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# MAN'S MICROBIC ENEMIES

BY

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## PREFACE

THIS little book is the outcome of much interest in Bacteriology shown by my wife, by personal friends, and by lay visitors to my laboratory. All have been keen to learn something of those mysterious germs that attack man's health in so many dreadful ways, yet remain invisible to the eye. It seemed likely, therefore, that a larger audience might be interested in the subject, and in the following pages I have tried to explain as simply as possible some of the wonders of Bacteriology. If I have succeeded I shall not only have fulfilled the object of the "Forum" series, but, in small measure, shall have repaid my teachers at Glasgow University, who first encouraged my own interest in the subject.



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## GLOSSARY

- Aerobic.* Requiring air : applied to those organisms which need oxygen.
- Agar.* A seaweed used, like gelatine, for solidifying culture media.
- Anaerobic.* Not requiring air : applied to those organisms which do not grow in the presence of oxygen.
- Anti-toxin.* A substance neutralising a toxin.
- Bacillus.* A rod-shaped organism.
- Coccus.* A spherical organism.
- Culture.* A growth of organisms on an artificial medium.
- Medium.* A preparation, either fluid or solid, supplying the necessary ingredients for the growth of organisms.
- Septicæmia.* Blood-poisoning : the presence in the blood-stream of dangerous organisms.
- Serum.* The clear part of the blood after all the blood cells have been removed. Usually the basis of artificially produced anti-toxins.
- Spirochæte.* An organism showing a number of waves in the length of its body.
- Toxin.* A poisonous substance produced by a dangerous organism.
- Vaccine.* A preparation used as a curative agent in bacterial disease : usually an emulsion of dead organisms.



## CHAPTER I

### The Discovery of Germs

MAN'S imagination has at all times filled the invisible air with a great variety of creatures—fairies, goblins, spirits, and ghosts—themselves for the most part as invisible as the air they inhabit or visible only under certain circumstances. In reality, however, these creatures of the imagination are nothing compared with the real and living things that fill the air and cover the earth, yet remain unseen by the eye of man. It is a fact that all around us—in the air, in water, in dust, on our bodies, clothes, and furniture—are millions of minute living creatures whose presence has been revealed to man only in comparatively recent times.

Bacteria, microbes, germs, or organisms, as these creatures are variously called, are so small that only under the magnifying power of a microscope can they be seen at all. Yet each is capable of living, of using up food-material, of multiplying, of producing poison, and even of killing men. The explanations offered centuries ago to account for men dying from no visible cause were no more fantastic than the reality.

The discovery of germs was made possible by the invention and perfection of the microscope, which allows of magnifications of many hundreds of times. The first man who really saw microbes was the Dutch investigator Leeuwenhoek, who made his own magnifying glasses by a method that even now is not understood. He was able, in 1675, to describe to the Royal Society of London the appearance of different microbes. Leeuwenhoek and most of those who came after him were concerned only with observing these minute creatures, and it was not until 1860 that Pasteur began to suggest that they might be of immense importance to man.

Pasteur was at first studying only the causes of putrefaction and the process of so-called "spontaneous generation," under which living matter was alleged to arise from dead matter. Pasteur showed that there existed invisible forms of living matter capable of growth under certain favourable conditions, easily killed by such a process as boiling, but always present in the normal air. This discovery was not at first connected with the diseases of mankind, and before we discuss this link of cause and effect we must learn more of the fundamental characteristics of germs and the manner of their discovery.

All living matter is composed of a highly complex substance called protoplasm. In animals and plants protoplasm is found divided up into tiny globules which we call cells. The smallest conceivable unit of life is a single cell, and of all single-cell creatures bacteria are the smallest and simplest. Yet their very size indicates a complexity that is astonishing, for within their tiny bodies goes on every process necessary to existence. To see these creatures we must use the compound microscope which, by a combination of different lenses, gives us a magnification up to many hundreds of times. In the ordinary examination of organisms we require to magnify them about one thousand times. The length of the most common germs is no more than one-five-thousandth of an inch; many are smaller. So small are they, indeed, that a special unit of measure is used to describe them. This is the micron or  $\mu$  (Greek *m*), which is 0.001 millimetre, or one twenty-five-thousandth of an inch. This order of magnitude is difficult to grasp, but something of its significance will be indicated by the calculation that if an organism 1  $\mu$  long were magnified to look like one inch, then an inch magnified to the same scale would be seven hundred yards long. The smallest germs are about 0.25  $\mu$  in length, while many are 3-5  $\mu$  long, though much greater lengths may be reached.

