards of the population. Thus, after World War I, 1914–1918, the incidence of tuberculosis in Europe rose markedly, and it is certain that the disease will be even more prevalent there now, after World War II, and probably will spread in a virulent, epidemic form among the unnumbered thousands of civilian refugees and other persons in many countries who are fatigued, ill-nourished, lacking even proper shelter, and otherwise suffering severe privations imposed by the brutalities of total war. In early 1945, there were unmistakable signs that a general, world-wide increase in tuberculosis was to be expected.

At the same time, however, preventive measures, and particularly methods for the early diagnosis of the disease, were improved during the war years. In the United States, a vigorous, well-financed anti-tuberculosis campaign on a national scale was getting under way in 1945, and the outlook for the eventual conquest of this ancient scourge was never brighter.

Varieties of tubercle bacilli. Tuberculosis is a common disease in various species of animals, as well as in man. The organisms responsible for these infections are not, however, exactly alike. Four distinct types of tubercle bacilli are recognized: (1) the *human,* (2) the *bovine* (cattle), (3) the *avian* (bird), and (4) the *cold-blooded* type. The latter causes a tuberculous disease in frogs, turtles, fish, and other cold-blooded animals. All are classified among the so-called higher bacteria, in the genus *Mycobacterium.* The human type is generally named simply *Mycobacterium tuberculosis,* but sometimes it is formally designated *Myco. tuberculosis* (*hominis*). The bovine bacillus is often named *Mycobacterium bovis.*

It was Robert Koch who, in 1882, discovered the human and bovine tubercle bacilli, and proved that they cause tuberculosis, but

the recognition of the cattle type as distinct from the human was due to other investigators. These two types, human and bovine, are the only ones which infect human beings.

Morphology and staining of Mycobacterium tuberculosis. The human tubercle bacilli are extremely slender, nonmotile rods, of variable length, but usually 2.5–3.0μ long. Many are slightly curved, and they appear less rigid than most other bacilli. Sometimes, in cultures, long filaments may form. In smears of tuberculous sputum, the organisms appear as relatively long and very thin threads, often in small groups or bundles, with the bacilli lying parallel, or at acute angles, to each other. There are no constant differences in morphology among the four types of tubercle bacilli.

One of the most outstanding characteristics of these organisms is their capacity to take an *acid-fast stain.* They belong to the group of *acid-fast bacilli*—organisms which are somewhat difficult to stain, but which, when once stained, are not decolorized either by alcohol or by acids. Among other members of the group are the *leprosy bacillus,* the *smegma bacillus* (a normal inhabitant of the human skin in the genital region), and certain harmless saprophytic acid-fast bacilli, including the *butter bacillus* and the *timothy-grass bacillus.*

The most widely used method of demonstrating acid-fast staining is the Ziehl-Neelsen method described on page 91. With this method, tubercle bacilli appear as bright red rods in a pale-blue field (Fig. 108).

Some lots of carbol fuchsin stain give the organisms a beaded appearance, as if they contained granules larger in diameter than the rod itself. The nature of these "beads" on tubercle bacilli has been debated for many years. Recent studies have finally explained the matter by showing that the granulations are really artifacts—that is, the granules are not actual structures natural to the tubercle bacilli, but only precipitations from the stain.

It is thought that non-acid-fast forms of the tubercle bacillus may occur in infected tissues.

Physiological properties of human tubercle bacilli. *Isolation.* *Mycobacterium tuberculosis* (*hominis*), the organism responsible for all but a small fraction of the cases of tuberculosis in human beings, is somewhat difficult to isolate in pure culture, because it multiplies much more slowly than the other bacteria with which it may be mixed, and does not adapt itself very readily to artificial

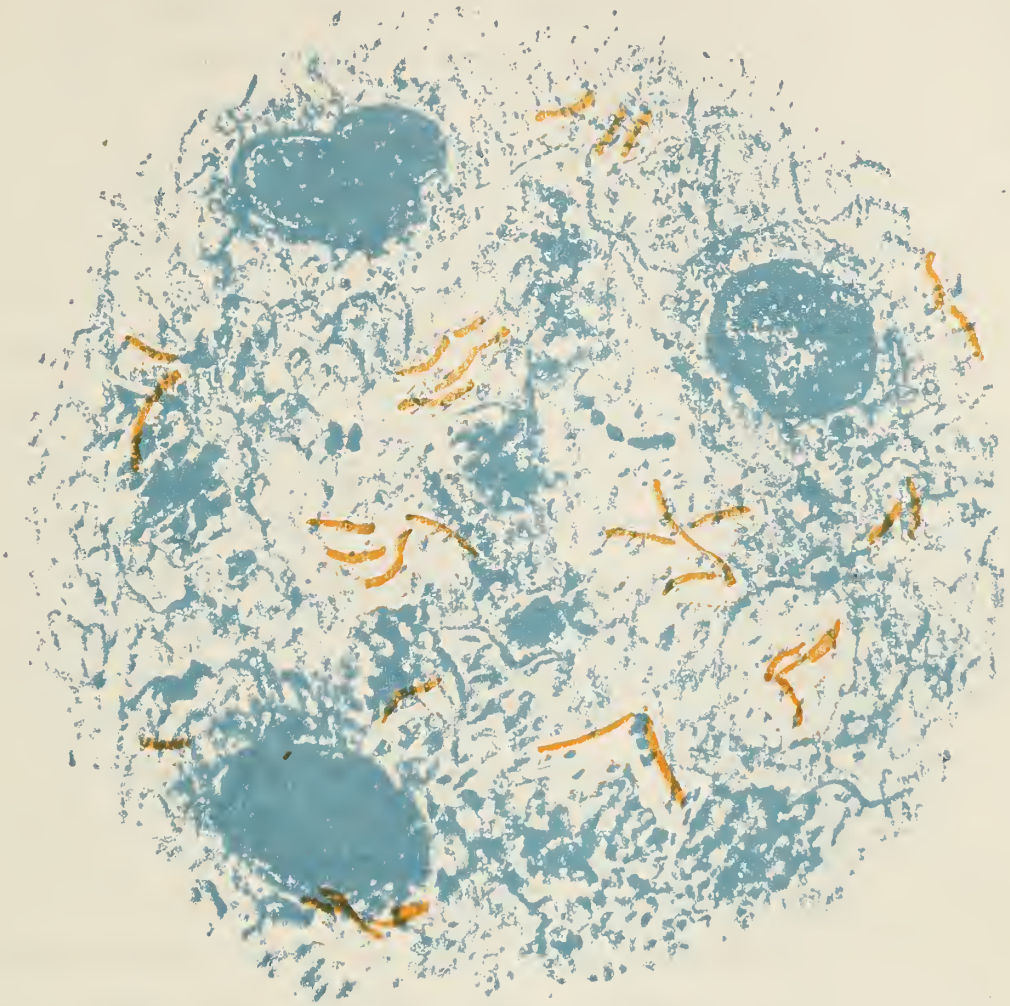


FIG. 108. *Mycobacterium tuberculosis* in a smear of sputum stained by the Ziehl-Neelsen acid-fast staining method.

conditions. Growth does not occur on ordinary media. The media most widely used for primary cultures are enriched with egg yolk or whole eggs, and with glycerine. Some also contain potato starch, and a small concentration of malachite green or other dye which will inhibit to some extent the development of organisms other than the tubercle bacilli. These egg media are coagulated, by heat, in the form of slants. Examples are *glycerine-egg* slants (a modification of the original *Dorset's medium*), *Petroff's* gentian-violet-egg *medium,* and *Lowenstein's medium.* The tubercle bacilli are aerobic, and may grow at temperatures between 30°–41° C, but multiply best at 37° C.

Isolation from raw sputum is especially difficult because of the presence of so many other rapidly growing bacteria, but advantage may be taken of the fact that tubercle bacilli are unusually resistant to alkali and acid. The germs may be concentrated in a sputum sample by first homogenizing the sputum with strong alkali or acid, then sedimenting the bacilli from the liquefied fluid by centrifugation. These treatments result not only in the collection, into a sediment, of all the tubercle bacilli present, but in the destruction of practically all the other kinds of bacteria in the sputum, so that inoculation of the sediment onto the surface of one of the coagulated egg media mentioned above (after it has been brought to a neutral reaction and washed), often yields a pure growth of the tuberculosis organism. Specimens of feces, gastric washings, pus, or other materials to be cultured for tubercle bacilli may be homogenized and concentrated in a similar manner before actual inoculation of media.

Another method for isolating tubercle bacilli, more certain than the direct inoculation of culture media, is to inject the original material into a guinea pig. The animal will develop tuberculosis, and in the characteristic tubercles the germs will be found almost free of contaminating organisms. If, now, a portion of this tuberculous tissue is carefully removed from the animal, with aseptic precautions, and rubbed over the surface of slants of Lowenstein's or a similar medium, a pure culture of the tubercle bacilli will usually be secured.

Cultural characteristics. The primary growth of human tubercle bacilli is always *slow;* the colonies may not appear for two *weeks,* or more, instead of in a day or two as in the case of ordinary bacteria. When, finally, the peculiar colonies become visible, they are

dry, irregular, heaped-up masses, resembling breadcrumbs, very tough and tenacious, and adhering closely to the medium. They are at first white, then later yellowish or buff-colored.

Once isolated, the human tuberculosis organisms grow rather well, though still slowly. Slants of glycerine-egg medium, or of infusion agar enriched with about 5% of glycerine, are commonly used. Culture tubes must be sealed, to prevent the drying out of the medium, and incubation is often continued for several weeks before the maximum growth is attained. In glycerol broth, the human tubercle bacilli characteristically grow as a wrinkled pellicle, covering the surface of the liquid.

Because of the comparatively luxuriant growth, in laboratory cultures, of tubercle bacilli of the human type, these organisms are said to be *eugonic* (capable of multiplying well), whereas bovine tubercle bacilli are *dysgonic* (capable of multiplying only poorly), as noted below.

Resistance. In common with other acid-fast bacilli, the human tubercle bacillus is unusually resistant to chemical germicides, and to drying, but it is as susceptible as other non-sporebearing bacteria to heat. Several hours are required to kill tubercle bacilli in sputum with a 5% carbolic-acid solution. We have already mentioned their resistance to strong acid or alkali. In dried sputum or dust, in the dark, some tubercle bacilli may remain alive for months. On the other hand, they are destroyed by an exposure of from twenty to thirty minutes to a temperature of 60° C. Pasteurized milk, then, is free of living tuberculosis germs. Direct sunlight and ultraviolet light destroy them quickly, so that sputum which has been exposed to the sun for an hour or two is not likely to be dangerous.

Properties of the bovine tubercle bacillus. *Mycobacterium bovis* cannot always be distinguished with ease and certainty from the bacilli of the human type. Typical strains, however, show certain properties which serve to differentiate the two varieties (Table XXIV).

The bovine tubercle bacillus not only is responsible for the tuberculosis of cattle, but is the type found most often in association with tuberculosis of other domestic animals, such as horses, pigs, dogs, and cats.

Characteristic features of tuberculous infection in man. Tubercle bacilli sometimes enter the body through the abraded skin, and somewhat more often they pass through areas of lymphoid

Table XXIV. Principal Differences Between Human and Bovine Types of Tubercle Bacilli

PROPERTY	HUMAN TYPE	BOVINE TYPE
Growth on egg media	Comparatively luxuriant ("eugonic"), dry, heaped-up, irregular, tough, and tenacious, not easily emulsified	Generally poor ("dysgonic"), thin, smooth, slightly moist, easily broken up
Use of glycerine	Glycerine aids growth	Glycerine does not aid growth
Pigment formation	Cultures eventually develop yellowish, orange, or brown pigments	No pigments
Pathogenicity for rabbits	Rabbits inoculated intravenously with a minimal dose usually show slight, local lesions only; rarely a generalized tuberculosis develops with death more than two months later.	Rabbits inoculated intravenously with a minimal dose (about 0.1 mgm of dried bacilli from a young culture), develop acute generalized tuberculosis, with death in 6–8 weeks

tissue along the upper alimentary tract (in the pharynx and tonsillar regions), or through the patches of similar tissue in the intestinal walls. Most commonly, however, the primary infection occurs somewhere along the lower respiratory tract, or in the lung itself. Once within the body, the bacilli may be carried by the lymph or blood to various internal regions, and they may spread by extension over contiguous surfaces. Almost any part of the body may be invaded. In children, the infection shows a tendency to generalize, though often active foci are found principally in the lymph glands of the abdomen or neck, or in the bones and joints. Occasionally, the skin is the site of infection (lupus). By far the most frequent form of the illness, however, especially in adults, is a chronic *pulmonary tuberculosis,* in which the lungs are the seat of the infection. The organisms in the lung lesions are almost invariably of the human type. In infants, and more rarely in adults, an acute, fatal, generalized infection may occur, known as *miliary tuberculosis,* in which tiny tubercles about the size of millet seeds (hence called *miliary* tubercles) are found scattered throughout the organs of the body. Another fatal form of tuberculosis in children is *tuberculous meningitis.*

Tubercles are fundamentally alike, wherever they are found. They form whenever tuberculous infection is present in any solid organ of the body. They begin as microscopic collections of epithelioid cells, arranged about a group of tubercle bacilli. Later, lymphocytes accumulate in this area. The microscopic tubercles enlarge, and coalesce with neighboring tubercles. Near their center, characteristic giant cells appear, and in these cells the tuberculosis germs are usually found. Often tubercles become so large that they are easily visible to the naked eye. They appear as tiny gray masses, about the size of a pinhead, and hard to the touch.

In the lungs, or other organs, when the disease is active, many tubercles appear and overlap one another, destroying the normal tissues. The cells in the center of these tubercles die and disintegrate into a soft, cheesy mass, and later, when this matter is cast out of the body, there is left behind an actual cavity in which all the normal tissue has been consumed. In this manner, almost the whole of a lobe of a lung may be destroyed; or another organ, such as a kidney, similarly may be reduced to little more than a shell. "Consumption" is an accurate term with which to describe a severe tuberculous infection (Fig. 109).

It must not be imagined, however, that tubercle formation and the consequent destruction of the tissues go on unimpeded. On the contrary, there is a characteristic cellular reaction about the tubercles which eventually results in the formation of a thick envelope of fibrous tissue around them. This seems to constitute the principal defense of the body against the germs; it is an attempt to wall off the infected area and to prevent the spread of the infection farther into the healthy tissues. When this defensive process is fully successful, the complete healing of the tubercles

FIG. 109. Portion of the lung removed at autopsy from a case of chronic pulmonary tuberculosis. The specimen has been cut through to expose the large cavities in which the lung tissue has been entirely consumed. There is no normal tissue remaining anywhere; the entire lobe is involved in an advanced tuberculous process.

follows—that is, all the germs present die, and the danger of future spread of the infection from that area is over. Often calcium salts are later deposited in these healed tubercles.

In many instances, however, the body reaction is not sufficient to bring about complete elimination of the germs, but instead the progress of the infection is simply *arrested*. The tubercle bacilli do not all die, but on the contrary, some remain alive in incompletely healed tubercles for long periods, perhaps for the whole of the individual's life. The infection may never advance any further, but there is always the possibility that the germs may start into renewed activity at some future time, when for some reason the resistance of the body is reduced and the cellular defense gives

way. The fact that infection with tubercle bacilli may thus remain *latent,* causing no illness, but may later develop into an actively progressing infection with accompanying symptoms of clinical tuberculosis, when resistance is lowered, is a very important and characteristic feature of this disease.

Prevalence of latent infection; tuberculous infection and tuberculous disease. A point of fundamental significance in the modern conception of tuberculosis is the knowledge that *almost every individual,* at some time, usually in childhood, *becomes infected with tubercle bacilli,* though only a comparatively few persons ever develop signs or symptoms of illness which we recognize as tuberculous *disease.* The majority of individuals have sufficient defensive powers so that the tuberculous infection, which they unconsciously suffer in early life, is confined to a relatively small area in the lymph glands or lungs, and heals spontaneously, or at least is held in check so well that it never advances far enough to produce recognizable symptoms of tuberculosis.

Two kinds of evidence have made the wide prevalence of tuberculous infection abundantly clear. Pathologists who have examined the lymph glands and lungs, at autopsy, of many hundreds of persons dying of diseases other than tuberculosis, have found healed or partly healed tubercles in a high proportion of individuals. A second kind of evidence is afforded by the results of *tuberculin tests.* Tuberculin is a preparation made from cultures of tubercle bacilli, as described below. Persons who *have been infected* with tubercle bacilli, *or are at the moment infected,* will develop a characteristic inflammatory reaction when a small amount of tuberculin is applied to the skin, whereas individuals who have never been infected will give no reaction. It has been found that the proportion of persons who react positively increases steadily with age, and that as high as 90% of adults may show this definite evidence of a past tuberculous infection.

Childhood infection is now not so nearly universal as it once was, but nevertheless it is still true that many people have a focus containing *Mycobacterium tuberculosis* somewhere in the body, and that most individuals acquire the germs originally during childhood. However, since by no means everyone develops the *disease* tuberculosis, it must be that the childhood infection cures itself or becomes latent in most persons, and it is clear that the normal human

body must possess a considerable degree of resistance to the tubercle bacilli.

Sources and modes of infection with human or bovine tubercle bacilli. *Spread of the human-type organisms.* Although the tuberculosis germs are somewhat more resistant to unfavorable external conditions than other common bacteria, and may survive, for example, for some time in dust, they have no natural existence outside the living body. The *human tubercle bacilli* are therefore passed from one individual to another through some form of personal contact, most often by way of fingers soiled with sputum or mouth secretions, or by droplet infection. Obviously, the *sputum* of persons with active pulmonary tuberculosis is an important source of infection for others. A patient may excrete sputum for months or years on end, every drop of which contains great numbers of virulent tubercle bacilli. The coughing that accompanies the lung infection sends germ-laden droplets into the air, and persons in the immediate vicinity may be infected by breathing in these *droplets* or *droplet-nuclei.* The organism may be present in other discharges, from tuberculous lesions elsewhere in the body, and they may be in the urine or feces. It is no wonder that tuberculous infection is so widespread, especially among crowded populations, and where good habits of personal hygiene are neglected.

Infections heavy enough to lead—often after a considerable time interval—to the appearance of clinical symptoms of tuberculosis are acquired through a rather prolonged contact, or by repeated contacts, with someone who is excreting virulent tubercle bacilli. People in crowded cities are exposed to the risk of contact with "open cases" of tuberculosis more often than is generally realized. Individuals with early, but active, undiagnosed pulmonary tuberculosis, unconsciously disseminating the germs, are likely to be encountered almost anywhere. Also, unfortunately, many persons having the disease in an advanced, easily communicable form remain at home with their families, mingle with friends and neighbors, or even stay at their work, for lack of proper medical supervision, or because there are no available hospital beds where they would be effectively isolated.

Tuberculosis of bovine origin. Infection with the *bovine type* of tubercle bacilli occurs mostly in children under five years of age, and the organisms enter the body through the alimentary tract as a

consequence of drinking *raw milk* from tuberculous cattle. The organisms are contained in the feces of the infected cows (since the sputum is swallowed rather than expectorated), and the bacilli get into the milk from the dirty flanks or udders of the animals.

Bovine organisms are sometimes responsible for a fatal miliary tuberculosis or meningitis in infants. Cases of bone and joint tuberculosis, and infections of the cervical or mesenteric lymph glands are often attributed to them. On the other hand, bovine bacilli very rarely give rise to pulmonary tuberculosis.

Cases of bovine infection occur with considerable frequency in some parts of the British Isles, but in the United States, at the present time, human tuberculosis of bovine origin is almost nonexistent, owing largely to the remarkable success of the efforts of public-health authorities to eliminate tuberculous cattle from dairy herds.

Factors which influence the development of active tuberculosis. Internists and pathologists recognize two varieties of tuberculous disease in man: (1) the original, *primary,* or *childhood type of infection,* and (2) the *reinfection,* or *adult type of tuberculosis.* The latter is modified in character, because it occurs in a person specifically sensitized and partially immunized by a previous tuberculous infection.

Tuberculosis in a young child is due, of course, to germs acquired by contact with other (infected) persons. But when an advancing tuberculous process and illness develop in an adolescent or an adult, this *may* be due to the lighting up of the focus of infection acquired in childhood, and the determining factor may be the loss of the general body resistance which had previously held the organisms in check. On the other hand, it may be that a *new* infection with virulent organisms is superimposed upon the original childhood infection. Epidemiological studies of recent years have indicated strongly that most active tuberculosis in adults arises in the latter way—that is, as a result of a *reinfection* from the outside. These patients have tuberculosis of the reinfection type, characterized by severe, localized tissue damage in the lungs or elsewhere, and by other signs of an infectious process occurring in a person already sensitized to the germ. Of course, as more and more people reach adolescence and early adult life *without* a childhood infection, more cases of tuberculosis of the primary type will be seen in these young persons.

In any case, whether the clinical disease is the manifestation of primary infection, or a reinfection, the *general body resistance* of the individual is a factor of unusual importance. Resistance seems to depend more upon general bodily vigor, and the capacity to perfect the local defenses against the spread of the germs in the tissues, than it does upon the action of specific antibodies as in other infections. Active tuberculosis is most likely to occur in persons whose normal resistance has been broken down by the weakening effect of other diseases, by malnutrition, alcoholism, too frequent childbearing, or fatigue and overstrain of one kind or another. It may be that some individuals inherit a constitution which makes them somewhat more susceptible to tuberculosis than other persons. But the disease itself is never directly inherited. Tuberculosis runs in families principally because the children in each generation can scarcely avoid receiving a heavy dose of the germs when they are obliged to live in close contact with an adult member of the family who has an "open case."

The occurrence of active tuberculosis is influenced by the general conditions under which people live and work. Dusty trades, for example (particularly those in which the worker inhales dust from metals or stone), predispose to tuberculosis. The disease is most frequent among the poorest families, where unhygienic habits combine with overcrowding, overwork, poor nutrition, alcoholism, and frequent attacks of other communicable diseases to create a favorable soil for the tuberculosis germs. On the other hand, tuberculosis is less likely to occur among those who live hygienically—that is, persons who from childhood can keep clean, well nourished, and well rested, can avoid serious weakening diseases, and can enjoy the blessings of sunshine, fresh air, wholesome recreation, and congenial work.

Of fundamental importance, however, is the matter of exposure. If one can *avoid exposure to a heavy dose of the germs,* and *also* can maintain continuously a high level of physical well-being, there is little likelihood of developing active tuberculosis.

Bacteriological diagnosis. In order to make a laboratory diagnosis of tuberculosis in men, it is necessary to demonstrate the presence of tubercle bacilli in the sputum (in cases of suspected pulmonary tuberculosis), or in the urine, joint fluid, spinal fluid, or other material from suspicious lesions. This may be done by: (1) *microscopic examination,* using the acid-fast staining method,

(2) *direct cultivation of the bacillus on artificial media,* or (3) *inoculation of the material into a guinea pig.*

Microscopic examination for tubercle bacilli. To examine sputum for tubercle bacilli, a cheesy or bloody portion of the sputum is selected, and spread in a large and rather thick film over a slide. When the smear is dry, it is fixed by heat, then stained by the Ziehl-Neelsen or some other acid-fast staining method, and searched for acid-fast bacilli. These may be very numerous in advanced cases, or very few in early cases. When once the typical acid-fast rods have been found, a diagnosis of tuberculosis is certain. This is one of the rare instances in bacteriology when a germ can be recognized by microscopic examination alone.

Use of the technique of fluorescence microscopy has been found of value in detecting tubercle bacilli.

In early cases of pulmonary tuberculosis, the organisms may be hard to find in smears of the sputum. Attempts may then be made to concentrate the organisms present by treatment of the sputum with strong acid or alkali, as previously described. When the liquefied specimen is centrifuged, the bacilli may be found in smears of the sediment.

Cultivation of the bacilli. Cultures may be made from such material as spinal fluid and similar *uncontaminated* specimens, by smearing the sediment directly over the surface of one or more of the special coagulated egg media designed for this purpose. Feces, sputum or other *contaminated* material must first be treated so as to kill off other bacteria and, at the same time, concentrate the tubercle bacilli present. This is done by addition of alkali, as previously mentioned.

Guinea-pig inoculation. This animal inoculation must always be done when there is serious possibility of tuberculosis and the tubercle bacilli cannot be found by other methods, and also whenever there is a question as to the identity of acid-fast bacilli that have been found. The guinea pig is so susceptible that it will develop tuberculosis even though the living tubercle bacilli in the injected material are very few indeed—so few that they cannot be found by any method of microscopic examination. Hence, this is a most delicate test for the presence of tubercle bacilli in any material. The injection is usually made subcutaneously, in the region of the groin. Four or five weeks later, the animal is killed, and examined for evidences of tuberculosis. If the typical tubercles

containing acid-fast bacilli are found, this is proof that the original material from the patient contained tubercle bacilli.

Immunity; tuberculin. We have already explained that resistance to the original infection with tubercle bacilli seems to depend principally upon the general bodily health, and particularly upon the capacity to wall off the tubercles and so prevent their advance, rather than upon the action of specific antibodies. Antibodies are formed, nevertheless, and can be detected by proper tests. These antibodies confer a certain degree of specific resistance against a fresh invasion of tubercle bacilli, so that *a person already infected is somewhat less susceptible* than an individual who has never harbored tubercle bacilli in his body. Nevertheless, as indicated above, reinfections certainly do occur, and they may bring about active tuberculous disease.

Koch's observations on immunity; old tuberculin (*O.T.*). Koch was the first to note that an animal already infected with tuberculosis is more resistant to a second injection of the germs than a normal animal. On the second injection of the bacilli into a tuberculous guinea pig, there is a prompt and severe reaction, which is due to the fact that the animal has become *hypersensitive* to the tubercle bacillus or its products. The inoculated organisms are destroyed in the course of this reaction, and do not set up a fresh infection. A tuberculous animal is, then, at once more sensitive and more resistant to tubercle bacilli than a normal animal. The situation is the same in human beings.

Koch thought that by inoculating persons deliberately with a preparation containing dead, disintegrated tubercle bacilli and their products, he might be able further to increase the resistance of tuberculous individuals and bring about a cure. With this in mind, he prepared *tuberculin*. He grew tubercle bacilli for six weeks in flasks of 5% glycerine broth, then evaporated the broth to 1/10th its volume by heating it in a water bath at 100° C (this heating, of course, killed the bacilli), and passed the concentrated broth through a bacteriological filter. The filtrate was a clear, brown liquid, free of living organisms, but containing all the disintegration products of the tubercle bacilli, any substances formed from the medium by the growing organisms, and the concentrated medium itself. This is the preparation now generally known as *Koch's Old or Original Tuberculin* (O.T.).

Koch found, as he expected, that tuberculous individuals give

a reaction when inoculated with tuberculin, whereas normal individuals do not. On his suggestion, physicians attempted to treat cases of tuberculosis with injections of tuberculin. The results were tragic, for a person infected with tubercle bacilli is so *hypersensitive* to tuberculin that severe illness develops at once, and even death may occur, when the dose is too large. If the dose is sufficiently small, however, the reaction to tuberculin is *local only* (confined to the site of the injection). Larger doses cause, in addition, a *focal reaction* (in the lungs or other infected areas), and a *general reaction* (evidenced by fever and other symptoms). The use of tuberculin for treatment is now practically abandoned, except for certain selected types of cases, and in the hands of experts.

Purified protein derivative (*P.P.D.*). Koch and others following him have prepared different forms of tuberculin, but only recently has a preparation been available that is definitely superior to O.T. This is a refined, concentrated product representing the active principle of the old tuberculin in almost a pure form. Strains of *Mycobacterium tuberculosis* are grown on synthetic medium, and the tuberculin is precipitated out of the heated filtrate by trichloracetic acid. It is then washed and concentrated by ultrafiltration. The purified protein derivative thus obtained is known as *Tuberculin P.P.D.* It is put up in tablet form in two strengths, and is now widely used.

Diagnostic tuberculin tests. Since only infected individuals react, the use of tuberculin preparations for *diagnosis* has naturally been tried. Among the various methods for making tuberculin tests, the procedure of choice is the *intracutaneous test of Mantoux. Patch tests* are also useful.

Mantoux method. The technique of the intracutaneous tuberculin test is as follows: The inner surface of the forearm is wiped with alcohol, then 0.1 cc of the tuberculin is injected *intradermally,* about an inch and a half below the bend of the elbow. When the injection is properly made, a sharply outlined, raised, whitish bleb will be visible when the inoculation is completed.

Since individuals vary considerably in their sensitivity to tuberculin, and unnecessarily severe reactions ought to be avoided, the strength of the first test dose should be low. In the case of *old tuberculin,* it is best to begin with a dilution of 1:1,000 (or even 1:10,000). If no skin reaction ensues in 48 hours, the test should be repeated (using the other arm), by injecting 0.1 cc of a 1:100

dilution of the O.T. If there still is no reaction within the next two days, the test may be considered negative.

When P.P.D. is used, the tablet of weaker strength is tried first; then, if a second test is necessary, the stronger tablet is employed. In each case, the tablet is dissolved aseptically in 1 cc of the buffer solution furnished, and 0.1 cc of this solution is injected intradermally. The dose from the less concentrated tablet contains 0.0002 mg P.P.D., whereas the stronger one has 0.05 mg (250 times as much) in one test dose.

A *positive* tuberculin test following such an intradermal injection is evidenced by the appearance of redness and edema, of varying intensity, after forty-eight hours. This is a delayed hypersensitivity reaction, and its occurrence is proof of the presence in the individual of antibodies that will react with tuberculin.

Tuberculin patch test (Vollmer). This method is especially useful for the routine testing of groups of children.

The skin is cleaned with acetone; then a small piece of filter paper, impregnated with tuberculin, is applied and covered with a patch of adhesive. The Vollmer patch test is supplied commercially in the form of a single adhesive strip on which are two tuberculin-impregnated pieces of filter paper, separated by a piece saturated with glycerine broth which serves as a control. The double test is merely a precaution against the possible failure of one test patch to maintain proper contact with the skin. The patches are allowed to remain in place for forty-eight hours; then they are removed, and a preliminary reading is made. The test site is inspected again after another forty-eight hours.

A *positive* reaction is indicated by the appearance of small, red papules and a more or less intense general reddening of the skin in the test area. The control patch will show no change, unless the patient is sensitive to adhesive, in which case it may not be possible to interpret the test satisfactorily. When the reaction is *negative,* the skin remains normal.

Meaning of tuberculin reactions. A positive tuberculin reaction always means infection, past or present, but it does not indicate the extent of the tuberculous lesions, nor whether the infection is progressing or latent. In adults, the diagnostic value of the test is decidedly limited, because so many persons have been infected and give a positive reaction, yet do not have *active* tuberculosis. An accurate measurement of the *degree* of tuberculin sensitivity can

be attained only through a series of injections of graded doses, and this is seldom attempted. In children, however, and in young people through the high school ages, a positive tuberculin reaction toward the usual test doses is often of real significance and a positive aid in diagnosis. Through the health departments in many communities, large groups of school children (all who are willing) are given tuberculin tests. Those who react positively are then given physical examinations, and x-rays are made of the chest.

This latter measure is of fundamental importance for diagnosis, for an expert can gain a good idea from the x-ray picture whether or not there is an active tuberculous process going on in the lungs. X-ray examination is needed to supplement tuberculin tests, since the latter may miss some infected individuals who, at the time of testing, are not sufficiently sensitized to react to the routine dose of tuberculin used. The recent development of satisfactory small x-ray films promises to make inexpensive x-ray examinations available to everybody in the near future. This important advance should result in the discovery of many early cases at a time when recovery, under proper care, may be most confidently expected.

From what has been said above, it would be expected that those persons who have never been in contact with tubercle bacilli, who have never been infected, and who therefore give a negative tuberculin test, would be highly susceptible to tuberculous infection. Some studies on this point have seemed, indeed, to show clearly that in the case of nurses, for example, active tuberculosis appears more often among those who have a negative tuberculin reaction when they enter training than among those with a positive reaction at that time. It must not be imagined, from this, that a positive tuberculin test is any guarantee against the liability of acquiring active tuberculosis. Young nurses and doctors, who are often exposed to tuberculous patients, must take care to prevent contact infection, and must avoid habits that predispose to tuberculosis, whether they are tuberculin-positive or not.

Tuberculosis in nurses, medical students, and hospital personnel. It is recognized that the nurses and other attendants in hospitals, where tuberculous patients are cared for, face a definite occupational hazard. Numerous authors have reported a disturbingly high incidence of active tuberculosis among senior and graduate nurses, senior medical students, and internes. It appears that many nurses and medical students become infected with tubercle

bacilli in the latter months of their training, when they begin to have contact with tuberculous patients. Proof of this is the rapidly rising percentage of positive tuberculin reactors among them, in the junior and senior terms. Moreover, in a certain number of individuals, this contact leads to the occurrence of progressing tuberculous disease. The liability of developing clinical tuberculosis is somewhat greater for those who are tuberculin-negative at the time of exposure than for those who give a strongly positive tuberculin test at that time.

More important, however, is the *relative amount of exposure.* Those students or graduate nurses and doctors whose duties bring them in repeated contact with open cases of tuberculosis are definitely in greater danger than those who are not so much exposed. All this fits in with what we have previously said. In young students, graduate nurses, and internes, we have a group of persons at a naturally susceptible age, some of whom have had no previous contact with tubercle bacilli, and consequently lack any degree of specific immunity, who are now exposed to an unusual degree to the virulent germs. In addition, long hours of work, and sometimes poor nutritional habits and inadequate rest, contribute their part by lowering the general level of body resistance.

A positive program designed to meet the threat of tuberculosis among students and hospital personnel has been adopted by some institutions, with good effect. Basic in this program is a constant vigilance over the health of faculty, students, and employees, and a continued effort to detect all active or incipient cases through routine tuberculin testing and chest x-ray examinations at frequent intervals. Routine use, on the wards and elsewhere, of all practicable methods to reduce the transfer of infection are enforced, and everyone concerned is made aware of the danger, and instructed as to the necessity for strict personal hygiene. The individual whose early pulmonary lesions are discovered through such a program is fortunate, for with prompt and conscientious treatment, full and rapid recovery is practically certain.

LEPROSY

Prevalence and importance. Few diseases have so powerful a hold on popular imagination as leprosy. Though the great majority of laymen have never seen a leper, nor read an authoritative

account of the illness, nearly everyone is convinced that leprosy is highly contagious, and horrible beyond description. Should a neighbor be discovered to be a leper, the most dire consequences would be expected to follow. This universal fear is evidence of the profound impression that this ancient disease has made on men's minds. Popular notions about leprosy, however, are totally wrong in one respect—contrary to the usual idea, this infection is *not* easily communicated. Instead, it occurs almost entirely among persons who have had long and intimate contact with a leprous individual under conditions favoring the transfer of the causative organisms. Actually, leprosy is one of the least contagious of all the infectious diseases.

The practice of good personal hygiene seems to protect against infection. Proof of this is the fact that not a single case of leprosy has ever developed among the nurses, doctors, or other employees at the National Leprosarium in Carville, Louisiana, during the fifty years of its operation, despite the continuous proximity of three hundred or more leprous patients. Moreover, the disease is apparently unable to perpetuate itself in the general population in modern communities, where cleanliness of person and habitation is the rule.

Leprosy has been known since ancient times. In the Middle Ages, it was common all over Europe. So great was the dread of the disease that lepers were often treated with a heartless cruelty. The unfortunate victims were made social outcasts, branded "unclean," and forced to beg for the means to sustain their miserable existence. During the fourteenth and fifteenth centuries, in Europe, the people began to make deliberate efforts to suppress leprosy by various drastic means, but principally by the segregation of all lepers in special institutions. These measures were so successful that the disease rapidly declined. At the present time, it is almost unknown in central Europe, though endemic foci still persist in a few localities, e.g., Spain and Italy. It is now most prevalent in tropical and subtropical areas, especially in Africa, China, India, and other Eastern countries. Numerous cases occur among the native populations of Hawaii, and in the Philippines, the West Indies, and Mexico. The disease is found also in Central America, and in the northern half of South America.

In the United States, there are perhaps as many as 1,000 cases of leprosy; the exact number is unknown. Most of the victims are

found among immigrants, especially Orientals; but an important fact is the existence of endemic foci of leprosy among the native white populations of Louisiana, Florida, and Texas. Whenever a case of leprosy is found in the United States, the patient is required to accept the care of the U. S. Public Health Service and to enter the National Leprosarium (U. S. Marine Hospital) at Carville, Louisiana. For years the total number of patients there has averaged approximately three hundred and fifty.

Spread of leprosy. The leprosy bacilli are transmitted through some form of personal contact. The primary infection may occur through the skin, or the nasal mucous membrane. The nodular lesions in the skin and the nose teem with the causative germs, and the organisms may be present in the saliva from lesions in the nose. Like tuberculosis, but to a greater extent, leprosy is a "house and family" disease. It is not inherited, but it does occur characteristically among the children of lepers, because of prolonged and close contact in the home. The leprous infection attacks children more readily than adults, and a high proportion of all patients probably acquire the original infection during childhood or adolescence. In any case, the frequency and duration of exposure to the germs is a factor of great importance.

Fundamental, also, in their influence on the spread of leprosy, are the general standards of living of the people. The disease does not spread now in the highly civilized parts of the world, even though imported cases are present in the community. The history of the disease in the United States illustrates this very well. Although cases of leprosy among immigrants are continually being discovered in New York and California, the disease is not communicated to the residents there. In the last half of the nineteenth century, about seventy lepers who were immigrants from Scandinavian countries (mostly Norway) settled in Minnesota. From this seemingly dangerous focus of leprosy, however, only seven cases developed among contacts of the original patients, and only one additional (doubtful) case has since been found that may have originated from contact with one of these. A similar experience has been recorded in California and Iowa, where smaller groups of lepers took up residence years ago. It is evident that in these states natural causes have operated virtually to eliminate the disease.

On the other hand, in the three states where leprosy remains truly endemic, new cases do appear among the native resident

population, though at a slow rate that seems to change little from year to year. Thus, of the leprous patients admitted to the National Leprosarium from Louisiana (259 between 1913 and 1937) and Florida (65 between 1911 and 1937), nearly all were infected within those states. In Texas (159 cases between 1920 and 1937), the situation is somewhat different, for a considerable proportion of the cases diagnosed in that state represent infections acquired in Mexico.

Clinical features. The incubation period in leprosy cannot be determined with exactness. It is commonly said to be about five years, but as many as twenty years may pass, after exposure to the disease, before definite signs or symptoms appear.

FIG. 110. *Mycobacterium leprae* in a *direct smear* from a skin lesion of a leper. Drawn from a single microscopic field. In such smears the irregular-shaped, acid-fast bacilli are usually very abundant, often occurring in large ball-like masses. Original preparation from U. S. Marine Hospital (National Leprosarium), Carville, La.

Ordinarily, the illness runs an extremely chronic course. There are two principal clinical forms of leprosy; both commonly appear in the same patient, though lesions of one or the other type predominate. In the *lepromatous* form, hard nodular swellings develop in the skin—especially on the face—which may greatly distort the features, giving a "leonine" expression to the countenance. The mucous membranes of the nose and throat, the eyes, and some-

times the internal organs, such as the liver, are the seat of similar, destructive lesions. In the *neural* form, the germs localize in the nerves, especially the peripheral ones, causing loss of sensation in extremities; as a result of secondary changes in the affected tissues, mutilation in some degree usually occurs, such as loss of fingers and toes, or even of whole limbs. The lepromatous type is the more acute, causing death, on the average, in about nine years. The neural type of disease is likely to predominate in persons with a relatively high resistance. As in tuberculosis, there is a tendency for leprosy to clear up spontaneously, and probably some cases of mild leprous infection never become evident clinically.

Mycobacterium leprae. The germ of leprosy is an acid-fast bacillus resembling, in appearance, the tubercle bacillus. It was first described by Hansen (1874), who recognized it in histological sections of skin from leprous nodules. The organisms are present in enormous numbers in these nodules, lying in bundles between the connective tissue cells, and crowded within the large round cells which make up the nodule (Fig. 110). Numerous attempts have been made to isolate these organisms in laboratory cultures; and bacteria of various kinds, some acid-fast and some not, have been grown by different investigators from leprous lesions. It is still very doubtful, however, whether the true leprosy bacillus has ever been cultivated. The organism is clearly an intracellular parasite.

Laboratory diagnosis. This depends upon demonstration of the leprosy bacilli by microscopic examination in scrapings from the nasal mucous membrane, or in bits of skin clipped from a leprous nodule. If there is any question as to whether the acid-fast organisms found in such material may be tubercle bacilli, it is only necessary to inoculate a guinea pig. If the organisms are tubercle bacilli, they will cause the development of tuberculosis in the animal, but if they are leprosy bacilli, no illness will be produced.

Control of leprosy. It is still the best policy to remove patients with leprosy from contact with healthy persons, and place them in leprosaria, where they may receive expert care. Little success has followed the treatment of leprosy with chaulmoogra oil—a substance at one time regarded as having curative powers. Recently the new drugs called promizole and diasone have proved effective in alleviating symptoms; their ultimate value is still uncertain. Other drugs and antibiotics are under trial. Lepers often suffer from concomitant diseases, especially tuberculosis, and their care is

consequently directed largely toward improving their general health and spirits.

REFERENCES

Tuberculosis

Baker, A. E. and Holoubek, J. F.: "Tuberculosis in Medical Students. A Survey of 298 Medical Students," *Am. Rev. Tuberc.,* Feb. 1941, 43:288.

Brean, H. P. and Kane, L. W.: "Pulmonary Tuberculosis in Harvard Medical Students," *N. Eng. J. Med.,* Sept. 5, 1946, 235:315.

Childress, W. G.: "Tuberculosis Among Hospital Personnel," *Am. Rev. Tuberc.,* June 1944, 49:501.

Furcolow, M. L., Hewell, B., Nelson, W. E. and Palmer, C. E.: "Quantitative Studies of the Tuberculin Reaction—I. Titration of Tuberculin Sensitivity and Its Relation to Tuberculous Infection," *Pub. Health Rep.,* May 23, 1941, 56:1082.

Griffith, A. S. and Mumo, W. T.: "Human Pulmonary Tuberculosis of Bovine Origin in Great Britain," *J. Hyg.,* Jan. 1944, 43:229.

Israll, H. L. and DeLien, H.: "Relation of Childhood Infection to the Development of Tuberculosis in Early Adult Life," *Am. J. Pub. Health,* Oct. 1942, 32:1146.

Long, E. R.: "Tuberculosis, Leprosy, and Allied Mycobacterial Diseases," *Science,* Jan. 14, 1938, 87:23.

Lurie, M. B.: "Experimental Epidemiology of Tuberculosis: The Prevention of Natural Air-Borne Contagion of Tuberculosis in Rabbits by Ultraviolet Irradiation," *J. Exper. Med.,* June 1944, 79:573.

Oppenheim, D. A. and Landes, J. H.: "Household Tuberculosis," *Am. Rev. Tuberc.,* Apr. 1944, 49:374.

Pottenger, F. M.: "Epidemiology of Reinoculation Tuberculosis," *Am. Rev. Tuberc.,* Aug. 1944, 50:112.

Rich, Arnold R.: *The Pathogenesis of Tuberculosis.* Springfield, Ill.: Chas. C Thomas, 1944.

Schwartz, S.: "Tuberculin Sensitivity and the Development of Tuberculosis in Nurses," *Am. Rev. Tuberc.,* Jan. 1943, 47:19.

Smith, H. R.: "Bovine Tuberculosis in the United States: Its Conquest and Its Effect on Public Health," *Am. Rev. Tuberc.,* Dec. 1944, 50:520.

Twinam, C. W. and Pope, A. S.: "Pulmonary Tuberculosis Resulting from Extra-Familial Contacts," *Am. J. Pub. Health,* Nov. 1942, 32:1215.

Yegian, D. and Baisden, L.: "Factors Affecting the Beading of the Tubercle Bacillus Stained by Ziehl-Neelsen Technic," *J. Bact.,* Dec. 1942, 44:667.

Leprosy

Faget, G. H.: "The Story of the National Leprosarium (U. S. Marine Hospital), Carville, Louisiana," *Pub. Health Rep.,* May 1, 1942, 57:97; "Pitfalls in Early Diagnosis," *N. Orleans Med. and Surg. J.,* March 1942, 94:432.

Frazier, C. N.: "Natural History of Leprosy," *J.A.M.A.,* Oct. 23, 1943, 123:466.

Kellesberger, E. R.: "Leprosy Today," *Clinics,* Dec. 1943, 2:842.

McCoy, G. W.: "Observations on the Epidemiology of Leprosy," *Pub. Health Rep.,* Dec. 18, 1942, 57:1935; "Chaulomoogra Oil in the Treatment of Leprosy," *Pub. Health Rep.,* Nov. 13, 1942, 57:1727.

Pardo-Castello, V. and Tiant, F. R.: "Leprosy: The Correlation of Its Clinical, Pathologic, Immunologic, and Bacteriologic Aspects," *J.A.M.A.,* April 17, 1943, 121:1264.

REVIEW QUESTIONS—CHAPTER XXXVI

1. Discuss the prevalence and importance of tuberculosis.
2. Name, and characterize briefly, the four types of tubercle bacilli. Who discovered the human tubercle bacillus, and when?
3. Describe the morphology and staining, methods of isolation, and cultural characteristics, of human tubercle bacilli. What is meant by *eugonic, dysgonic*?
4. Discuss the resistance of tubercle bacilli outside the body.
5. What are the principal differences between the human and the bovine types of tubercle bacilli?
6. What are some of the clinical forms of tuberculosis in human beings? Explain the nature of tubercles, and the reaction of the tissues to their presence.
7. Discuss the prevalence and importance of latent tuberculosis. Distinguish between tuberculous infection and tuberculous disease. What two kinds of evidence prove the wide occurrence of tuberculous infection?
8. Explain how the germs of human tuberculosis are transmitted from person to person. What accounts for the almost universal tuberculous infection among city populations?
9. Discuss the origin, importance, and prevention of infection of human beings with the bovine tubercle bacillus.
10. Discuss the factors which influence the development of active tuberculosis. What two varieties of tuberculous disease are recognized by pathologists and internists?
11. What is now considered the most probable explanation of the origin of active tuberculous disease in an adult?
12. Explain the methods employed for the laboratory diagnosis of tuberculosis. When is it necessary to use animal inoculation?
13. What were Koch's observations on immunity in tuberculosis? What is O.T.? P.P.D.? May tuberculin be used in the treatment of active tuberculosis? How does the body react to different doses of tuberculin?
14. Outline the Mantoux method and the patch method of making tuberculin tests. Describe a positive tuberculin reaction with each kind of test.
15. Explain the meaning of tuberculin reactions. Contrast the fundamental mechanism involved in: (a) tuberculin tests versus (b) Schick tests.
16. Discuss the value of tuberculin tests in helping to discover cases of active tuberculosis.
17. Is a person who gives a positive tuberculin test more, or less, susceptible to a new infection? Why?

18. Discuss the problem of tuberculosis among nurses, medical students, and hospital personnel.
19. Discuss the historical importance, and present prevalence, of leprosy. Is this disease highly contagious?
20. Mention some facts about leprosy in the United States.
21. What are the principal clinical forms of leprosy? What can be said about the incubation period? other clinical features?
22. Name and describe the causative organism of leprosy. How does it differ from, and how does it resemble, the tubercle bacillus?
23. How is a laboratory diagnosis of leprosy made?

CHAPTER XXXVII

RICKETTSIAL INFECTIONS

As we have previously explained, the rickettsiae have a number of peculiarities which entitle them to recognition as a special class of microorganisms. Moreover, the illnesses they cause in human beings have similar features, and differ in several respects from most infections caused by ordinary bacteria.

Properties of rickettsiae. The organisms now known as rickettsiae were first observed in 1909 by Ricketts, who saw them in the blood of patients with Rocky Mountain spotted fever. Although the germs looked like small bacteria, they would not grow out on artificial media. Similar bodies were again found by Ricketts and Wilder, in 1910, in smears from the blood of patients with typhus fever, and from lice that had fed on typhus patients. These findings were confirmed by Prowazek, during his study of typhus in Siberia in 1913, and shortly thereafter by other investigators in other parts of the world. In 1916, De Rocha-Lima introduced the genus name, *Rickettsia,* when he designated the causative organism of epidemic typhus fever *Rickettsia prowazeki* in honor of Ricketts and of Prowazek, both of whom had died of typhus while investigating the disease.

The outstanding properties which distinguish the pathogenic rickettsiae as a group are: (1) their minute size and characteristic staining, (2) their peculiar habitat in ticks, lice, and other arthropods, (3) their apparent development, in the infected human or animal body, only within living tissue cells, and (4) their complete failure to grow in the laboratory in lifeless artificial culture media.

Morphology and staining; filtrability. In appearance, rickettsiae suggest very small cocci or bacilli, and it is generally believed that they have evolved from ordinary bacteria. Electron-microscope pictures confirm the impression of a bacterium-like structure (Fig. 111). These photographs show that rickettsiae cultivated in the yolk sac of chick embryos are rounded or rod-shaped bodies, having a diam-

eter of about 0.5μ and a maximum length of about 1.5μ. They appear to have an outer cell wall, and to multiply by transverse fission, like ordinary bacteria. In smears made from infected animal tissues the organisms tend to be smaller, averaging approximately 0.3μ x 0.6μ, and they are often highly pleomorphic (Fig. 111). Chains and masses of diplococcoid forms are seen. These small organisms approach the limits of vision possible with the ordinary microscope,

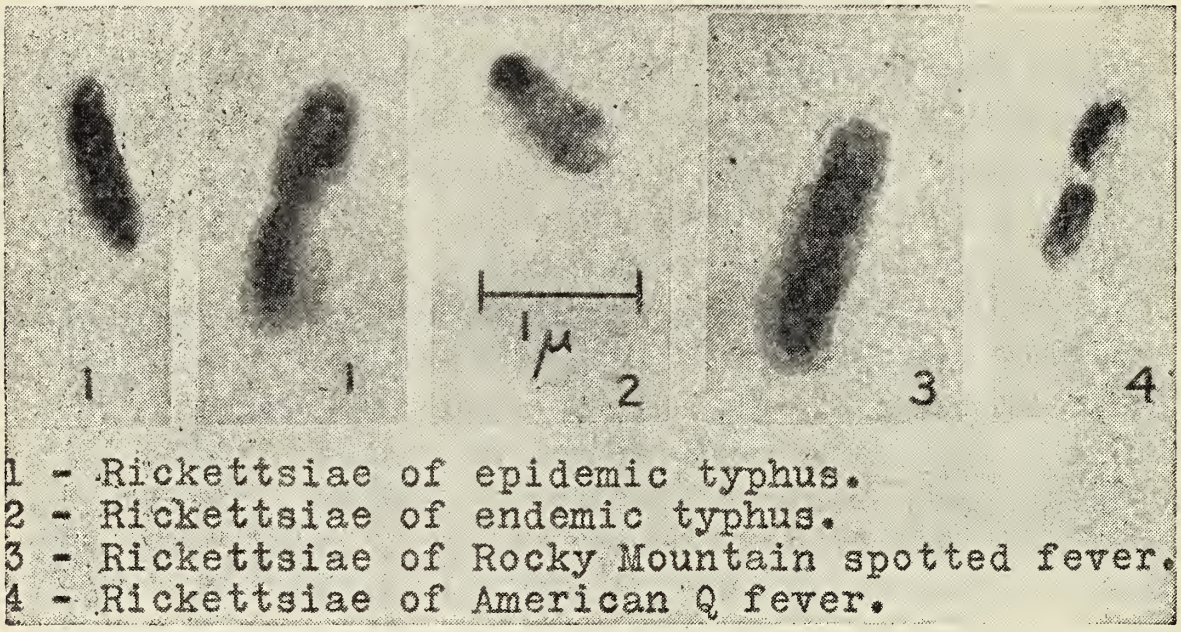


Fig. 111. Electron microscope pictures of rickettsiae. These photographs show the organisms from cultures in the yolk sacs of embryonated eggs. The four species of rickettsiae are evidently similar to one another in morphology, and all of them look much like ordinary bacteria, except, of course, for their minute size. (From Plotz, H., Smadel, J. E., Anderson, T. F., and Chambers, L. A., "Morphological Structure of Rickettsiae," *J. Exper. Med.*, 77:355, April, 1943.)

and it may be that some individual rickettsiae are too tiny to be visible in the usual microscopic preparation. There is an obvious similarity between the rickettsiae and the elementary bodies of filtrable viruses. The Q fever rickettsiae *are* filtrable, but the other varieties do *not* pass the usual bacterial filters. It seems fair to regard rickettsiae as occupying an intermediate position, in the great family of microbes, between the true bacteria on the one hand and the viruses on the other.

Rickettsiae generally stain faintly with the usual dyes, and they are Gram-negative. Special stains, such as Giemsa's, are used to demonstrate the organisms in infected tissues.

Habitat. The rickettsiae may be said to be habituated primarily to existence within the bodies of various species of insects and

arthropods. There are good grounds for the assumption that they were originally parasites of insects or arthropods only, and have become secondarily parasitic in various mammals and in man, as a consequence of the blood-sucking habits of the infected ticks, fleas, etc. These insects and arthropods now act as the *vectors* of rickettsial infection from animal to animal, animal to man, and man to man.

Intracellular growth. As we now encounter them, the pathogenic rickettsiae are all highly specialized *intracellular parasites.* They may be present in the blood and organs of the infected individual, but they are much more abundant within the cytoplasm, or, in the case of Rocky Mountain spotted fever, within the nucleus, of certain cells, especially those lining the peritoneum and the walls of the smaller blood vessels. They apparently multiply only within suitable living body cells (Fig. 112).

Laboratory cultivation. We cannot grow the pathogenic rickettsiae in lifeless laboratory media. They may be cultivated, however, in artificial tissue cultures, where they develop within the growing cells of the culture, just as they do under natural conditions in the body. They also may be grown on the chorio-allantoic membrane of the embryo chicken by the procedure successfully used for the propagation of the filtrable viruses. The most successful method, however, and the one most widely used at present, is cultivation in the yolk sac of the growing chick. The rickettsiae multiply in the cells in the wall of the sac—not in the yolk itself.

Rickettsial diseases. Four natural subdivisions may be recognized among the principal human rickettsial diseases: (1) the *typhus fever* group, (2) the *Rocky Mountain spotted fever* group, (3) *tsutsugamushi disease,* and (4) *Q fever* (Table XXV).

Included in the typhus group are two distinct forms: (1) *epidemic typhus,* caused by *Rickettsiae prowazeki,* the classical European typhus of olden times, transmitted from man to man by the *body louse,* and (2) *endemic* or *murine typhus,* caused by the almost identical organism now called *Rickettsia mooseri,* a natural infection of rats, carried to man by the rat *flea.*

The Rocky Mountain spotted fever group includes not only the spotted fever of the western mountain states of the United States, and the somewhat milder form of the infection in southern and eastern states, but the similar, variously named *tick-borne* diseases in Central and South America, the Mediterranean countries, Africa, and elsewhere.

TABLE XXV. Rickettsial Diseases in Man

DISEASES	CAUSATIVE RICKETTSIAE	NATURAL HOST	INSECT OR ARTHROPOD VECTOR	WEIL-FELIX REACTION		
				OX19	OX2	OXK
Epidemic Typhus	*Rickettsia prowazeki*	Man	Human body lice* (*Pediculus humanus*)	++++	+	+ / —
Endemic (Murine) Typhus	*Rickettsia mooseri*	Rats and mice	Fleas, especially *Xenopsylla cheopsis*	++++	+	—
Rocky Mountain Spotted Fever and Related Diseases	*Rickettsia rickettsi* (*Dermacentroxenus rickettsi*)	Probably various animals, especially rodents; ticks	Ticks, especially *Dermacentor andersoni, Dermacentor variabilis, Amblyomma americanum*	++++	++	+ / —
Tsutsugamushi Disease (Scrub Typhus)	*Rickettsia nipponica* (*R. orientalis* or *R. tsutsugamushi*)	Field mice and other rodents	Mites of genus *Trombicula*	—	—	+++
Q Fever	*Rickettsia burneti* (*R. diaporica*)	Bandicoot, opossum, probably various other mammals; ticks	Ticks* (*Dermacentor andersoni, Amblyomma americanum,* and others)	—	—	—

* Air-borne infection through inhalation of contaminated dust from dried feces of infected lice (or ticks) may account for some human cases.

Under the head of tsutsugamushi disease are the various forms of *mite-borne* infection, including *scrub typhus,* prevalent in southern Asia and on islands of the Southwest Pacific.

A peculiar characteristic of all these rickettsial infections, with the exception of Q fever, is that they stimulate in patients the formation of agglutinins for certain strains of *Proteus vulgaris.* The agglutination of *Proteus* bacilli by the serum of patients with typhus or other rickettsial disease is known as the *Weil-Felix* reaction. The *Proteus* strains called OX19 and OX2 are acted upon by the blood serum of typhus and of spotted-fever patients, but not by patients with tsutsugamushi disease. Victims of the latter infection, however, form agglutinins for another strain, called OXK. These remarkable reactions are obviously of practical diagnostic importance. No strain of *Proteus* has yet been found that will be acted upon by the serum of Q-fever patients.

Epidemic typhus fever. *Prevalence and importance.* This ancient malady has played a conspicuous part in the gloomy history of epidemic disease. Typhus is one of those pestilences with a high mortality that plagued European and Asiatic peoples for centuries before general living conditions and standards of personal cleanliness improved sufficiently to limit their spread. This louse-borne "jail-fever," to give it one of its common names, has always been associated with war, famine, overcrowding, filth and human misery. It always tends to increase in populations disorganized by war, among unfortunate people crowded together where lousiness prevails, in the poor sections of the cities, and in concentration camps, asylums, and prisons. Typhus outbreaks occur principally in cold weather.

Epidemic typhus was a serious menace to troops and civilian populations in war areas during World War II. It occurs in parts of Mexico, but, although it has been introduced at seaports from time to time, it has never found a permanent foothold in the United States or Canada.

Clinical features; mode of transmission. In epidemic typhus fever, the incubation period is commonly about ten days. The illness usually begins abruptly with rapidly rising fever, chills, headache, and body pains. The fever remains high for about two weeks, then falls rather quickly. Often patients are profoundly prostrated; there are marked signs of injury to the brain, heart, and other body tissues. The most distinctive feature of the disease is a skin rash which

appears on the fourth to sixth day of illness, and which has a characteristic appearance and distribution.

The mortality rate in severe epidemics may be as high as 60%, but is generally much lower, especially among young people. Recovery is followed by a strong and lasting, specific immunity.

The causative rickettsiae (*Rickettsia prowazeki*) are carried from person to person by body lice (*Pediculus humanus*). The rickettsiae are present in great numbers in the feces of infected lice, and it is probable that they gain entrance to the body when the bite-wound or other skin abrasions are contaminated with fecal material. If lice are crushed on the skin, rickettsiae may be rubbed in. It is likely, also, that air-borne dust, containing particles of dried, infected louse feces, may be a source of infection, by inhalation, under some circumstances. This latter method of transmission is thought to explain the frequent occurrence of epidemic typhus fever among doctors and nurses in attendance on patients, and among laboratory workers handling infectious materials. These infections occur despite the fact that the persons concerned are not infested with lice.

Bacteriological diagnosis. For routine diagnosis, the most useful procedure is the *Weil-Felix agglutination test with the patient's blood serum.* Dilutions of the patient's serum are mixed in test tubes with an antigen consisting of a suspension of a nonmotile X strain of *Proteus vulgaris,* usually the strain OX19. Then the mixtures are incubated, and examined for agglutination.

Agglutinins for *Proteus* OX19 appear in the blood of most typhus patients during the second week of illness, and increase in concentration until about the time convalescence begins, then disappear rather rapidly. At least two blood samples should be titrated, one taken early in the illness, and the other taken toward the close of the second week. If a definite rise in the titer of agglutinins in the later sample is observed, this is strong evidence for the existence of rickettsial infection. Final titers may be as high as 1:10,000, or more; to be diagnostic the titer should be at least 1:320.

The *Proteus* strain OX2 is also agglutinated by the serum of typhus patients, but not with equal regularity, and this strain is not used routinely. Serums of patients with Rocky Mountain spotted fever give an equally strong Weil-Felix reaction, and consequently this test does not help distinguish between the two diseases.

It has recently been found, however, that *complement-fixation tests* with patients' serum, set up with typhus rickettsiae as antigen,

are positive in typhus fever only—not in spotted fever. The specific complement-fixation reaction in typhus becomes positive during the second week of the disease, and remains positive for years after recovery.

Specific prophylaxis. A vaccine made up of a suspension of the rickettsiae grown in the yolk sac of the developing chick embryo, and killed by formalin, is now used. The effectiveness of the yolk-sac-culture vaccine in the face of a typhus epidemic has not as yet been tested adequately, but laboratory workers have been found to be almost completely protected by it.

General preventive measures. The delousing of the population in areas where the disease is occurring is a necessary measure for the control of epidemic typhus. This is best done at special establishments where the clothing (to which most of the lice cling) can be freed of lice by steaming or other treatment, and where the louse-infested individual can be bathed and the hair on the head and body clipped. Failing this, lice can be largely, if not entirely, eliminated by dusting the body and clothing liberally with the remarkable new powdered insecticide, DDT. Doctors, nurses, and others in attendance upon typhus patients should wear louseproof clothing, use louse-repellent powders, and take every precaution against droplet or air-borne infection.

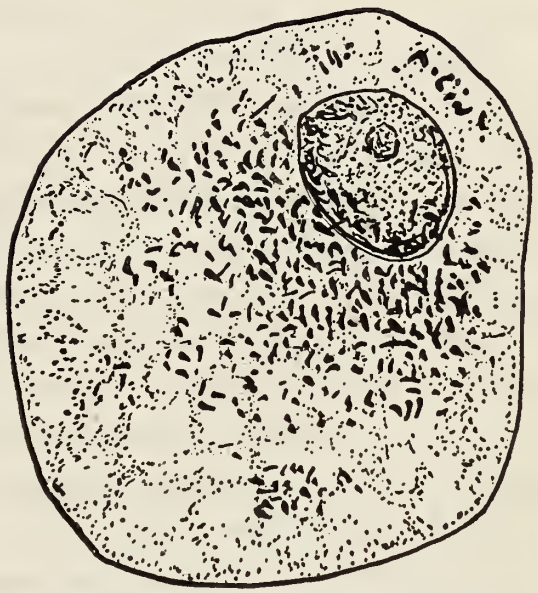


FIG. 112. A tissue cell from a guinea pig experimentally infected with the rickettsiae of endemic typhus. The pleomorphic rickettsial bodies occur in large numbers in the cytoplasm.

Endemic (murine) typhus fever. *Prevalence and importance.* This form of typhus infection is of immediate concern in this country. The existence of this flea-borne disease, of which the rat is the animal reservoir, was not generally realized until about 1923, when Maxey called attention to it through his extensive investigations in southeastern United States. Soon it was found that this form of typhus was endemic in many communities along the South Atlantic coast, the Gulf coast, and in the Rio Grande valley, and

that it occurred also occasionally in southern California. Its prevalence in Mexico was established, and numerous cases were reported in residents of the United States near the Mexican border.

In recent years, the disease has apparently spread northward along the Atlantic seaboard, and also inward to towns in the interior of Texas, Georgia, Alabama, and other coastal states. Wider recognition of the illness by physicians has resulted in a marked increase in the total of cases reported to health authorities. Also, since the identification of endemic typhus in the United States, its worldwide occurrence has been demonstrated, and it is known to appear with special frequency in seaports. It is thought that flea-borne typhus probably occurs at the beginning, and during louse-borne epidemics, though it may not be recognized as such.

Clinical features; mode of transmission. Clinically, endemic typhus does not differ from epidemic typhus, except that usually the illness is less severe. Death occurs in less than 5% of cases, and principally in older persons. The clinical features may closely resemble those of the milder cases of Rocky Mountain spotted fever, typhoid fever, or other acute infections.

Endemic typhus is referred to as typhus of the *murine type* because it is primarily an infection of rats. Rat fleas and rat lice carry the causative rickettsiae from rat to rat. Human beings are infected when feces of infected rat fleas are rubbed into the skin around the fleabite. The causative rickettsiae (*Rickettsia mooseri*) multiply actively in the fleas, but do not harm the insects. (In contrast, human body lice that transmit epidemic typhus from man to man invariably succumb, themselves, eventually to the rickettsiae that they harbor.) The species of flea important in transmission of endemic typhus from rat to man is the same as is concerned in the spread of plague—*Xenopsylla cheopis*.

The life cycle and feeding habits of rat fleas determine the epidemiological features of endemic typhus in man. The disease occurs among persons who live or work where there are rats and rat harbors. Hence, cases are especially frequent among warehouse men, grocery men, and others engaged in food-handling establishments. The infection is not associated with poverty or personal uncleanliness, and it does not spread from person to person. Most cases occur in the late summer and in the fall.

Bacteriological diagnosis. As in epidemic typhus, the Weil-Felix reaction (agglutination of Proteus OX19, and usually also of OX2)

becomes strongly positive in the course of endemic typhus, and this test is relied upon principally for laboratory diagnosis. The patient's serum will also show a positive complement-fixation test with an antigen made from the causative rickettsiae.

Specific prophylaxis. Vaccines prepared by the same techniques used to make those for epidemic typhus are available. They are effective immunizing agents, and should be used by persons likely to be exposed to murine typhus.

General preventive measures. The prevention of endemic typhus, like the prevention of plague, is principally a matter of *reducing the rat population* as much as possible, by trapping, poisoning, and especially by building out, these dangerous and destructive pests.

Rocky Mountain spotted fever. *Prevalence and importance.* Until about 1930, it was generally believed that this disease was confined to the Rocky Mountain region of the United States. It was especially often encountered in Montana and Idaho, with occasional cases in other western states. In 1930, it was established that the infection occurs also in the eastern and southern states. Since that time, the disease has been diagnosed in almost all parts of the country, except in New England, Wisconsin, and Michigan. Like endemic typhus, it is apparently increasing in prevalence.

Clinically similar tick-borne diseases, long known in other regions of the world, in particular a form of "typhus" occurring in Sao Paulo, Brazil, and in Colombia, are now recognized as varieties of the same infection. Closely related, also, are the so-called boutonneuse fever of the Mediterranean countries, the Kenya "typhus" of East Africa, and the "tick typhus" of South Africa.

Clinical features; mode of transmission. The incubation period in spotted fever varies from 2–15 days, but is commonly about 7 days. As in typhus, the onset is usually sudden, with chills and rapidly rising temperature, body pains, and prostration. Often the fever persists for as long as 21 days, then terminates quickly. A characteristic rash appears, usually on the third or fourth day. This has a different appearance and location than the rash of typhus. It is marked by the severe, hemorrhagic character of the skin lesions. The typical Rocky Mountain spotted fever of the western states is a highly fatal disease, whereas most cases reported in eastern states have been milder, but exceptions to this generalization have been observed frequently in recent years. The mortality increases

with age. Dyer states that the crude fatality rate for reported cases in the United States is 18.4%.

Although several other varieties of ticks are probably capable of transmitting the rickettsiae, three species of man-biting ticks have been proved to be natural vectors of Rocky Mountain spotted fever in the United States. These are the common *wood tick* of the northwestern states (*Dermacentor andersoni*), the *dog tick* (*Dermacentor variabilis*) of the eastern states, and the *Lone Star tick* (*Ambylomma americanum*) of Texas and adjacent southwestern states. These ticks infest a wide variety of mammals in rural and wooded places, and readily attach themselves to human beings—farmers, foresters, surveyors, campers, picnickers and others—who venture into areas where the ticks are abundant. Comparatively few individual ticks are infected, but once the rickettsiae are picked up, a tick remains infected throughout the several years of its life; moreover, the organisms survive in the eggs and pass into newly hatched ticks, so that the parasite is perpetuated from one generation to the next without the necessity of passing through a mammalian host.

Infection in man is acquired by contamination of the tick bite or skin abrasions with crushed ticks or with their feces. The longer an infected tick remains attached to the skin, the greater the likelihood that the rickettsiae will be transferred. Persons have been infected by crushing ticks with their fingers while removing them from dogs or other animals.

Bacteriological diagnosis. The Weil-Felix reaction is positive in most cases of Rocky Mountain spotted fever, and the rise and fall of agglutinins in the blood, during and after the infection, is similar to that seen in typhus patients. Some proved cases of spotted fever have failed to show a positive Weil-Felix test with either *Proteus* OX19 or OX2; hence, a negative reaction does not necessarily exclude the disease. Inoculation of the patient's fresh blood into guinea pigs is sometimes done. In these animals, virulent spotted fever rickettsiae produce a severe disease, with scrotal swelling, and a high mortality. The most sensitive and specific diagnostic test, however, is a complement-fixation reaction with the patient's serum and a spotted-fever-rickettsiae antigen.

Specific prophylaxis. As a vaccine for Rocky Mountain spotted fever, Spencer and Parker made an emulsion of infected ticks by grinding them in salt solution containing phenol. This vaccine, as prepared by the U. S. Public Health Service Laboratory at Hamil-

ton, Montana, has been widely used. A second type of vaccine, made, like the typhus vaccine, from cultures of spotted-fever rickettsiae in the chick embryo yolk sac, is now available. Both of these preparations apparently give considerable protection to vaccinated individuals.

General preventive measures. The eradication of all the tick vectors of spotted-fever rickettsiae is hardly possible. Obviously, unnecessary visits to known areas where there are infected ticks ought to be avoided. Individuals who must enter such areas should take care to dress appropriately, and to examine clothing and body frequently for ticks. Dogs and other pets should be freed of ticks every few days. Ticks should not be removed with the bare fingers, but in a bit of paper held by the fingers, or by means of forceps.

Scrub typhus. This is the name now commonly used in American medical literature in referring to the disease otherwise known as *tsutsugamushi, Japanese river fever,* or the *tropical typhus* of Malaya, Sumatra, the Philippines, and other islands of the Southwest Pacific. It is caused by *Rickettsia nipponica* (also called *R. orientalis* or *R. tsutsugamushi*) and is conveyed to man from infected rodents, chiefly field mice, by the larval form of the mite *Trombicula akamushi.* These mites resemble the chiggers, or red bugs, that are familiar pests in many parts of the United States. The rickettsiae are apparently transmitted from one generation of larvae to the next.

Scrub typhus became a matter of concern to the U. S. Navy and Army medical men during World War II, when outbreaks of the disease occurred among our troops on New Guinea and other Southwest Pacific islands. It was necessary for the men to sleep and work in the clear areas found here and there in the midst of dense tropical forests. These areas were covered with kunai grass, a coarse grass that grows to a height of ten feet or more, and is so thick that the ground under it is constantly wet. This grass was a fine harborage for the mite-infested field mice and other rodent carriers of *R. nipponica.*

The illness begins abruptly after an incubation period of about two weeks and is commonly severe, with symptoms similar to those of the other rickettsial infections described above. The death rate is about 15%. A characteristic clinical feature is the appearance of a small ulcer, or eschar, on the skin at the site of the bite.

The serum from patients who have been ill for a week or more

will usually agglutinate *Proteus* OXK in dilutions of 1:160 or higher, but not the OX19 or OX2 strains. In some cases, the Weil-Felix reaction remains negative.

The tsutsugamushi rickettsiae are distinct immunologically from those of typhus and spotted fever, so that vaccination against the latter diseases will not offer any protection against scrub typhus.

Q fever. This infection stands somewhat apart from the other rickettsial diseases. The causative rickettsiae (*Rickettsiae burneti* or *R. diaporica*) are filtrable, and patients do not develop a positive Weil-Felix reaction.

The first human cases of this new disease were recognized in Australia in 1937. These were shown to be caused by a kind of rickettsia which was given the name *R. burneti*. About the same time a new strain of rickettsiae was isolated from ticks in Montana and named *R. diaporica*. Later it was proved that these two infectious agents are identical.

The Australian cases occurred among workers in slaughterhouses, as well as among foresters and dairy employees, presumably as a result of bites by infected ticks. In Australia a reservoir of the infection resides in bandicoots and opossums.

In the United States, an outbreak among laboratory personnel has been reported. This was apparently due to the inhalation of dust contaminated with the causative rickettsiae from infected animals or from dried feces of infected ticks. In these cases, the symptoms were like those of so-called atypical pneumonia, and lasted from 10 days to 3–4 weeks. No fatalities from Q fever have been observed.

REFERENCES

Anigstein, L.: "The Present Status of the Spotted Fever Problem," *Clinics*. Philadelphia: J. B. Lippincott Co., 1943, Vol. II, p. 890.

Bengston, I. A.: "Complement Fixation in the Rickettsial Diseases—Technique of the Test," *Pub. Health Rep.*, Mar. 24, 1944, 59:402.

Blake, F. G., Maxen, K. F., Sadusk, J. T., Jr., Kohls, G. M. and Bell, E. J.: "Studies on Tsutsugamushi Disease (Scrub Typhus, Mite-Borne Typhus) in New Guinea and Adjacent Islands," *Am. J. Hygiene,* May 1945, 41:243. See also Kohls, C. M., Armbrust, C. A., Irons, E. N. and Philip, C. B.: *Ibid.*, p. 374.

Dyer, R. E.: "The Rickettsial Diseases," *J.A.M.A.*, Apr. 22, 1944, 124:1165.

Hull, T. G.: *Diseases Transmitted from Animals to Man.* Springfield, Ill.: Chas. C Thomas, 1941.

Pinkerton, H.: "The Pathogenic Rickettsiae, with Particular Reference to Their Nature, Biologic Properties, and Classification," *Bact. Rev.*, Mar. 1942, 6:37.

Pollard, M. and Augustson, G. F.: "Serological and Entomological Survey of Murine Typhus," *Am. J. Trop. Med.*, Jan. 1945, 25:31.

SHERRARD, G. C.: "A Plan for Rodent Control in Cities," *Pub. Health Rep.,* May 28, 1943, 58:825.

Virus and Rickettsial Diseases, Harvard School of Public Health Symposium Volume. Cambridge, Mass.: Harvard Univ. Press, 1940.

REVIEW QUESTIONS—CHAPTER XXXVII

1. How and when were the organisms known as rickettsiae discovered and named? State four outstanding properties which distinguish the pathogenic rickettsiae as a group.
2. Describe the morphology, habitat, growth habits, and requirements of the rickettsiae. By what means are rickettsiae maintained for study in the laboratory?
3. Name the four principal groups of rickettsial diseases. What two forms of typhus occur?
4. Give: (1) the scientific name of the causative rickettsiae, (2) the natural host, (3) the insect or arthropod vector concerned in each of the five principal kinds of rickettsial diseases.
5. What is the Weil-Felix reaction? To what extent does this reaction serve to distinguish between the common rickettsial infections?
6. Describe the prevalence and importance, clinical features, and transmission of epidemic typhus fever.
7. Outline practical methods used for the bacteriological diagnosis of epidemic typhus.
8. Describe practical measures for the prevention of epidemic typhus.
9. Describe the prevalence and importance, clinical features, and transmission of endemic typhus fever. How does this disease differ in its epidemiological features from epidemic typhus?
10. Outline practical methods for the bacteriological diagnosis and prevention of endemic typhus.
11. Describe the prevalence and importance, clinical features, and transmission of Rocky Mountain spotted fever.
12. How may a bacteriological diagnosis of spotted fever be made?
13. Discuss practical measures for the prevention of spotted fever.
14. Explain the nature and importance of scrub typhus. How may bacteriological diagnosis be made?
15. Explain the nature and importance of Q fever. In what two ways may the causative organisms be transmitted?

CHAPTER XXXVIII

VIRUSES AND BACTERIOPHAGES

Discovery of filtrable infectious agents. We have already described how the invention of filters which do not allow the passage of ordinary bacteria led to the discovery that the causative agents of certain diseases will pass through such filters in invisible form. The first to observe this was Iwanowski, in 1892, when he found that the agent responsible for the mosaic disease of tobacco leaves is filtrable. The *filtrate* from an emulsion of diseased leaves was found to be entirely free of bacteria, and no form of living thing could be seen when it was examined with the ordinary microscope. Nevertheless, *when some of this clear filtrate was placed upon healthy tobacco leaves, these leaves soon sickened* and showed every sign of the typical mosaic disease.

In 1898, Loeffler and Frosch demonstrated that an infection of animals sometimes seen in man, and called *foot and mouth disease,* is also caused by a filtrable, invisible "virus."

Since these pioneer studies, a large number of other disease conditions, including some of the most common and serious of human ills, have been shown to be due to infection with similar filter-passing agents. In each of these diseases, blood samples from the patient, or washings from the nasal passages, or extracts from body discharges or from infected tissues, when passed through a bacterial filter yield a filtrate which is free of any organisms visible by *ordinary* methods of microscopical examination, and which gives no growth of any kind on artificial culture media, yet contains an active element—the virus—capable of reproducing the same disease in other individuals.

For want of a better name, the elusive infectious agents of this sort are now generally referred to as *filtrable viruses,* or are called, merely, *viruses*—terms which do not imply any definite notion as to their real nature.

Fundamental properties of viruses; a definition. The viruses are universally regarded as a distinct class of infectious agents, and the word *virus* has as definite a meaning as bacterium or spirochete. The most experienced expert, however, finds it difficult to give a fully satisfactory definition. The evidence is strong that certain of the viruses are *living, ultramicroscopic organisms,* but *others seem to lack the usual attributes of life,* and appear like inanimate substances, in the nature of large-molecule, self-reproducing proteins.

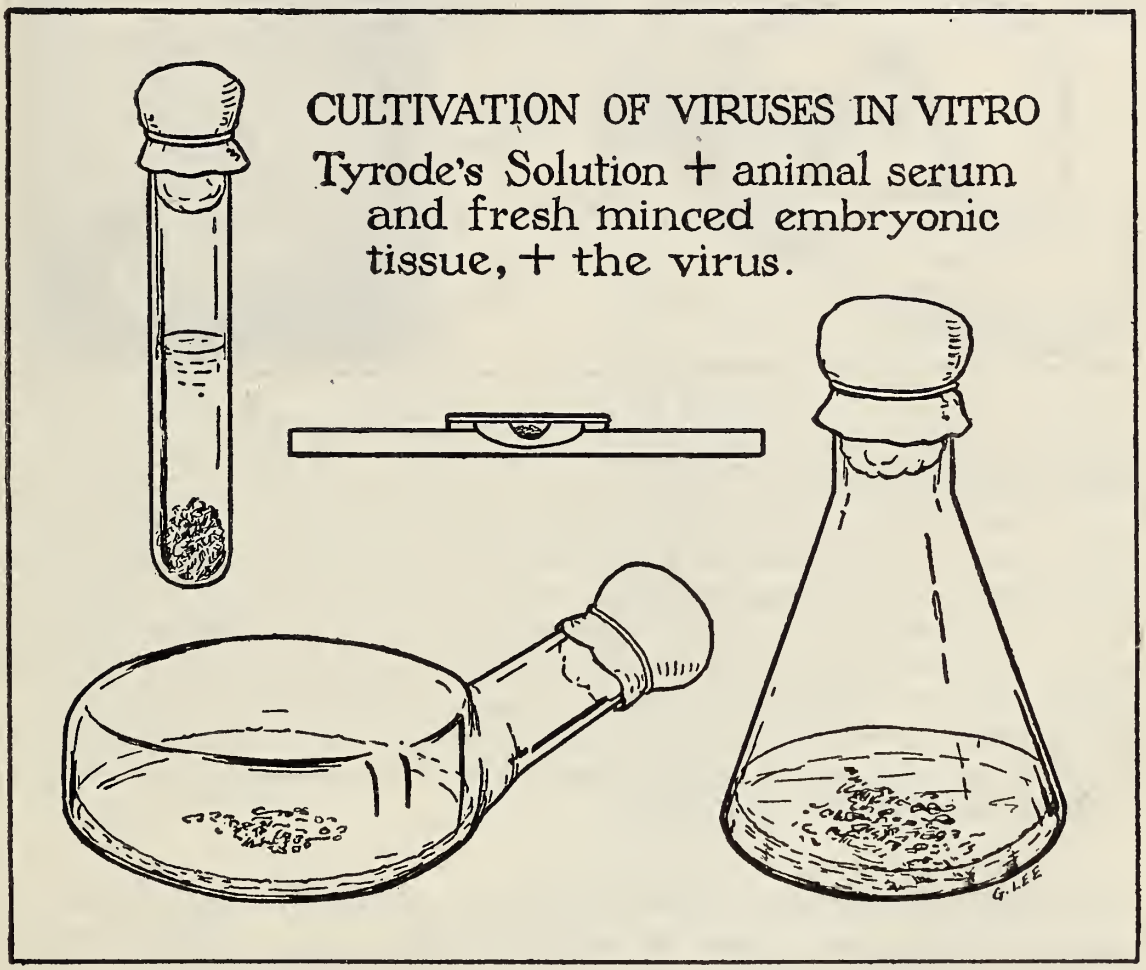


FIG. 113. Methods commonly used for growing filtrable viruses in tissue cultures. The virus multiplies only when the tissue cells are living and of a suitable kind.

While the question of the living or nonliving character of the viruses is still debated, it is necessary, for purposes of definition, to fall back upon the few general properties which all the viruses share in common. These may be summarized as follows:

(1) Viruses are smaller than bacteria or rickettsiae, and are invisible by ordinary methods of microscopic examination.

(2) They pass through filters which hold back ordinary bacteria (though some of them go through with difficulty).

(3) They cannot be grown in artificial media without the presence of living cells (Figs. 113, 114, 115, 116).

(4) They exist in the most intimate relationship with the living body tissues which they damage, and they multiply (or regenerate) only within young, susceptible living cells.

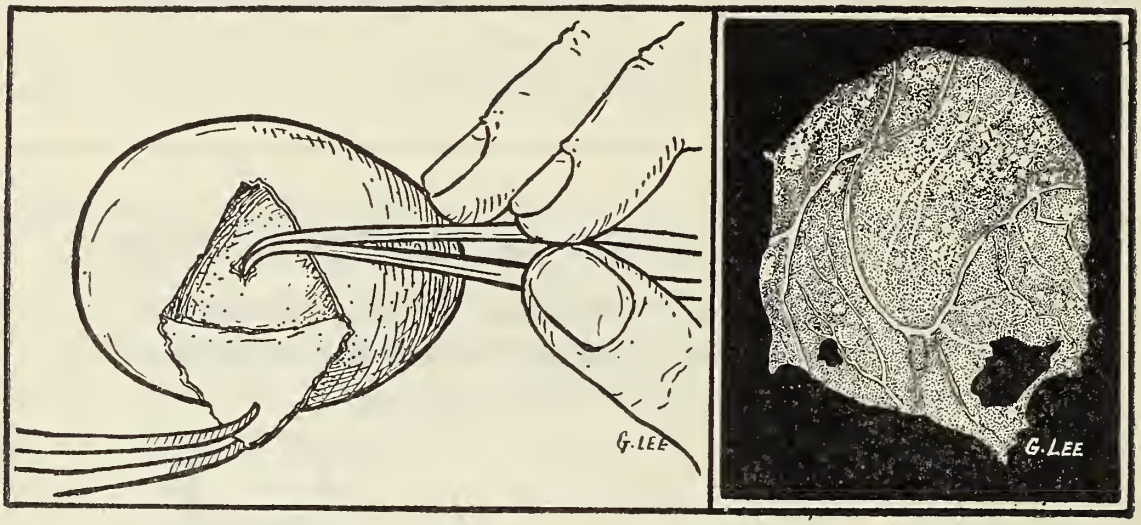


FIG. 114. Cultivation of viruses on the chorio-allantoic membrane of the developing chick embryo. *Left,* one method of inoculating; a bit of infected membrane from a previous egg culture is being introduced through the exposed shell membrane of the fertile egg on to the chorio-allantoic membrane that lies just beneath. A more usual procedure is to remove entirely a much smaller square of the egg shell, then introduce the fluid virus by capillary pipette or needle and syringe. The opening in the shell is then sealed by a sterile coverslip and paraffin or by paraffin alone. On return of the inoculated egg to the incubator, the virus multiplies on the still-growing fetal membrane of the embryo chick. *Right,* a chorio-allantoic membrane removed after growth of a virus, showing the white, opaque areas produced in it. From such infected membranes viruses may be recovered readily for further studies.

The first- and last-mentioned qualities, especially, characterize the filtrable viruses as a class. In general terms, then, we may define them as *ultramicroscopic infectious agents, which appear to be intracellular parasites, and which are clearly dependent for multiplication upon an intimate association with living cells.*

Virus diseases. At the present time, a large number of diseases are attributed to infection with a virus. The number continually increases as new virus diseases are recognized. Some of the more important and best known are listed in the following table:

Diversity among virus diseases; the distinctive character of individual viruses. These lists, though still incomplete, indicate how extensive is the field of study concerned with virus infections. It seems probable that no form of life is exempt from virus maladies.

Table XXVI. Some Virus Diseases

In Bacteria
- Transmissible lysis (action of bacteriophages)

In Plants
- Mosaic diseases of tobacco, tomato, and potato plants
- Ring-spot of tobacco leaves
- "Breaking" of the solid color of normal tulip petals

In Insects
- "Silkworm jaundice" (an affection of the caterpillar of the silkworm moth) and similar diseases of other caterpillars

In Animals
- Hog cholera
- Distemper in dogs
- Foot-and-mouth disease
- Vesicular stomatitis in horses
- Sheep pox
- Fowl pox
- Rabies
- Pseudorabies
- Fox encephalitis
- Encephalomyelitis of horses
- Louping ill of sheep
- Psittacosis in parrots
- Swine influenza
- Virus III infection of rabbits
- Myxomatosis and fibromatosis of rabbits
- Infectious ectromelia in mice
- Rous sarcoma of chickens
- Fowl leukemia
- Fowl plague
- Cattle plague
- Salivary-gland disease of guinea pigs

In Human Beings
- Smallpox
- Chickenpox
- Herpes simplex
- Herpes zoster
- Molluscum contagiosum
- Common warts
- Measles
- German measles
- Mumps
- Psittacosis
- Dengue fever
- Yellow fever
- Common colds
- Influenza
- Rabies
- Poliomyelitis
- Encephalitis—(St. Louis type; equine encephalomyelitis; Japanese B encephalitis)
- Lymphocytic chorio-meningitis
- Louping ill
- Lymphogranuloma venereum
- Infectious keratoconjunctivitis

Even bacterial cells are subject to injury and lysis (dissolution) by the filtrable agents known as *bacteriophages.* These are so important that we give them special consideration in a later section of this chapter.