The microscope which enables us to see such minute

bodies is of course a remarkable instrument, and many optical problems had to be solved before we evolved it. Leeuwenhoek used a single lens of great magnifying power; in the modern microscope a number of lenses are combined. To prevent distortion, to allow as much light as possible to come through, and to give the necessary high magnification, these lenses have to be carefully chosen, carefully ground and combined; and in addition the microscope must be solid enough to prevent vibration yet sufficiently light to enable the smallest detail to be accurately focussed. All these conditions have been met by the makers of microscopes.

Leeuwenhoek (1675), Joblot (1711), Spallanzani (1750) and many others had to work with instruments lacking the precision of those of the present day. Nevertheless they laid the foundations of all modern bacteriology. The first problem they had to tackle was the question of where germs came from, how they lived, and whether they multiplied. They found that germs required moisture, a certain temperature, and certain food-stuffs; given these, they would multiply at an astonishing rate. They showed that germs could be killed by boiling and that they could arise only from previously existing germs.

Pasteur's experiments on this point were conclusive. It was recognised that if a liquid such as a sugar solution, or beetroot juice, or an infusion of vegetables was exposed to the air it would decompose and germs would appear in it. Pasteur took some narrow vertical flasks and partly filled them with such a fluid. By heating the glass he was able to draw the neck out into a series of curves. He then boiled the flasks until steam came through the curved neck. When such a flask was left standing the fluid did not decompose and did not grow any germs. Although ordinary air was free to move in and out of the flask, the curves in the neck prevented germs, being solid particles, from entering.

To-day, with the general acceptance of the fact that microbes arise only from previously existing microbes, it is possible to prepare fluids containing all that is necessary for bacterial life, and keep them uncontaminated for ever. If, however, even a single germ is left alive in such a fluid, or allowed to enter it, microbial growth will at once commence and proceed at a rate that is more than astonishing. The more common organisms grow at such a rate that they become, in the mass, clearly visible in twenty-four hours. A bacillus will divide into two bacilli in about twenty minutes; each of these new organisms will at once begin to grow, and in twenty minutes division will again take place. This means that, under ideal conditions, *a single bacillus would in eight hours produce 16,000,000 descendants.* The weight of a single organism is infinitesimal, but in twenty-four hours it would, if allowed complete growth, produce about 500 tons of bacilli.

It follows that if ideal conditions were available to bacteria the world would be swamped by their growth. There are, however, many factors operating against this possibility. Only the commoner organisms grow at the rate mentioned, and many require days or even weeks to produce any appreciable weight of material.

The early bacteriologists were not aware of these facts. They did not get beyond observing that germs existed and could be grown in fermentable fluids. It was only after these and many other facts had been discovered that Pasteur first suggested that germs had a connection with human disease. The great French investigator had begun by being interested in the part played by germs in the production of alcohol, but soon he realised that germs had a greater part in the life of men. As we shall see, bacteria play a part in many living processes and mechanisms of value to man. The most important point, however, is that these tiniest globules of protoplasm are capable of causing disease and death in the higher animals.

## CHAPTER II

### The Growth and Identification of Bacteria

MODERN bacteriology has almost innumerable methods of examining bacteria, and many tests have been devised to enable the various germs to be identified. The first thing necessary is that we should be able to grow the germs under examination. To do so we must provide them with easily assimilable food-stuffs. All bacteria require carbohydrates, a source of nitrogen and certain salts; given these, most of them are easily grown.