The lists serve to emphasize the important fact that there is as much diversity among the virus diseases as there is among bacterial diseases. *The different filtrable viruses are definite entities, differing as sharply from one another as do the separate species of fungi or bacteria.*

The activity of each virus is specific. For example, ordinary

measles and German measles, though similar, are nevertheless quite distinct diseases. Virus diseases of insects and animals occur only in certain particular species. Also, in human diseases of this kind, the viruses show a specific action upon certain tissues only. The virus of

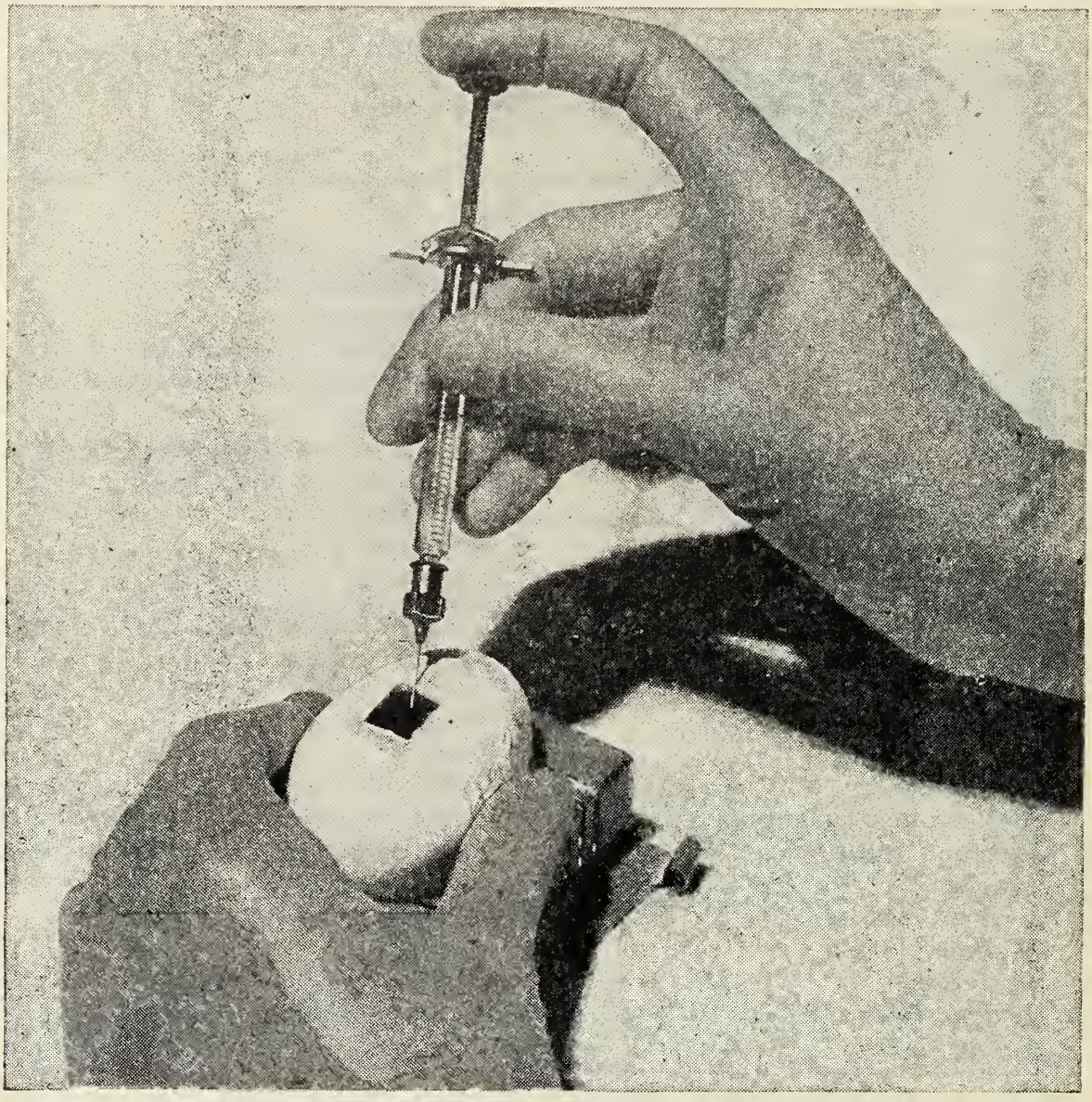


FIG. 115. Inoculation of the chorio-allantoic membrane of the chick embryo by injection of virus-containing material through a window cut into the egg shell. (Photographed at the Texas State Board of Health Laboratory, Austin, Texas. Reproduced through the courtesy of Dr. George W. Cox, State Health Officer, and of Dr. S. W. Bohls, Director of Laboratories.)

rabies (hydrophobia), for instance, does no harm until it comes in contact with the tissues of the spinal cord and brain.

Different viruses, as they naturally occur, have a marked affinity for particular hosts and for particular tissues, but most of them may be induced experimentally to develop in various other hosts

and in various other tissues. Many viruses, for example, including those of yellow fever and other diseases which do not primarily affect the nervous system, have been made to grow in the brains of mice, and are regularly maintained for study in many laboratories in this way.

In the process of *adaptation* of a virus to different hosts the original virulence may be considerably changed. For example, the smallpox (*variola*) virus when inoculated into calves becomes modified into cowpox (*vaccinia*) virus. Now when this altered virus is reinoculated into human beings it causes not smallpox, but only the local vaccination skin lesion. Its virulence for man has been greatly reduced. When the virus of rabies obtained from the saliva of a mad dog is first introduced into the brains of rabbits, it is not highly

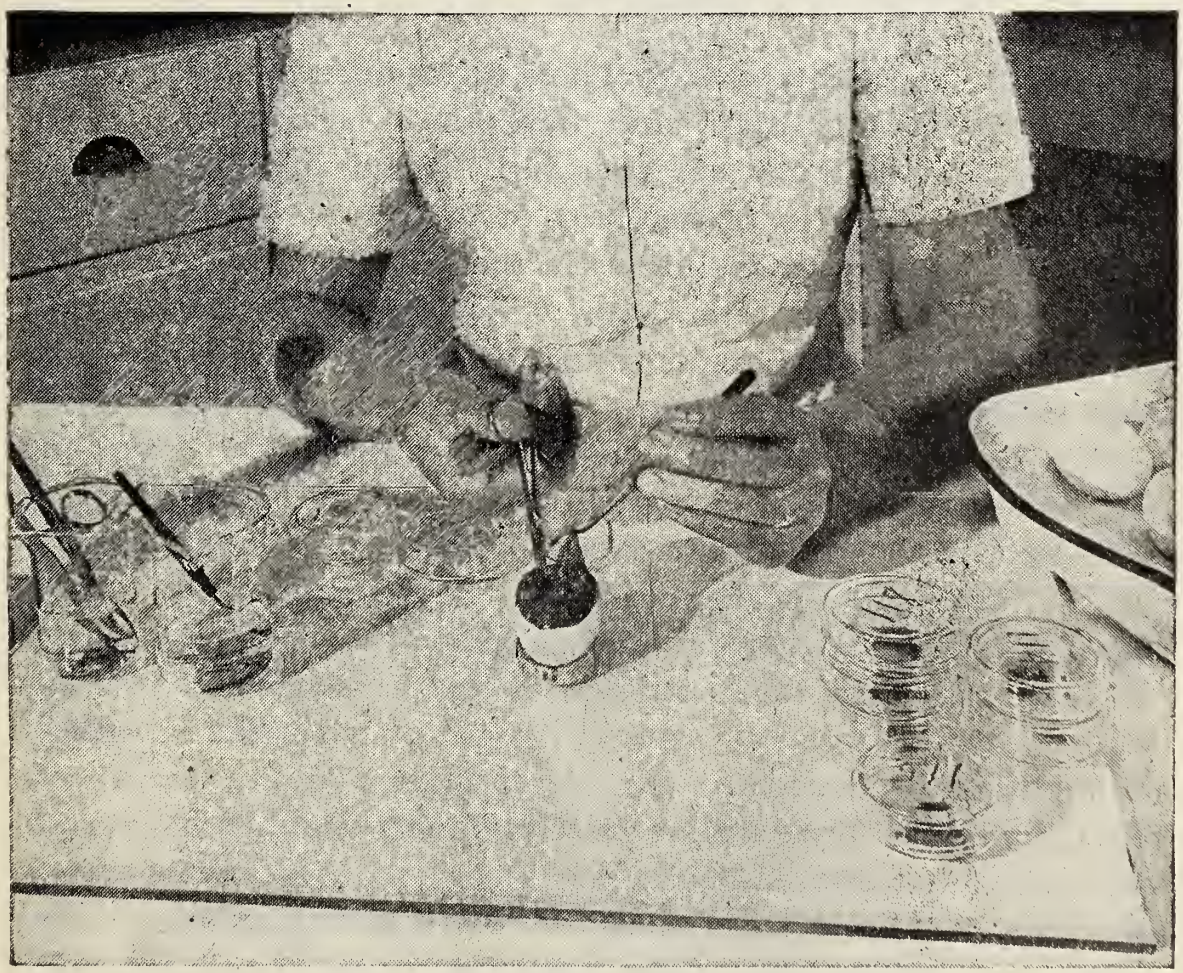


FIG. 116. Harvesting the virus-infected membranes from a chick embryo egg-culture. The infected membrane thus aseptically obtained may be ground up and used for further egg inoculations, for vaccine preparation, or for studies of the virus particles (elementary bodies). (Photographed at the Texas State Board of Health Laboratory, Austin, Texas. Reproduced through the courtesy of Dr. George W. Cox, State Health Officer, and of Dr. S. W. Bohls, Director of Laboratories.)

virulent for these animals, but when the virus is repeatedly passed from rabbit to rabbit it becomes so active that a very small dose will invariably cause death in a few days. At the same time the virus from rabbit brain is less virulent for dogs.

Throughout all such manipulations, however, and despite changes in virulence, the *individual viruses maintain their identity.*

Inclusion bodies and elementary bodies. About the same time that the filtrable viruses themselves were first recognized, another important discovery concerning the virus diseases was made. It was observed that, in the tissues affected by certain viruses, there occurs a remarkable and characteristic change inside some of the cells. When stained, these cells were seen to contain, within the cytoplasm or the nucleus, bodies of various sizes and shapes not found in normal cells. These we now call *inclusion bodies.*

As early as 1887, Pfeiffer noticed granular structures in the skin cells affected by smallpox virus. These inclusions were studied more fully (1892) by Guarnieri, and have since been known as the *Guarnieri bodies.* They are constantly found in epithelial cells infected with the virus of smallpox or of cowpox. In 1903, Negri described characteristic inclusions in the cells of the brain infected with the virus of rabies—the *Negri bodies* (Fig. 117). Subsequently, inclusion bodies were demonstrated in tissues affected by many other viruses, and we know now that the majority, though not all, of the virus diseases are accompanied constantly by the appearance of some type of inclusion in the particular body cells injured by the virus. *Such inclusions are not found in tissues invaded by ordinary bacteria.* The inclusion bodies in many of the virus diseases have a peculiar form and character, so that when they are seen in any of the body cells, they are a sure sign of the presence there of a particular virus. Diagnosis of rabies in dogs, for example, is regularly accomplished by searching for Negri bodies in the brain cells. The nature of all these intracellular inclusion bodies is not certain, but modern research supports the theory that, in many cases at least, they are essentially *colonies of the infecting virus.*

Observers noted, many years ago, that *inside the inclusions* of smallpox, and of some other virus diseases, numerous very tiny granular elements may be discerned by special methods of examination (using the regular visible light microscope, or one illuminated by ultraviolet light); and recent investigations have shown clearly that these granules, the so-called *elementary bodies,* when freed from the inclusion body, *act as if they were particles of the*

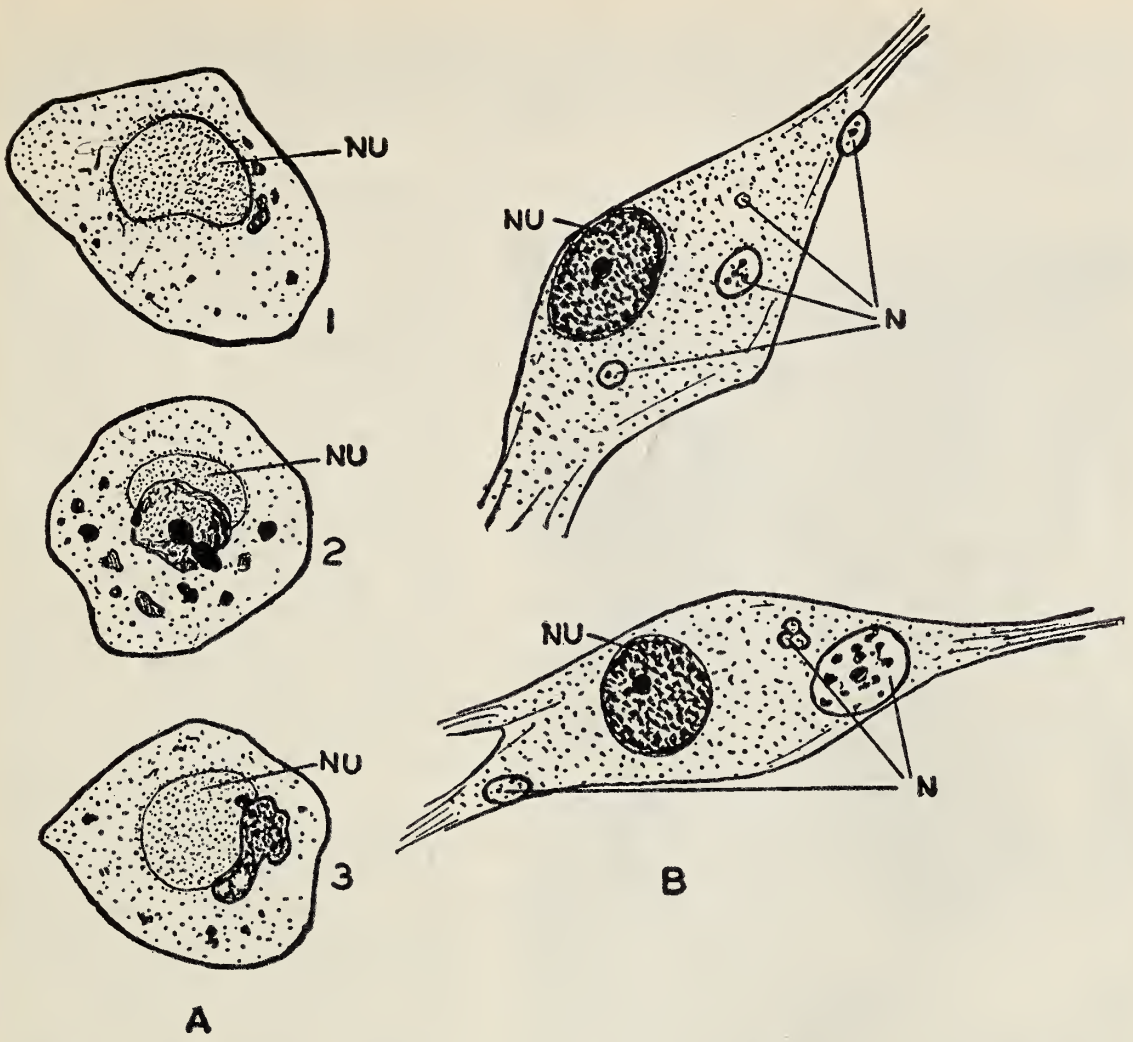


FIG. 117. Examples of "inclusion bodies" which develop in certain body cells in many diseases caused by filtrable viruses. A: *Guarnieri bodies,* characteristic of infection with smallpox virus. 1, 2, 3: cells from the cornea of a rabbit after inoculation of the eye with smallpox vaccine virus (cowpox virus). The dark, irregular-shaped bodies close to the nucleus (NU) are the Guarnieri bodies. These are just beginning to appear in 1, and are further developed in 2 and 3. (After Cowdry, in Rivers.) B: *Negri bodies* in pyramidal cells from the brain of a mad dog. These bodies (N) are a constant and characteristic finding in cases of rabies. (A: adapted from T. M. Rivers *Filtrable Viruses,* Baltimore: Williams & Wilkins Co., 1928.)

virus itself. Modern research is now so far advanced that much of the present-day work with a number of the viruses is conducted not with the crude filtrates of earlier times, but with purified and concentrated suspensions of elementary bodies (Fig. 118).

In certain important virus diseases, however, such as poliomyelitis, neither inclusion bodies nor elementary bodies have been demonstrated.

Additional properties of some well-studied viruses. In recent years, many workers have subjected purified preparations of different viruses to intensive study, in order to determine the size and

other physical characteristics, chemical nature, and antigenic composition of the active agents. We can mention here only some of the outstanding findings with respect to a few of the better-known viruses.

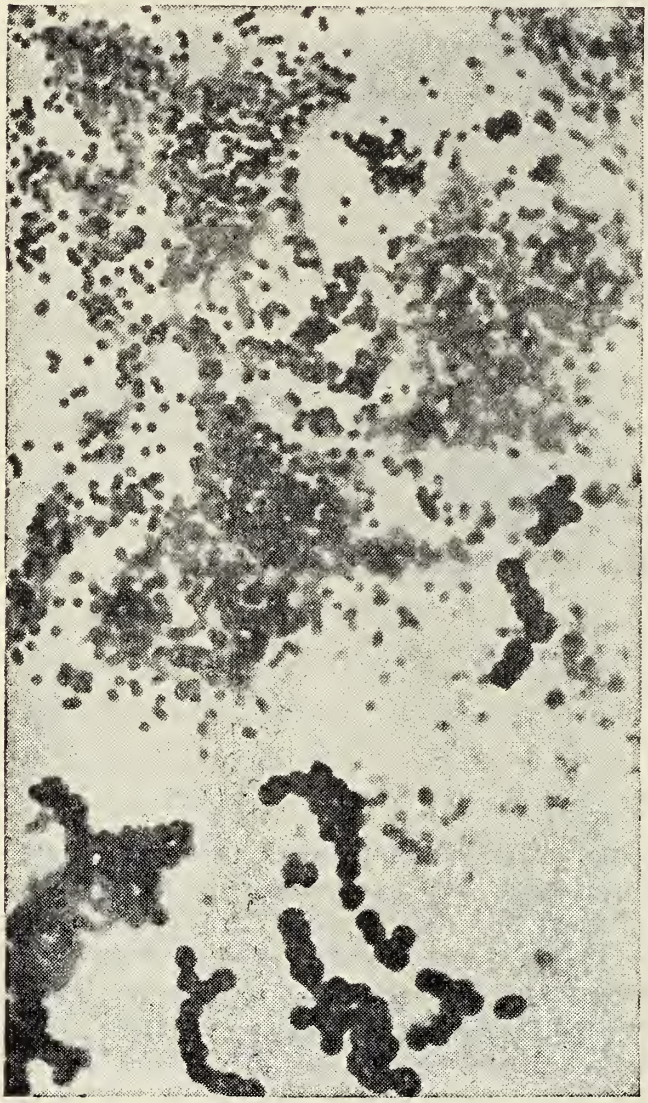


FIG. 118. To make this preparation, a single inclusion of *fowl-pox* has been ruptured and stained. The tiny elementary bodies (Borrel bodies) contained in it are thus revealed. Streptococci have been added to the lower part of the smear to afford comparison of size. (Courtesy of Dr. Ernest W. Goodpasture, Vanderbilt University School of Medicine, Nashville, Tenn.)

Plant viruses. Stanley, in 1935, applying the techniques of advanced protein chemistry to a study of plant viruses, succeeded in obtaining the virus of tobacco mosaic disease in chemically pure form. Starting with the infected juice from the diseased tobacco leaves, the final product was a *crystallizable nucleoprotein of high molecular weight.* Although all possible tests indicated that this material contained nothing but lifeless protein, a very minute amount of it would cause the appearance of the typical mosaic disease when inoculated upon healthy tobacco leaves; and it obviously multiplied, or reproduced itself in some way, in the infected plants. Such a protein could not be isolated from healthy tobacco plants. Subsequent exhaustive studies have served to establish beyond question that this nucloprotein is, in fact, the tobacco-mosaic virus.

By the use of similar methods, several other plant viruses have been separated from the host tissues and identified with large-molecule proteins.

Animal viruses. The viruses causing disease in human beings and animals, however, have proved to be too unstable to be purified by the physical and chemical procedures employed for isolation of the plant viruses. Evidently they are, as a rule, of more complex chemical composition. The infectious agents of influenza, yellow fever, and poliomyelitis, for example, are quickly destroyed by chemical manipulations. For concentration and purification of animal viruses, reliance has been placed chiefly upon the high-speed, air-driven ultracentrifuge (Fig. 119). Suspensions of virus particles almost entirely free of extraneous cellular débris and other inert matter have been prepared with the aid of these remarkable machines.

These particles have been shown to be infectious, reproducing the typical disease when inoculated into susceptible animals. Also, they are agglutinated, or otherwise acted upon specifically, by the serum of animals or men known to be immune to the particular virus concerned. They may be confidently regarded as the active units of these viruses.

Size of virus particles. There are good reasons for the belief that *all* viruses are in the form of particles, whether definite elementary bodies demonstrable under the ordinary microscope have been recognized or not; and moreover, *each kind of virus appears to exist as ultramicroscopic particles having a constant and characteristic shape and size.* Sizes are estimated from the results of ultrafiltration experiments, in which the viruses are tested for their capacity to pass through collodion membranes having pores of known diameter (Fig. 59), from the behavior of the virus materials in the ultracentrifuge, and from pictures made with the electron microscope.

In Figure 120 the probable sizes of certain viruses are shown diagrammatically. For comparison, the diagram also indicates the sizes of some of the larger protein molecules; and to emphasize the almost infinitesimal dimensions of all these particles, they are represented within the outline of a single bacterium—a staphylococcus—itself only 1 micron in diameter. The figures given are expressed in millimicra, or thousandths of a micron ($m\mu$).

It will be noticed that there is a fairly regular gradation in size over a wide range, from the virus of psittacosis (275–300$m\mu$) and the relatively large vaccinia virus (225$m\mu$), through the viruses of medium size, including those of herpes, rabies, and influenza (150–100$m\mu$), to the extremely small ones, among which are found the

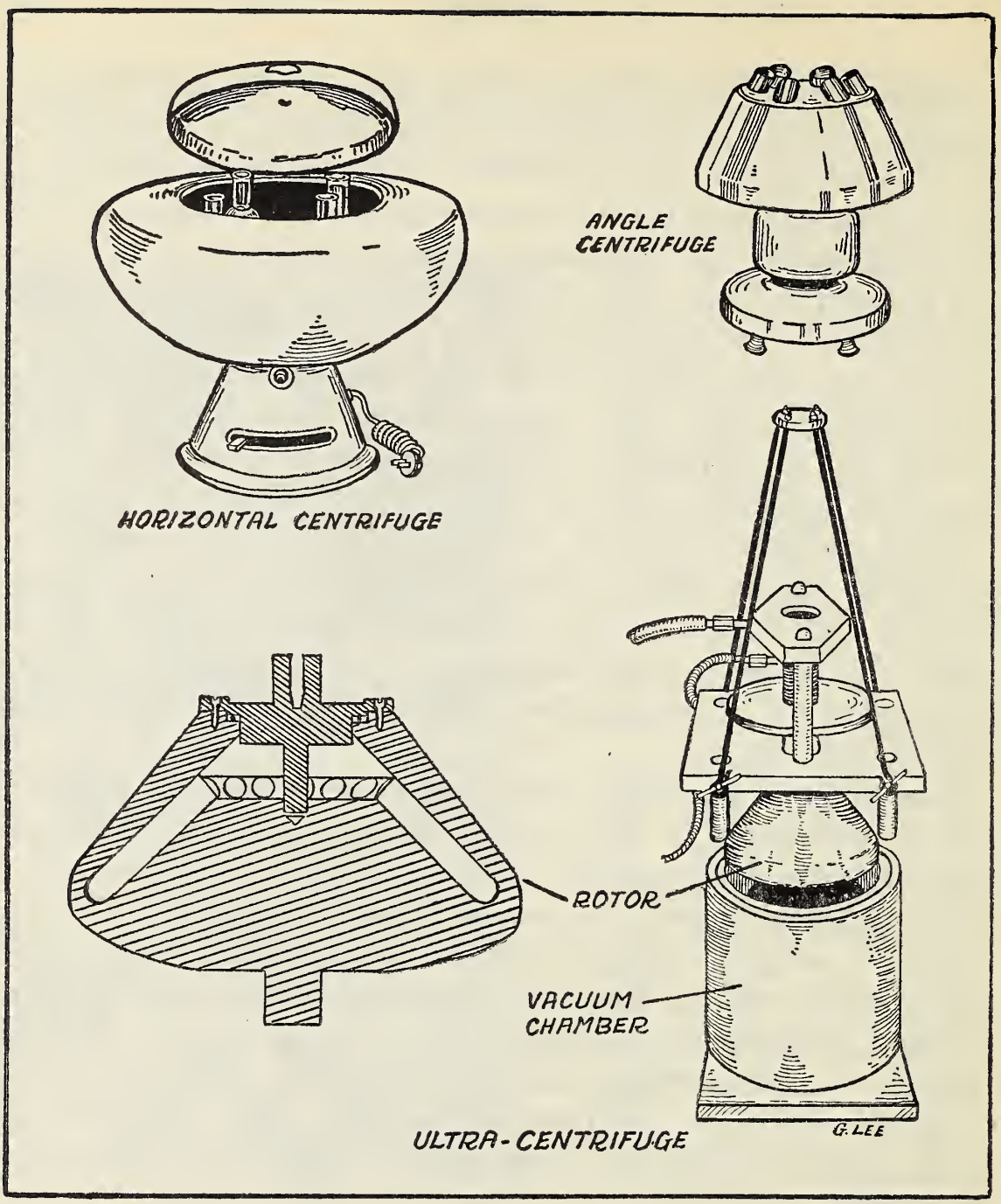


FIG. 119. Centrifuges. In the ordinary Horizontal Centrifuge (illustration shows an International Clinical model) the tubes are in a horizontal position while spinning. In the Angle Centrifuge they are held constantly at an angle of somewhat less than 50 degrees while they revolve. This makes for greater efficiency, and smaller particles can be thrown down.

Ultracentrifuges combine the angle principle with great speed; the tubes (made of celluloid because glass would break) are held at an angle within a solid metal rotor, driven by compressed air, which revolves at the rate of 25,000 or more revolutions per minute within a vacuum chamber. Right, the rotor and driving mechanism (simplified) of an ultracentrifuge developed at the Rockefeller Institute, New York, shown lifted out of the vacuum chamber. Left, cross section of the rotor. Drawn from the illustrations in the article by Bauer and Pickels, *J. Exper. Med.*, 64:503, 1936.

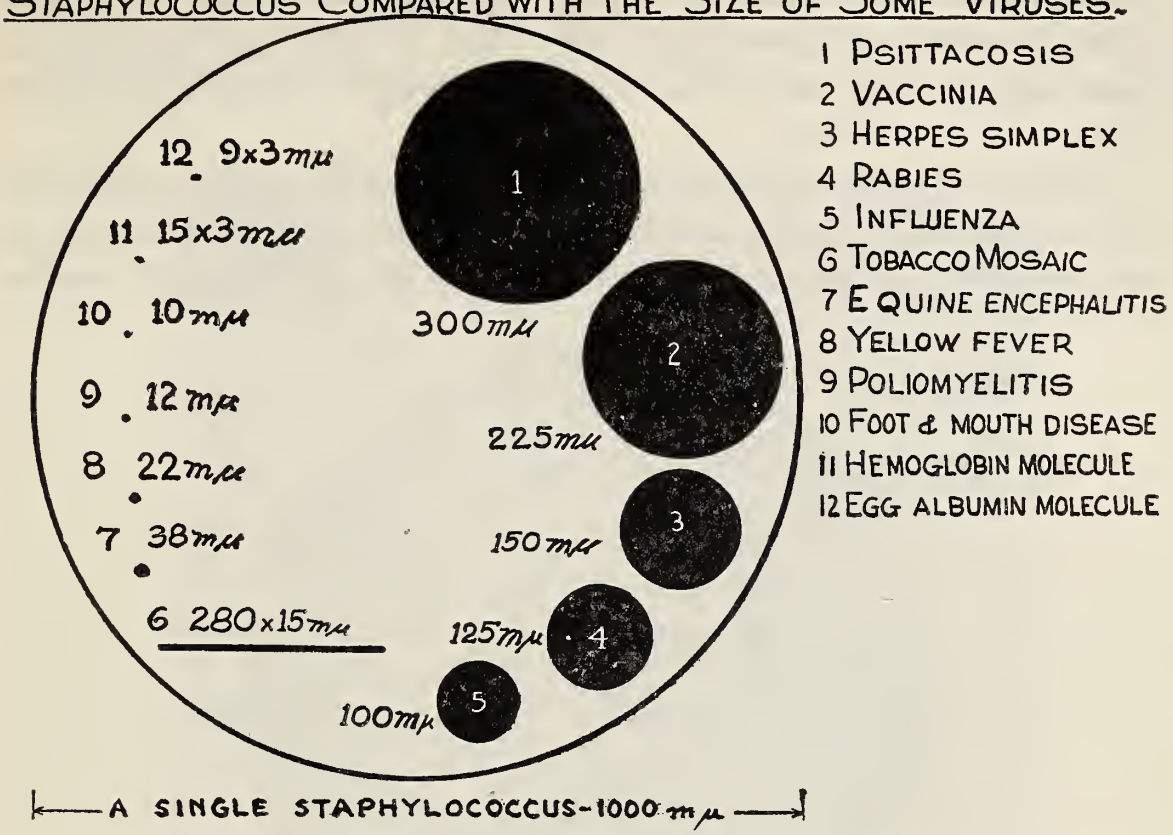


FIG. 120. The large circle represents the outline of a single staphylococcus, having a diameter of 1μ, (or 1,000mμ). Shown superimposed are smaller, blacked-in, circular and rod-shaped drawings, showing the comparative sizes, and the approximate shapes, of some of the viruses and certain protein molecules. Most virus particles are roughly spherical or cuboidal, but the tobacco mosaic virus occurs in long needle-shaped forms, as shown. Compare with Figs. 86, 118, 121, 122, and 123.

viruses of yellow fever and poliomyelitis (22–12mμ). These tiniest of virus particles are only slightly larger than molecules of hemoglobin or egg albumin!

Shape and structure of virus particles. Elementary bodies of some of the larger viruses, such as vaccinia, may be seen under an ordinary microscope in fixed, dried smears when colored by special stains (Fig. 121), but most viruses are too small to be made out by this means. It was not until the *electron microscope* became available that the true shapes of virus particles were revealed. Now we have learned from electron micrographs that the larger viruses, e.g., those of the psittacosis-lymphogranuloma group, and vaccinia, consist of organized bodies, of characteristic form, which are essentially like ordinary living bacteria, or other cells, except that they are ultra-microscopic. The individual units of vaccinia virus, for

example, consist of cubical or rectangular, brick-like structures, about 200mμ across, usually containing relatively dense inner portions of unknown significance (Fig. 122). All that we know about them supports the view that these tiny bodies are *living, autonomous, midget-microbes.*

Other human and animal viruses appear to be roughly spherical bodies. Influenza virus particles, for example, may be described as spheres with a diameter of about 100mμ (Fig. 86). The still smaller

FIG. 121. Elementary bodies of vaccine virus, concentrated by centrifugation, from the experimentally infected chorio-allantoic membranes of embryo chicks. Stained by a silver impregnation method and photographed with the ordinary light microscope. Compare with Fig. 122. (Courtesy of Dr. Joseph E. Smadel, Rockefeller Institute for Medical Research. Smadel and Wall, *J. Exper. Med.*, 66:325, 1937.)

particles of equine encephalomyelitis virus are also spherical, and have a relatively dense inner zone surrounded by a vaguely outlined peripheral portion; the whole particle is only about 40mμ in diameter. Possibly viruses about this size are the smallest that have essentially a cellular structure.

Some of the plant viruses are spherical, but others have an elongated, rectangular form. The highly purified tobacco-mosaic virus occurs as needle-shaped or rod-shaped crystals, 15mμ wide by 120–280mμ long (Fig. 123). It is now well established that these virus particles of tobacco mosaic are "macromolecules" of pure nucleoprotein.

Resistance to environmental factors. In general, viruses are destroyed outside the body by the same physical and chemical agencies that kill bacteria. They resist cold, and may be kept for long periods of time without loss of activity when frozen and stored at

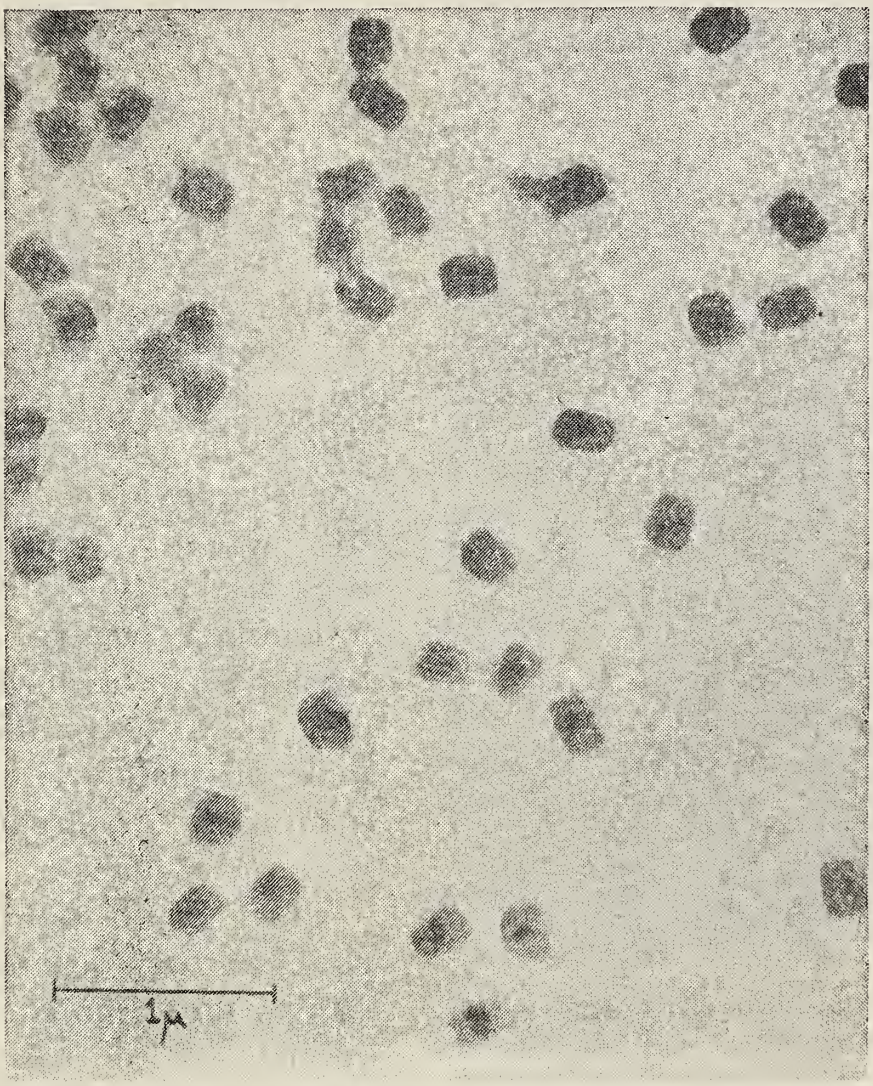


FIG. 122. The elementary bodies (virus) of vaccinia, photographed with the electron microscope. Note that the brick-like, or dice-like, vaccine bodies occur not only singly, but in pairs and in short chains, joined by their cell walls. Each individual virus particle contains about five internal dark spots. These areas of relatively greater density are of unknown significance. (From Green, R. H., Anderson, T. F., and Smadel, J. E., *J. Exper. Med.*, 75:656, 1942.)

–70° C. They may be preserved by desiccation in a vacuum after rapid freezing. On the other hand, viruses are readily destroyed in a few minutes by moderate heat, at temperatures of 55°–65° C; they will not survive, therefore, in pasteurized milk. Formalin, phenol, and iodine inactivate them quickly; so also does ultraviolet light. Most viruses have a notable resistance to pure glycerine. It is a

common practice to use a 50% glycerine-physiological-salt solution for collection and preservation of virus-infected tissues.

Nature and origin of viruses. At the present time, the true nature of all the viruses cannot be definitely decided. It may well be that not all of them have the same fundamental character. There is no reason why all the agents that cause infectious diseases should necessarily be living organisms of the types familiar to us.

FIG 123. The virus of tobacco mosaic disease, photographed with the electron microscope. This picture shows the characteristic rod-shaped particles of the purified virus. (Stanley, W. M. and Anderson, T. F., *J. Biol. Chem.*, 139:325, 1941.)

Rivers has suggested that the larger viruses may indeed be living midget-microbes, of which the elementary bodies are the individual units, while the viruses of intermediate sizes may be forms of life of unfamiliar pattern, on the borderline of organized living cells as we know them, and those of smallest size may be inanimate, self-reproducing incitants of disease.

There is no valid reason, however, for separation of viruses into animate and inanimate on the basis of size alone. No one knows how small a chemical unit may be, and still be living. The yellow fever virus, one of the smallest of the viruses, is just as adaptable, acts as if it were just as foreign to the tissues it invades (stimulating a high immunity), and otherwise has as much of the general attributes of living organisms as the vaccinia virus or any other of the larger viruses. It is hard to imagine it as nonliving while other viruses are regarded as living. At present, the weight of evidence is against the living nature of the plant viruses that have been isolated as pure proteins, but in view of all their known properties, most authorities lean to the

view that all the other viruses, irrespective of their size, have something essentially "alive" about them.

The character of this "life" in some cases may be different, however, from the organized cells we are accustomed to associate with animate beings. To imagine what it might be like, we are led to contemplate the misty borderline between the living and the nonliving, and to speculate on no less fundamental a question than what constitutes the very essence of life. Twort thinks that body cells as we know them have evolved from precellular units, and suggests that the viruses may be precellular units of life which have remained independent, yet cannot grow except in association with functioning body cells.

Other authorities have expressed a more appealing theory of the origin of viruses. Goodpasture, Greene, and others have pointed out that *intracellular parasitism* is by no means an uncommon phenomenon, and that other infectious agents besides viruses are adjusted to an intracellular existence—for example, the malaria parasites and other protozoa, all the rickettsiae, and even certain bacteria, such as the leprosy bacilli. *The viruses, however, seem to have carried dependence upon other living cells to the* n*th degree.* Perhaps the ancestors of our present-day viruses were larger, living microbes which adopted an exclusively intracellular life, and then, in the course of time, became more and more completely dependent upon their host cells. In consequence, they lost to a greater or lesser degree their original form and structure, so that now some of them consist of no more than a few molecules. At the same time, their original physiological activities were largely given up, so that now they have little or no independent metabolism, depending upon the vital activities of their living host cells to furnish the few necessary elements they need to maintain and reproduce themselves. This conception would be in line with the principle that the assumption of a fully parasitic existence always involves more or less *loss of substance and of physiological independence.* This principle we have already mentioned and we have seen how well it is illustrated among the protozoa, spirochetes, and fungi. It is a theory only, but an attractive one, which would regard the viruses as a group of "superparasites," essentially foreign to the tissues they invade, which have evolved from larger intracellular parasites, and now are reduced to extremely simple forms, existing only by suffrance of the very cells they damage.

THE BACTERIOPHAGES

Discovery. The English investigator Twort, in 1915, and the French bacteriologist d'Herrelle, in 1917, were the first to call attention to a most remarkable and interesting phenomenon. They discovered that bacteria themselves may suffer a filtrable virus disease. D'Herrelle emusified in broth a bit of feces from a case of dysentery, then passed the emulsion through a bacteriological filter. He found that the clear filtrate (free of any visible microbes) contained an agent of some kind which would *destroy dysentery germs.* If a trace of this filtrate were added to a young broth culture of dysentery bacilli, all or nearly all of the organisms were dissolved (lysed) and the previously cloudy culture became clear in a few hours. Moreover, if a trace of this culture was placed in another young broth culture of dysentery organisms, the same thing occurred, and by successive transfers the mysterious bacteria-dissolving agent could be carried along indefinitely. When a drop of a filtrate containing the unknown agent came in contact with a young growth of dysentery germs on a solid medium, such as an agar slant or plate, the growth became glassy and transparent and the organisms in these transparent areas were found to have been killed. D'Herrelle thought he was dealing with an invisible living microbe which was a parasite upon bacteria. He called this hypothetical ultramicroscopic organism *Bacteriophagum intestinale,* and the term *bacteriophage* (meaning literally "bacteria-eating agent") has come into general use. Often a bacteriophage is referred to simply as a *phage* (Fig. 124).

Chief properties of bacteriophages. Since the original observations of the bacteriophage phenomenon, a great many investigators have studied the subject. It has been found that phages can be isolated from a variety of substances, but are most easily obtained from polluted water or from other material contaminated with the intestinal discharges of higher animals or man. They are always filtrable—that is, they are found in filtrates of various materials, the filtrate always being free of any visible, or cultivable, microbes.

There are many different bacteriophages, and the action of each is specific; that is, it affects a particular type or species of bacterium only. Phages have been isolated for bacteria of many kinds, includ-

ing saprophytic and nonpathogenic species, as well as parasitic and pathogenic varieties.

Nature of the bacteriophages. Modern research has shown clearly the close similarity of the bacteriophages to the recognized filtrable viruses affecting animals and men. Like these viruses, the

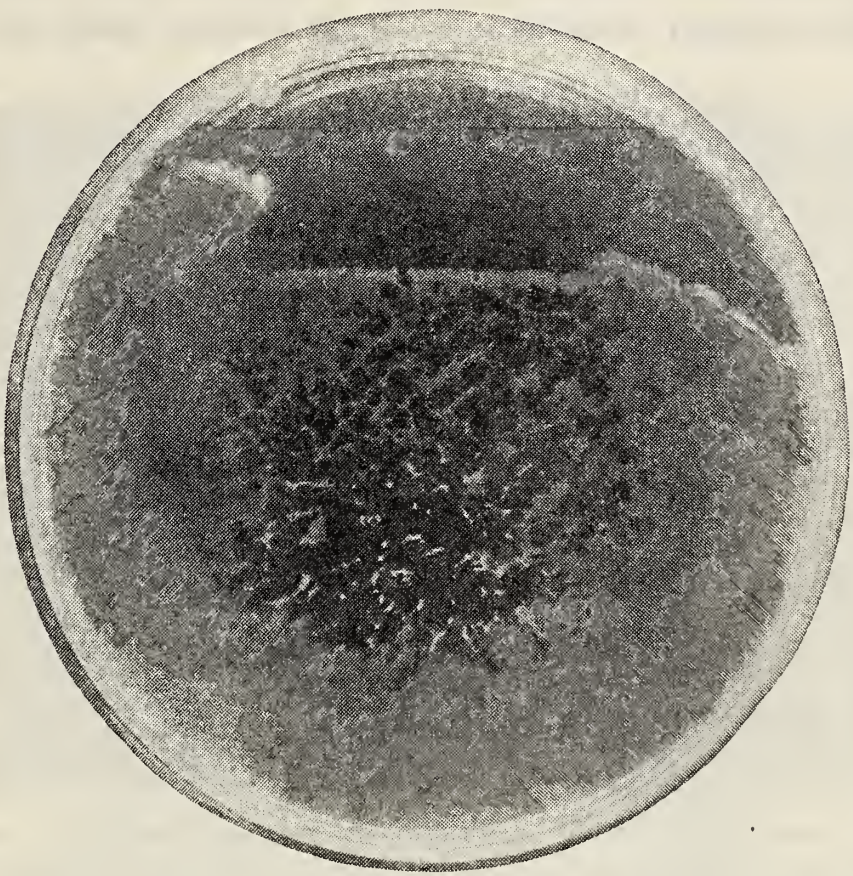


FIG. 124. An agar plate culture showing the effect of bacteriophage. The bacteria have grown as a continuous film over the surface of the agar (grayish-white in the photograph), and the irregular dark areas are the places where the growth has been "eaten away" by a bacteriophage. (Courtesy of Dr. Alvin Wells.)

phages are extremely minute, filtrable bodies, invisible by ordinary methods of microscopic examination, not cultivable on lifeless media, and increasing only in the presence of the particular living, growing bacteria cells which are susceptible to their action. Particle sizes of different phages have been found to range from about 40mμ to 100mμ.

In connection with the bacteriophages, the revelations of the electron microscope have been truly spectacular. Luria, Delbrück and Anderson, studying two strains of phage active against *E. coli*, found them to be tiny bodies of definite structure, with roundish heads and tail-like appendages, looking amazingly like miniature

spermatozoa. The particles of one of the phages had a round head 45–50mμ in diameter, to which was attached a straight or slightly curved tail, not more than 15mμ thick and about 150mμ long. The other phage particles had oval heads, 65 by 80mμ, and straight tails 120mμ long and 20mμ thick (Fig. 125).

A staphylococcus phage has been described that has a head 100mμ in diameter and a tail about 200mμ long. Still other

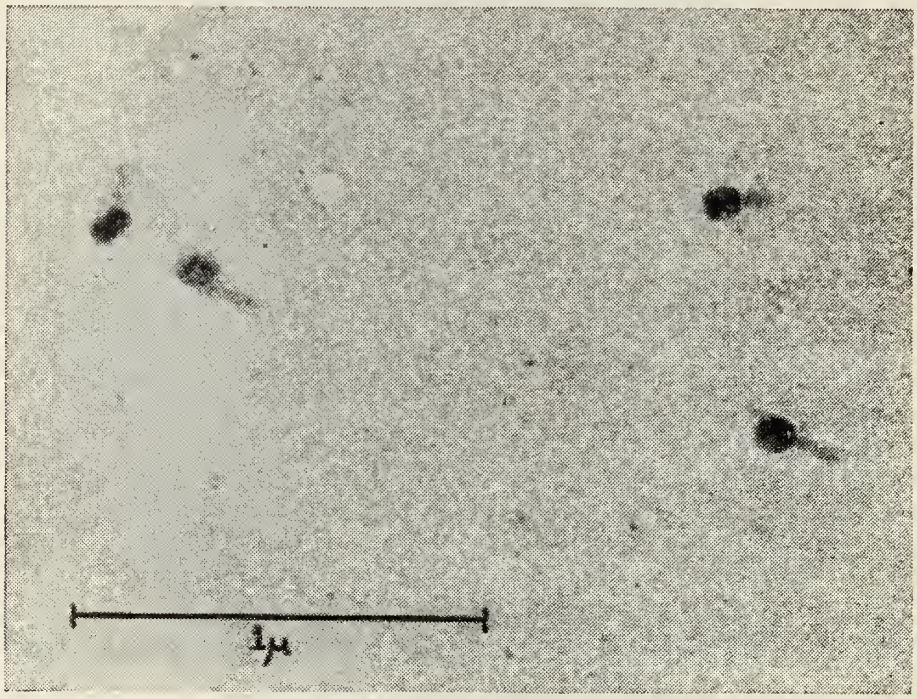


FIG. 125. Particles of *E. coli* bacteriophage, photographed with the electron microscope. Note the tails, and the curious spermatozoon-like appearance. (From Luria, S. E., Delbrück, M., and Anderson, T. F., "Electron Microscope Studies of Bacterial Viruses," *J. Bact.*, 46:39, 1944.)

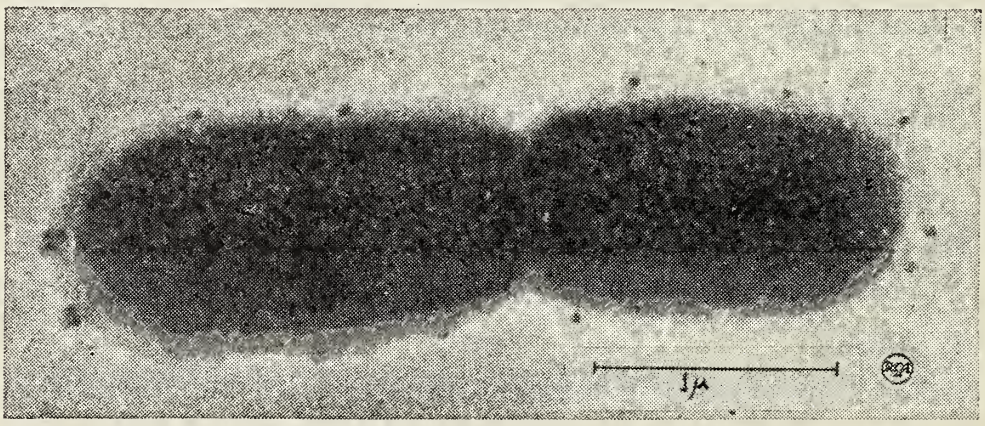


FIG. 126. Electron micrograph showing cells of *E. coli* being attacked by *coli* bacteriophage. Note that numerous particles of the phage have been adsorbed upon the surface of the organisms. This is the first stage of the attack. (From Luria, S. E., Delbrück, M., and Anderson, T. F., "Electron Microscope Studies of Bacterial Viruses," *J. Bact.*, 46:39, 1944.)

phages have been found to be round or oval bodies, apparently without tails.

Equally remarkable is the manner in which these bacterial viruses behave in the presence of susceptible bacteria, as revealed in electron micrographs. The initial event in the phenomenon of phage activity is the specific adsorption of phage particles to the surface of the bacterial host cells (Fig. 126). The next step is apparently

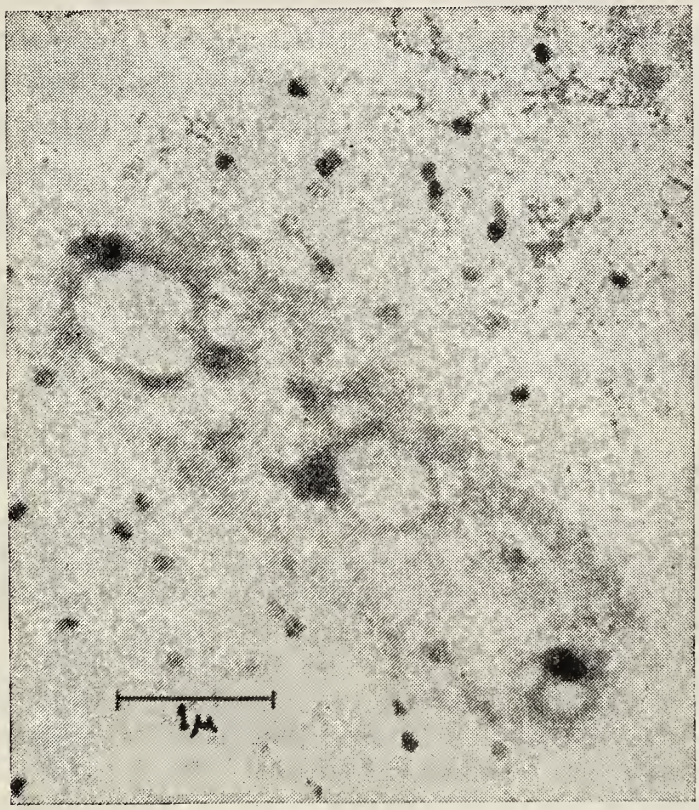


FIG. 127. A cell of *E. coli* 3 minutes after contact with *coli* bacteriophage. The organism has been reduced almost completely to a ghost form, surrounded by cellular débris; later the cell will break up completely. (From Luria, S. E., Delbrück, M., and Anderson, T. F., "Electron Microscope Studies of Bacterial Viruses," *J. Bact.*, 46:39, 1944.)

the penetration of the bacterial cell by a single phage particle. This seems to alter the bacterium in such a manner that it at once becomes refractory to penetration by other phage particles of the same kind. (There is an obvious analogy here to the fertilized egg of higher animals, which does not allow the entrance of more than a single spermatozoon.) The invading phage particle then multiplies within the bacterium, and the organism usually absorbs water, and eventually is lysed and destroyed, releasing the new phage particles. Sometimes, however, a phage multiplies in a bacterium without causing lysis. The true mechanism of phage multiplication, and of

the lysis that usually accompanies it, still awaits elucidation through further research (Fig. 127).

Use of phages in treatment of disease. If bacteriophages destroyed bacteria as readily in the body as they do in a test tube, they would have a very important influence upon the course of germ diseases. Some have claimed that it is indeed the activity of bacteriophages which largely determines whether we die or recover. But experimental studies have not borne out this claim, and it is probable that phages have no appreciable effect upon the outcome of infections. Preparations containing active phages have been used to treat certain germ diseases for many years, but their value is very doubtful. Such beneficial results as have been reported were probably not due to any direct action of the phage itself, but to the fact that the phage-containing filtrates, which always contain some dissolved bacterial substance, were good vaccines, stimulating resistance to the particular organism involved.

REFERENCES

Baylor, M. R. B., Severens, J. M. and Clark, C. L.: "Electron-Microscope Studies of the Bacteriophage of *Salmonella pullorum*," *J. Bact.*, Mar. 1944, 47:277.

Green, R. C.: "On the Nature of Filtrable Viruses," *Science*, 1935, 82:443; "On the Nature of Virus Adaptations," *Science*, 1942, 95:602.

Krueger, A. P. and Scribner, E. J.: "The Bacteriophage, Its Nature and Its Therapeutic Use," *J.A.M.A.*, May 10, 1941, 116:2160; May 17, 1941, 116:2269.

Laidlaw, P. P.: *Virus Diseases and Viruses.* New York: The Macmillan Co., 1939.

Lennette, E. H.: "Recent Advances in Viruses," *Science*, 1943, 98:415.

Luria, S. E., Delbrück, M. and Anderson, T. F.: "Electron-Microscope Studies of Bacterial Viruses," *J. Bact.*, July 1943, 46:57.

Rivers, T. M.: *Viruses and Virus Diseases.* Stanford University, Cal.: Stanford Univ. Press, 1939.

Rivers, T. M., Stanley, W. M., Kunkel, L. O., Shope, R. E., Horsfall, F. L. and Rous, P.: *Virus Diseases.* Ithaca, N. Y.: Cornell Univ. Press, 1943.

Kalmanson, G. M. and Bronfenbrenner, J.: "Restoration of Activity of Neutralized Biologic Agents by Removal of the Antibody with Papain," *J. Immunol.*, Nov. 1943, 47:387.

REVIEW QUESTIONS—CHAPTER XXXVIII

1. Explain how the mosaic disease of tobacco plants was shown to be caused by a filtrable infectious agent.
2. Outline the basic properties of viruses, and give a definition.
3. Name some of the filtrable virus diseases of plants, insects, animals, and men. Are bacteria affected by viruses?
4. Are the individual viruses distinct entities? Illustrate their adaptability.

What usually happens to their virulence when they become adapted to new hosts?

5. Define and illustrate what is meant by *inclusion bodies*. What are *elementary bodies*? Have these bodies been found in all virus diseases?
6. Describe the nature of the plant viruses studied by Stanley and his associates.
7. Why has it not been possible to isolate and purify the virus particles of animal viruses by purely chemical methods? What means are used to secure pure, concentrated suspensions of animal viruses?
8. What properties have been shown by the purified virus-particle suspensions, obtained by ultracentrifugation, which indicate that these particles are actually the active units of the viruses concerned?
9. How are sizes of virus particles determined? Give examples of the largest, medium-sized, and smallest viruses; state the sizes in millimicrons.
10. What new research tool has aided greatly in understanding the shape, size, and nature of viruses? Describe the outstanding facts about the shape and make-up of vaccinia, influenza, equine encephalomyelitis, and tobacco mosaic viruses.
11. What resistance do viruses have outside the body? How do they differ from bacteria in susceptibility to the newer therapeutic drugs?
12. Outline current ideas regarding the origin and nature of the viruses.
13. Who discovered that bacteria may suffer a filtrable virus disease, and when was this discovered? What is the origin of the term *bacteriophage*? How did d'Herrelle demonstrate the action of a phage?
14. How do phages resemble the viruses described above? Outline recent discoveries about the shape and structure of phages. Describe the action of phages on susceptible bacteria, as indicated by recent studies with the electron microscope.
15. What is the value of bacteriophages in the treatment of infectious diseases?

CHAPTER XXXIX

IMPORTANT VIRUS DISEASES OF MAN

Classification of virus diseases. In Table XXVII we indicate how the human virus diseases may be divided into groups, according to the regions of the body which are most conspicuously affected. More specifically, some viruses are commonly said to be *dermotropic*, since they have an evident affinity for epidermal tissues, causing visible lesions chiefly, or wholly, in the skin. Other viruses characteristically involve the respiratory tract, and have sometimes been called *pneumotropic*. Still others localize in the eyes, while the virus of *lymphogranuloma venereum* primarily affects the genitalia. In another, sharply defined group, each virus is *neurotropic*, involv-

TABLE XXVII. Groups of Human Virus Diseases

1. **With characteristic lesions in the skin** (caused by dermotropic viruses) (though the infection may be generalized)
   Smallpox, chickenpox, herpes zoster, herpes febrilis, common warts, measles
2. **With characteristic involvement of the respiratory tract** (though sometimes generalized)
   Common colds, measles, mumps, psittacosis, influenza, atypical pneumonia
3. **With characteristic localization in the eyes**
   Herpes infection, inclusion conjunctivitis, trachoma, infectious keratoconjunctivitis
4. **With characteristic involvement of the genitalia** (though sometimes generalized)
   Lymphogranuloma venereum
5. **With primary involvement of the central nervous system** (caused by neurotropic viruses)
   Encephalitis, poliomyelitis, rabies, lymphocytic choriomeningitis
6. **Generalized infections** (caused by viscerotropic or pantropic viruses)
   Smallpox, yellow fever, dengue fever

ing nervous tissue primarily. Finally, there are the viruses of *smallpox, yellow fever,* and *dengue fever,* which ordinarily produce a generalized, systemic infection; these viruses are sometimes said to be *viscerotropic* (affecting various internal organs) or *pantropic* (attacking many different tissues).

The student will realize that such a classification as we have just outlined is artificial, and does not give a strictly accurate picture of many of the viruses. Mumps, for example, though listed as a disease of the respiratory-tract group, is often generalized, with symptoms resulting from growth of the virus in the brain. Other viruses, also, have wider tissue affinities than indicated. Nevertheless, this classification is useful, and on the basis of it we have already decribed the majority of the virus infections in previous chapters of this book. Remaining to be discussed are smallpox, yellow fever and dengue, and the principal diseases caused by the neurotropic viruses.

SMALLPOX

Smallpox, or *variola,* has been known throughout history as one of the great scourges of mankind. It was prevalent in ancient times, and swept in a series of devastating epidemics over medieval Europe. It has attacked men of all races and all ages, everywhere. In our own time it is still prevalent in the Oriental countries, but, owing to the almost universal practice of vaccination, it is now a comparatively rare disease in most civilized lands. The United States has a poor record in this respect, for in recent years, there has been more smallpox in this country than anywhere else, except in Asia.

Clinical forms of smallpox; vaccinia. Before the days of vaccination, which did not become general until the early part of the nineteenth century, smallpox was not only exceedingly common, but very severe, and fatal in a high proportion of cases. Practically everyone had the disease at one time or another, usually during childhood, and it is said that parents did not count a child a member of the family until it had survived its expected attack of smallpox. The patient with virulent smallpox presents a most hideous appearance (Fig. 128). He suffers from fever, headache, and backache, and his body becomes covered with loathsome pustules. If he is lucky enough to recover, he is marked forever with unsightly scars. In the United States and other countries where vaccination

has been widely practiced, smallpox in late years has been mostly of a mild type, but there is evidence that the disease tends to become malignant whenever it gets an opportunity to spread rapidly among susceptible persons.

The disease known as *alastrim,* which occurs in the West Indies, Brazil and other subtropical areas, is generally regarded as a mild form of smallpox.

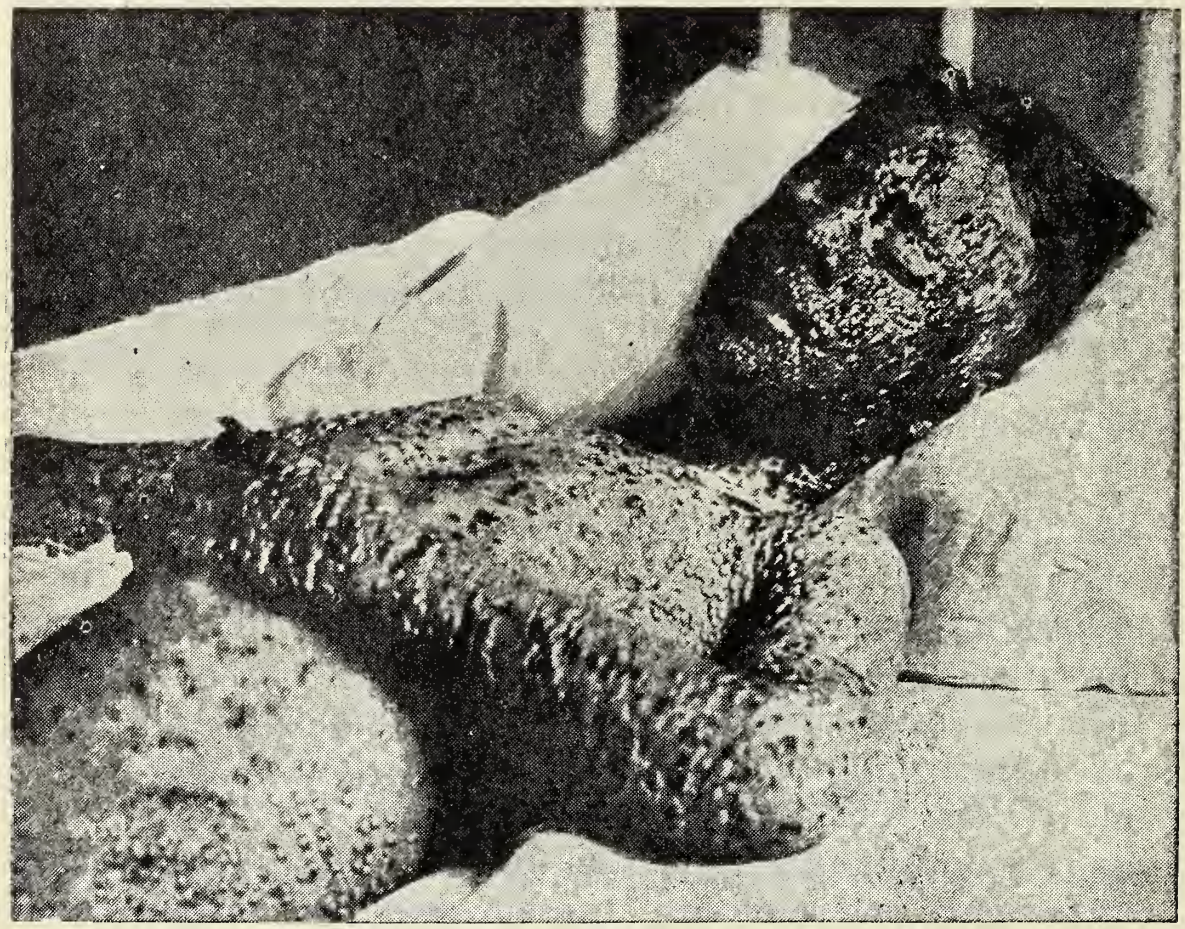


FIG. 128. A severe case of smallpox.

The localized infection produced in the skin of man by inoculation of *cowpox* material, as in vaccination, is called *vaccinia.* The virus of vaccinia (*vaccine virus* or *vaccinia virus*) is nothing more nor less than smallpox (*variola*) virus which has become permanently modified in virulence for man by adaptation to animal (cattle) tissues.

Spread of smallpox. The virus of smallpox is present in the secretions of the nose and throat, in the fluid of the vesicles in the skin, and in the scabs or crusts from the skin lesions. It may also be present in the feces or other body discharges. As in other virus diseases,

the actual virus particles exist in enormous numbers in the infected tissues. From the smallpox patient, they probably become scattered into the air in the form of insensible droplet nuclei. The infection spreads with remarkable ease and rapidity, and seems to attack practically every individual who is not immune. The usual incubation period is about twelve days.

The virus of smallpox and of vaccinia. Smallpox virus may be recovered in filtrates of nasal secretions and other materials, including the blood, from variola patients. It may be cultivated in tissue cultures and on the chorio-allantoic membrane of the developing chick embryo. Most laboratory animals are not susceptible to the experimental inoculation of the virus, but in monkeys, and usually also in rabbits, a local pock, similar to the skin lesion of smallpox in man, may be produced.

When *variola virus* is inoculated into rabbits or monkeys, then into calves, and is passed through a series of these animals, it becomes converted permanently into *vaccinia* (cowpox or vaccine) *virus*. If now introduced into the human skin, it produces only the local lesion familiar as the vaccination sore.

It is now well established that the characteristic inclusions which appear in tissue cells affected by either variola or vaccinia virus, first described by Guarnieri in 1894 and known as the *Guarnieri bodies,* represent intracellular aggregations or colonies of the active particles. These particles, or elementary bodies, in this particular case are called *Paschen bodies;* there is every reason to believe that they constitute units of the actual causative agent, the virus itself.

Description of vaccinia virus. This infectious agent has been extensively studied, and is as well understood as many ordinary microbes. The elementary bodies have been obtained in highly purified form, analyzed in various ways, and photographed with the electron microscope (Fig. 122, p. 603).

It has been shown that a single one of these elementary bodies is all that is needed to initiate a lesion in a fully susceptible animal. The brick-shaped bodies have a diameter between 236mμ and 252mμ. They are somewhat less dense to electrons than bacteria, but usually contain five darker, inner portions. They seem to have an external limiting membrane and some sort of internal structure.

Chemically, the vaccinia bodies are complex. They contain the constituents of ordinary protoplasm, including nucleoprotein, fat,

and carbohydrate. They possess several distinct antigenic elements: including a heat-labile antigen (L), a heat-stable antigen (S), an antigenic complex referred to as LS, and a separate nucleoprotein (NP) antigen.

The virus is quickly destroyed by heat, and is killed in a few minutes by common disinfectants, such as phenol, tincture of iodine, lysol, and mercuric chloride.

Immunity to smallpox; prophylactic vaccination. *Inoculation with smallpox as a means of immunization.* It has been common knowledge for centuries that recovery from an attack of smallpox renders the individual immune from subsequent attacks. In China, Turkey, and other countries, long before the present method of vaccination was worked out, it was the common practice to *inoculate* healthy persons deliberately with smallpox matter from a mild case, in the hope that the inoculated individual would have a light attack of the disease and so become immune. In 1717, Lady Mary Wortley Montagu introduced the practice of smallpox inoculation into England. It was, of course, an extremely dangerous procedure, and many of the inoculated persons had an attack of typical, severe smallpox, instead of the mild infection they desired, and some died. Furthermore, the person with inoculation smallpox was just as likely to spread the infection as one who had contracted the disease in the natural way, and, while the practice of inoculation did succeed in making some individuals immune, it was also responsible for keeping smallpox more prevalent among the population than it might have been otherwise.

Jenner's discovery of vaccination. Credit for the discovery of a safe and efficient method of immunization against smallpox belongs to Edward Jenner (1749–1823), an English country physician (Fig. 129). Jenner developed the method of *vaccination* against smallpox, which consists in the introduction into the human skin of the virus of *cowpox,* or *vaccinia.* Cowpox is a disease which, in Jenner's time, commonly appeared in dairy cattle in the form of pustules on the teats. Similar lesions sometimes developed on the hands of persons who milked infected cows. These cowpox sores resembled closely the lesions of smallpox, but they remained localized, and the individual experienced general symptoms of only the mildest character. Cowpox did not spread to other persons. It was a matter of common observation, among country people, that a person who had had cowpox would not take smallpox, and the inference was drawn that cow-

pox, a very mild, localized infection, must furnish immunity to the more serious disease, smallpox.

As a student, Jenner was impressed with these beliefs, and as soon as opportunity was afforded him, he began to study the ques-

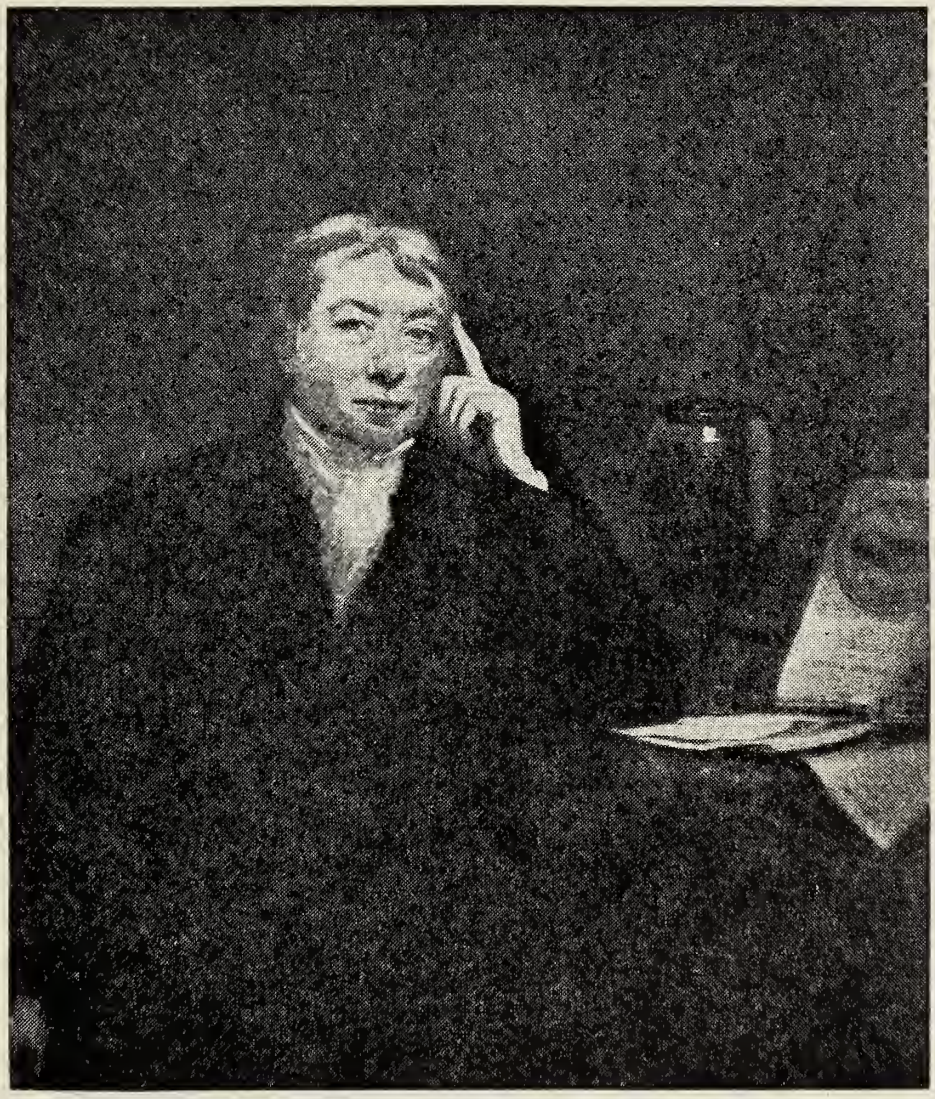


Fig. 129. Edward Jenner, 1749–1823.

tion very thoroughly. After several years of investigation, he was convinced that cowpox does, in truth, protect against smallpox, and he determined to put the matter to the test. In 1796, Jenner took some of the fluid from a typical cowpox lesion on the hand of a dairymaid, Sarah Nelms, and transferred it to the skin of a boy, James Phipps. The boy had what we would now recognize as a typical vaccination "take." Several weeks later, Jenner inoculated this same boy with matter taken directly from a pustule of a smallpox patient, but no disease resulted. The boy was immune. Later, Jenner made other experiments and collected additional data, all of which

supported the claim that inoculation with cowpox is a safe and certain way of producing immunity to smallpox. This supremely important discovery marks one of the most significant advances ever made by man toward the conquest of disease. Though vaccination met with much opposition at first, and has always been opposed by some ignorant or misguided persons, it has stood the test of time, and has proved its value over and over again.

Preparation of smallpox vaccines. During the early days of vaccination, it was customary to inoculate human beings with material taken from the pustules of persons previously vaccinated. This practice of arm-to-arm vaccination sometimes led to undesirable complications, and it has long since been abandoned. The vaccine is now prepared by inoculating healthy young calves, and individual doses of the purified virus from the lesions on these animals (vaccine virus) are made up to be used for the vaccination of human beings.

For the original inoculation of the calves, virus obtained directly from human vaccination vesicles is sometimes employed. By another method, virus from human vaccination lesions is first inoculated into a calf, and then into rabbits, and the pulp from the rabbit lesions is then emulsified in glycerin and used for the vaccination of calves to produce the regular supply of vaccine for human vaccinations. Young calves known to be free of disease are selected, thoroughly cleaned and washed, and the abdominal surface is shaved and disinfected. A number of scratches are made across the abdomen, and into these scratches the virus is rubbed. The vaccinated animals are kept in specially constructed stalls, and every effort is made to keep their surroundings as clean as possible. On the eighth day, when the characteristic pustules are well formed, the vaccinated area is washed with sterile water, and the crusts are removed. The soft pulp remaining in the lesions is then collected with a sterile curette, transferred to a sterile container, and mixed with a 50% solution of glycerin containing a small amount of carbolic acid.

This glycerinated pulp is then stored in the cold for several weeks. During this time, bacteria in it die off, while the vaccine virus remains active. Cultures are made from the material repeatedly until it is certain that no living bacteria remain, and tests by animal inoculation are made to exclude the possibility that the virus of foot-and-mouth disease, or tetanus spores, might be present. When the vaccine is thus proved to be safe, its potency is tested by inoculation of rabbits. Then the vaccine is put up in amounts sufficient for

a single vaccination in small, sterile capillary tubes with a rubber bulb attached. Throughout the entire process of manufacture, strict aseptic precautions are observed, and the vaccine is never touched by human hands. The virus will remain active for at least three months, *provided the vaccine is kept continuously cold.*

Rivers has shown that vaccinia virus cultivated in *tissue cultures* may be safely inoculated intradermally into human beings, and that it produces a strong immunity comparable to that developed after vaccination with the usual calf vaccine. Goodpasture and others have described a practical method of making a vaccine from vaccinia virus grown on the *chick-embryo membranes.* It is probable that in the future these newer forms of smallpox vaccines will in part, at least, replace the calf vaccine now used.

Modern methods of vaccination. It was formerly the custom to vaccinate human beings by rubbing the virus into a large scarified area of the skin, but this produced unnecessarily large sores, which were liable to contamination, and left large, ugly scars. In modern methods of vaccination, the insertion of the virus is restricted to a *very small area;* the resulting lesion is smaller, but just as effective in producing immunity.

The skin of the upper arm over the deltoid muscle is washed with soap and water, then with *acetone,* which is allowed to evaporate. The tip of the capillary tube containing the vaccine is held in a fold of sterile gauze and broken, and the virus is expelled onto the prepared skin. Then with the *side* of a sterile needle, held almost parallel to the skin surface, a number of minute pricks are made into the superficial skin through the drop of virus (Fig. 130). This is the so-called *multiple-pressure method.** The *scratch method,* in which the virus is rubbed into a single scratch in the skin *not more than one-eighth inch long,* is also permissible. With either method, care must be taken to avoid drawing blood. In a minute or two, the excess vaccine is gently wiped off with sterile gauze. *No dressing* at all need be used, though often the patient is better satisfied if the area is covered with a *loose* fold of sterile gauze.

There is no longer any reason why anyone should be exposed to the danger and inconvenience of vaccination on the leg. By the modern technique, only a very small area of the superficial skin is inoculated, and the resulting scar is no more than a "sanitary

* Leake, J. P. "Questions and Answers on Smallpox and Vaccination." *U. S. Public Health Reports,* Reprint No. 1137, 1927.

dimple," not unattractive in appearance. Vaccination should be thought of as a minor surgical operation, and should be performed only by persons who understand aseptic methods.

Local reactions to vaccination, and their significance. Provided the vaccine used is potent, and properly applied, *there is always a*

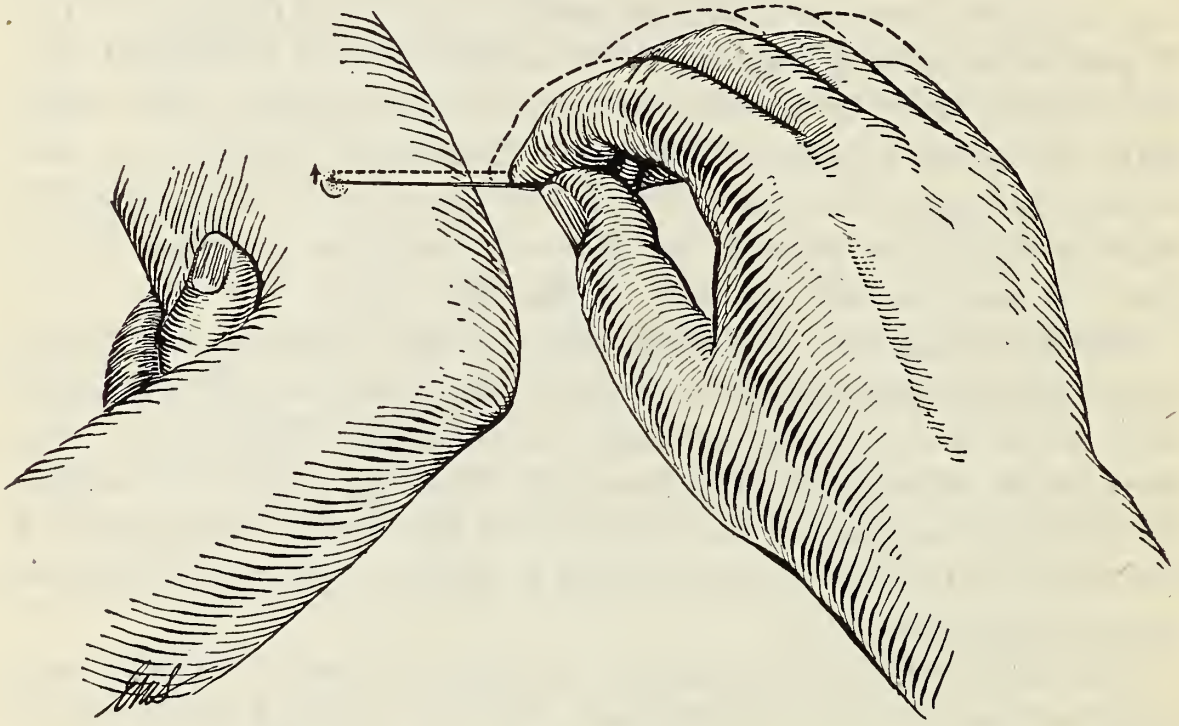


Fig. 130. The "multiple pressure" method of vaccination against smallpox. The sterile needle is repeatedly pressed against the skin through the drop of vaccine on the arm, with a rapid-up-and-down motion. The tip of the needle each time makes a tiny rip in the superficial skin, and carries some of the vaccine virus under the skin surface.

local reaction of some kind following vaccination. Failure of the vaccination to cause any reaction whatever is most commonly due to faulty technique, or to the fact that the vaccine used was not fresh, or had been allowed to get warm, so that the living virus originally present in it had been destroyed. Three types of reactions following a successful vaccination may be recognized: (1) *primary vaccinia* (the typical "take"), (2) the *vaccinoid* (or accelerated) reaction, and (3) the *immune* (or immediate) reaction (Fig. 131).

Primary vaccinia occurs in individuals who have never been previously vaccinated, and have not had smallpox. During the first three days, nothing is to be seen, then a papule appears, which by the seventh day becomes a raised, whitish vesicle. This enlarges

somewhat during the next few days. By the twelfth day it begins to dry up, and a crust forms over it, which drops off during the third or fourth week, leaving a characteristic scar. An area of redness (erythema) in the skin about the vesicle, which is at first narrow, begins to spread rapidly about the seventh day, and reaches a maximum diameter of about six inches on the ninth or tenth day. At this time there may be, also, a general reaction, with some fever and malaise, lasting for a few days.

The *vaccinoid* reaction occurs in persons who have a partial immunity—for example, in persons vaccinated eight or ten years previously. The reaction is less severe, and it is *accelerated,* i.e., it develops more quickly, reaches its maximum sooner, and disappears more rapidly. The height of the reaction usually occurs on the fourth to the eighth day, instead of the ninth or tenth day. This is really an allergic reaction, due to the fact that the tissues are in some degree hypersensitive to the virus.

The *immune reaction* occurs in individuals who have a relatively high immunity, as a result of recent vaccination or recent recovery from smallpox. *It is this reaction which is often mistaken for a failure of the vaccine to take,* for unless the arm is examined on the first or second day after vaccination, the reaction may be missed entirely. The immune reaction consists of a small area of redness—reaching its maximum in about forty-eight hours—and possibly a small papule, but there is no vesicle and no diffuse area of erythema as in reactions of the other types.

Care of the vaccination wound. The vesicles produced by modern vaccination methods are small and tough, and will usually dry without rupturing. In case the pustules, at the height of their development, seem likely to burst, the sore may be covered by a loose fold of sterile gauze. Alcohol, or other mild disinfectants, may be applied at this time, since the reaction has reached its peak, and the desired immunization has already been well started. *No mechanical shield of any kind* should be used over the sore, for this acts only to keep the surrounding skin soft and moist, making it more liable to secondary infection; and a tight bandage over the vaccination site should never be permitted.

Care must be taken not to allow the vaccinated person to contaminate his fingers with the virus, which then may be reinoculated by rubbing it into the eyes or nose, or into other places on the skin, with embarrassing if not serious results.

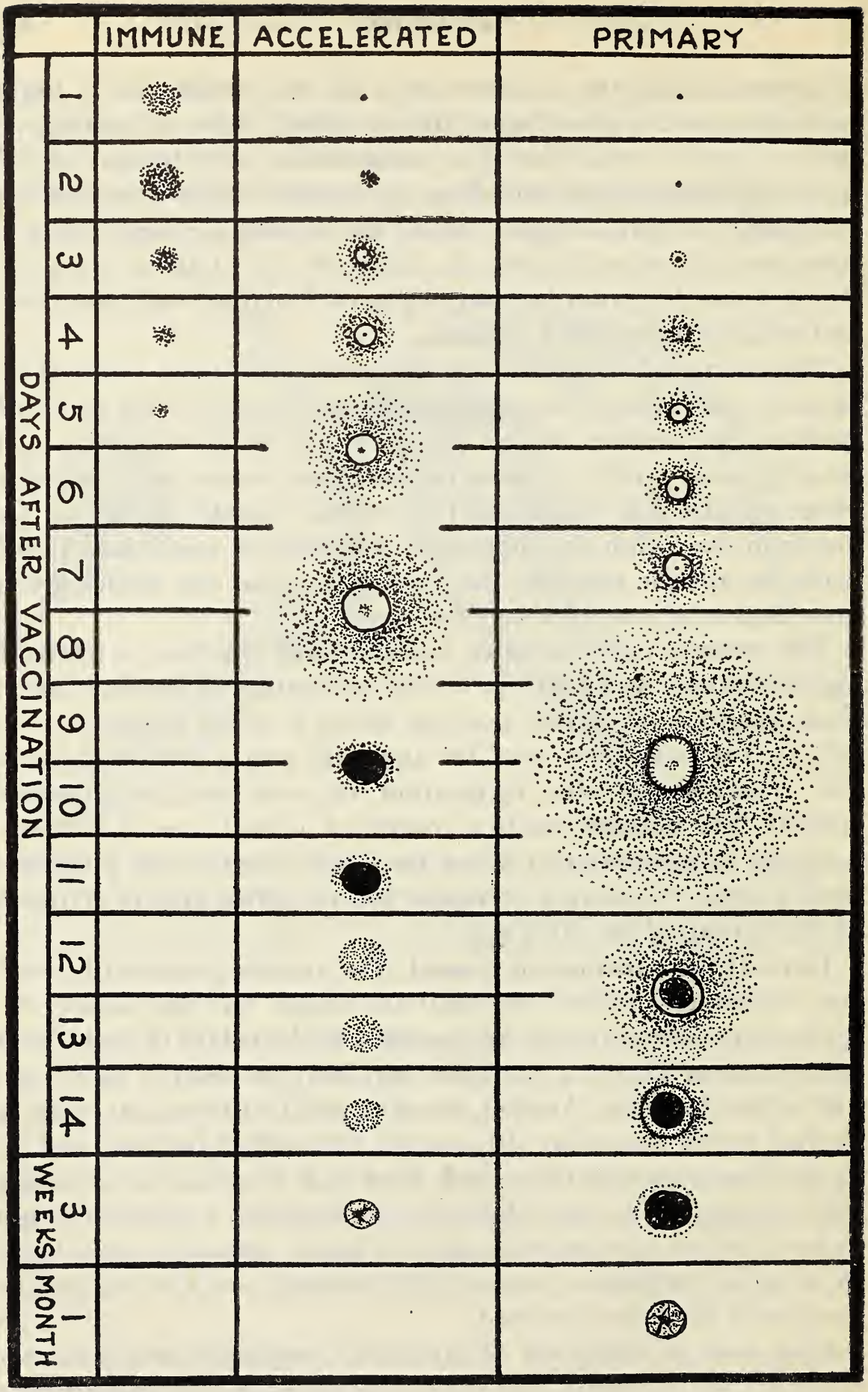


FIG. 131. Types of local reaction which may follow vaccination against smallpox. The dotted parts represent the area of redness in the skin.

A rare complication of vaccination is *post-vaccinal encephalitis.* The true cause of this extremely uncommon condition is not definitely known. Another, somewhat more often reported, but still infrequent, complication (occurring perhaps once in 100,000 vaccinations) is *generalized vaccinia.* In this case, the vaccine virus not only causes the single sore at the site of inoculation, but spreads to produce multiple pocks elsewhere in the skin, and the patient has an illness like smallpox. There is evidence that persons who are highly allergic, and particularly children who have eczema or other manifestations of hypersensitivity of the skin, are especially liable to suffer this generalized infection following vaccination. Recognition of the occasional occurrence of such rare accidents should not be allowed to obscure the fact that millions of vaccinations are performed safely without any complications at all.

The value of vaccination for the prevention of smallpox. The safety of the smallpox vaccines and the prophylactic value of the vaccination procedure have been demonstrated beyond a doubt, and can be denied only by those who willfully refuse to believe the evidence. Analysis of the records of many years shows the following significant facts. The amount of smallpox in any country has always declined abruptly when vaccination has been introduced and widely practiced. At the present time, the prevalence of the disease in any locality is in inverse proportion to the completeness with which the population is vaccinated; in those states and cities of the United States where compulsory vaccination is enforced, the disease is rarely seen; whereas, in other communities, where vaccination laws are lax, serious outbreaks occur repeatedly (Fig. 132).

In these epidemics the vast majority of cases (about 95%) are in individuals who have never been vaccinated, a small proportion (about 3 to 7%) occur in persons vaccinated more than seven years previously, and not more than 1 or 2 cases in a hundred occur in those who were vaccinated within seven years' time. Deaths of persons recently vaccinated are extremely rare. That a certain number of cases of smallpox should occur among vaccinated persons is not in the least surprising, and does not lessen the value of vaccination in general. *Absolute* immunity in every individual following vaccination, or, for that matter, following an attack of natural smallpox itself, is not to be expected. Immunity to any infectious disease to which human beings are naturally susceptible is always *relative,* and never absolute. As a matter of fact, smallpox vaccination comes as near to giving 100% protection as any known method of active immunization.

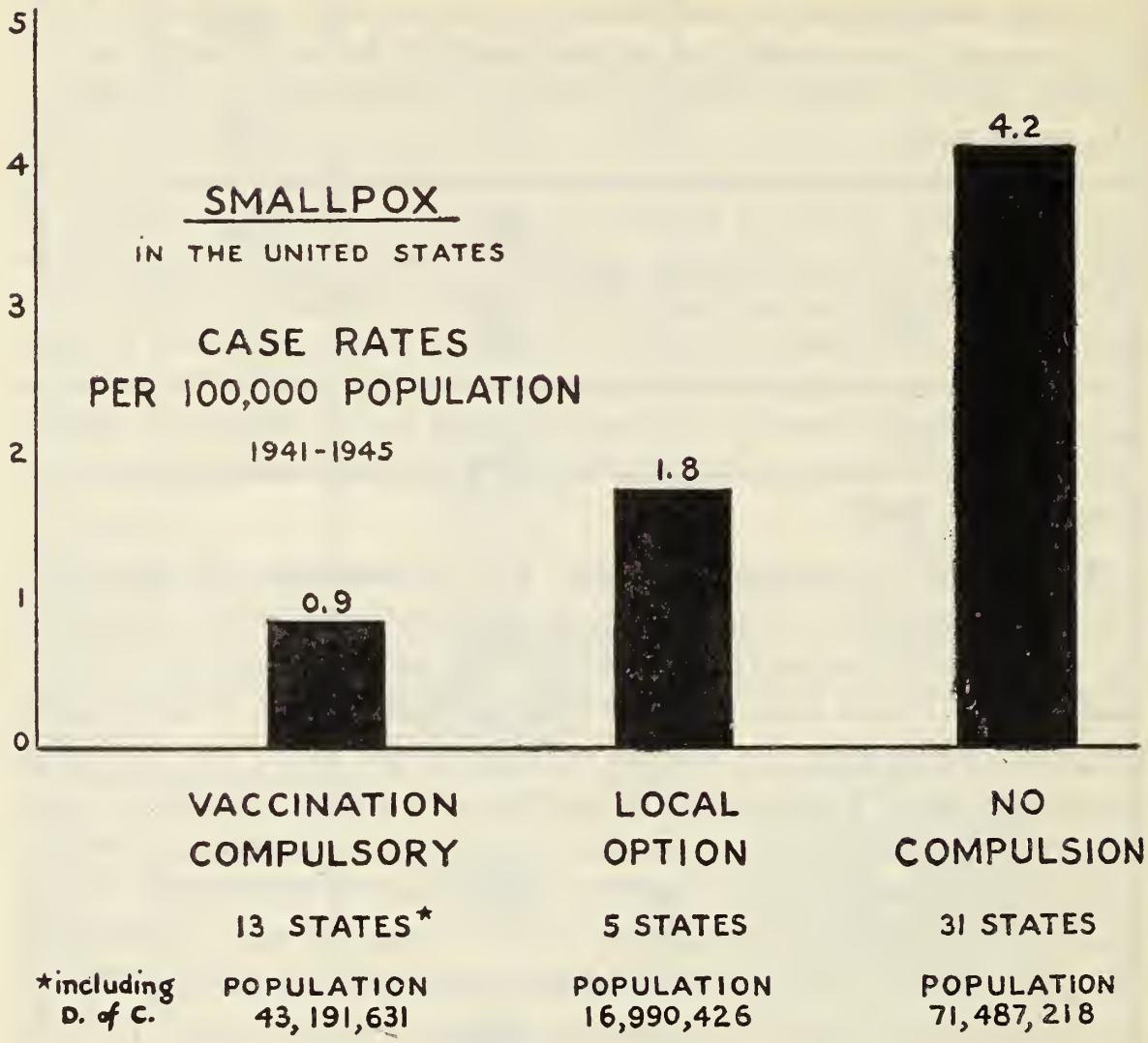


FIG. 132. Cases of smallpox per 100,000 population in the United States during the five-year period, 1941–1945. "Local Option" means that communities within the states may decide independently whether to make vaccination compulsory or not; in consequence, vaccination is not carried out universally. The majority of the states included in the column marked "No Compulsion" have no laws requiring smallpox vaccination. A few of these states actually forbid health officers to compel anyone to be vaccinated. (Data from *U. S. Public Health Reports,* compiled by Dr. Roy F. Feemster, Director, Division of Communicable Diseases, Massachusetts Department of Public Health.)

Every infant should be vaccinated when a few months old. The vaccination should be repeated at the school age, and at any subsequent time when there is danger of exposure to smallpox.

YELLOW FEVER

Principal features. Yellow fever is an acute infectious disease of tropical and subtropical countries, transmitted by the bite of infected mosquitoes, and caused by a specific filtrable virus. The ill-

ness comes on abruptly, within 3–6 days after the mosquito bite, with pain in head and back, congested face, and high fever, and soon the patient develops jaundice, and severe gastrointestinal symptoms; there are signs of kidney damage, and there is a marked tendency for hemorrhages to occur, especially from the stomach, causing "black vomit." Death may occur on the fifth or sixth day.

Historical importance; early work on the cause and prevention of yellow fever. Of all the serious epidemic diseases which have affected peoples of the Western Hemisphere, yellow fever has perhaps the greatest historical importance. The story of the virtual conquest of this disease makes one of the most dramatic chapters in the history of medicine. It was yellow fever (together with malaria) that made almost uninhabitable for the white man (before about 1904) large areas of Central and South America, and the West Indies. For a while, these demoralizing infections defeated attempts to build the Panama Canal. Prior to 1906, severe epidemics of this fearsome disease repeatedly appeared along the southern seaboard of the United States, and with especially devastating effect in New Orleans, where in 1878, for example, among a population of 210,000 persons, there were 27,000 cases and 4,046 deaths.

The first definite progress toward an understanding of yellow fever was made by the United States Army Commission, composed of Reed, Carrol, Lazear, and Agramonte, working in Havana, Cuba, in 1900. With the aid of human volunteers from the ranks of the Army, this commission established the following fundamental points:

(1) Yellow fever is caused by an invisible, filtrable virus, present in the blood of patients during the first few days of the disease.

(2) The disease is transmitted from man to man solely by the bite of infected mosquitoes (*Aedes egypti;* at that time called *Stegomyia calopus*), and not by even the most intimate contact with patients, nor with the bedding or clothing soiled by the excreta of patients.

The investigators proved that, to become infective, the mosquito must bite the patient within the first three days of the illness, and that about twelve days must pass before this mosquito becomes capable of transmitting the disease to another human being. The idea that yellow fever is carried by *Aedes* mosquitoes had been strongly championed by Dr. Carlos Finlay since 1881.

The practical preventive measures suggested by these findings were soon tried out, with remarkable success. In Havana and Panama, Army sanitarians, under Gorgas, undertook a vigorous

campaign to destroy mosquitoes and to protect the populace from their bites, with the result that yellow fever was eradicated from these areas. Similar success followed antimosquito work elsewhere, and it was generally thought that all the necessary knowledge was at hand to eliminate yellow fever permanently from the face of the earth.

Modern studies; the menace of jungle yellow fever. As late as 1927, however, little was known about the causative virus. In that year, a commission (Stokes, Bauer, and Hudson) sent by the Rockefeller Foundation to study the disease in West Africa, succeeded for the first time in producing yellow-fever infection experimentally in animals, when they found the Asiatic monkey susceptible. By animal experimentation they confirmed the conclusions of the Reed Commission, and in particular reëstablished the fact that the causative agent is a specific filtrable virus. A further practical advance was made when Theiler in 1931 adapted the yellow fever virus to mice by intracerebral inoculations, thus making possible the laboratory study of the virus by the same methods so successful with other viruses. Since an attack of yellow fever confers a lasting immunity, investigators were not surprised to find that the serum of persons who had recovered from the disease, even though the illness was suffered years before, possessed the power to neutralize the virus specifically in mouse-protection tests.

The extensive use of such tests to determine past incidence and present prevalence of yellow-fever infection, combined with field observations, soon brought out several important new facts. The disease was found to exist over much wider areas than was formerly supposed. Most important was the discovery by Soper (1932), and other investigators, that there exist two vast circumscribed endemic foci of yellow fever, one in the jungles of South America, and another in Africa. This *jungle yellow fever* occurs naturally in monkeys, and possibly other jungle animals, and from them is transmitted to man, as is proved by the occurrence of the disease among natives living near the jungle. The jungle disease occurs *without the presence of Aedes egypti,* and is presumably transmitted by other varieties of mosquitoes, several of which have been shown to be potential carriers, and possibly by other biting insects.

It appears from this that yellow fever, far from being conquered entirely, is still a menace, for a permanent reservoir of the infection hides in the jungle. The possibility that yellow fever may be reintro-

duced into communities in the United States or elsewhere where *Aedes* mosquitoes abound is a real one, and is a matter of concern to international health authorities. The rapid increase of air travel all over the world, greatly accelerated by the demands of global war, makes it imperative that precautions be observed to prevent the unwitting transfer of infected mosquitoes on airplanes. The United States Public Health Service has instituted suitable practices and regulations, and is prepared to go into action at once should a single case of yellow fever appear in this country.

The virus of yellow fever. The causative agent of yellow fever is one of the smallest of the viruses, the particles having an estimated diameter of no more than 19–22mμ. It is easily filtrable through the finest of filters. It has been cultivated in tissue cultures and in chick embryos.

In the liver cells from monkeys and human beings dying of the disease, intranuclear inclusion bodies have been found. The virus is highly infective, and is said to be able to penetrate the unbroken skin and conjunctiva of monkeys. At least five American investigators, including Noguchi and Stokes, have died while studying the disease.

Active immunization. Immunizing injections of a vaccine consisting of a mixture of attenuated yellow-fever virus (from cultures in chick embryos) and human blood serum were given to some 2,000,000 United States' troops during the early days of World War II. The appearance in 1942 of many cases of jaundice among these men, however, forced the abandonment of this particular type of vaccine. It was found that the jaundice was caused by some unknown element in the human serum used in some batches of the preparation. A vaccine not containing serum, developed in the meantime by the United States Public Health Service laboratories, was substituted, and is now the accepted preparation. This aqueous-base vaccine consists of a distilled-water extract of 10–11-day-old chick embryos infected with the so-called 17D strain of yellow-fever virus. A subcutaneous injection of 0.5 cc is given to soldiers and others in need of protection. It has been shown that an effective resistance to yellow fever is induced by the vaccine, and that this immunity lasts for at least four years.

DENGUE FEVER

This disease is similar, in many respects, to yellow fever. It is a virus infection, transmitted by the same mosquito (*Aedes egypti*) that carries the yellow-fever virus. It is of more immediate interest, however, for epidemics of dengue have recently occurred in South Atlantic and Gulf states of the United States, as well as in warmer countries overseas. Moreover, numerous cases were encountered among troops in the Philippines and on South Pacific islands during World War II.

From the military point of view, dengue is one of the most serious of the epidemic diseases. It is not a fatal infection, but the patient is suddenly acutely ill and, after the acute phase has passed, remains rather profoundly depressed, mentally and physically, for several days. Thus, the individual is made unfit for combat or other strenuous duty for a period of at least two weeks. Epidemics of this incapacitating disease struck our troops with dramatic suddenness, involving a high proportion of all the men at certain stations.

The onset of the illness is typically very abrupt, after an incubation period of about six days. There is an original fever period of about three days, then a remission, followed by a brief terminal fever, higher than before. Often there is a skin rash; blood counts show a leukopenia. A characteristic clinical feature is pain referable to the eyeballs, and aggravated by movement of the eyes; the sense of taste is altered. The pains in the back and joints, felt in severe cases, account for the popular name "breakbone fever."

In dengue fever, as in yellow fever, the causative virus is present in the blood only during the first three or four days of the illness. About ten days after biting a patient, the *Aedes* mosquito becomes capable of transmitting the infection to other persons. Little is known about the dengue-fever virus itself, except that it is filtrable, and distinct from that of yellow fever.

RABIES (HYDROPHOBIA)

Rabies is an acute and highly fatal disease, most common in dogs and cats, and in wolves and other carnivorous wild animals, but transmissible to human beings. It is caused by a *filtrable virus,* which is present in the saliva of rabid animals. Most cases in man

follow the bite of a rabid dog or cat. Rabies is prevalent among dogs, and occasional human cases occur in most countries, except in England, where laws requiring the muzzling of dogs, and a strict supervision over imported animals (180-day quarantine), have been rigidly enforced, with the result that rabies has been practically eradicated there. In many parts of the United States rabies becomes disgracefully prevalent from time to time and, during these outbreaks, fatalities in human beings bitten by rabid animals are likely to occur in alarming numbers.

Rabies in dogs. It is principally the homeless, stray dog that keeps rabies infection alive among the canine population. Such an animal may bite, and so infect, many other dogs and cats before it succumbs. The disease in dogs begins gradually, from two to eight or more weeks after infection has occurred. The virus may be present in the saliva five to eight days before the onset of definite symptoms. Thus, a dog may seem to be well, and yet his bite may carry rabies infection. However, symptoms of the disease always appear in a dog in less than fourteen days after the virus first appears in the saliva. A bite incurred fourteen days or more before a dog becomes ill will consequently *not* carry the rabic virus, and there is no danger that it will lead to hydrophobia.

A dog coming down with rabies is at first merely restless, and perhaps more affectionate than usual; then the restlessness increases, the animal barks and howls in a peculiar manner, begins to snap at objects about it, and may soon become furiously "mad." In this stage, the unfortunate creature runs aimlessly about, frothing at the mouth, and biting viciously at man or beast or anything else in its path. Finally, paralysis sets in, beginning usually in the hind legs, and within a short time the animal is dead. Sometimes a rabid dog shows paralytic symptoms from the first ("dumb rabies"), and does not pass through the furious stage. If an animal suspected of having rabies is securely confined so that it can be safely observed, and symptoms of the kind described above develop, there is no doubt as to the diagnosis. Death will always occur before the tenth day, and usually within five days.

Diagnosis of rabies in animals. *Demonstration of Negri bodies.* The experienced veterinarian has little difficulty in recognizing rabies in dogs or cats, from the symptoms and manner of death, and it is important that an animal suspected of having the disease be captured and watched carefully to note these clinical evidences.

When a supposedly rabid animal dies or is killed, it is possible, by examination of the brain for the characteristic *Negri bodies* (Fig. 117, p. 597), to determine whether or not it was really rabid. To detect Negri bodies, smears are made directly from certain portions of the gray matter of the brain, stained with Giemsa or some special stain, and examined under the microscope; or a portion of the brain (cerebellum) is hardened and histological sections are made, appropriately stained, and examined. When these Negri bodies are seen, this is definite proof that the animal was rabid.

Animal inoculation. In case Negri bodies are not found, a final test is to inoculate an emulsion of the brain tissues intracerebrally into laboratory animals, preferably several young white mice. If the inoculated material contains the rabies virus, these animals will become ill in a few days, and sections of the brains will show the presence of Negri bodies.

Rabies in man. *Factors of infection.* Not every person who is bitten by a rabid animal will develop hydrophobia. It is estimated that perhaps 16% would die of rabies if no preventive measures of any kind were taken. Much depends upon the location of the bite, whether it occurred on the bare skin or through the clothing, and the depth and extent of the wounds. Most of the saliva may have been caught on the clothing. The most dangerous type of bite is one through the bare skin, which causes a deep or lacerated wound. The importance of the location of the bite will be clear when it is remembered that the virus is *neurotropic,* that is, it has a strong affinity for the nervous tissue. *It travels slowly along the nerves to the brain,* and when the central nervous system is reached, the symptoms of rabies appear. Naturally, then, a bite upon a part of the body which is abundantly supplied with nerves, such as the hands, is liable to lead to the disease, and the nearer the bite to the brain, the shorter will be the incubation period. Bites on the face are therefore especially dangerous.

It must be remembered that there is always a possibility of infection whenever the saliva of a rabid animal has come in contact with the broken skin, and the question of taking the "Pasteur treatment" (prophylactic immunization) described below must be seriously considered in every case of such contact, even if the animal did not actually bite.

Symptoms. In human beings, rabies is invariably fatal. Once the symptoms have begun, there is no available treatment which will

save the unfortunate victim from a horrible death. Beginning with depression and irritability, and difficulty in swallowing food, there follow two or three days of intense excitement, then paralysis ending in death. The patient's unwillingness to take water ("hydrophobia") is due to painful reflex spasms when he attempts to swallow fluids.

The long incubation period. The fortunate feature of rabies in man is the long period, after the virus has been introduced, before symptoms begin. This incubation period is rarely less than four weeks, and is usually from six to eight or more. Cases developing rabies five or six months, and *rarely* as much as eleven months, after infection, have been reported. It was Pasteur who developed the lifesaving method of giving each person who has been exposed to a possible rabic infection a series of treatments with a vaccine, by means of which an active immunity to rabies virus is built up *during the incubation period.*

The virus of rabies. The rabic virus is a typical example of a *neurotropic* virus—one that propagates in the susceptible host entirely in the nervous tissue. It is easily filtrable through all grades of filters. It is pathogenic for many animal species, and fatal rabies is readily induced experimentally by the intracerebral inoculation of guinea pigs, rats, mice, and rabbits. The virus, as it occurs in the saliva of the mad dog, i.e., in a natural infection, is called *street virus,* but when this virus has been fully adapted to the rabbit by passage through a series of these animals, until its virulence can no longer be increased, and it kills rabbits always in the same number of days, it has been converted into the so-called *fixed virus.* All the several forms of vaccines now used for prevention of rabies are prepared from a fixed virus.

The rabic virus has been made the subject of investigation by the newer methods of virus study only within recent years. Webster first reported (1937) its cultivation in tissue culture. By adapting the virus successfully to the mouse he has also developed a practicable method, based on mouse-protection tests, for evaluating the efficiency of rabies vaccines. More recently, the virus has been grown in chick-embryo cultures, and efforts are now being made to increase the yield of the virus from egg cultures in order that vaccines may be made from such material.

Prevention of rabies; general measures, treatment of the wound. Measures which will effectively control rabies among the animal population are well known, but they are not always success-

fully applied, because they require public support and cooperation, particularly among the owners of dogs. In places where rabies has been common, marked success has followed vigorous efforts: (1) to eliminate, so far as possible, all stray dogs, and (2) to enforce an ordinance requiring all other dogs to be licensed by their owners, the license to be issued only after the dog has been vaccinated against rabies. The *vaccination of the dogs* is accomplished by a single injection of rabic vaccine made from chloroform-inactivated virus; it must be repeated yearly.

When a person is bitten or scratched by a dog, possibly rabid, immediately action is called for in two directions. The wound should be thoroughly cleaned at once, and a physician called who will probably cauterize it with *fuming nitric acid.* This drastic and disfiguring treatment has long been regarded as necessary. Recent studies indicate, however, that a safe and welcome substitute for the nitric acid is a 20% solution of soft soap. Immediate and thorough washing out of the wound *is imperative;* it should always be done, even though immunizing injections are to be taken later.

Efforts must then be made to determine whether the dog was really rabid. It is best to capture it, confine it securely, and observe its behavior for fourteen days. But if, in the interest of safety, it is necessary to kill the animal, the head should be packed in ice and sent to the nearest laboratory where the brain may be examined for Negri bodies. The immunizing injections of rabic vaccine are ordinarily delayed a few days until it is known whether the dog is rabid. If so, the animal will die within ten days. But it may be considered advisable to begin the inoculations at once, for they may later be stopped, and no harm done, if the animal does not prove to be rabid.

Prophylactic vaccination against rabies. *The Pasteur treatment.* The method of immunization against rabies first worked out by Pasteur (1880–1885) is still widely used.

As a preliminary to the actual preparation of a vaccine, Pasteur experimented with the effect of the rabic virus on laboratory animals, and learned to convert the "street virus" to a "fixed virus" which would kill rabbits regularly on the seventh day.

The next step was the development of a satisfactory method of attenuating "fixed virus," so that it could be safely used as a vaccine. Pasteur found that, when spinal cords of rabbits containing

the virus were dried in the air over potassium hydroxide, the virulence of the virus was gradually lost. By inoculating dogs with emulsions of cords dried for varying periods, beginning with the least virulent and working up to the fully virulent cord, he succeeded in immunizing the animals against rabies. The first trial of this preventive vaccination in a human case was made in 1885 upon a nine-year-old boy, Joseph Meister, who had been severely bitten by a mad dog, and without the treatment would surely have died of hydrophobia. Pasteur's method of immunization was completely successful; the boy remained well, and this bold experiment served as the starting point for a system of preventive treatment for hydrophobia which has since been used the world over.

In the scheme of treatment now most widely employed, a series of subcutaneous injections of rabies vaccine is given on twenty-one consecutive days. Each inoculation consists of an emulsion in salt solution of a small piece of a rabbit spinal cord (from a rabbit killed by "fixed virus") which has been dried for a specified number of days. The first dose of the vaccine is prepared from a cord dried for eight days; subsequent doses are made from cords dried seven days, then six, five, four, and three days. This rabies vaccine is readily secured from state health laboratories or special "Pasteur Institutes," almost everywhere.

Newer forms of rabic vaccines. A number of methods simpler than those used by Pasteur for the attenuation of fixed rabies virus have been tried. Rabies vaccines prepared by the Semple method are most extensively used at the present time. The material injected is an emulsion of infected brain tissue from rabbits killed by fixed virus, which is diluted with saline and contains 0.5% phenol. Before injection into man, the material is tested by intracerebral inoculation into rabbits, to show that the virus has been completely inactivated, and to prove its sterility. Such a phenolized vaccine has a number of advantages over the classical dried-cord vaccine of Pasteur. It is apparently an effective immunizing agent, and its routine use is attended with fewer complications than any other type of rabies vaccine. Rarely, paralysis has occurred as a result of injections of other forms of vaccine, but experience indicates that the possibility of this serious accident is practically eliminated by the use of a phenolized vaccine.

Webster has suggested a vaccine containing cultured virus inactivated by irradiation with ultraviolet rays.

Complications; value of antirabic vaccination. Ordinarily the course of prophylactic inoculations against rabies is accompanied by no general reactions or marked discomfort of any kind. During the second week of injections, adults commonly show inflammations about the site of the inoculations, due to the development of a local hypersensitivity; these disappear quickly when the treatments are completed.

The value of this prophylactic immunization against hydrophobia is well established. Only a small fraction of 1% of persons who take the treatment ever develop rabies. These fatalities are usually in people severely bitten about the head or neck.

ENCEPHALITIS

Types of virus encephalitis in human beings. Of particular importance in North America at the present time are three forms of infectious encephalitis caused by viruses. These are: (1) the so-called *St. Louis encephalitis,* (2) the *Western-type equine encephalomyelitis,* and (3) the *Eastern-type equine encephalomyelitis.* In addition, a nonepidemic form of brain disease, long known as *lethargic encephalitis* (or von Economo's disease), is occasionally seen.

In other countries, clinically similar human encephalitides occur; for example, *Japanese B encephalitis,* and *Russian spring-summer encephalitis;* and there have been infections among laboratory personnel caused by the virus of *Venezuelan equine encephalomyelitis.*

St. Louis encephalitis. This infection was first recognized as an epidemic disease when more than one thousand cases occurred in St. Louis, Missouri, in the late summer of 1933. In this epidemic, after an incubation period estimated to vary from about nine to fourteen days, patients developed an acute illness, characterized by severe headache, stiff neck, and high fever, and usually fell into a somnolent state. Recovery within about ten days, without signs of residual nervous impairment, was the rule. The total mortality was about 20%.

This outbreak was thoroughly investigated, and one important result was the isolation, from patients, of a specific filtrable *virus* which reproduced the disease in monkeys and was later adapted to mice. The virus has been cultivated in embryonated eggs, and

extensively studied. The size of the virus particles has been estimated to be 22–33mμ.

Since 1933, in the south-central and western United States there have been several smaller epidemics of encephalitis due to this virus, and much has been learned of its general distribution and manner of spread. In the recent outbreaks, the St. Louis virus has sometimes been found alone, while in other epidemics it has been accompanied by the virus of western equine encephalomyelitis, which was causing many cases of human encephalitis at the same time. These two viruses have much in common. Both have been shown to produce an inapparent (symptomless) infection in many different vertebrate animals, especially often in domestic fowl. Both have been isolated from *Culex tarsalis* and other common species of mosquitoes caught in endemic areas, and the capacity of these insects to transmit the viruses from animal to animal has been experimentally proved beyond question. Both diseases are therefore thought to be transmitted primarily by mosquitoes, and chickens and other domestic animals are regarded as important reservoirs of infection for both the St. Louis and equine viruses. Thus, these virus infections must be added to the growing list of arthropod-borne diseases. It is possible, however, that the viruses are also spread by personal contact; at present, this is an unsettled matter.

A method of preparing an effective vaccine for St. Louis encephalitis by use of ultraviolet irradiation has recently been reported.

Equine encephalomyelitis infection. In horses, epizootics of an illness characterized by extreme lethargy have been noted, from time to time, for many years in the United States and elsewhere. A virus was first isolated in 1930, in California, from horses dead of this disease. Later (1933) the disease appeared in Atlantic coast states, and a virus was again isolated, but it proved to be different serologically from the virus of the western equine infection. The The first sizable outbreak of encephalomyelitis among horses in the the East occurred in Massachusetts in 1938. At this time, also, the first human cases were observed, and proved to be caused by the same virus infecting the horses—the eastern equine virus. The human cases were severe; of the 30 patients, 8 died. It has since been shown that there exists a variety of natural animal reservoirs of the eastern equine encephalomyelitis virus, just as in the case of the western variety, and mosquito transmission also is probable.

The importance of the western equine virus as a cause of human

disease has been indicated above. The most extensive epidemic occurred in 1941 in North Dakota; there were 1,080 cases and 96 deaths. It is evident that the wide distribution of these dangerous neurotropic viruses presents a continual menace, and creates a difficult problem of control. Effective vaccines are available for both the eastern and the western type of equine encephalomyelitis viruses, however, and they may prove valuable in the face of a threatened epidemic.

Both of the equine viruses have been cultivated and studied by ultracentrifugation procedures, and the electron microscope. The infective particles appear to consist of rounded bodies, with some suggestion of an internal structure, averaging about 40–42mμ in diameter.

Lethargic encephalitis. In 1913, von Economo first called attention to a peculiar disease of the central nervous system characterized by the development, in most patients, of a marked and often long-continued state of somnolence. Following the great epidemic of influenza in 1918, sporadic cases and small outbreaks of this disease, which became known as "sleeping sickness" or *lethargic encephalitis,* were observed rather frequently in most countries, including the United States. Since about 1925, however, the affection has become increasingly rare everywhere. The cause of this form of encephalitis has never been definitely determined, but is probably a filtrable virus.

POLIOMYELITIS (INFANTILE PARALYSIS)

Nature; clinical features. Poliomyelitis is an acute, communicable disease, caused by a specific neurotropic filtrable virus. The illness may take a number of clinical forms, but is characterized principally by paralysis of various groups of muscles. It attacks chiefly young children—hence the common name, infantile paralysis—but it also occurs in older persons. The disease is seldom fatal, but in a certain proportion of patients a partial paralysis persists for long periods, sometimes permanently, and the unfortunate individuals may be seriously crippled for life.

The spread of poliomyelitis. Cases of infantile paralysis are reported throughout the year, but epidemics have a definite seasonal occurrence—they develop in the late summer months, and fade off with the arrival of frosty weather. A fully satisfactory explanation of this feature of poliomyelitis has not been made, and indeed there

are a number of other puzzling points about this disease which await elucidation.

The distribution of recognizable cases among any group of people is very irregular. The typical disease appears in comparatively few of the persons who are certainly exposed to the virus. There is seldom more than one case in a family. Perhaps a special susceptibility is necessary. However, in epidemic times, many individuals show vague symptoms of variable nature, without paralysis, which are almost certainly due to infection with the poliomyelitis virus in a mild or atypical form.

The virus is present in the nasopharynx of patients in the early stages of the disease, and it may be demonstrated even more regularly and abundantly in the *feces*. Excretion of the virus in the stools continues for two or three weeks after the onset of symptoms in the majority of cases, and for still longer in a smaller percentage. Sewage, and the night-soil in the unsanitary privies of rural and suburban communities, where poliomyelitis is endemic, often contain the virus.

Infection may occur through *inhalation* of infected droplets or dust, or by *ingestion* of food heavily contaminated with the virus. The portal of entry is apparently the mucous membranes of the upper respiratory and alimentary tracts. After penetrating these membranes and reaching the nerves that are close to the surface, the virus then travels along the nerves to the nearest ganglion, and thence to the central nervous system.

Flies trapped in areas where poliomyelitis is epidemic are often found to carry considerable amounts of the virus, and it has been shown experimentally that such flies may contaminate food to such an extent as to produce typical paralysis in monkeys to which it is fed. The probable participation of flies in the transmission of the disease helps to explain the seasonal incidence of poliomyelitis epidemics. At the same time, there is good reason to feel that the virus is widely spread among the population by personal contact. Which of the possible modes of spread are of greatest importance is still an unsettled matter.

The virus of poliomyelitis. The causative agent of poliomyelitis is present in filtrates of the brain stem and spinal cord tissue, and in the feces, and may be found in filtered washings from the nasopharynx of human patients. With these filtrates, the disease may be reproduced in monkeys by various routes of inoculation, including

introduction of the material intranasally. Considerable difficulty is encountered in the laboratory study of this virus. Armstrong has recently succeeded in adapting a single strain of poliomyelitis virus to white mice (Lansing strain), but this development has not led to much practical advance as yet. Other strains have not been adapted to mice or other laboratory animals. The virus has been grown in tissue cultures. Its size has been estimated to be only about 10–20mμ. No inclusion bodies have been identified.

Prevention of poliomyelitis. When poliomyelitis is prevalent in a community it is wise for all individuals to avoid excessive physical exertion and fatigue, since susceptibility is apparently increased thereby. Operations for removal of tonsils should not be performed during epidemic periods.

An attack of infantile paralysis leaves at least a temporary immunity, and the serum from recovered individuals possesses the power to neutralize poliomyelitis virus.

A vaccine has recently been prepared by ultraviolet irradiation of the Lansing strain. Its value for human protection is still to be demonstrated.

LYMPHOCYTIC CHORIOMENINGITIS

In 1934, Armstrong and Lillie first isolated a virus which is now accepted as the cause of this peculiar form of meningitis in human beings. The disease, which has been recognized in both Europe and the United States, usually runs a short, mild course. It is said to resemble clinically the milder, nonparalytic cases of poliomyelitis. The clinical manifestations are variable, and often the patient feels only weakness and temporary mental confusion. The spinal fluid contains an increased number and proportion of *lymphocytes,* rather than the pus cells (polymorphonuclear leukocytes) which are so characteristic of the common forms of acute meningitis caused by bacteria.

The virus has been cultivated on the egg membranes. Its size has been estimated to be about 70mμ. Intranuclear inclusion bodies have been described in cells of the meninges and brain cortex of experimentally inoculated animals.

REFERENCES

MUDD, S.: "Pathogenic Bacteria, Rickettsias and Viruses as Shown by the Electron Microscope: Their Relationships to Immunity and Chemotherapy —II. Relationships to Immunity," *J.A.M.A.,* Nov. 4, 1944, 126:632.

OLITSKY, P. K. and CASALS, J.: "Concepts of the Immunology of Certain Virus Infections," *Bull. N. Y. Acad. Med.,* July 1945, 21:356.

REIMANN, H. A.: "Infectious Diseases: Tenth Annual Review of Significant Publications," *Arch. Int. Med.,* Oct. 1944, 74:280.

SULKIN, S. E. and HARTFORD, C. G.: "Laboratory Diagnosis of Virus Diseases," *J.A.M.A.,* July 3, 1943, 122:643.

VAN ROOYEN, C. E. and RHODES, A. J.: *Virus Diseases of Man.* London: Oxford Univ. Press, 1940.

Smallpox

BUDDINGH, G. J.: "Pathogenic and Antigenic Properties of Dermal Vaccine Virus Propagated in the Chorio-allantois of Chick Embryos," *Am. J. Hyg.,* Nov. 1943, 38:310.

GREEN R. H., ANDERSON, T. F. and SMADEL, J. E.: "Morphological Structure of the Virus of Vaccinia," *J. Exper. Med.,* June 1942, 75:651.

JUBB, A. A.: "Generalized Vaccinia," *Brit. Med. J.,* Jan. 23, 1943, 1:91.

NORTH, E. A., BROBEN, J. A. and MENGOUI, A. H.: "Use of Chorio-allantois of Developing Chick Embryo in Diagnosis of Smallpox," *Med. J. Australia,* May 13, 1944, 1:437.

TOP, F. H. and PECK, L. E.: "A Small Outbreak of Smallpox in Detroit," *Am. J. Pub. Health,* May 1943, 33:490.

Yellow fever

ANIGSTEIN, L.: "Yellow Fever and Dengue," *J. Ped.,* May 1943, 22:612.

SAWYER, W. A.: "Public-Health Implications of Tropical and Imported Diseases: Yellow Fever and Typhus, and the Possibility of Their Introduction into the United States," *Am. J. Pub. Health,* Jan. 1944, 34:7.

WILLIAMS, C. L.: "Yellow Fever Control During the War," *Am. J. Trop. Med.,* July 1944, 24:245.

Dengue

CARSON, D. A.: "Observations on Dengue," *U. S. Naval Med. Bull.,* May 1944, 42:1081.

EWING, D. Q.: "The Dengue Fevers," *Med. Clin. N. Am.,* Nov. 1944, 28:1471.

KISNER, P. and LISANSKY, E. T.: "Analysis of an Epidemic of Dengue Fever," *An. Int. Med.,* Jan. 1944, 20:41.

Rabies

KERN, R. A.: "Rabies: Some Practical Questions and Answers," *Med. Clin. N. Am.,* Nov. 1944, 28:1406.

PALIK, E. E. and MOSS, E. S.: "A Ten-Year Survey of the Pasteur Institute of Charity Hospital of Louisiana at New Orleans," *New Orleans Med. & Surg. J.,* Oct. 1944, 97:153.

SHAUGNESSY, H. J. and ZICHIS, J.: "Prevention of Experimental Rabies; Treatment of Wounds Contaminated by Rabies Virus with Fuming Nitric Acid, Soap Solution. Sulfanilamide or Tincture of Iodine," *J.A.M.A.,* Oct. 30, 1943, 123:528.

SULKIN, S. E. and HARFORD, C. G.: "Concerning the Infectivity of Saliva in Human Rabies," *An. Int. Med.,* Aug. 1943, 19:256.

Encephalitis

CASALS, J.: "Immunological Relationships Among Central Nervous-System Viruses," *J. Exper. Med.,* Apr. 1944, 79:341.

HAMMON, W. McD. and REEVES, W. C.: "Recent Advances in the Epidemiology of the Arthropod-borne Virus Encephalitides," *Am. J. Pub. Health,* Oct. 1945, 35:994.

HENDERSON, J. L.: "The Arthropod-Borne Encephalitides of North America," *New Orleans Med. & Surg. J.,* July 1944, 97:22.

HOWITT, B. F.: "The Complement-Fixation Test with Human Sera Against the Viruses of St. Louis Encephalitis and Equine Encephalomyelitis," *J. Immun.,* Oct. 1943, 47:293.

LEVINSON, S. O. and OTHERS: "Production of Potent Inactivated Vaccines with Ultraviolet Irradiation," *J.A.M.A.,* June 24, 1944, 125:531.

SABIN, A. B.: "The St. Louis and Japanese B Types of Epidemic Encephalitis," *J.A.M.A.,* June 19, 1943, 122:477.

SHARP, D. G., TAYLOR, A. R., BEARD, D. and BEARD, J. W.: "Morphology of the Eastern and Western Strains of the Virus of Equine Encephalitis," *Arch. Path.,* Aug. 1943, 36:167.

SMITH, M. G., BLATTNER, R. J. and HEYS, F. M.: "The Isolation of the St. Louis Encephalitis Virus from Chicken Mites (*Dermanyssus hallinae*) in Nature," *Science,* Oct. 20, 1944, 100:362.

Poliomyelitis

AYCOCK, W. L. and KESSEL, J. F.: "The Infectious Period of Poliomyelitis and Virus Detection," *Am. J. Med. Sci.,* Mar. 1943, 205:454.

BROOKS, N. J.: "The Rôle of Non-Specific Immunity Factors in the Etiology of Poliomyelitis," *Arch. Ped.,* Aug. 1943, 60:381.

FRASER, A. W.: "Some Recent Developments in the Epidemiology of Poliomyelitis," *Arch. Ped.,* May 1943, 60:256.

HORSTMANN, D. M., WARD, R. and MELNICK, J. L.: "Persistence of Virus Excretion in the Stools of Poliomyelitis Patients," *J.A.M.A.,* Dec. 23, 1944, 126:1061.

HOWARD, R. E.: "Relationship of Poliomyelitis to Tonsillectomy," *An. Otol., Rhin. & Laryng.,* Mar. 1944, 53:15.

SHERMAN, M. S.: "The Natural Course of Poliomyelitis; A Report of 70 Cases," *J.A.M.A.,* May 13, 1944, 125:99.

WARD, R. and MELNICK, J. L.: "Spread of Infantile Paralysis," *J.A.M.A.,* Feb. 26, 1944, 124:595.

WARD, R. and SABIN, A. B.: "The Presence of Poliomyelitis Virus in Human Cases and Carriers During the Winter," *Yale J. Biol. & Med.,* May 1944, 16:451.

Lymphocytic Choriomeningitis

FARMER, T. and JANEWAY, A.: "Infections with the Virus of Lymphocytic Choriomeningitis," *Medicine,* 1942, 21:1.

MILZER, A.: "Neurotropic Virus Infections in Chicago, 1939–1941. Nine Cases of Lymphocytic Choriomeningitis," *Proc. Soc. Exper. Biol. & Med.,* Dec. 1943, 54:279.

REVIEW QUESTIONS—CHAPTER XXXIX

1. Give a classification of the virus diseases according to the tissue or body-regions primarily affected.
2. Discuss the prevalence of smallpox in historical times, and at present. What are the principal clinical features of the disease? What is variola, alastrim, vaccinia? What is the relation of smallpox to cowpox? How does smallpox naturally spread?

3. How is variola virus converted to vaccinia virus?
4. Name and describe the inclusion bodies and elementary bodies of vaccinia virus.
5. What method of preventing severe smallpox was practiced before the days of vaccination?
6. Who developed the method of vaccination, and when? Distinguish between *inoculation smallpox* and *vaccinia*. Explain how the principle of vaccination was discovered and proved to be sound. What is the origin of the word *vaccine*?
7. Outline the modern method of preparation of smallpox vaccine from calves. What are two other sources of vaccinia virus for possible use in vaccines?
8. What principles are followed in modern methods of vaccination? Describe the multiple-pressure method.
9. Name and describe the types of local reaction that may follow smallpox vaccination, and explain the significance of each.
10. What are the important points to be remembered in the proper care of the vaccination wound? Mention two possible complications of vaccination.
11. Defend the necessity and value of vaccination for the prevention of smallpox.
12. Describe the principal features of yellow fever.
13. Discuss the historical importance of yellow fever, and outline the early work on the cause and prevention of this disease. Name the principal insect vector.
14. Outline the nature of modern investigations of yellow fever, and the new facts which have been discovered.
15. Describe the virus of yellow fever. Is artificial active immunization practicable?
16. Describe the nature, prevalence, causative virus, and manner of spread of dengue.
17. What is the cause of rabies? Describe rabies in dogs. What are Negri bodies? How can a diagnosis of rabies be made after the death of an animal?
18. Describe rabies (hydrophobia) in man. What are some of the factors which influence the chances of acquiring rabic infection from a dog bite? What feature of the infection permits time for active immunization?
19. Describe the properties of the virus of rabies and the characteristic inclusion bodies. What are the principal general measures that have been found effective in controlling rabies among the dog population?
20. What local treatment must be given the wounds made by the bites or

scratches of a possibly rabid animal? What should be done with the animal?

21. Who developed the original method of vaccination for the prevention of rabies in human beings, and when? Outline the important steps in the preparation of Pasteur's vaccine, and the scheme of anti-rabic "Pasteur treatment" usually followed. Describe the newer forms of rabies vaccines.
22. What complications of rabic vaccination may be observed? Is the immunization of proven value?
23. Name four types of encephalitis in human beings that may occur in the United States. What is lethargic encephalitis?
24. Describe St. Louis encephalitis. How may it be transmitted? What other virus encephalitis has similar epidemiological features?
25. Describe the occurrence and spread of equine encephalomyelitis virus infection in horses, and in man.
26. Describe the clinical features, probable modes of spread, and prevention of poliomyelitis.
27. What is known about the virus of poliomyelitis?
28. Discuss the nature, and causative agent, of lymphocytic choriomeningitis.

CHAPTER XL

THE ACTINOMYCETES AND THE TRUE FUNGI

ORDER ACTINOMYCETALES

In this large and important order of microbes are grouped organisms having properties resembling, on the one hand, the true fungi, and on the other, the true bacteria. In the forthcoming 6th Edition of Bergey's *Manual of Determinative Bacteriology,* a new scheme for the classification of these organisms, worked out by Waksman and Henrici, will probably be adopted. This arrangement is shown in Table XXVIII.

Actinomyces. This is the most typical genus of the *Actinomycetaceae,* and the name is often loosely applied to all the various members of this family. The organisms grow in the form of branched filaments, thus forming a true mycelium like that of a common mold; moreover, in some kinds, short aerial hyphae extend into the air above the growth, and from the tips of these are pinched off round or rod-shaped reproductive elements (conidia) like the arthrospores of some true fungi. When the aerial mycelium and the spores are well developed, the surface of a colony on a solid medium has that powdery look which we associate with typical molds. The saprophytic species, found in the soil and elsewhere, are especially characterized by this mold-like development. But even these differ in several respects from the typical molds, and many varieties, especially the pathogenic strains, have marked similarities to the true bacteria.

The mycelium is always made up of fine, *narrow* filaments, only about 1μ wide (i.e., about the width of most bacterial cells), and therefore much thinner than the hyphae of molds. Also, the filaments are homogeneous throughout, without cross-walls or any visible internal structures. Moreover, most *Actinomyces* show, early in the growth, a *fragmentation of the mycelium* into round or rod-

Table XXVIII. Order Actinomycetales*

A. Mycelium rudimentary or absent	Family Mycobacteriaceae
Acid-fast bacilli	Genus *Mycobacterium*
B. True mycelium produced	
I. Vegetative mycelium fragments into bacillary or coccoid elements	Family Actinomycetaceae
a. Anaerobic or microaerophilic, parasitic, not acid-fast	Genus *Actinomyces*
b. Aerobic, partially acid-fast or non-acid-fast	Genus *Nocardia*
II. Vegetative mycelium does not fragment into bacillary or coccoid elements	Family Streptomycetaceae
a. Multiplication by conidia in chains from aerial hyphae	Genus *Streptomyces*
b. Multiplication by single terminal spores on short sporophores	Genus *Micromonospora*

* "Nomenclature and classification of the Actinomycetes" (S. A. Waksman and A. T. Henrici) *J. Bact.*, 46:337, Oct. 1943.

shaped segments that have the size and aspect of ordinary bacteria. Smears from actively growing cultures of this kind reveal only *bacteria-like elements* of various shapes scattered through the field (Figs. 12, p. 46; 18, p. 66; and 133: A, C).

The word *actinomyces* means "ray-fungi," and was suggested to Harz, who originally named these organisms in 1877, by the appearance of the organisms in the sulphur-yellow *granules* that appear in the discharge from tissues infected with the pathogenic variety, *Actinomyces bovis* (Fig. 133: B).

Unlike the true fungi, the actinomyces are sensitive to acid, and require a neutral or alkaline culture medium, such as would be used for the ordinary bacteria. The pathogenic species are cultivated in the laboratory with some difficulty and, for them, blood agar and other enriched media, like those used for pathogenic bacteria, are necessary.

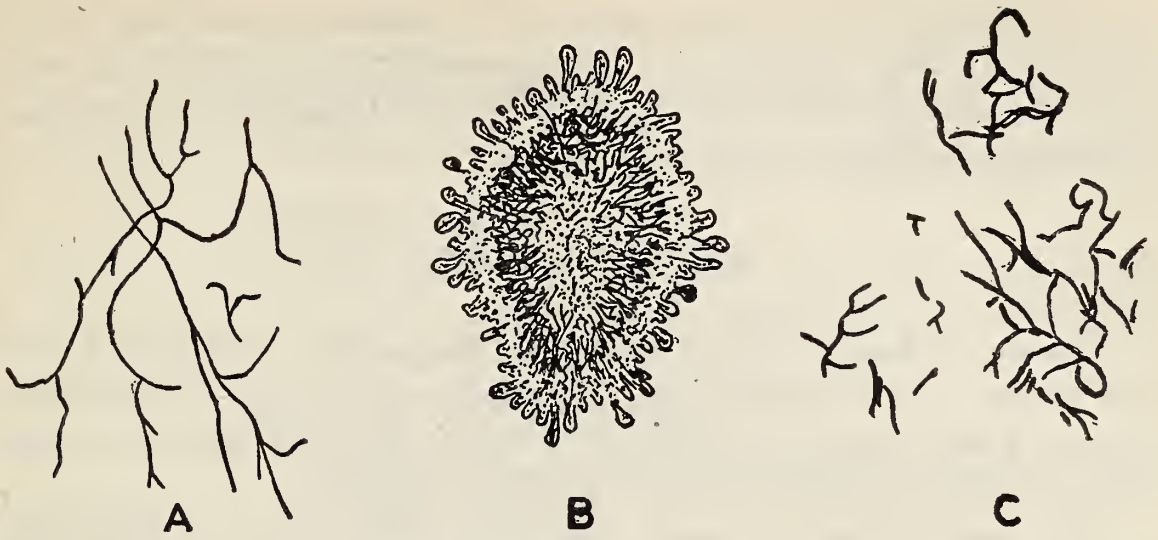


FIG. 133. *Actinomyces.* A: appearance of organisms in smear from a granule. B: an actinomyces granule as it appears when crushed upon a slide, showing the characteristic radiating structure with "clubs" at the periphery. C: actinomyces in smear from a culture.

Saprophytic actinomycetes. As much as 30% of the microorganisms that may be cultivated from the soil consist of various species of "actinomyces," principally those that are now placed in the genus *Streptomyces.* These organisms probably play an important rôle in the decomposition of dead plant and animal matter. Since these saprophytic varieties produce spores (conidia) which get into the air, it is not surprising that their colonies sometimes appear as contaminants on Petri-dish cultures. These colonies are usually recognizable by their tough, almost cartilaginous consistency, their close adherence to the agar, and the brownish discoloration in the surrounding medium.

Actinomyces in the normal mouth and throat. A number of investigators have recently cultivated several varieties of *Actinomyces* from the normal human mouth or throat. These organisms have been found most often in the crypts of the tonsils and in pyorrheal pockets about the teeth. Some of the cultures have been shown to be indistinguishable from the pathogenic species called *Actinomyces bovis* that causes actinomycosis.

Pathogenic actinomyces. There are several varieties of *Actinomyces* that are pathogenic for man and animals. We may recognize three principal pathogenic species and three main types of disease, as follows: (1) *Actinomyces bovis,* the most important species, and the cause of actinomycosis in cattle and in man; (2) *Actinomyces madurae,* the cause of some cases of *madura foot* and other forms of *mycetoma*—infections clinically similar to actinomycosis, but usually involving the foot, and seen mostly in the tropics; and (3) *Actinomyces asteroides,* and related strains, which cause relatively

rare infections of the lungs, and other tissues, resembling tuberculosis clinically, and also may be responsible for an infection similar to the actinomycosis caused by *A. bovis.* These latter organisms are *acid-fast* (Chapter XLI).

THE TRUE FUNGI

Classes of fungi. The true fungi (*Eumycetes*) are divided into four orders: (1) *Phycomycetes,* (2) *Basidiomycetes,* (3) *Ascomycetes,* and (4) *Fungi imperfecti* (Table XXIX). The last group is commonly called *Hyphomycetes* in medical writings.

This classification is based, first, upon differences in the structure of the filaments, when present. An individual filament is called a *hypha,* and the mass of branched hyphae that makes up the bulk of a filamentous fungus constitutes the *mycelium.* In most fungi the mycelium is *septate,* that is, the hyphae are divided by cross-walls (*septa*) into a series of cylindrical cells, end to end. But in one order (*Phycomycetes*), the filaments in the growing organisms are *nonseptate.* These nonseptate hyphae allow the multinucleated cytoplasm to *flow* without interruption throughout the filaments, and consequently the mycelium is said to be *coenocytic,* i.e., made up of protoplasm *shared in common* by the whole mold. Secondly, classification is based on the manner of reproduction, especially the way in which sexual spores, if any, are formed. Table XXIX shows the principal differential characteristics. The meaning of the terms used will be explained more fully later.

Phycomycetes. Included in this order are the common water molds that sometimes appear in goldfish aquaria, and related forms that parasitize water plants and fish. These are primitive fungi, apparently closely related to certain varieties of protozoa and algae. *Phycomycetes* means literally "seaweed fungi." Also in this class belong some of the most widely distributed and best known of the *common molds*—those white, cottony growths so often seen on barnyard manure, decaying foodstuffs, and as contaminants in Petri-dish cultures. The most familiar of these molds belong to the genus *Mucor,* or *Rhizopus.*

Basidiomycetes. Here belong the large, fleshy fungi, such as the toadstools, puffballs, and mushrooms, and also smaller forms, which are important plant parasites, known as smuts and rusts. Many of the latter type have complicated life cycles, involving a sexual phase

Table XXIX. Orders of the True Fungi

Eumycetes (true fungi)	Nonseptate mycelium 1. **Phycomycetes**—asexual spores (sporangiospores) formed in a closed structure (sporangium); produce zygospores (a kind of sexual spore). Septate mycelium (when present) 2. **Basidiomycetes**—sexual spores borne on special club-like stalks (basidia). 3. **Ascomycetes**—reproduce most commonly by asexual spores (conidia), but all are capable of forming sexual spores at times (ascospores) in an especially developed little sac (ascus). 4. **Fungi imperfecti**—(*Hyphomycetes*)—no sexual spores; reproduce by various types of asexual spores, including blastospores (budding).

in one plant host, and asexual phases in a different plant host. None of these fungi is pathogenic for man or animals.

Ascomycetes. This is the largest order of fungi, containing numerous important species. The many varieties included here differ materially from one another, but have one feature in common —namely, they all form, after a kind of fertilization process, sexual spores called *ascospores*. These are developed within an especially evolved sac, known as an *ascus*.

Common possession of this one structure brings together into this botanical group fungi which represent the opposite extremes of complexity—the typical, single-celled, true *yeasts* (genus *Saccharomyces*) on the one hand, and the common, highly organized, multicellular *molds* (such as those of the genus *Penicillium* and *Aspergillus*) on the other hand. Since the actual process of ascospore formation is much the same in all the many varieties of *Ascomycetes,* and is *very rarely observed* in any case, subdivisions among the molds within this great group are based upon the appearance, and manner of formation, of the *asexual* spores (*conidia*).

Fungi imperfecti, or **Hyphomycetes.** In this large order are placed those fungi which do not form ascospores, or other types of

sexual spores—or at least, such spores have not been observed. Hence these organisms have an imperfect—or imperfectly known—life history. The study of these "imperfect" fungi is difficult. We encounter among them bewildering irregularities in form and development; many have an abundant mycelium at times, while at other times they grow as yeast-like cells, and reproduction occurs through asexual spores elaborated in a confusing variety of ways. The pathogenic forms are notoriously hard to name and classify, and eminent mycologists disagree as to the proper designation of important species. We must utilize a working classification of the pathogenic fungi based only in part on botanical considerations.

It is no accident that *we find the majority of fungi pathogenic for man and animals among the Fungi imperfecti,* for it is a general principle, as we have previously noted, that, with the adoption of a parasitic, or semiparasitic existence, microbes regularly lose those specialized reproductive and protective structures which characterize related, free-living, saprophytic species.

Morphological features of the fungi. The structure of various fungi is illustrated in Figs. 134 and 136, and also in Figs. 16, p. 62; and 17, p. 63. These drawings, and the accompanying descriptions, should be consulted while the organisms themselves are being studied in the laboratory, and while reading the paragraphs which follow.

THE COMMON MOLDS

The molds which most often appear upon food and in agar cultures which have been opened to the air belong to several different genera. Among the best known of these common contaminants are members of the genus *Rhizopus, Mucor, Aspergillus, Penicillium, Alternaria,* and *Hormodendrum.*

Rhizopus nigricans. This is perhaps the most frequently seen of all the molds. It is often found on moldy bread, and as an air contaminant in Petri-dish cultures in the laboratory. When the spores settle upon an agar plate, they soon germinate and a meshwork of white filaments develops, which may fill the entire dish. This mold grows rapidly over the whole surface of the medium by means of its stolons—branches which extend away from the main body of the growing mold, like the runners of a strawberry plant. The mycelium is nonseptate (coenocytic), and rapid flowing of the

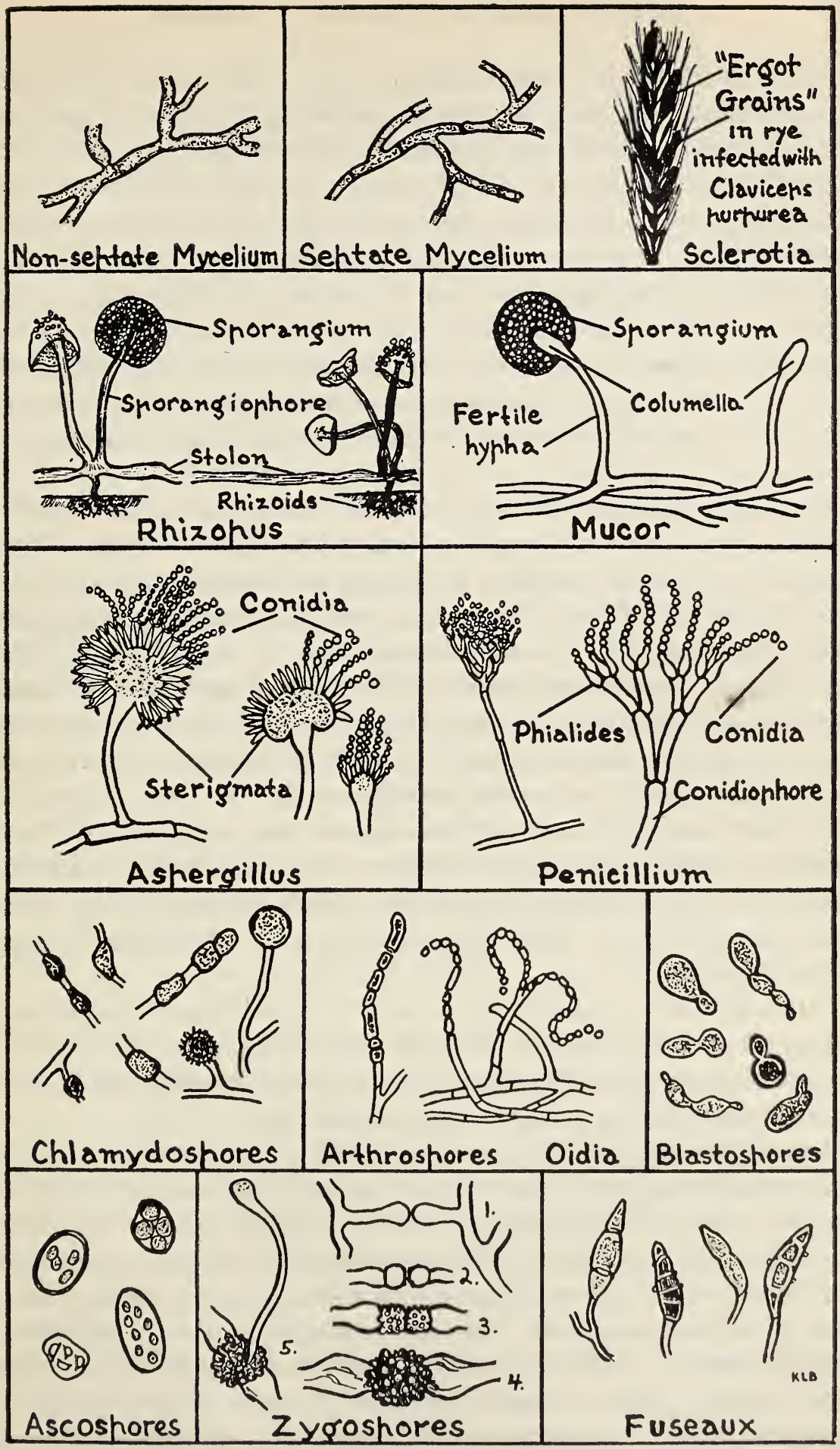


Fig. 134. Some of the morphological features of the fungi.

protoplasm through the filaments can be clearly seen. Where the tips of the stolons come in contact with the medium, a group of short, root-like, submersed hyphae (rhizoids) develops. Often the stolons climb the sides of the Petri dish and run along the under side of the lid, the rhizoids attaching themselves to the glass. At the points in the runners where the rhizoids appear, fertile aerial hyphae (sporangiophores) rise, and eventually bear the black spore cases (sporangia) within which the asexual reproductive spores (sporangiospores) are formed. The swollen tip of the sporangiophore, the columella, extends into the sporangium, and is clearly seen when the wall of the sporangium breaks open, releasing the spores.

Mucor mucedo. Of the many species of the genus *Mucor,* this is the best known. It can always be obtained by incubating fresh horse manure in a moist chamber. Its spores are common in dusty air. Like *Rhizopus* it forms a luxuriant, and rather coarse, nonseptate, white mycelium and black sporangia, and it may likewise fill a Petri dish in twenty-four hours. There are no stolons, or rhizoids, however, and the mold does not attach itself to the lid of the dish. Sporangiophores, branched and of unequal length, arise from all parts of the mold, and to the naked eye the sporangia appear as black dots scattered uniformly throughout the mycelium. The sporangium of different varieties of *Mucor* is built about a spherical, cylindrical, or pear-shaped columella, which becomes visible when the spore sac bursts open, allowing the mass of elliptical spores to be scattered.

In both *Mucor* and *Rhizopus* molds, sexual spores (zygospores) are occasionally formed by the union of two hyphae arising from neighboring growths. The zygospores are usually large, black structures covered with a rough, warty membrane.

Aspergillus niger. This common mold grows preferably on materials rich in sugar and of an acid reaction, but may be found on a great variety of substances. A white, or gray, septate mycelium forms, and soon the mold becomes recognizable by its characteristic conidiophores. These are long, unbranched hyphae, arising from a foot-cell in the mycelium. They are enlarged at their ends into a rounded body (vesicle), from which small, flask-shaped stalks (sterigmata) radiate. These primary sterigmata branch, in this species, to make still smaller projections, and on the tips of these are borne chains of black or brown conidia. The chains of spores are so

closely packed that the supporting structures often are hidden from view, and we see only the large, black sporeheads made up of a globular mass of pigmented spores. These "heads" are easily visible to the naked eye.

Other varieties of *Aspergillus* have spores of other colors, such as green or yellow, and also the mycelium may be variously colored. Some species develop rounded bodies called perithecia which contain asci and ascospores. These microbes generally prefer a somewhat higher temperature than other common molds, and many grow especially well at body temperature (37° C). They are often found on the human body; and at least one species, *Aspergillus fumigatus,* is pathogenic, as noted in the following chapter.

Penicillium glaucum. This is the name commonly applied to the familiar blue-green, powdery molds. These are often seen on fruits, and also may contaminate laboratory cultures, though not quite so frequently as the other molds already mentioned. They are readily distinguishable by the peculiar structure of their conidiophores. These branch toward their ends and finally terminate in a radiating cluster of flask-shaped cells (sterigmata), from the tips of which the small, round conidia develop. These spores are formed by successive divisions of the terminal cell and are pushed outward, so that the oldest spore is at the outermost end of the chain. The whole sporehead, under low power magnification, looks somewhat like a tassel, or brush, and this is the reason for the name *Penicillium,* derived from the Latin *penicillus,* a brush. The mass of newly formed spores gives the mold a powdery appearance to the naked eye.

There are numerous varieties of *Penicillium;* many have distinctive colors because of pigments contained in the conidia. Species of special interest are *P. roqueforti,* which is grown in Roquefort cheese to give it its characteristic flavor; *P. camemberti,* the mold in Camembert cheese; *P. expansum,* the cause of spoilage of apples; *P. digitatum* and *P. italicum,* the species responsible for spoilage of oranges and other citrus fruits; and *P. notatum,* from which the wonder-working antibiotic *penicillin* is derived.

Alternaria. Molds of this genus are often encountered as contaminants. They form dark, olive-green, brown, or black felt-like colonies, growing close to the agar surface. A loose, grayish-white aerial mycelium may eventually cover the original dark sporulating mycelium. Identification of these organisms is easy, because of the

large and characteristic dark-brown multichambered spores. These peculiar oval-shaped conidia are produced in chains, each formed by budding from the one immediately below. Branching spore-chains often occur.

Hormodendrum. Colonies of members of this genus somewhat resemble in appearance those of *Penicillium,* but the reverse side is gray or black. The conidiophores support branching chains of elongated, oval conidia, forming characteristic tree-like clusters. The conidia are formed in a peculiar manner, by a process of continuous budding. The first spore buds out to make a second one, then a third, and so on; the terminal spore in the chain is thus the youngest (Fig. 136: E).

Importance of the common molds. Some types of molds will grow on almost any object, including the walls and floors of a building, in the absence of sunlight and in the presence of moisture. In localities where the atmosphere is warm and humid, molds may develop on soiled, damp towels or clothing in the laundry bag, on the old shoes stored away in the closet, and on the books on the library shelves.

Molds sometimes grow extensively in the humid rooms of bakeries, and in creameries they may contaminate the butter churns. Certain kinds cause deterioration of textiles and the dry rot of lumber. Aspergilli and other common varieties cause much economic loss when they grow upon strawberries and other fresh fruits as these are being shipped to market. Foods which are well preserved from decomposition by *bacteria,* may nevertheless be spoiled by the growth of molds, because the latter develop (though slowly) at low temperatures in materials with a high concentration of sugar or salt, and in substances that are strongly acid. So we find that such foods as preserved fruit and jellies, butter, pickles, sauerkraut, vinegar, and salted and smoked meats may become moldy, even though kept in a cool place. The housewife often finds that some of her jars of home-canned foods, not having been sealed perfectly, in time show a growth of molds.

In the home and the hospital, the growth of molds is reduced by general cleanliness and by removal of dust. It is well to remember that dry objects do not "mold" easily, and that sunlight and cold keep molds down.

All the common types of molds are occasionally found in inflamed ears, eyes, nose, or skin of human beings, though there is

usually no clear evidence that they are in any degree responsible for the inflammation. The *Aspergilli* have been observed especially often in lesions of the lungs, and the species *Aspergillus fumigatus* is definitely pathogenic. Molds are the cause of some cases of *asthma* in individuals who are hypersensitive to the spores when these are inhaled.

Molds are helpful in some circumstances. Those in the soil contribute, along with the bacteria present, to the breakdown of complex organic waste into simpler nitrogenous compounds, which may be utilized by plants for food. Certain varieties are widely used in the Orient for the conversion of rice starch to sugar, as the first step in making alcoholic beverages. The preparation of citric acid from cane sugar by action of molds is an example of the commercial use of these organisms. We have already mentioned that some species of *Penicillium* are responsible for the characteristic flavor of certain popular types of cheese.

It may well be that future research will discover molds besides *Penicillium notatum* that are capable of producing valuable antibiotic substances.

THE YEASTS

The yeasts make up a group of fungi whose activities have always been of great practical importance in human affairs. Their usefulness in the preparation of bread, beers, and wines is well known to all. These microbes are widespread in nature; they are especially abundant wherever much sugar is present. Some varieties are always to be found, for example, in foodstuffs, especially cream and other dairy products, in honey, in the nectar of flowers, in the exuded sap of trees, and upon ripe fruits, notably grapes. They occur also in soil, on vegetation, and as commensals in insects and animals.

Chief characteristics. The yeasts stand out from all the other fungi, because they occur constantly as single, budding cells, and do not form the branching filaments (mycelium) which characterize the molds. Many other fungi *temporarily* assume a single-celled, budding form, but eventually develop a mycelium under proper conditions. Only the genuine yeasts never do. The yeasts have almost certainly originated from molds, by losing permanently the power to form a mycelium (Fig. 17, p. 63).

The typical yeast cell is a colorless, oval, or round body, which

is about 10–15 microns in diameter. The size varies greatly. Many yeasts have thick walls, giving the cells a double contour or outline. Each organism has a nucleus (though this is not often visible). The cytoplasm, especially in older individuals, commonly contains many granules and vacuoles, and often large droplets of fat.

A few kinds of yeasts multiply by splitting of the cell into two parts (fission) like the bacteria, but all the common varieties reproduce by *budding*. In the budding process, a small swelling first appears at the edge of the cell. The swelling gradually increases in size, and becomes constricted at the base, so that it has a very narrow connection with the parent cell. This knob-like protrusion is the bud, or blastospore. The bud finally becomes entirely separated from the parent organism and develops into a full-sized yeast. Then it, too, may form buds and so continue the reproductive process. When growth is rapid, a series of buds may appear, which do not fully separate, but form clusters or short chains of oval units.

Two main groups are recognized among the yeasts: (1) the common, harmless bread, beer, and wine yeasts (mostly placed in the genus *Saccharomyces*), which occasionally form *ascopores* in addition to reproducing by budding, and (2) a group of yeasts in the genus *Torula* (or *Cryptococcus*) that multiply by budding, but do *not* form spores. Among the *Torulae* there are both harmless and highly pathogenic species.

Saccharomyces. These kinds of yeasts have been extensively studied because of their great importance in the manufacture of beers, wines, and bakery goods, as well as their value in other industries which depend upon a process of fermentation (decomposition of sugars and similar substances). The relation of the yeasts to fermentation was discovered as early as 1837 by Schwann, but the complete demonstration of the fermenting powers of yeasts was first given by Pasteur, about 1865. The studies of the Danish investigator Hansen, about 1880, added much to our practical information about the yeasts, and led to the now universal practice of using, in fermentation industries, pure strains of yeasts, cultivated in the laboratory and especially selected for their efficiency in bringing about a particular type of fermentation.

The most important of the fermentative changes produced by yeasts is the breaking down of sugar to form *alcohol* (so-called alcoholic fermentation). The reaction is represented by the following equation:

$C_6H_{12}O_6$ (sugar) $\rightarrow 2C_2H_5OH$ (alcohol) $+ 2CO_2$ (carbon dioxide)

Formation of alcohol goes on in this way when living yeasts grow in a sweet solution—for example, in the juice of ripe grapes. Either the wild yeasts naturally present on the grape skins, or cultivated yeasts deliberately added to the mixture, may be used to bring about the alcoholic fermentation, but the latter are preferable because especially chosen wine yeasts are not only good alcohol formers, but they are resistant to rather high concentrations of alcohol, so that they continue to be active while the alcohol content climbs to 10 or 12% or higher. The actual transformation of sugar to alcohol occurs through the action of the enzyme "zymase," which may be expressed from the yeast cells by pressure.

Saccharomyces cerevisiae is the name most often applied to the common bread and beer yeasts.

The familiar yeast cake is composed of compressed living yeast cells, mixed with a little starch. In the making of bread, the first gentle heating of the dough causes the added yeasts to multiply. They ferment the sugar present and cause the release of carbon dioxide. This harmless gas escapes through the dough, leaving countless small holes. Thus the dough rises and the bread becomes light and porous.

Torula (Cryptococcus). These are distinguished from the yeasts above described by the fact that they fail to form ascospores (or other types of spores) under any circumstances. They also differ in other respects. They are smaller, spherical cells, less widely distributed, and not of practical use in fermentation industries. The most commonly encountered of the harmless saprophytic torulae are the so-called red torulae. These are seen not infrequently as contaminants in bacterial cultures, and are especially numerous in cream and other dairy products. Their colonies on solid media are sticky and mucoid, and have a red or pink color.

The medical importance of these organisms arises from the fact that a species of torula, called *Torula histolytica* (*Cryptococcus neoformans*), is highly pathogenic for human beings, and may cause a fatal infection of the brain, as described in the following chapter.

LABORATORY STUDY OF FUNGI

The general methods used in the study of bacteria are employed also in mycology. Isolation and identification of fungi require, how-

ever, a number of special techniques not ordinarily made use of by the bacteriologist.

Isolation and cultivation. In securing a pure culture of molds or yeasts, advantage is taken of their peculiarly high resistance to acid, and their preference for a medium containing a large amount of fermentable sugar. Good media for isolation purposes may be made

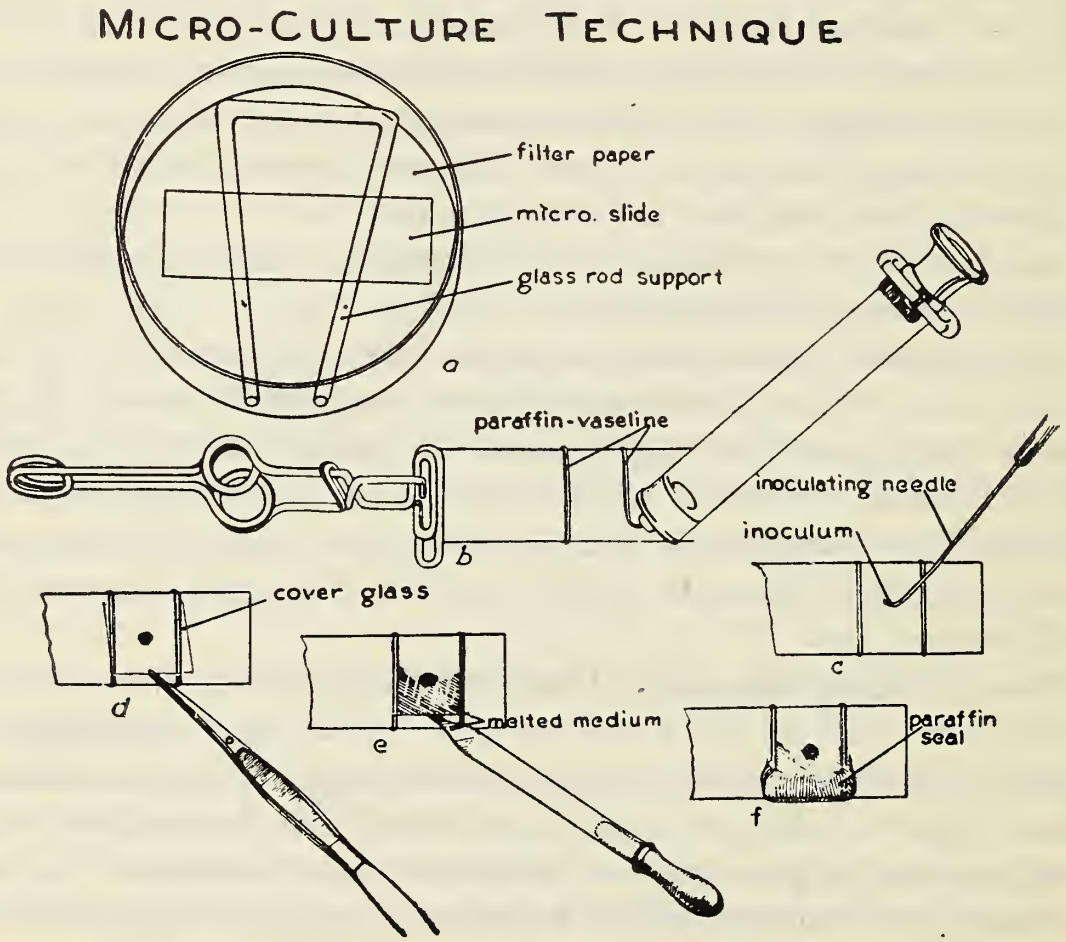


FIG. 135. Steps in the preparation of a microculture for the study and identification of fungi. The paraffin-vaseline mixture contains 30 parts of paraffin to 70 parts of petroleum jelly by weight. The recommended medium is potato-dextrose agar. (From Johnson, E. A., "An Improved Slide Culture Technique for the Study and Identification of Pathogenic Fungi," *J. Bact.*, 51:689, June 1946.)

from the ordinary nutrient broth or agar, as used for bacteria, by adding about 5% of dextrose, and by making the medium strongly acid—so acid that bacteria are inhibited, while the relatively slow-growing fungi have a chance to develop unhindered.

A medium widely used for obtaining the first growth of fungi is a solution of peptone (1%), and crude maltose or glucose (4–5%) in water, of acid reaction (pH 5.5), to which agar in the usual proportions (1.5%) may be added. The original formula for this medium

was developed by Sabouraud, a French mycologist who has contributed much to our knowledge of the fungi. *Sabouraud's medium* is especially useful for cultivation and identification of the fungi causing skin diseases in man. Other pathogenic fungi may require a richer medium; and in attempting to isolate any fungus directly from human or animal hosts, inoculations should be made upon a variety of different nutrient substances, including *blood agar* and other media commonly used for pathogenic bacteria. Duplicate cultures should be incubated at 37° C and at room temperature.

Once separated from other organisms in a pure culture, most fungi grow readily, though rather slowly, on the usual bacteriological culture media. Mycologists often use, in addition, concoctions made from vegetables (potato agar, cornmeal agar, etc.) and from other natural substances. Sometimes a fungus will show fruiting bodies on some of these more complex media when it will fail to fructify on the simpler ones. Yeasts are often cultured in beer wort, whey, or similar liquids.

Two special types of cultures are of great value in studying fungi: (1) *slide cultures* and (2) *giant colony cultures.*

Slide cultures. These may be made in several different ways, with either liquid or solid media. In these miniature cultures on slides, the development of a fungus from day to day may be observed directly under the microscope. A type of slide culture developed by Johnson is suitable for the identification of pathogenic fungi, as well as for the study of saprophytic species, and it permits the preparation of permanent, stained slides of the growth (Figs. 135, 136).

Giant colonies. These are made by inoculating the very center of the surface of agar medium contained in an Erlenmeyer flask or in a Petri dish. From this central spot a single, round, giant colony develops which may cover almost the whole surface (Fig. 136). When properly protected from drying, such cultures may be kept for months.

The naked-eye appearance of giant colonies is sometimes so characteristic as to permit recognition of a fungus at once. Frequently the underside of a colony has a distinctive look or color. Further study of the growth is made with a magnifying glass, or wide-field microscope. Under low magnification the chief types of common molds are easily recognizable.

Microscopic examination. For the finer details of structure, it is necessary to make slide preparations for examination with the

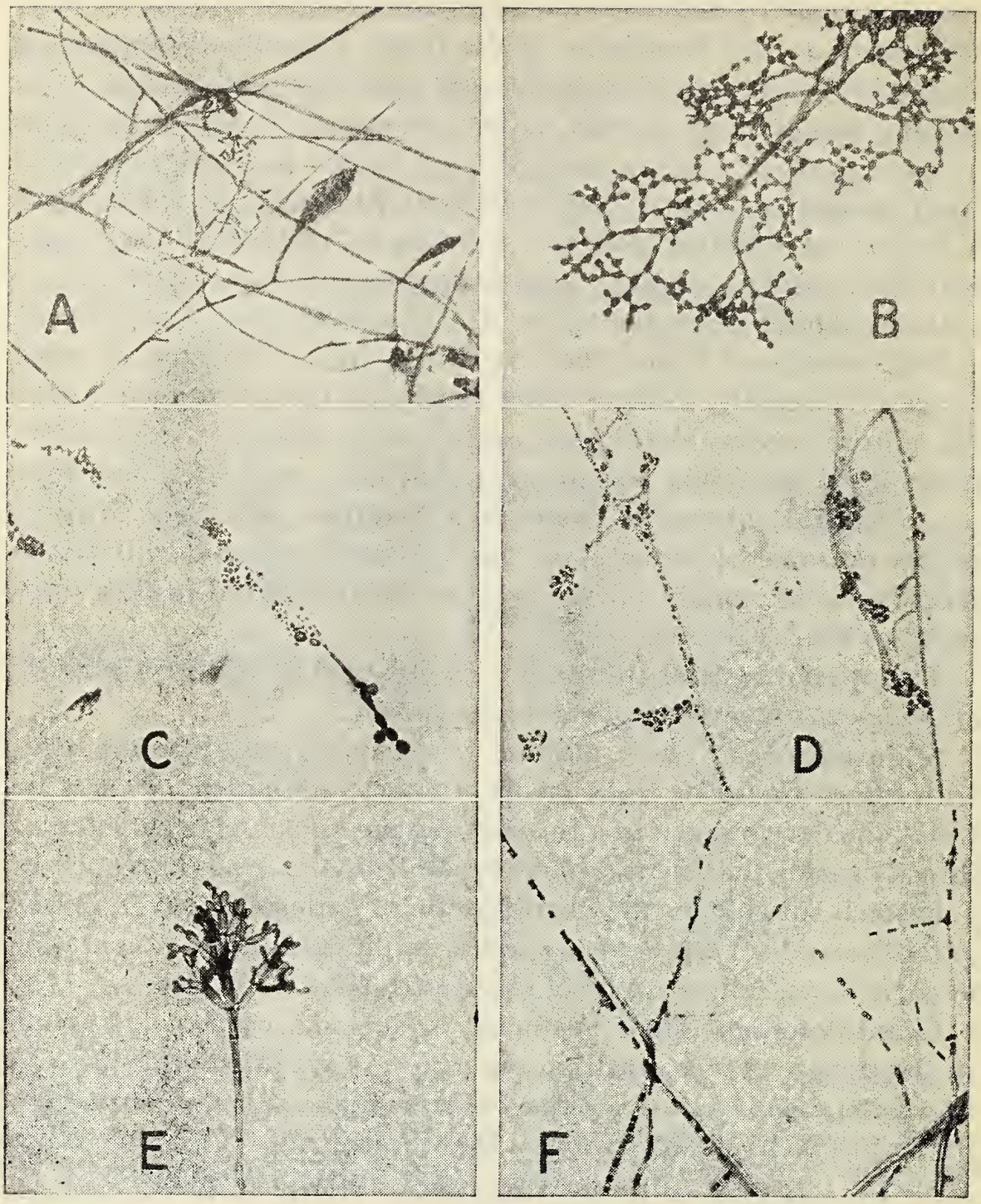


FIG. 136. Photomicrographs of various fungi as they appear in permanent, stained preparations made from microcultures on potato dextrose agar, incubated at room temperature for 3 to 14 days (Johnson's method). A: *Microsporum gypseum,* (x400); B: *Monilia sitophila,* (x200); C: *Candida albicans,* (x400); D: *Sporotrichum schenkii,* (x900); E: *Hormodendrum compactum,* (x400); F: *Coccidioides immitis,* (x400). The characteristic reproductive structures are shown as follows: A: macro- and microconidia; B: blastospores (conidia formed by continuous budding); C: blastospores and terminal chlamydospores; D: conidia in clusters; E: branching conidia; and F: arthrospores. (Photographs by C. G. Breckenridge.)

ordinary microscope. Microscopic techniques in mycology differ from the routine bacteriological methods in one important respect—the fungi are regularly examined in *wet* preparations, rather than in dried, stained smears as customarily used in the study of bacteria. Only in exceptional cases is a dried smear of any value.

To make a preparation from a laboratory culture of a mold, some of the growth may first be gently removed with a stiff needle, or

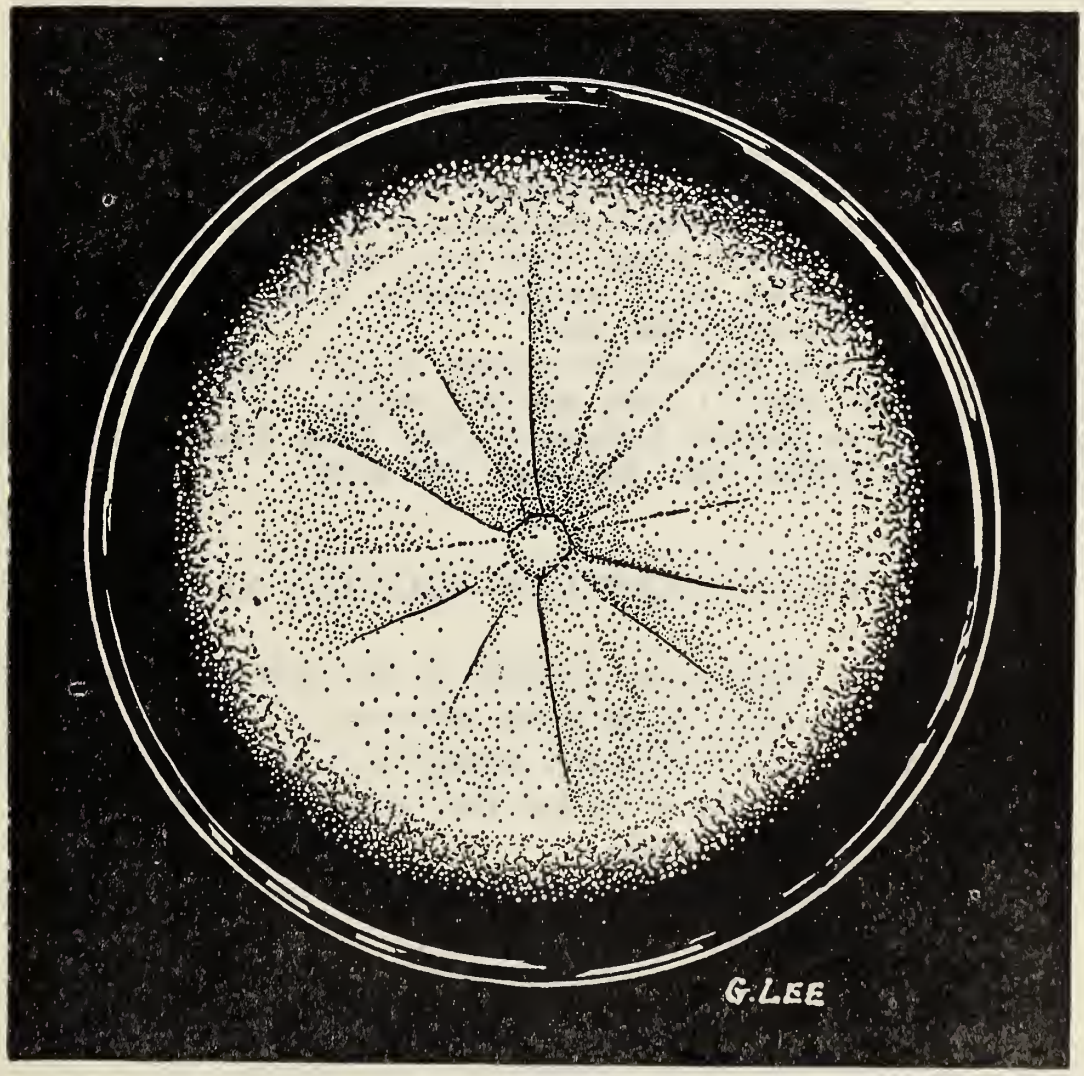


FIG. 137. Giant colony of a fungus (*Microsporum audouini*) in a Petri dish.

better, a particular hypha or group of hyphae (with the associated reproductive elements) may be picked carefully out of the growth with a pair of small forceps, while watching through a low-power microscope. This material must then be transferred to a drop of mounting fluid on a slide, covered with a coverslip and examined, with reduced light, by the low- and high-power objectives. The fungus must be gently handled, for otherwise the spores and other structures will be displaced from their normal positions.

As a mounting fluid for fungi from cultures, ordinary physiological salt solution is satisfactory, but water, alcohol, and various other fluids are used, and especially often a 5 or 10% sodium hydroxide solution. The latter has the effect of clearing the fungus, making it more transparent. The mounting fluid may or may not have an added dye, such as dilute eosin. Yeast cells from a yeast cake are commonly suspended in saline, to which dilute iodine solution is added, so as to color blue the starch granules present.

REFERENCES

Conant, N. F., Martin, D. L., Smith, D. T., Baker, R. D. and Callaway, J. L.: *Manual of Clinical Mycology*. Philadelphia: W. B. Saunders Co., 1944.

Henrici, A. T.: *Molds, Yeasts and Actinomycetes*. New York: John Wiley and Sons, Inc., 1930; and "The Yeasts," *Bact. Rev.*, 1941, 5:97.

Lewis, G. M. and Hopper, M. E.: *An Introduction to Medical Mycology*. Chicago: Year Book Publishers, Inc., 1943.

Mackinnon, J. E. and Artagaveytia-Allende, R. C.: "The So-Called Genus *Candida berkhout*, 1923," *J. Bact.*, Apr. 1945, 49:317.

Rosebury, T.: "The Parasitic Actinomycetes and Other Filamentous Microorganisms of the Mouth," *Bact. Rev.*, Sept. 1944, 8:189.

REVIEW QUESTIONS—CHAPTER XL

1. What kinds of microbes are classified in the Order *Actinomycetales*? Describe the organisms of the genus *Actinomyces*.
2. What properties characterize the saprophytic actinomyces of the soil? What is the importance of the fact that actinomyces may be found in the normal mouth or throat?
3. Name three species of pathogenic actinomyces and state the disease conditions with which each is associated.
4. Define *Eumycetes, nonseptate, coenocytic*. Name the four classes of fungi. What is the basis for this classification?
5. Describe briefly the fungi classified as *Phycomycetes* and *Basidiomycetes*.
6. What feature is shown by all members of the class *Ascomycetes*? Name some common fungi classified in this group.
7. What are the *Fungi imperfecti*? Discuss the significance of the fact that many pathogenic fungi belong to this group.
8. How many special morphological features of the fungi can you name and describe?
9. Name six genera of common molds; describe, briefly, typical members of each genus. What are the differences between *Rhizopus* and *Mucor*?
10. Discuss the importance of the common molds. Give some instances in

which mold growth is harmful, and other instances in which molds are helpful. Name the species of mold from which penicillin is derived.

11. Describe the chief characteristics of the yeasts. What distinguishes them from common molds?
12. Name the genus in which the common bread yeasts belong. Explain the useful activities of these organisms.
13. Name a genus of pathogenic yeasts. How do these differ from the common harmless yeasts?
14. Outline methods used in the isolation and cultivation of fungi in the laboratory.
15. What special methods are used in the microscopic study of fungi?

CHAPTER XLI

ACTINOMYCOSIS AND THE PRINCIPAL FUNGOUS INFECTIONS

ACTINOMYCOSIS

Clinical features. The human disease known as *actinomycosis* is fairly common and of world-wide occurrence. A similar infection is frequently seen in animals, especially in cattle, horses, and swine.

The human infection is customarily classified clinically as: (1) cervicofacial, (2) thoracic, or (3) abdominal, according to the site where the infection begins. The first-mentioned form of the disease is most common. Beginning around the posterior part of the lower jaw, or in the region of the tonsils, the infection characteristically involves the tissues of the face and neck, at the angle of the jaw. Subcutaneous nodules form over this area; the infected region shows a hard, brawny swelling. Later, the swollen areas soften and pus drains from multiple openings in the skin. (In cattle, the nodular swellings around the jaw account for the popular name for the disease, "lumpy jaw.")

Usually the disease progresses very slowly, but there is severe injury to the affected parts. The bones, as well as the soft tissues, may be destroyed. The infection spreads through the tissues by continuity; unlike the case in tuberculosis and syphilis, the distant lymph vessels and glands draining the area are not involved. Usually, if the mouth parts only are infected, the disease is slowly overcome, but death may be caused by streptococci or other invasive germs which have invaded the injured tissues.

Sources and modes of infection. Just how actinomycosis is acquired is not known precisely. But since pathogenic strains of *Actinomyces bovis* have been found in the tonsils and around the teeth of apparently normal persons, it seems most probable that infection occurs when these organisms are given the opportunity to enter the tissue because of the loss of the usual level of local or

general resistance. A history of a preceding tooth infection and extraction is often obtained, and poor oral hygiene undoubtedly increases the liability to the disease.

Infection of the lungs presumably arises from aspiration of infected material from the mouth; and the intestinal infection, which usually begins in the ileocecal region, is probably due to *Actinomyces* that have been swallowed with the saliva.

Actinomyces bovis. This is an anaerobic, Gram-positive organism which grows as a tangled mass of branched filaments, each about 1μ wide, but which readily fragments into short rods that look much like diphtheroids. In the pus from the lesions of actinomycosis, it is usually organized into yellowish granular bodies, the so-called "sulphur granules." These are about 2 mm in diameter, and may be recognized with the naked eye. When a granule is crushed under a cover slip on a glass slide and examined without staining, it appears as a rosette-like mass, made up of a dense network of slender filaments which radiate outward from the center—hence, the name "ray-fungus" (Fig. 133: B, p. 645). The curious structure of the granule is best seen if a little 20% sodium hydroxide solution is added to the preparation. The free ends of the filaments at the outer edge of the granule are usually thickened, forming what are called actinomyces "clubs." These thickened, clubbed ends probably represent the reaction of the organism to the body fluids which surrounded the granule. If we apply the Gram stain, the central mass of filaments stains Gram-positive, but the clubs may appear Gram-negative.

Actinomyces bovis can be isolated and cultivated in the laboratory, but only with considerable difficulty. Shake cultures made in deep columns of beef or veal infusion glucose agar are often used. In such cultures, incubated at 37° C, colonies appear in 4 or 5 days as irregular, white masses 5 to 10 mm below the surface.

Laboratory diagnosis. A diagnosis of actinomycosis is made when the characteristic sulphur-yellow granules, made up of the ray fungus as above described, are found in the pus. The yellow granules must be searched for, fished out with a loop or capillary pipette, crushed under a cover slip, and examined unstained. Gram-stained preparations should also be made. When pus is not available from a suspected actinomycotic lesion of the skin, a sterile saline gauze compress may be placed over the infected area. When the compress is removed after about twelve hours, sulphur granules may be found clinging to the gauze.

If cultures are attempted, they should be made with material from a previously unopened abscess.

Actinomycosis due to Actinomyces asteroides. Occasionally actinomycosis has been shown to be caused not by the anaerobic *Actinomyces bovis,* but by the related aerobic, acid-fast organisms named *Actinomyces* (or *Nocardia*) *asteroides.* Infection with this species is especially likely to occur in the lungs, resulting in a clinical picture that may closely resemble tuberculosis.

These organisms may often be found in the sputum, or in pus from lesions elsewhere, in smears stained by the acid-fast method, where they appear as delicate, branching acid-fast filaments. Cultures may be secured by making a heavy inoculation upon Sabouraud's glucose agar slants.

PRINCIPAL FUNGOUS DISEASES OF MAN

Mycotic infections in human beings are common. Sometimes they are a serious threat to life; but more often they cause, on the body surface, unsightly and uncomfortable lesions which are stubbornly resistant to treatment.

Superficial mycoses. The commonest of the fungous diseases are *superficial infections,* involving only the skin or the mucous membranes. These mycoses fall into two large groups: (1) the various forms of *ringworm* (or *tinea*), in which filamentous fungi invade the external skin layers, hairs, or nails, but not the internal organs; and (2) the several clinical varieties of *moniliasis,* caused by a yeast-like organism. In addition, there are the less frequently seen skin infections called: (3) *tinea* (or *pityriasis*) *versicolor,* (4) *erythrasma,* (5) *otomycosis,* and (6) *chromoblastomycosis* (Table XXX).

These superficial mycoses are more common and more severe in places where the climate is warm and humid; they are especially prevalent in the tropics. Many of our troops acquired fungous skin diseases ("jungle-rot") while on duty in the Pacific theater during World War II.

Deep mycoses. A rather small group of mycotic infections, in contrast to those mentioned above, must be classified as *deep mycoses*—that is, diseases in which the causative fungi characteristically invade the lungs or other internal parts of the body. The infection is either actually systemic (generalized) or potentially so. These mycoses are serious, and not infrequently fatal. They are all relatively rare, however. Even so, some of them are sufficiently

common in the United States to be considered as a possible diagnosis in many cases of infection of obscure nature. Principal mycoses of this kind are: (1) *coccidioidomycosis,* (2) *blastomycosis,* (3) *torulosis,* (4) *sporotrichosis,* (5) *histoplasmosis,* (6) *mycetoma,* (7) *aspergillosis,* and (8) *rhinosporidiosis* (Table XXX).

RINGWORM, OR TINEA

Most common of the fungous diseases of man are those infections of the skin, hair, or nails, generally referred to as *ringworm* or *tinea.* On the skin, the lesions frequently take a circular form, thus making a ring of infected tissue. Many of the ringworm infections, especially those common in children, are communicable, and must be guarded against in schools, children's hospitals, and similar institutions. Ringworm is a common disease in dogs, cats, horses, and other domestic animals, and many cases in human beings are acquired by contact with infected animals.

The ringworm fungi. The microorganisms responsible for ringworm infection, known as the *dermatophytes,* all belong to the *Fungi imperfecti.* They do not form distinctive conidiophores for the bearing of reproductive spores, but small, pear-shaped *microconidia* develop in most cases along the sides, or in clusters at the ends, of undifferentiated hyphae. In addition to these small spores, many of the dermatophytes form those curious, elongated, spindle-shaped, multichambered *macroconidia* called *fuseaux* (Fig. 134). These fungi all grow slowly, eventually forming dense colonies which usually become covered with a downy or powdery growth of aerial hyphae.

Because of their complexity and variability, their systematic classification on purely mycological grounds is difficult and unsatisfactory. It is customary to follow the suggestions of Sabouraud, who met this difficulty by arranging these fungi into groups according to their appearance in or about infected hairs. He recognized as one group the organisms of favus (*Achorion*)—forms that invade the hair but also, as we shall see, form characteristic yellow crusts at the base of the hairs. The fungi associated with the more common ringworm diseases he then placed in the following three genera:

(1) *Microsporum*—forms which develop a mass of small, round spores forming a collar about the base of the hair, just beneath the skin surface.

Table XXX. Principal Mycotic Infections of Man

NAME OF DISEASE	NATURE OF THE INFECTION	PRINCIPAL CAUSATIVE FUNGI
	THE SUPERFICIAL MYCOSES	
Ringworm (Tinea) Tinea capitis	Ringworm of the scalp	*Microsporum audouini, M. lanosum*
Tinea barbae	Ringworm of the beard	*Trichophyton gypseum*
Tinea glabrosa	Ringworm of the smooth skin	*M. lanosum, T. gypseum, T. purpureum*
Tinea cruris	Ringworm of the groin (eczema marginatum; dhobie itch)	*Epidermophyton floccosum (inguinale)*
Favus	Chronic fungous infection, usually of scalp, with yellow crusts	*Achorion (Trichophyton) schoenleini*
Dermatophytosis	Fungous infection of the hands, feet, or nails	*T. gypseum, T. purpureum, E. floccosum*
Moniliasis	Localized infections of skin, of mucous membranes of the mouth, vagina, etc., or of the nails; rarely systemic disease	*Monilia (Candida) albicans*
Tinea versicolor	Chronic fungous infection of the most superficial layer of skin	*Malassezia furfur*
Erythrasma	Superficial fungous infection of skin, usually in groin or axilla	*Actinomyces minutissimus*
Otomycosis	Chronic or subacute infection of external ear canal	*Aspergillus, Penicillium, Mucor, Monilia, etc.*
Chromoblastomycosis	Superficial skin infection, usually of legs, characterized by large, colored, warty or cauliflower-like nodules	*Hormodendrum pedrosoi*

THE DEEP MYCOSES (SYSTEMIC OR POTENTIALLY SYSTEMIC)		
Coccidioidomycosis	Commonly occurs as a benign, self-healing lung infection, but may become a progressing, generalized, fatal infection	*Coccidioides immitis*
Blastomycosis	Chronic infection, chiefly of skin, lungs, and bones; may be localized in skin, or generalized	*Blastomyces dermatitidis*
Torulosis	Subacute disease, may involve lungs or skin, but characteristically appears as fatal infection of brain and meninges	*Cryptococcus neoformans* (*Torula histolytica*)
Sporotrichosis	Chronic infection of lymph vessels, usually of arm, with characteristic nodular swellings which soften and ulcerate	*Sporotrichum schenkii*
Histoplasmosis	Generalized chronic fungous infection, with emaciation, anemia, fever, various clinical manifestations (Rare)	*Histoplasma capsulatum*
Mycetoma	Chronic fungous infection of feet (Madura foot)	*Various actinomyces* (*Nocardia,* etc.) and true fungi (*Madurella mycetomi,* etc.)
Aspergillosis	Inflammatory, granulomatous lesions of lungs, skin, external ear, etc.	*Aspergillus fumigatus*
Rhinosporidiosis	Infection of nose, eyes, ears, larynx, etc., characterized by development of friable tumors (polyps) (Rare)	*Rhinosporidium seeberi*

(2) *Trichophyton*—all other varieties that invade the hair.

(3) *Epidermophyton*—fungi that do not attack the hair, but invade the epidermis only.

Ringworm of the scalp, beard, and smooth skin. *Tinea capitis.* *Microsporum audouini* is recognized as the species responsible for the classical form of ringworm of the scalp (tinea capitis) in children, known as the "gray patch." This rather common, and sometimes epidemic, disease is thought to be entirely of *human* origin. The affected areas lose their normal hair, and become covered with grayish scales and the short stumps of diseased hairs. When one of these infected hairs is removed, we find its base surrounded by a whitish collar of small spores (2 to 3μ in diameter). These are often arranged in a regular mosaic pattern, like the tiles in a floor. The shaft of the hair is not invaded. Once established, this fungus is likely to persist in the scalp for many months. There is little tendency for the infection to spread to other parts of the body.

In children, and adults as well, infection of the scalp or of the smooth skin with *Microsporum lanosum* is often of *animal* origin. Young dogs, cats, and horses are usually the sources of infection. Ringworm due to this species tends to clear up spontaneously in two or three months.

Other cases of tinea capitis are caused by fungi of the genus *Trichophyton,* especially *T. violaceum, T. crateriforme* or *T. gypseum.* A very persistent form of infection of the scalp, known as "black dot ringworm," is caused by *T. violaceum.* The infected areas of the scalp show tiny dark spots where the hairs have broken off close to, or just below, the surface.

The contagiousness of ringworm of the scalp is sometimes not appreciated by laymen. It is important that precautions be taken to prevent the spread of this disease, and particularly that efforts be made to protect children from those persistent forms of human ringworm that are transmitted by direct contact of one child with another. Contaminated combs, hats, or other articles may carry these fungi. Animal pets ought to be examined frequently for ringworm and, if necessary, promptly treated.

Mycological diagnosis of ringworm of the scalp is made by carefully removing infected hairs from the area involved—usually hairs that come out most easily are the diseased ones—mounting them in 10% sodium-hydroxide solution under a cover glass, and noting the size, position, and arrangement of the fungous spores.

Tinea glabrosa. Ringworm of the smooth skin is due to spread of the fungi to the skin of the trunk, face, or limbs, from a focus of ringworm infection elsewhere on the body, as on the scalp, nails, inguinal region, or feet. All the dermatophytes mentioned above are capable of causing this kind of mycosis. Most cases are due to *Microsporum lanosum, Trichophyton gypseum,* or *T. purpureum.* The typical skin lesion is roughly circular, in the form of a ring of raised, reddened, scaly skin, which gradually increases in diameter. To rid a patient permanently of this kind of ringworm, attention must be directed toward discovering and treating effectively the original focus of the infection in the scalp or elsewhere on the body.

Tinea cruris. This is a clinically distinct fungous disease of the superficial skin, also known as *eczema marginatum,* which is typically confined to the inner surfaces of the thighs in the region of the groin. The lesions are red, with a scaly surface and a distinct border. An especially severe form of this infection is known in the tropics as "dhobie itch." The disease is sometimes spread among young men by contaminated athletic clothing or suspensories.

The causative fungus is named *Epidermophyton inguinale* (or *E. floccosum*). It differs from those mentioned above, in its failure to invade the hairs. Instead, the infection is confined to the superficial layers of the epidermis.

Favus. This disease may be regarded as a form of tinea capitis, though the causative fungus, *Achorion schoenleini,* differs somewhat from the common ringworm fungi. Favus is characterized by the appearance of a yellow, cup-shaped crust (scutulum) about the base of the diseased hair. These crusts, which consist of dense masses of mycelium and spores, press upon the underlying skin, causing inflammation, scarring, and permanent loss of the hair. The infection may also involve the smooth skin, or the nails. The disease is rare among native Americans, and in the United States is seen most commonly in immigrants from eastern Europe.

Dermatophytosis. This is one of the general names commonly given to the superficial fungous infections involving, primarily, the sites on the body where opposing skin surfaces come together, as in the webs between the toes or fingers, and in the axilla or groin. Secondary infections of the hands and nails, and the allergic skin manifestations called *dermatophytids,* may also be mentioned under this heading.

Athlete's foot. The condition known as *"athlete's foot"* (also

spoken of as ringworm of the feet) is the outstanding example of a dermatophytosis. The lesions on the feet usually begin between the toes, then spread to the soles. As many readers doubtless know from personal experience, the infection is most persistent and annoying. It is apparently spread in schools, gymnasia, swimming pools, and similar places where persons go about barefooted. In all public swimming pools and like places, there should be facilities for disinfection of the feet, in order to reduce the possibility of spreading the disease.

When microscopic preparations are made from the infected skin between the toes, mycelial threads and chains of spores are usually found. A careful study of cultures is required for identification of the fungi. The majority of cases are due to *Epidermophyton floccosum, Trichophyton gypseum,* or *T. purpureum.*

Simple precautions to prevent this all-too-common fungous infection are worth while. The toes should be carefully dried after bathing, and then an appropriately medicated dusting powder should be applied. Socks should be changed every day or so, and walking barefooted in bathrooms, shower rooms, and similar places should be avoided as much as possible.

A chronic infection of the *nails* of the feet or hands, in which the nails become lusterless, yellowish, thickened, and brittle, is a common sequel to fungous disease of the feet. This lesion is very resistant to treatment, especially when the causative organism is *T. purpureum.*

Dermatophytids. An important feature of the superficial skin infections with *Trichophyton,* and other dermatophytes, is the frequent development of a high degree of cutaneous *sensitivity* to the fungi or their products. This hypersensitivity manifests itself spontaneously in some patients by the development of secondary skin eruptions on the hands, or elsewhere, owing to the dissemination of the fungi or their antigenic products by way of the blood stream. Such an eruption, occurring in sensitized individuals, is known as a *dermatophytid.* Often this eruption takes the form of rows or clusters of small vesicles along the sides of the fingers or on the back of the hands. These "id" lesions are free of fungi; the organisms can be found ordinarily only in the primary focus of the infection, as, for example, on the feet. The dermatophytid usually disappears after the primary lesions are treated and cleared up.

MONILIASES

Clinical types of moniliasis. There is a large group of fungous infections of the mucous membranes, skin, and other tissues, which are attributed to yeast-like organisms classified in the genus *Monilia.* (Martin and some other recent authors prefer the genus name *Candida.*) The best known of these infections is called *thrush.* This is an acute inflammation of the mucous membrane of the mouth, gums, tongue, or pharynx, characterized by the appearance of a whitish, membranous patch over the inflamed area, resembling the membrane of diphtheria. In shreds of this membranous material, the causative organisms are easily found. Thrush is quite common in ill-nourished and ill-kept children. The infection may be carried readily from child to child in a hospital ward, unless care is exercised.

Besides the typical thrush, a similar infection with *Monilia* may be seen in the angle of the mouth, about the genital and anal region, or between the fingers and the toes, especially in infants and young children. The causative fungi are common inhabitants of the intestine, and they may get upon the body surfaces. Apparently, most patients are infected with *Monilia* which they themselves are carrying, and any local or general condition (such as diabetes) that lowers body resistance predisposes to moniliasis.

In adults, *Monilia* have been found associated with a variety of pathologic clinical states resulting from infection of the skin, of the mucous membranes of the *mouth* or *vagina,* and occasionally of the *bronchi* or *lungs.* Lesions of the hands have most often been observed in dishwashers, bartenders, and others whose hands are softened by continual immersion in water. A vulvovaginitis due to *Monilia* is rather common in pregnant Negro women of the poorer classes.

Monilia albicans. This is the name most commonly applied to the fungus associated with the infections mentioned. It is also known as *Candida albicans* (Fig. 136: C, p. 658).

These organisms appear, in scrapings from the lesions, as a network of slender, branched, septate threads and clusters of spores. Thin-walled, oval budding cells, 2 to 4μ in diameter, may be seen. A Gram-stained smear may assist in the recognition of these forms.

The fungi may be cultured readily upon Sabouraud's glucose agar where they form smooth, moist, creamy colonies. The organisms

ferment glucose, maltose, and sucrose, while lactose is not fermented. Agglutination will occur in the presence of a specific antiserum. An important property is the high virulence of these fungi for the rabbit. Intravenous injection causes the formation of abscesses throughout the body of the animal, and death within four or five days.

COCCIDIOIDOMYCOSIS

Clinical features. This is a specific infection caused by *Coccidioides immitis.* This disease is more dangerous than most other fungous infections, for it often causes death within a few months or years after its onset. Recent studies indicate, however, that mild cases ending in full recovery are relatively more frequent than fatal ones. These mild cases may show skin lesions (erythema nodosum), but most characteristic is an infection of the *lungs,* with clinical signs and symptoms which often suggest tuberculosis. In the fatal cases, the skin or the lungs may be the site of the initial infection, but the organisms are soon carried by way of the blood stream to internal organs, and the infection rapidly becomes generalized. This severe disease was formerly called *coccidioidal granuloma.*

Sources and modes of infection. The majority of reported cases of coccidioidomycosis in the United States have originated in the San Joaquin valley in California. A few cases have been noted in other states, and the disease has also been recognized in Mexico and Brazil. The manner of infection is not definitely known, but it seems likely that the dried spores of the fungus are inhaled, or are introduced from dirt or plant material through skin abrasions. An especially large proportion of cases occurs among men who are workers in the fields or vineyards. The disease is not transmitted from one person to another.

Coccidioides immitis. This fungus, first described by Vernicke (1892), was originally regarded as a protozoon of the genus *Coccidium;* hence, the name. It was later shown to be a fungus which, like the *Blastomyces,* appears in the tissues as a round body without filaments, but develops a mycelium in culture (Fig. 136: F). In the thick pus from the lesions the organisms are easily recognized as cyst-like, spherical bodies, varying in size from about 5μ to as much as 60μ or more in diameter, and having a thick, refractive, doubly contoured capsule. Within the rounded elements may be seen replicas

of the parent organism, consisting of tiny, round structures enveloped in a capsule. These forms represent endospores, and indicate the method by which the organism reproduces itself in the tissues. These fungi somewhat resemble the *Blastomyces,* but are distinguished by the *presence of endospores* and by the fact that *there are no buds.*

They may be isolated readily on a variety of culture media. They grow best at 37° C and on blood agar, in brain broth, or in similar enriched media. The cyst-like structures described above are never seen in culture; instead, the organism develops at once a filamentous growth like an ordinary mold. The colonies which appear in a few days on solid media are at first creamy-white plaques, but they soon become fuzzy with the development of an abundant aerial mycelium. Microscopically, the growth appears as a tangled network of long, profusely branched, septate filaments, about 3μ wide, with many chlamydospores.

Laboratory diagnosis. The characteristic thick-walled, cyst-like structures, with endospores and without buds, are so characteristic and numerous in the pus that their recognition is easy. Cultures on glucose agar will reveal the organism in its filamentous phase. These cultures must be handled with care, for laboratory infections have occurred from inhalation of the spores. An allergic skin test has been developed, using as an antigen the filtrate from cultures of the fungus, but its practical usefulness is limited. The infection may be reproduced in monkeys, rabbits, guinea pigs, and mice by the inoculation of cultures. The virulence of *Coccidioides immitis* for guinea pigs is a property that helps differentiate this organism from *Blastomyces,* which is relatively harmless for these animals.

BLASTOMYCOSIS

Clinical features. Blastomycosis, sometimes called Gilchrist's disease, is a specific infection caused by a particular fungus, generally known as *Blastomyces hominis* or *Blastomyces dermatitidis.* In its most common form, blastomycosis is chronic, and involves the *skin* only. Beginning as a small papule on the hand, face, neck, or other exposed part where there has been a previous cut, bruise, or other slight traumatic injury, it spreads slowly and irregularly into the surrounding skin. In these cases there is usually little systemic illness, and after several years, recovery may ensue.

In other cases, however, the internal organs are invaded by the organisms, and abscesses may form in various locations. Usually the first lesions are in the *lungs*. Later the kidneys, bones, central nervous system, and other regions are involved, and the patient succumbs to an overwhelming infection. The clinical course may suggest tuberculosis, syphilis, or sporotrichosis, and exact diagnosis depends upon laboratory examinations.

Blastomyces hominis. The causative organism of blastomycosis is a typical example of a fungus with properties intermediate be-

FIG. 138. *Blastomyces hominis* in pure culture.

tween those of the common filamentous molds on the one hand, and those of the simple, budding yeasts on the other. In the body tissues the fungi appear as yeast-like budding cells, but in laboratory cultures they develop a filamentous growth—a moderately luxuriant mycelium (Fig. 138). In the pus from the lesions (or in sections of the tissue) the organisms are seen as round or oval bodies, 7 to 20μ in diameter, with thick, doubly contoured walls. They are often surrounded by a capsule. Many of the cells show *buds* forming from them.

The *Blastomyces* may be cultured readily on glucose agar, or other media, at room or incubator temperature. In culture, the organisms show considerable variations in the manner of growth, with the passage of time, and on different types of media. The colonies are

originally smooth and creamy, somewhat similar to those of staphylococcus, but eventually become covered with a spiny or downy overgrowth which represents an aerial mycelium.

The morphology of the organisms is irregular, and is correlated with the colony form. At first, they appear as they do in tissue, as yeast-like bodies reproducing by budding, but soon there appear many elongated cells which seem to be preliminary to the development of a filamentous growth. Still later a coarse, irregular, branched mycelium appears.

Laboratory diagnosis. Microscopic examination of the pus from the lesions (or in pulmonary cases of the blood-stained sputum) will show the characteristic encapsulated, budding forms. For complete diagnosis, the fungus should be isolated in pure culture and studied for several weeks to establish its properties. The organism will cause abscess-formation in mice, or rats, but guinea pigs and rabbits are resistant.

TORULOSIS

This is a relatively rare disease, caused by infection with a pathogenic yeast—a species of torula, called *Cryptococcus neoformans,* or *Debaryomyces neoformans.* An older name is *Torula histolytica.* The organisms bring about skin inflammations occasionally, but their usual portal of entry into the body is probably the upper respiratory tract; a few patients have definite lung involvement. The fungi cause the greatest damage, however, when they reach the central nervous system, where they produce a *chronic infection of the brain and meninges,* which is eventually fatal. The symptoms may suggest brain tumor, tuberculous meningitis, or encephalitis. The patient progressively loses weight, and finally dies of respiratory failure after weeks or months of illness.

Cryptococcus neoformans, like ordinary yeasts, reproduces by budding, and never forms mycelium. In the infected meninges little masses form, containing groups of the organisms, each enclosed in a gelatinous capsule (Fig. 139). These masses may be large enough to be seen with the naked eye. They resemble the tubercles of tuberculosis, but are larger and more opaque.

Laboratory diagnosis may sometimes be made by finding the yeast-like cells, which have a diameter of 3–4μ, in the spinal fluid, but more commonly the organisms are first demonstrated in smears

from the brain or in sections of the tissue obtained at autopsy. Cultures on glucose agar or other solid media yield white or yellowish, smooth, glistening, heaped-up colonies, similar to those formed by the saprophytic yeasts. Unlike the latter, however, the torulae are virtually inert in culture; they do not ferment carbohydrates. Mice and rats, also guinea pigs and rabbits, are susceptible to the injection of pure cultures.

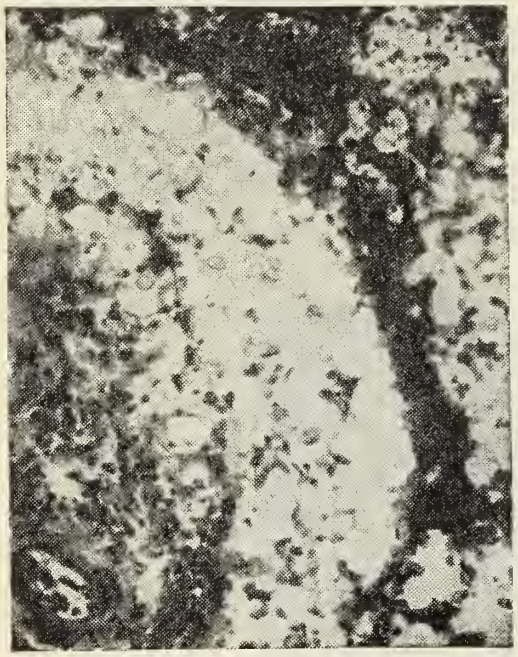


FIG. 139. *Cryptococcus neoformans* (*Torula histolytica*) in a section of the human brain from a case of cryptococcosis (torulosis). The pale-staining, oval bodies of these pathogenic yeasts are seen lying in clear spaces amid the darker-staining brain tissues. These spaces were in life filled with a gelatinous capsular material.

SPOROTRICHOSIS

Clinical features. This is a specific infection of the skin and underlying tissues, usually of the arm, characterized by gumma-like swellings and ulcerations along the course of lymph vessels, and caused by the fungus *Sporotrichum schenkii*. The disease begins with a small sore on the back of the hand, or on other exposed portions of the body. This initial lesion can often be traced to the prick of a thorn, and undoubtedly is the point of inoculation of the fungus. The infection usually runs a protracted, mild course, extending slowly along the lymph channels. At points along the vessels, swellings develop which are at first hard, then later soften and discharge pus. Sometimes the fungus becomes more widely disseminated in the body and may involve various tissues, such as muscles, bones and joints, and the lungs. Clinically the disease may be confused with syphilis or tuberculosis. Most patients respond well to treatment with iodides.

Sources and modes of infection. Not all the ways in which infection with *Sporotrichum* may be acquired are definitely known. Apparently, in most cases, infection occurs when plant material bearing the causative organism is mechanically carried into the skin, as by a thorn prick or other superficial wound.

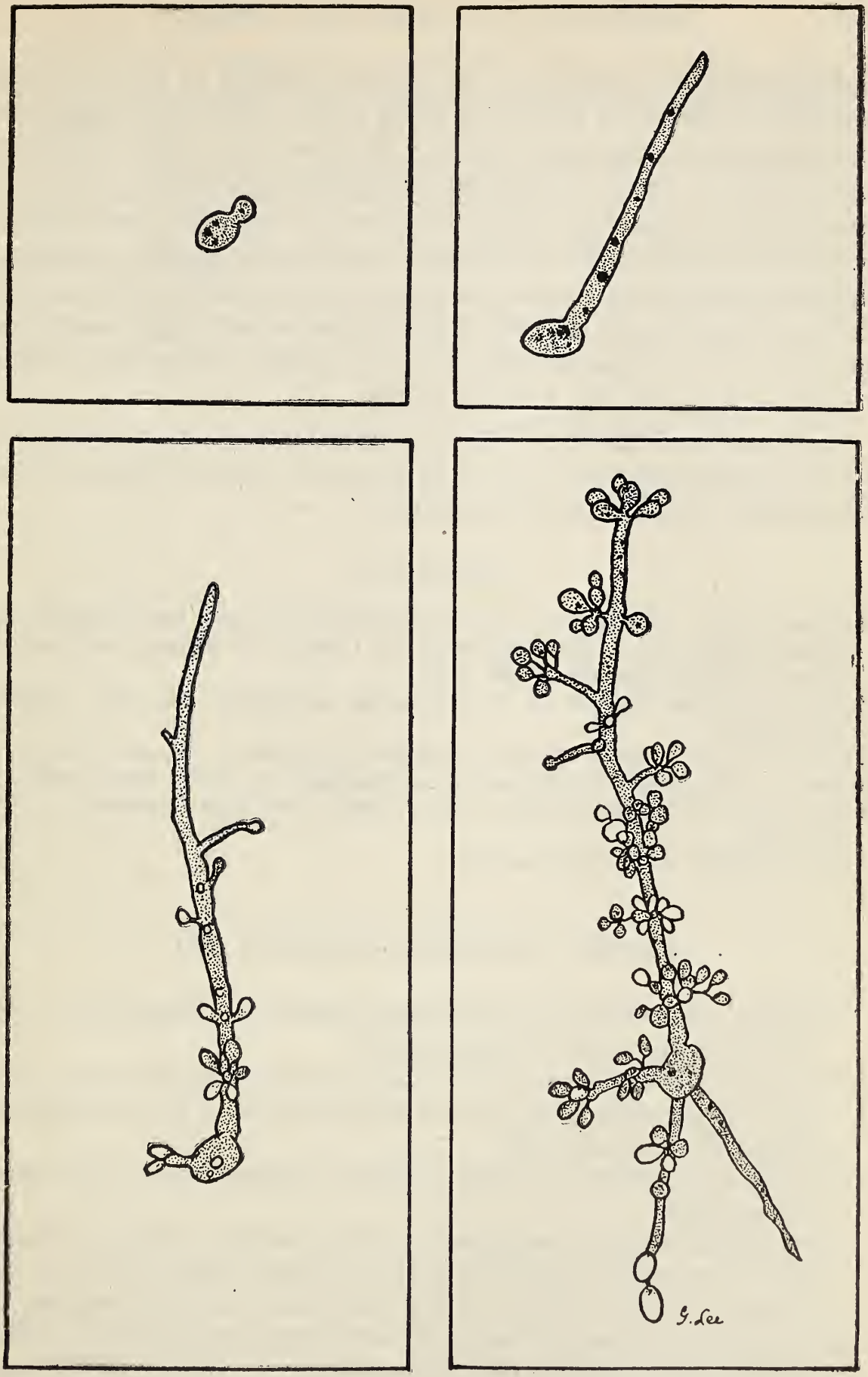


FIG. 140. *Sporotrichum schenkii*. Stages in the growth from a chlamydospore. (Original preparations by Dr. John R. Schenken.)

Sporotrichum schenkii. The causative fungus of sporotrichosis was first described by Schenk in 1898. In the pus of the lesions, the organisms are ordinarily too few in number to be seen. They grow out readily, however, in cultures on Sabouraud's medium, at room temperature. Colonies appear in three or four days, and microscopic examination reveals a delicate, septate, branching mycelium, approximately 2μ in diameter, to which are attached numerous oval or pear-shaped microconidia. These spores occur singly along the mycelium, or in cloverleaf-like clusters at the extremity of short branches (Fig. 140; Fig. 136: D, p. 658).

Laboratory diagnosis. This depends on culturing the organisms by obtaining pus from a previously unopened lesion. White rats are susceptible to experimental inoculation.

REFERENCES

BENHAM, R. W.: "Fungi and Their Pathogenicity for Man and Animals," in *Agents of Disease and Host Resistance* (p. 1109), by F. P. Gay, and pub. by Chas. C Thomas, Springfield, Ill., 1935.

DRAKE, C. H. and HENRICI, A. T.: *"Nocardia asteroides," Am. Rev. Tuberc.,* 1943, 48:184.

JACOBSON, H. P.: *Fungous Diseases.* Springfield, Ill.: Chas. C Thomas, 1932.

SLACK, J.: "The Source of Infection in Actinomycosis," *J. Bact.,* 1942, 43:193.

SMITH, C. E.: "Coccidioidomycosis," *Med. Clinics.* New York: Saunders, p. 790, May 1943.

See also References to Chapter XL.

REVIEW QUESTIONS—CHAPTER XLI

1. Describe the clinical and pathological features of actinomycosis. What is the probable origin of human cases?
2. Describe *Actinomyces bovis* as it appears in pus from the lesions. How can it be isolated? What practical methods are used in the laboratory diagnosis of actinomycosis?
3. Name and describe an aerobic acid-fast *Actinomyces* that may cause actinomycosis.
4. Distinguish between superficial and deep fungous infections. Name the two commonest forms of superficial mycoses. Name four other less frequent fungous infections of the skin. Name eight deep mycoses.
5. Describe the ringworm fungi, and name four principal genera; define *dermatophytes.* Describe the clinical features of ringworm of the scalp, and of the smooth skin; name five species of fungi likely to be responsible. What is *tinea cruris*?
6. What is the disease called favus? Name the causative organism.

7. What fungous infections are included under the heading *dermatophytosis*? Name three of the fungi that may be responsible for so-called athlete's foot.
8. What is the nature of the so-called *dermatophytid*? Under what circumstances are these "ids" seen in human beings?
9. Name the principal clinical types of moniliases. What species name is given to the pathogenic strains of *Monilia*? Describe these organisms.
10. Describe the clinical features; name and describe the causative fungus; and outline methods of laboratory diagnosis of (a) coccidioidomycosis, (b) blastomycosis, (c) torulosis and (d) sporotrichosis.

CHAPTER XLII

PROTOZOAL DISEASES. AMEBIASIS. MALARIA

Classes of protozoa. We have previously outlined chief characteristics of the *Protozoa,* and have described some of the common free-living varieties of these unicellular animals (Chapter V). It will be recalled that all the protozoa, whether free-living or parasitic, may be divided into four main classes: (1) *Sarcodina,* the most important subgroup of which is the *Amebae,* (2) *Flagellates* (or *Mastigophora*), (3) *Infusoria* (or *Ciliates*), and (4) *Sporozoa* (Figs. 12, p. 46; 13, p. 54; and 14, p. 56).

Pathogenic protozoa; human protozoal diseases. In each of these classes there are kinds of protozoa that are pathogenic for man.

Two of these protozoal diseases are of outstanding importance in the United States and in most of the rest of the world; namely, *amebiasis* and *malaria.* In the tropics and subtropics, *leishmaniasis* and *trypanosomiasis* are common and serious diseases. Less important are *giardiasis, trichomoniasis,* and *balantidiasis* (Table XXXI).

AMEBIASIS

Clinical and pathological features. Infection with the pathogenic ameba *Endameba histolytica* is called *amebiasis.* This organism invades the wall of the large intestine, producing an ulcerative colitis, which, if sufficiently severe, causes an acute diarrhea and the painful symptoms of *amebic dysentery.* When cysts of this ameba, swallowed in contaminated food or drink, reach the small intestine, their walls become weakened and the imprisoned organisms escape and undergo division, resulting in the formation of eight active amebae from each cyst. These then pass into the large intestine, and finally find lodgment somewhere in the mucous membrane

Table XXXI. Human Diseases Caused by Protozoa

CLASS	DISEASE	CAUSATIVE ORGANISMS	TRANSMISSION
Amebae	Amebiasis	*Endameba histolytica*	Cysts of amebae passed in feces from cases, carriers; spread by *feces, flies, fingers, food, water*
Mastigophora (Flagellates)	**Leishmaniasis** Visceral (kala-azar) Cutaneous (oriental sore) Mucocutaneous (espundia)	 *Leishmania donovani* *Leishmania tropica* *Leishmania braziliensis*	Transmitted principally by *sandflies* of the genus *Phlebotomus;* probably some forms of the infection spread by direct contact with nasal secretions
Mastigophora (Flagellates)	**African trypanosomiasis** (African sleeping sickness)	*Trypanosoma gambiense* *Trypanosoma rhodesiense*	Spread by bite of *tsetse* flies: *Glossina palpalis* carries *T. gambiense* (W. Africa, etc.); *Glossina morsitans* carries *T. rhodesiense* (E. Africa, etc.)
Mastigophora (Flagellates)	**American trypanosomiasis** (Chagas' Disease)	*Trypanosoma cruzi*	Transmitted by winged bugs of the family *Reduviidae*
Mastigophora (Flagellates)	**Giardiasis** (Diarrhea)	*Giardia* (*Lamblia*) *intestinalis*	Cysts are passed in feces; spreads in same way as amebiasis
Mastigophora (Flagellates)	**Trichomoniasis** (Vaginitis)	*Trichomonas vaginalis*	Presumably spread by personal contact; parasites may be present in male urethra
Infusoria (Ciliates)	**Balantidiasis** (Diarrhea; clinically similar to amebic dysentery)	*Balantidium coli*	Swine are commonly infected, excrete cysts; human beings ingest food contaminated by these cysts
Sporozoa	**Malaria** Tertian (benign tertian) Quartan Estivo-autumnal (subtertian) Ovale (rarely seen)	 *Plasmodium vivax* *Plasmodium malariae* *Plasmodium falciparum* *Plasmodium ovale*	Transmitted by mosquitoes of the genus *Anopheles* (*A. quadrimaculatus* in southern U. S.); sexual cycle of the parasites in mosquitoes, asexual cycle in r.b.c. and cells of the reticulo-endothelial system in humans

along the intestinal wall, most often in the region of the cecum or rectum. Aided by tissue-dissolving ("histolytic") ferments, the amebae penetrate the intestinal epithelium in a narrow column down to the underlying muscularis mucosae, then the colony usually spreads out fanwise as it infiltrates the deeper tissues. Not infrequently, the amebae penetrate the small mesenteric blood or lymph vessels and are carried to the liver, where they set up an amebic *liver abscess.* Rarely, the lungs or brain, or other regions of the body, are the site of secondary localizations of the organisms.

The onset of amebic dysentery is usually less abrupt, and the symptoms are generally less severe, than in bacillary dysentery. The incubation period is variable, but commonly about three weeks. The disease usually runs a protracted course, periods of freedom from general illness alternating with episodes of more or less acute gastrointestinal distress. Appendicitis is a common diagnosis, especially in the tropics.

There is much evidence that cases of amebiasis with mild symptoms only, or with no noticeable symptoms at all, far outnumber those showing frank, clinically typical dysentery. Strains of amebae vary in degree of virulence, and different individuals show a marked difference in resistance to this infection. Once infected, and in the absence of adequate treatment, an individual is likely to remain a carrier of the amebae indefinitely.

Prevalence; sources and modes of infection. Amebiasis is prevalent in most of the warmer countries of the world. Like typhoid fever and bacillary dysentery, it tends to be more common wherever sanitation is poor.

The only way one can acquire infection with *E. histolytica* is to swallow the *cysts* of the ameba. The real source of amebic infection, then, is the excreta from convalescent patients and symptomless carriers, for these are the individuals who are excreting cysts. Cooks and other food-handlers contaminate food or water with the fingers. Flies also may convey the cysts to food. A water supply may be contaminated. The cysts of this dangerous ameba are more resistant than the pathogenic intestinal bacteria; they are not killed, for example, by the concentration of chlorine ordinarily used in drinking water. Often, in such places as mental hospitals, orphan asylums, or prisons, the infection is spread by direct contact from person to person through gross neglect of environmental sanitation and personal cleanliness.

The dire possibilities of water-borne amebic infection in a limited area was illustrated in dramatic fashion by the extraordinary outbreak of more than 1,000 cases of amebiasis (with 70 deaths) originating in Chicago during the Century of Progress Exposition there, in 1933. This epidemic occurred among the employees and guests of two of the large hotels. Several of the cooks and waiters were found to be carriers of the amebae, but it was not primarily the contamination of food by these carriers that caused the spectacular outbreak. Investigations disclosed that the really important source of infection was the drinking water within the hotels. The water was being contaminated through forgotten cross-connections and unsuspected leaks in the maze of sewerage and water pipes making up the complicated, and in part antiquated, plumbing system. Guests in these hotels were drinking water polluted with the cyst-laden excreta of other residents there. It is no wonder that even a brief sojourn turned out to be unhealthful for so many people.

Endameba histolytica. In the mucus of the diarrheal stools from the acute case of amebic dysentery, and in material obtained with the aid of the proctoscope from the base of ulcers in the rectum, or in preparations from the walls of amebic abscesses, the active, ameboid, growing forms of the organism—the *trophozoites*—may be found. These are about 20–30μ in diameter. They reproduce in the tissues by fission. When observed unstained, they show a grayish, finely granular, translucent endoplasm, and a definitely delimitated, clear, highly refractile outer zone, or ectoplasm. There is a single, delicate nucleus, hard to make out unless especially stained. The organisms are actively phagocytic and their cytoplasm contains *red blood cells* and sometimes other tissue elements, but, unlike the saprophytic amebae, no bacteria (Fig. 141).

Unless expelled at once, amebae that leave the infected tissues and are caught in the intestinal contents undergo encystment. *Cysts* are found in the feces in subacute or symptomless stages of amebic dysentery. The process of encystment proceeds in several stages, finally resulting in a heavy walled round or oval body, 7–9μ (or in other strains 15–20μ) in diameter, and containing *four* small nuclei.

Laboratory diagnosis. The definite diagnosis of amebiasis demands that the presence of either the trophozoites or the cysts of *Endameba histolytica* be demonstrated in the intestinal discharges of the patient. Repeated examinations of a series of stool specimens may be required. A differentiation must be made between this

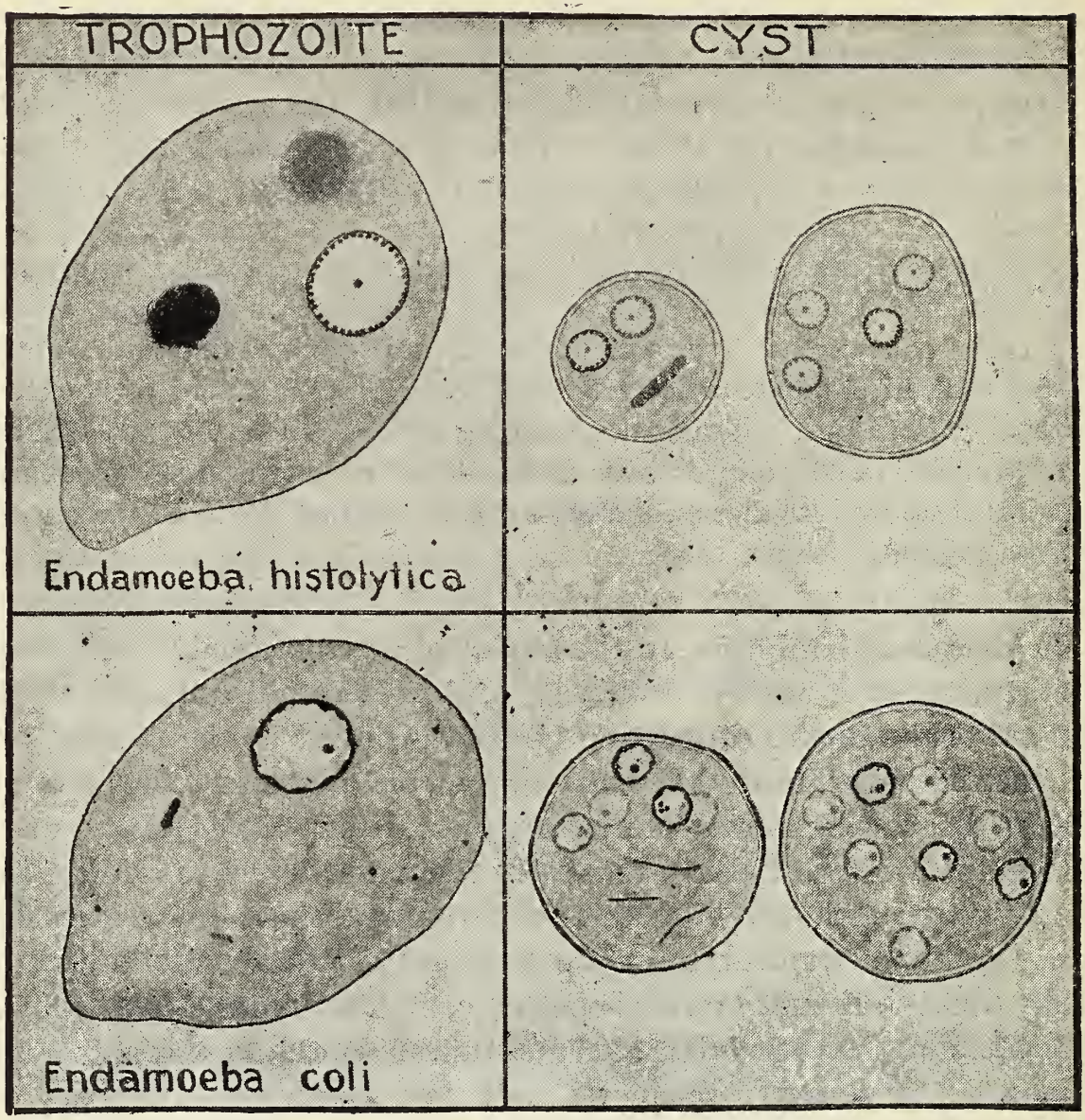


FIG. 141. Illustrating the principal differences between the trophozoites and cysts of *Endameba histolytica* and of *Endameba coli*. (From Moss, Emma S., *Am. J. Clin. Path.*, Tech. Supp., 2:43, March, 1938.)

pathogenic ameba and *Endameba coli,* the harmless ameba normally present in the feces (Chapter V). This is not always easy, and considerable experience and judgment are required. The principal points of difference are illustrated in Figure 141.

Complement-fixation tests, using the patient's serum and an alcoholic extract of cultured *Endameba histolytica* as antigen, have a limited use for diagnosis of carriers and atypical cases.

MALARIA

Prevalence and importance. Malaria is one of the most common and widespread of all human ills. It would not be an exaggeration

to call it the most important parasitic disease now suffered by human beings. It is especially frequent and severe in tropical and subtropical countries, but it occurs in localized endemic areas throughout most of the world, and is annually responsible for an estimated 3,000,000 deaths, and for perhaps 100 times as many cases of a debilitating illness. Malaria has played an important part in the history of nations, and it is still so common that it constitutes one of the most serious drawbacks to the development of peoples in many places.

In India alone, it is said to cause 100,000,000 cases and 1,000,000 deaths each year. Its evil potentialities were dramatically illustrated a few years ago (1938), when a new species of malaria-carrying mosquito (*Anopheles gambiae*), introduced in 1930 from Africa into Brazil, reached the northeastern part of the latter country in great numbers. Suddenly there occurred a devastating epidemic of severe malaria which struck down an estimated 90% of the human population in the invaded regions, and killed no less than 14,000 persons in six months' time.

In war, malaria has always been a menace to troops operating in endemic areas, and for the American soldiers and their Allies who were detailed to the Pacific theaters, it was a major problem of World War II. In all the strange tropical and subtropical places where evil necessity forced our troops to go, only bacillary dysentery and mycotic infections of the skin approached malaria in prevalence and importance as causes of disability.

In the United States, malaria was a great scourge in pioneer days, and it remained exceedingly common throughout most of the nineteenth century. Toward the end of that time, it had begun to disappear from the more northerly states, while it continued to be rife in the South. Although the percentage of fatal cases was generally low, malaria was so nearly universal an affliction in many southern communities, thirty years ago, that a large fraction of the population was made chronically ill, and, as a result, the whole region suffered a tremendous economic loss. Malaria and hookworm have been major factors in retarding economic development in parts of the South.

During the past ten years or so, however, there has been a marked improvement in the malaria picture in the United States, as control measures have been instituted more widely. The war has added new problems, since the return to their homes throughout the country

of hundreds of veterans of World War II, who have a relapsing type of malaria, may conceivably result in the reappearance of the disease in communities from which it has long been banished. This danger is fully appreciated by Federal and local health authorities, however, and the vastly improved antimalaria measures now available are expected to keep this menace well in hand. Indeed, there is good prospect that malaria may be entirely driven out of the continental United States in the not-too-distant future.

The malarial parasites and their life history. The germs of human malaria are protozoa belonging to the class *Sporozoa,* and the genus *Plasmodium.* They are exclusively parasitic, and lack any special organs for locomotion or for the ingestion of food. They multiply asexually (by schizogony), principally in human red blood cells. They produce a characteristic brownish pigment from these cells. Probably they undergo some initial development, also, in body cells of the reticulo-endothelial system, or possibly extracellularly in the lymph spaces. Sexual forms appear in the circulating blood, and these pass through the sexual phase of the life cycle within *Anopheles* mosquitoes.

Species of malarial parasites. There are three principal species, and a fourth variety of relatively slight importance. *Plasmodium vivax* causes the form of the disease called *tertian, benign tertian,* or *vivax* malaria. This is the most widely distributed species, by far the commonest outside of the tropics. The chills and fever occur at about 48-hour intervals. Patients with *vivax* malaria are prone to develop a chronic disease, characterized by repeated relapses, when suppressive treatment with atabrine or quinine is discontinued. *Plasmodium falciparum* is the cause of the severe, and often fatal form, common in the tropics, called *estivo-autumnal, malignant tertian, subtertian,* or *falciparum* malaria. In this dangerous type the interval between attacks of fever is irregular, but often averages about 48 hours. *Plasmodium malariae* is the species responsible for the so-called *quartan* type of malaria, in which paroxysms occur at intervals of about 72 hours. Quartan malaria is relatively uncommon. A fourth species, *Plasmodium ovale* is found so rarely that it need not be considered further here.

The differences in time intervals between successive attacks of chills and fever in infections caused by the different species of malarial parasites are reflections of the time required for the organisms to complete a cycle of development within the red blood cells. It

takes *P. vivax* and *falciparum* about 48 hours, and *P. malariae* about 72 hours, to mature; and the chill comes on at the end of the schizogony cycle, when numbers of young forms (merozoites) are simultaneously liberated from the many parasitized red blood cells. Persons are often bitten more than once, and on successive days, by infected mosquitoes, so that, at the beginning of the primary *vivax* or *falciparum* malaria infection, there may be two or more sets of parasites maturing at different times, and for the first few days attacks of chills and fever are likely to occur every day (*quotidian* malaria). Later a rhythm is established which brings on the paroxysm on alternate days only.

The life cycle of all the species of malaria organisms is essentially the same. There are two distinct phases: (1) in the human body, principally in the red blood cells, and (2) in the mosquito.

Asexual cycle in human red blood cells. The parasites are present in the secretions of the salivary glands of infected *Anopheles* mosquitoes. They pass, in this secretion, into the proboscis of the mosquito at the time of biting, and thus are injected into the human blood stream. The organisms then disappear from the blood for a number of days, and it is during this period, when the parasites are hidden away, that they are thought to undergo some initial development in the tissue lymph spaces or within reticulo-endothelial cells. After a week or ten days, however, the regular cycle of schizogony begins in at least a few of the red blood corpuscles. Only one plasmodium enters a single erythrocyte in most cases, except in *falciparum* infections, in which two or more organisms may penetrate one cell.

Almost at once after entering the corpuscle, the parasite assumes a "signet-ring" form; then, later, it grows into a larger, irregular-shaped body (the *schizont*), which eventually nearly fills the entire cell (Fig. 142). (In *falciparum* infections the stages in development of the plasmodia beyond the ring form cannot ordinarily be seen by examination of the peripheral blood, since nearly all the infected corpuscles accumulate in internal organs. In malaria due to the other species, however, erythrocytes containing parasites in all stages of the cycle continue to circulate.) Finally, the mature schizont becomes divided by multiple asexual divisions (schizogony) into a number of tiny forms, the *merozoites*. These young parasites, like the one which originally entered the erythrocyte, are capable of penetrating other red blood cells. The infected corpuscle by this

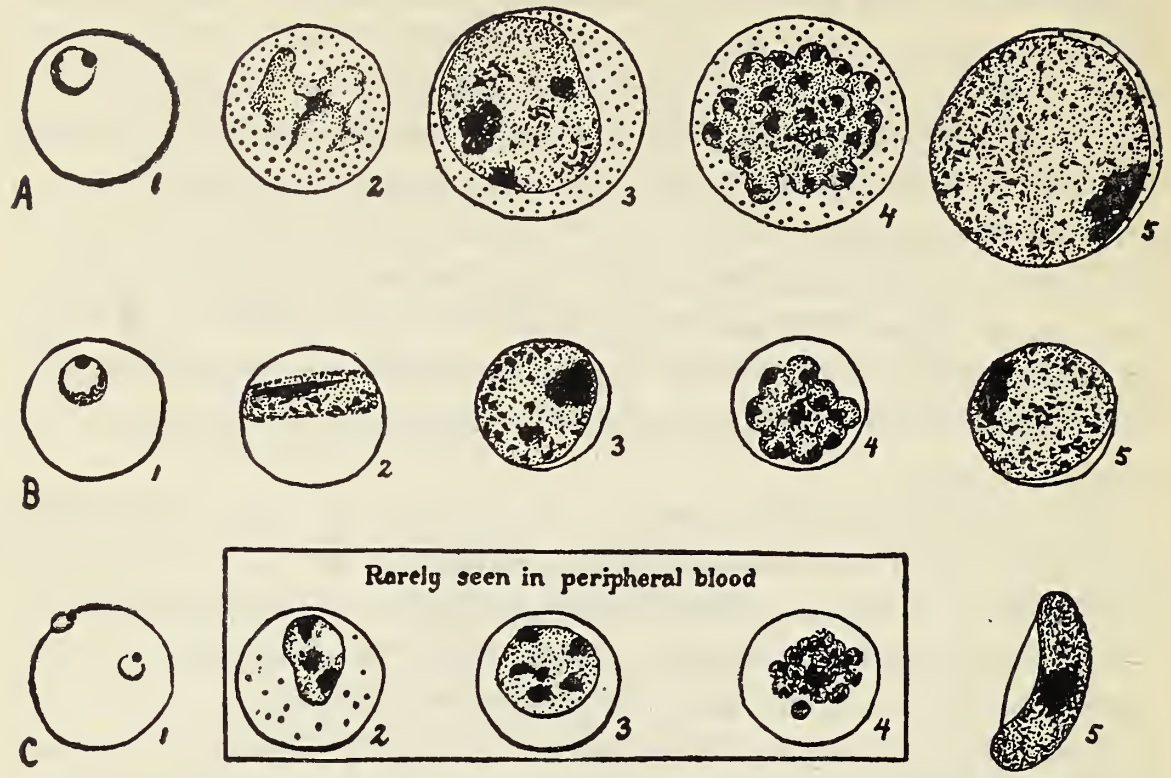


FIG. 142. Comparison of the three species of malarial parasites, illustrating diagnostic characteristics in each stage. A: *Plasmodium vivax;* B: *P. malariæ;* C: *P. falciparum; 1,* "ring" stages; *2,* growing schizonts; *3,* grown schizonts with dividing nucleus; *4,* segmenting parasites nearly ready to leave corpuscle; *5,* female gametocytes. (From Chandler, A. C., *Introduction to Parasitology,* New York: John Wiley & Sons, Inc., 7th Ed., 1944.)

time is entirely destroyed, and it ruptures, setting free the merozoites into the blood stream. Many of these probably die at once or are phagocytized by leukocytes, but some succeed in invading other red blood cells and so start the cycle all over again. It is supposed that, at the time the infected red cells break up, releasing the young parasites, toxic waste products are set free, and these are thought to cause the characteristic chill.

This *asexual cycle* (Fig. 143: upper part) goes on continuously, more and more blood cells being infected, until, after ten days or two weeks (the usual incubation period of the clinical disease), several thousand cells in every cubic millimeter of blood are infected. The classical symptoms then appear.

After the asexual development of the organisms, as described above, has gone on for a number of days, new forms begin to appear within a small proportion of the infected red blood cells. These new forms are *gametocytes,* the *sexual forms* of the parasites. These develop rather slowly; some become male cells (*microgametocytes*),

others female cells (*macrogametocytes*). They continue to circulate in the blood for several weeks. They do not multiply or otherwise change, once fully matured in the human red blood cell, but they undergo a developmental cycle of their own within the body of *Anopheles* mosquitoes (Fig. 143: lower part). Persons with gametocytes in the peripheral blood, where these mosquitoes may get them, constitute the essential reservoir of malarial infection.

Sexual cycle in the mosquito. When the sexual forms are taken into the stomach of the mosquito, as she bites a malaria patient, the male cell throws off tiny bodies resembling human spermatozoa, which have an active movement. One of these bodies enters and fertilizes a female cell, which, in the meantime, has matured to receive it. The fertilized cell now becomes embedded in the stomach wall of the mosquito and proceeds to grow into a large, round, cyst-like body, the *oöcyst.* A single mosquito may have scores of these oöcysts in the stomach wall. In eight or ten days, the oöcysts become filled with hundreds of delicate spindle-shaped nucleated forms, each about 15μ long—the *sporozoites.* (These are the "spores," which give the name *Sporozoa* to this class of protozoa. Their formation is referred to as *sporogony* in contrast to schizogony, the asexual process by which merozoites are formed.) The sporozoites soon escape into the body cavity of the mosquito and become distributed throughout the body of the insect, many of them lodging in the salivary glands. Then, when the mosquito bites, these sporozoites are introduced into the human blood stream and soon are ready to start once more the asexual cycle in the human red blood cells.

It takes from ten to fifteen days for the development of the parasites within the mosquito and, until these changes are complete, the insect is not able to infect human beings. Once the parasites have reached the salivary glands, however, a mosquito may infect many persons in succession during a period of several weeks. The mosquito does not seem to be harmed in any way by the presence of the malarial parasites.

Laboratory diagnosis. Often an acute case of malaria may be diagnosed with confidence from the clinical signs alone, but good medical practice requires that laboratory studies *always* be carried out.

A definite diagnosis of malaria is made when the parasites are found microscopically in blood smears. A drop of blood from the ear or finger is spread in a *thin smear* over a clean slide by using

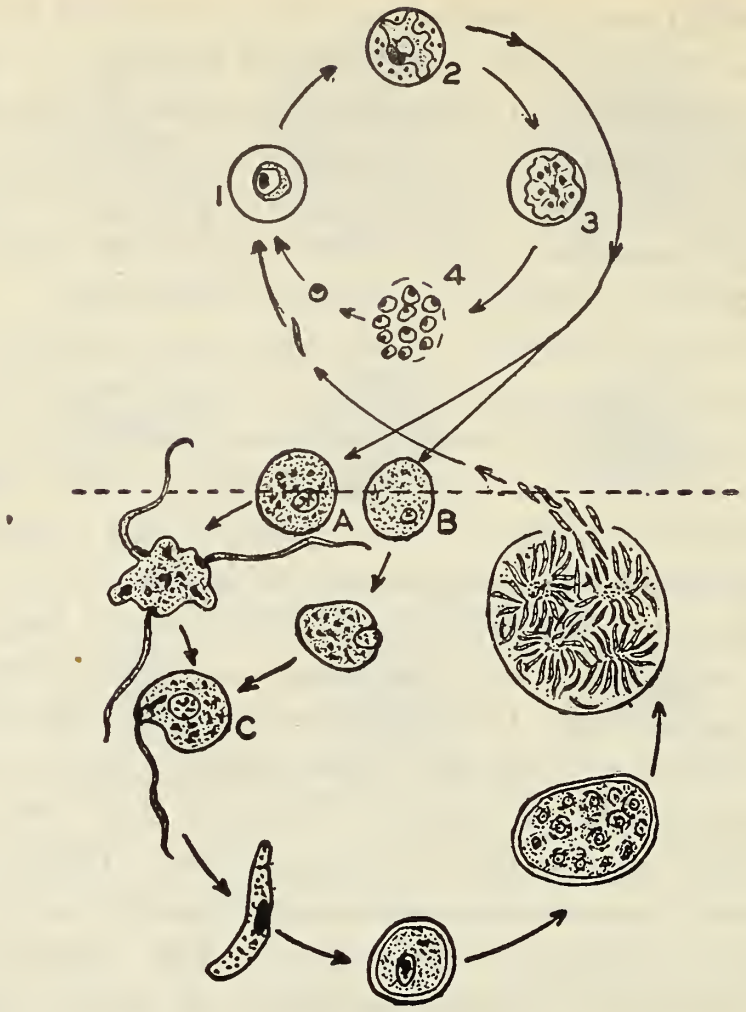


FIG. 143. Diagram illustrating the life-cycle of the tertian malarial parasite (*Plasmodium vivax*). The stages *above* the dotted line occur in the blood of man, whereas those *below* the line are found only in the mosquito. 1, 2, 3, 4: stages of the asexual cycle in human red blood cells. 4: the merozoites, produced by asexual multiple division (schizogony). A: the microgamete (male cell), and B: the macrogamete (female cell), sexual forms of the parasite. After fertilization of the female cell within the mosquito (C), a series of changes occur which eventually result in the production of very many tiny spindle-shaped forms, the sporozoites, by the process called sporogony. These forms reach the salivary glands of the mosquito, and, when this infected mosquito bites again, these sporozoites are inoculated into the blood and invade red blood cells, thus completing the cycle from man to mosquito and back again. (Modified from Hegner, Cort and Root, *Outlines of Medical Zoology,* New York: Macmillan Company, 1927.)

the edge of another slide, as previously described (Fig. 25), or a so-called *thick smear* is made by allowing a large drop to dry in the air on a half-inch-square area of the slide. A thick smear is valuable when the parasites are scarce, and it saves time by permitting the systematic examination, under the microscope, of a relatively large amount of blood in a small space. Thin smears are usually stained with Wright's stain, and thick smears with a modified Giemsa's stain.

Immunity in malaria. Human malaria is not regularly transmissible to lower animals; hence, the study of immunity to these parasites cannot be carried out by experimental animal inoculations. Investigators have therefore turned to bird malaria as a natural disease analogous to the human infection, and have studied it experimentally.

It has been found that in birds, after the acute attack, during which an enormous number of parasites are present in the circulating blood, a long latent period occurs, when the parasites are *apparently* entirely absent from the blood cells; yet their persistence in small numbers is proved by the fact that a lowering of the resistance of the bird, by chilling or otherwise, brings on a second attack. During the latent period, birds are strongly resistant to reinfection with the same species of *Plasmodium*. This state of immunity to fresh invaders declines gradually, but persists in some degree so long as any parasites remain within the body. If a really complete cure is effected, and the bird becomes entirely free of the organisms, then resistance is rather quickly lost, and reinfection with the same strain of parasite is possible.

The situation is believed to be the same in human beings. Persons who do not receive treatment adequate to eliminate all the parasites at the time of their first attack may harbor, for long periods, a latent malaria infection (especially if they have *vivax* malaria). They are subject to relapses whenever their resistance is lowered for any reason—for example, by intercurrent infection, excessive fatigue, or shock, or merely by the upsetting circumstances associated with a change in climate. At the same time, a persistent latent infection builds up considerable specific immunity to a new infection with the same strain of *Plasmodium*.

Continual exposure to the bites of infected *Anopheles* mosquitoes acts as a kind of vaccination, so that older children and adults in heavily infested districts often become highly resistant to the prevalent local strains, and have no fever or other symptoms, even though their spleens are enlarged and a few parasites may be found in the blood. This immunity apparently depends upon increased activity of phagocytic cells in destroying the parasites.

Treatment and prevention of malaria. *Chemotherapy.* Malaria is one of the diseases for which we have long had specific chemical remedies, the principal drugs being *quinine* and related derivatives of chinchona bark. Quinine has been known and used since the

seventeenth century. Two synthetic products have more recently been developed for malaria therapy—*atabrine,* which may be substituted for quinine, since it also attacks the asexual parasites actually causing the illness in men; and *plasmochin,* which has practically no effect upon those forms, but does have a remarkably destructive action on the sexual parasites (gametocytes) in human blood.

Atabrine, introduced in 1933, proved itself a true lifesaver in World War II, after the Japanese seized nearly all the lands where the world's supply of quinine is produced. Used in the armed forces on a scale never before attempted, this drug was found to be safe and effective, and to have a number of advantages over quinine. When used in moderate amounts, it will suppress the development of symptoms in exposed persons and prevent the appearance of relapses, while in larger doses it will have curative action and will even kill the gametocytes of the *vivax* or *malariae* species.

Plasmochin is more toxic than atabrine, and is little used at present. During World War II extensive studies were made of new antimalaria drugs; some of these show great promise.

Preventive measures. Prevention of malaria would seem, on the face of it, to be a fairly simple matter, depending chiefly on the destruction of *Anopheles* mosquitoes. In practice, however, effective malaria control often turns out to be a difficult, complicated and expensive job. No two communities present quite the same problem, and all the local factors affecting the incidence of malaria must be carefully analyzed before anything is done. Antimalaria work must be a community effort, applied over an area of sufficient size to produce perceptible results.

The principal measures used in successful malaria-control work include: (1) adequate treatment of all cases, and efforts to suppress, with drugs, the gametocytes in the blood of human carriers; (2) protection of the people against mosquito bites by screening of dwellings and by killing of adult mosquitoes, with insecticidal sprays, inside houses; and (3) destruction of mosquito larvae in water, and, so far as possible, permanent elimination of their breeding places.

Recent studies indicate that DDT will prove of supreme value in the control of *Anopheles* mosquitoes. It is efficient as a larvicide when distributed as an oil spray from boats, or as a dust or aerosol

from airplanes, over water where the larval forms of the insects are growing, and also as an agent for the destruction or repelling of adult mosquitoes within houses. This hopeful development comes just at the time when the need is urgent for effective measures of controlling and suppressing quickly any localized epidemic of malaria that may develop in consequence of the return to camps and homes in the United States of soldiers who are carriers of relapsing types of malaria.

LEISHMANIASIS

Clinical varieties; transmission of leishmaniasis. Infections caused by the pathogenic flagellates of the genus *Leishmania* are usually classified into three types: (1) *visceral leishmaniasis* or *kala-azar,* a generalized, often fatal, malaria-like disease in which the parasites grow in the cells of the reticulo-endothelial system, causing marked enlargment of the liver and spleen; (2) *cutaneous leishmaniasis,* or *Oriental sore,* a localized infection of the skin, without fever or general symptoms; and (3) *mucocutaneous leishmaniasis,* or *espundia,* which is similar to Oriental sore, except that the mucous membranes of the nose and mouth are involved in destructive ulcerations with resulting severe disfigurement. These infections occur principally in the Orient, in the Middle East, and in South America.

The usual method by which these diseases are carried from man to man is through the bite of *sandflies* belonging to various species of the genus *Phlebotomus.* It is likely, however, that the leishmania are sometimes transmitted by direct personal contact with other infected persons or with dogs, especially in the case of the cutaneous and mucocutaneous forms of leishmaniasis.

Leishmania. These organisms are named after two British investigators, Leishman and Donovan, who discovered the parasites while studying kala-azar in India, in 1903. Leishman-donovan bodies, as they occur in the human body, are minute, rounded, oval, or torpedo-shaped bodies, about 2–5μ in diameter (Fig. 14, p. 56). In the infected host, the leishmania invade the large endothelial cells of the blood and lymph vessels, and the macrophages of the spleen, liver, bone marrow, and other tissues; a few may develop within the monocytes of the circulating blood.

In the digestive tract of the sandflies which act as intermediate

hosts, the organisms become transformed into actively motile, spindle-shaped, flagellated forms about 14–20μ long. They have a similar morphology in laboratory cultures on a special blood agar medium.

Three species are recognized. *Leishmania donovani* is the parasite of kala-azar; the cause of Oriental sore is named *Leishmania tropica;* and the organism of South American mucocutaneous leishmaniasis is called *Leishmania braziliensis.*

TRYPANOSOMIASIS

Trypanosomes. Many different species of these protozoa are found as parasites in all kinds of vertebrate animals, including fish, amphibians, reptiles, and birds, as well as horses, cattle, and other mammals, including man. The trypanosomes live in the lymph, blood, or tissues of their hosts, and often, when host and parasite have become fully adapted to each other, they cause no illness, even though present in large numbers. On the other hand, when this adaptation is incomplete, as in the case of the trypanosomes that infect man, they give rise to serious and often fatal disease. These parasites are carried from host to host by bloodsucking insects or arthropods—those causing African trypanosomiasis being transmitted by various species of *tsetse flies* (genus *Glossina*), and those responsible for the South American form of the infection (Chagas' disease) by *bugs of the family Reduviidae.*

Typical trypanosomes have a narrow, elongated, leaf-like body, pointed at the anterior end, and more or less blunted at the opposite pole. The flagellum arises from the blepharoplast, or a basal granule near by, at the blunted end, and passes along the body, marking the outer margin of a well-developed undulating membrane, and extending outside the body at its anterior end (Fig. 14: D, p. 56). There is a single large nucleus. The organisms have an active, wriggling, eel-like locomotion, swimming in the direction of the pointed end of the body.

African trypanosomiasis (sleeping sickness). This serious disease is a menace to all natives and travelers in the "tsetse-fly belts," which extend over most of central Africa. The parasites, introduced through the bite of one of these flies, cause a more or less acute disease, marked by irregular fever and increasing debility. Finally, after some weeks or months, the organisms usually invade the

cerebrospinal fluid and the tissues of the brain and spinal cord, with the resulting appearance of the characteristic depression, drowsiness, and coma of sleeping sickness. Soon the patient's miseries end in death.

The trypanosomes of African sleeping sickness are generally classified into two species, *Trypanosoma gambiense* and *Trypanosoma rhodesiense*. These actually are closely related varieties. *T. rhodesiense* parasitizes wild game (antelope) and domestic animals, as well as man; it is transmitted by tsetse flies of the species *Glossina morsitans*. It is generally more virulent for humans than *T. gambiense*. The latter seems to be more fully adapted to man—there is apparently no natural animal reservoir; instead, *gambiense* infection is carried directly from man to man by the species of tsetse fly named *Glossina palpalis*.

American trypanosomiasis (Chagas' disease). In 1909, Chagas discovered a new type of human trypanosomiasis in Brazil. This infection occurred among natives living in houses infested with the voracious, bloodsucking, winged bugs of the family *Reduviidae* (species *Triatoma megista),* and these bugs were shown to be carriers of the causative trypanosomes. It was subsequently found that trypanosomes of the same kind, now called *Trypanosoma cruzi,* parasitize many small mammals, especially armadillos and opossums, and that natural infection with *T. cruzi* exists widely among these animals and in the reduviidae bugs, from Argentina all the way north through Central America and Mexico to the southwestern United States. Despite the wide distribution of the reservoir animals and potential insect vectors, however, human cases of Chagas' disease have not appeared in any numbers except in those parts of Brazil and Argentina where *Triatoma* bugs habitually live in human habitations and customarily feed on human blood. Although Packchanian has produced an experimental human infection with a strain of *T. cruzi* from a species of *Triatoma* bug in Texas, no cases of naturally acquired Chagas' disease have been reported in the United States.

GIARDIASIS

This is an infection of the small intestine and duodenum with the flagellate *Giardia* (*Lamblia*) *intestinalis* (Fig. 14: E, p. 56). There is a recurrent diarrhea and abdominal distress. Giardiasis is

especially common in the tropics, and also among ill-kept children in orphanages and similar places.

Although they may be present without symptoms it is generally agreed that these flagellates are truly pathogenic. They may be exceedingly numerous in the upper part of the small intestine, and in the stools of patients with diarrhea of no other known cause. Even if not capable by themselves of imitating disease they undoubtedly aggravate any coexisting intestinal inflammation.

TRICHOMONIASIS

Infections of the intestine, or of the genital organs with flagellated protozoa of the genus *Trichomonas* give rise to the disease called trichomoniasis. Vaginal trichomoniasis is the most important clinically.

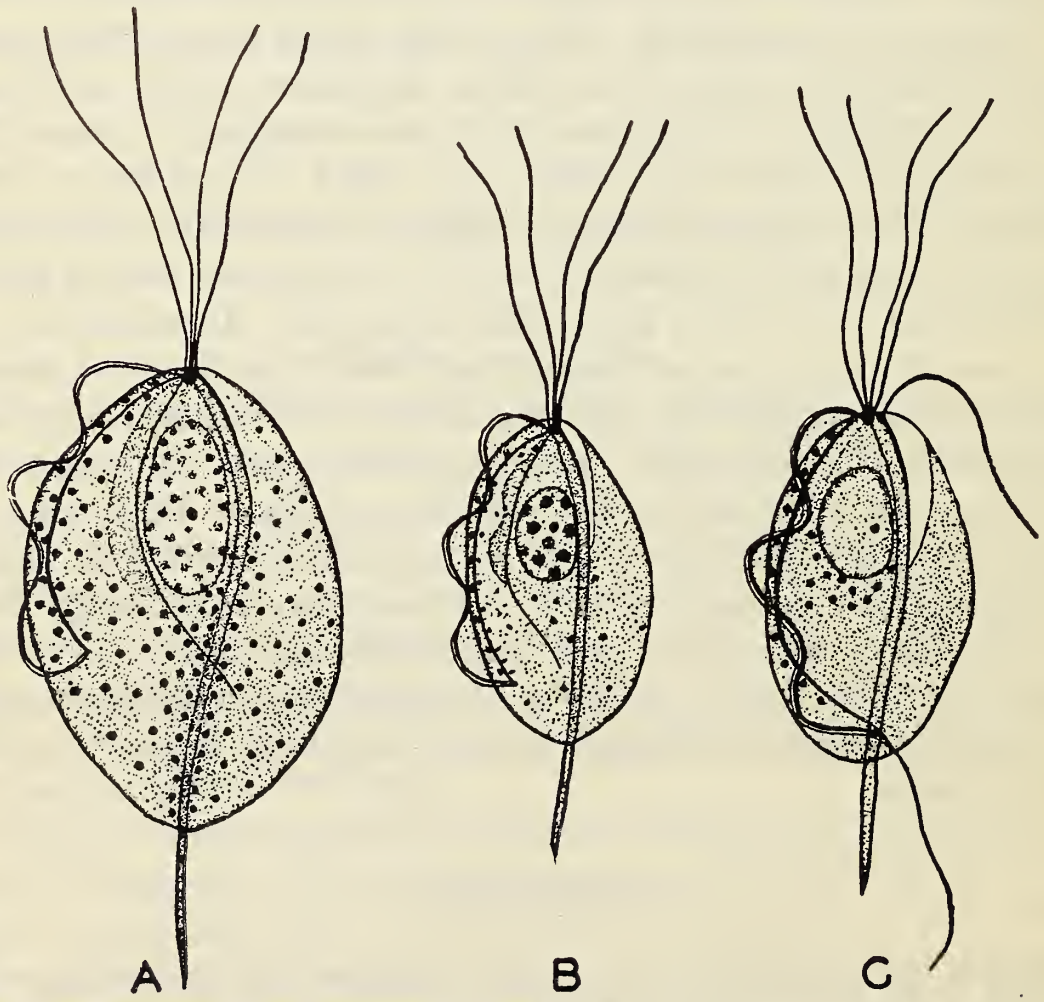


FIG. 144. Diagrammatic representations of *Trichomonas vaginalis* (A), *T. tenax* (B), and *T. hominis* (C). (From Wenrich, D. H., "Comparative Morphology of the Trichomonad Flagellates of Man," *Am. J. Trop. Med.*, 24:39, 1944.)

The trichomonad flagellates. The various species of *Trichomonas* are spoken of collectively as trichomonads. All have a similar structure (Fig. 13, p. 54). By the action of the undulating membrane and the free flagella, these organisms move forward somewhat jerkily, wobbling from side to side, and revolving on the long axis.

Three species of trichomonads are found in man; these differ in morphology and in physiological habits, and each is closely adapted to its particular habitat, which, for *Trichomonas tenax* is the mouth; for *T. hominis,* the intestine; and for *T. vaginalis,* the vagina (Fig. 144). Only the latter is of medical importance.

Vaginal trichomoniasis. It has long been recognized that *T. vaginalis* is a common parasite of the human vagina. Recently, evidence has been found that it exists not infrequently in the male urethra also, and doubtless the organisms are transmitted through sexual intercourse. When present in the vagina, these flagellates often grow freely, and although they do not invade the uterus, they do set up a persistent low-grade inflammation, with the appearance of a whitish, acid discharge (leukorrhea). This form of vaginitis is of special importance as a complication of pregnancy, and a relatively higher morbidity rate following childbirth has been noted in *Trichomonas*-infected women. A considerable proportion of the cases of nongonorrheal urethritis in *men* may also be attributed to *T. vaginalis* infection.

Laboratory diagnosis is best made by microscopic examination of fresh, wet preparations (moist films or hanging drops).

REFERENCES

General

BELDING, D. L.: *Textbook of Clinical Parasitology.* New York: D. Appleton-Century Co., 1942.

CHANDLER, A. C.: *Introduction to Parasitology.* New York: John Wiley & Sons, Inc., 7th Ed., 1944.

CRAIG, C. F. and FAUST, E. C.: *Clinical Parasitology.* Philadelphia: Lea & Febiger, 2nd Ed., 1940.

MANSON-BAHR, SIR PHILIP: *Synopsis of Tropical Medicine.* Baltimore: Williams & Wilkins Co., 1943.

Amebiasis

CRAIG, C. F.: *The Etiology, Diagnosis and Treatment of Amebiasis.* Baltimore: Williams & Wilkins Co., 1944.

FAUST, E. C.: "Some Modern Concepts of Amebiasis," *Science,* Jan. 21, 1944, 99:45 *idem.,* Jan. 28, 1944, 99:69; "Some Biological Interrelationships," *Am. Scientists,* Jan. 1945, 33:13.

SMITH, GEDDES: "The Case of the Plumber's Patch Work," in *Plague On Us.* New York: Commonwealth Fund, 1941, p. 238.

Malaria

BISPHAM, W. N.: *Malaria: Its Diagnosis, Treatment and Prophylaxis.* Baltimore: Williams &. Wilkins Co., 1944.

BOYD, M. F.: "An Historical Sketch of the Prevalence of Malaria in North America," *Am. J. Trop. Med.,* 1941, 21:223; "Present-day Problems of Malaria Infections," *J.A.M.A.,* Apr. 22, 1944, 124:1179.

FREEBORN, S. B.: "Problems Created by Returning Malaria Carriers," *Pub. Health Rep.,* Mar. 17, 1944, 59:357.

TALIAFERRO, W. H.: "Infection and Resistance in the Blood-Inhabiting Protozoa," *Science,* June 17, 1932, 75:619.

WILCOX, A.: "Manual for the Microscopical Diagnosis of Malaria in Man," *Nat. Inst. Health Bull. No. 180,* 1943.

Trichomoniasis

BLAND, P. B., GOLDSTEIN, L. and WENRICH, D. H.: "Vaginal Trichomoniasis in the Pregnant Woman," *J.A.M.A.,* Jan. 17, 1931, 96:157.

FEO, LOUIS G.: "The Incidence and Significance of *Trichomonas vaginalis* Infestation in the Male," *Am. J. Trop. Med.,* May 1944, 24:195.

STABLER, R. M. and FEO, L. G.: "Survival Time of Intravaginally Implanted *Trichomonas hominis," Am. J. Trop. Med.,* Nov. 1942, 22:633.

WENRICH, D. H.: "Comparative Morphology of the Trichomonad Flagellates of Man," *Am. J. Trop. Med.,* Jan. 1944, 24:39.

Other Diseases

ELKELES, G.: "Experimental Studies and Critical Considerations Regarding the Life Cycle of *Trypanosoma cruzi," Am. J. Trop. Med.,* Mar. 1945, 25:141.

JAMES, H., RAKE, G. and HAMRE, D.: "Cultivation of Leishmania in the Yolk Sac of the Developing Chick Embryo," *Am. J. Trop. Med.,* Nov. 1944, 24:381.

PACKCHANIAN, A.: "*Trypanosoma cruzi* Infections in Texas Triatomidae," *Pub. Health Rep.,* 1939, 54:1547; 1940, 55:1300; "Infectivity of the Texas Strain of *Trypanosoma cruzi* to Man," *Am. J. Trop. Med.,* May 1943, 23:309.

REVIEW QUESTIONS—CHAPTER XLII

1. Name seven human diseases caused by protozoa. Which are the more important?
2. Outline the clinical and pathological features of amebiasis, and name the causative organism.
3. Discuss the prevalence of amebiasis, and explain the sources and modes of infection.
4. Describe *Endameba histolytica.* Define *trophozoite, cyst.* How is a laboratory diagnosis of amebiasis made?
5. Discuss the importance of malaria, and its prevalence and significance as a world problem today.
6. Name the four types of malaria parasites and characterize the infection produced by each type. What explains the difference in the time intervals between attacks of chills and fever in infections caused by the different species?
7. What happens in the first few days after malaria parasites have been introduced into a human being by the mosquito bite? Trace the subse-

quent asexual cycle of development of the parasites in the human red blood cells.

8. Describe the sexual forms that appear in the blood, and explain their importance. Define *schizogony, ring forms, schizonts, merozoites, gametocytes, microgametocytes, macrogametocytes.*
9. Trace the development of the sexual forms of the parasite within the mosquito. How long is it after a mosquito has taken in the organisms before she can infect another person? Define *oöcyst, sporogony, sporozoites.*
10. What methods are used to demonstrate the presence of the parasites in the patient's blood?
11. Discuss immunity in malaria. What species of *Plasmodium* tends to induce a relapsing type of infection?
12. What long-known drug has a specific curative effect in malaria? What new synthetic drug has been found to be a good substitute?
13. Discuss the problem of malaria prevention.
14. Name the three clinical varieties of leishmaniasis. Where do these diseases occur? What is the usual means by which the causative organisms are transmitted?
15. Describe the *Leishmania,* and name the species associated with each of the three clinical forms of the infection.
16. Discuss the occurrence of *trypanosomes* in nature. Describe a typical trypanosome.
17. Describe the disease called African sleeping sickness. Name and compare the two species of trypanosomes responsible. How are the causative organisms transmitted from animal to man, or from man to man?
18. What is *Chagas' disease,* and how is it transmitted? Name the causative trypanosome.
19. What is *giardiasis*? Name and describe the causative organism.
20. Name and describe the three species of *Trichomonas* that occur in human beings. Discuss the importance of vaginal trichomoniasis. How is a laboratory diagnosis made?

APPENDIX

COMPARISON OF METRIC AND ENGLISH UNITS OF MEASURE AND CENTIGRADE AND FAHRENHEIT TEMPERATURE SCALES

In ordinary life in English-speaking countries we measure length by inches and feet, but in scientific work linear measure is based

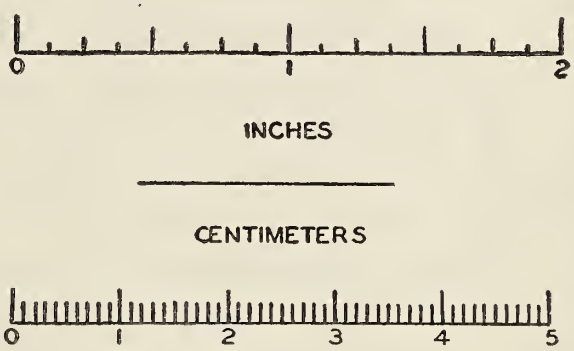


FIG. 145. Scales for comparison of English and metric units of linear measure. The smallest divisions of the lower scale are millimeters.

upon the *metric system,* and length is expressed in terms of *meters* and multiples and fractions of a meter. The standard meter has a length of 39.37 inches. In microscopical sciences, such as bacteriology, the metric units in most common use are *centimeters* (a hundredth part of a meter), and *millimeters* (a thousandth part of a meter). A centimeter is about $\frac{3}{8}$ of an inch, and a millimeter about $\frac{1}{25}$ of an inch. A useful mental picture of these units of measure, as compared with a familiar scale in inches, may be gained by study of the accompanying diagram (Fig. 145). In the metric scale here shown the smallest divisions are in millimeters, and the numbered divisions in centimeters.

Bacteriologists have found it necessary to adopt a unit of measure still smaller than the millimeter in order to express the extremely minute size of bacteria and other microorganisms. This is the *micron* (μ), or micromillimeter, which is a *thousandth part of a*

millimeter. Most of the bacilli are no more than one micron (1 μ) wide, which means that a thousand of them could be laid side by side across a millimeter space.

In the metric system the unit of *weight* is a *gram.* This is the weight of *one cubic centimeter* of water. The unit of *volume* is a *liter,* which is *1,000 cubic centimeters,* or a little more than a quart.

In all scientific work, and, in fact, even for ordinary purposes practically everywhere except in English-speaking countries, temperature is expressed according to the *Centigrade* scale rather than the more familiar *Fahrenheit* scale. In the latter the freezing point of water is 32° and the boiling point 212°, while according to the *Centigrade* scale water freezes at 0° and boils at 100°. Thus, the range of temperature from the freezing to the boiling point on the *Fahrenheit* scale is divided into one hundred and eighty degrees and the same range of temperature on the *Centigrade* scale is divided into only one hundred degrees, and each degree *Fahrenheit* is $\frac{5}{9}$ of a degree *Centigrade.* To change a *Fahrenheit* temperature reading to its equivalent on the *Centigrade* scale subtract 32, then divide by 9, and multiply the result by 5. To change a *Centigrade* reading to its equivalent in the *Fahrenheit* scale divide by 5, then multiply by 9, and add 32. The accompanying figure will help to familiarize the student with the relation between these two scales (Fig. 146).

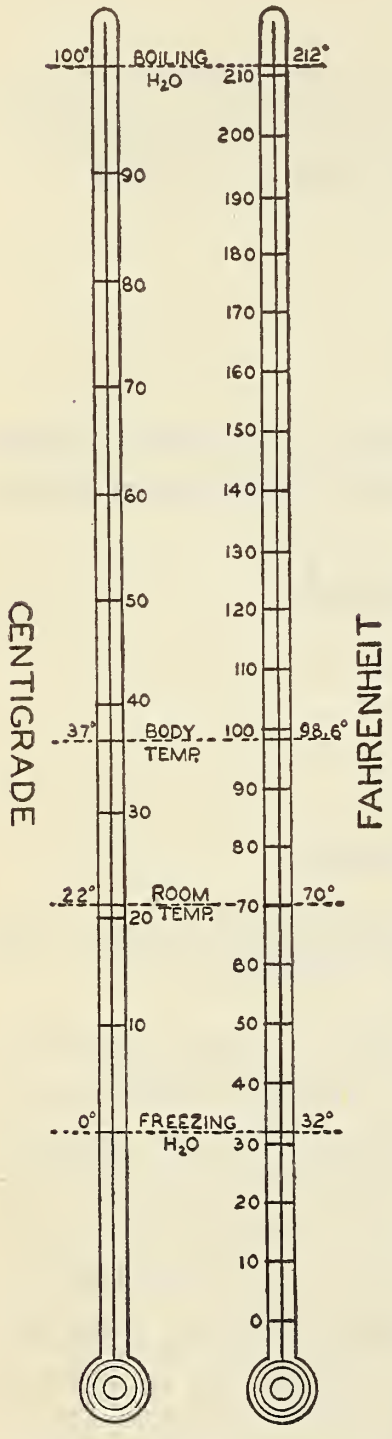


FIG. 146. Laboratory thermometers, for comparison of the *Fahrenheit* and *Centigrade* scales.

Clinical thermometers are made with either the *Fahrenheit* or the *Centigrade* scales, and some physicians prefer one, and some the other. The normal body temperature is 98.6° F. or 37° C, and a temperature of 104° F is equivalent to 40° C. The diagram (Fig. 147) permits a ready comparison

of the two scales. It is so drawn that the equivalent temperatures are directly opposite each other throughout the scale.

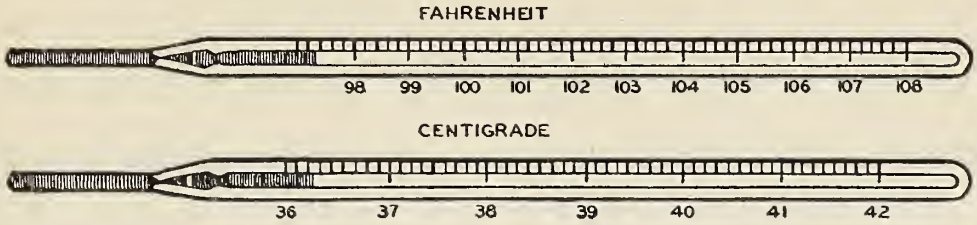


FIG. 147. *Fahrenheit* and *Centigrade* clinical thermometers. The equivalent temperatures are directly opposite each other throughout the scale.

INDEX

Figures in **bold face** refer to pages on which illustrations may be found.