Bacteriologists use, as the most common type of "medium" for the growth of bacteria, a broth prepared from heart muscle soaked in water, to which is added a protein, commonly peptone, and some common salt. This combination gives a fluid in which most organisms will grow. Fluid media are, however, not suitable for every purpose; to obtain solid media gelatine may be added to the broth, giving a mixture which is solid at ordinary temperatures. But as germs usually grow best at body temperature ( $37.5^{\circ}\text{C.}$ ), at which gelatine is fluid, the mixture described is not always suitable. In its place is used a substance called "agar," prepared from seaweed found especially in the Chinese Seas. This gives a medium which is solid at incubator temperature; broth so solidified is the most common culture medium in use. It is generally kept in glass test-tubes in the form of "slopes," made by allowing the agar to solidify while the tube is lying almost flat. The tubes are closed with plugs of cotton wool and the whole apparatus is sterilised so that no germ may be present.

## 6 GROWTH AND IDENTIFICATION OF BACTERIA

Sterilisation in this case consists of placing the medium in what is called a Koch's Steriliser, which provides a temperature of 100° C. by means of steam. Steam heat is used instead of dry heat in order to prevent damage to the medium from evaporation. The medium is left in the steriliser for twenty minutes, by which time most of the

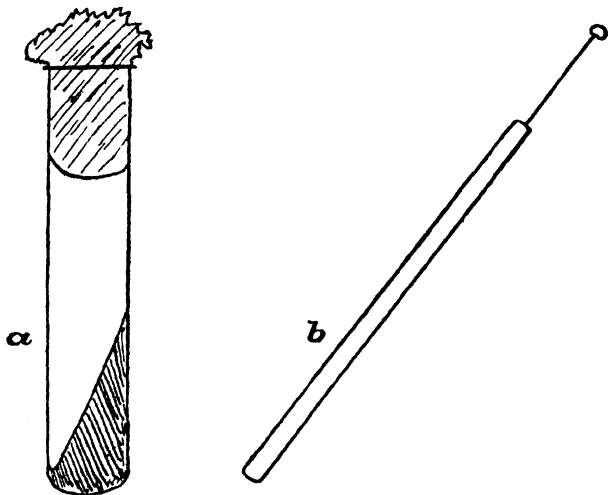


FIG. 1.—(a) A "SLOPE" OF CULTURE MEDIUM IN A COTTON-WOOL PLUGGED TEST-TUBE.

(b) A PLATINUM "LOOP."

ordinary germs will be killed. But it is possible that spores may still survive, so the process is repeated on three successive days. During the periods between the successive sterilisations any spores present are likely to have germinated and changed to forms susceptible to heat.

When we wish to grow some germs we inoculate or plant such a sterilised "slope" tube with the matter thought to contain the bacteria. This is done by means of a bacteriological needle or, as it is usually called, a "loop," which

is a piece of fine platinum wire about two inches long, mounted in a metal or glass holder. The end of the wire is bent into a closed loop, platinum being used because of its comparative indestructibility and because it is very easily heated to red heat. If placed in an open flame such a wire becomes instantly red-hot and is therefore completely sterilised. If we dip the loop into a fluid containing germs, it takes up some of the fluid, which can then be transferred to the surface of the medium. We take out the cotton-wool plug and pass the mouth of the tube through the flame so that no germs may drop in from the edge of the glass. Then, holding the tube at an angle so that air-borne germs may not enter, we pass in the platinum loop and gently rub it over the surface of the culture medium. We replace the cotton wool and put the tube in an incubator, heated by gas or electricity and kept constantly at a temperature of  $37.5^{\circ}$  C.

In thus inoculating the tube we may have deposited many organisms on the surface, but there will be nothing to see. The medium remains clear and its surface smooth. Twenty-four hours later, however, we find a change. Wherever an invisible organism was placed, there has arisen a small mass visible to the naked eye; such a mass is a "colony," and each has developed from a single organism. Colonies increase in size for some time but become stationary when all the available food-stuffs have been used, or when poisons produced by the germs themselves become too powerful. If this poisoning process occurs the organisms will die unless the conditions are changed.

We may again take the platinum loop and, touching one of these colonies with it, transfer the bacteria to another tube of medium, making what is called a sub-culture. Here the germs will go on growing again and produce new colonies. By repeated sub-cultures we can examine the behaviour of any germ under different conditions, and it is on such behaviour that organisms are classified.

## 8 GROWTH AND IDENTIFICATION OF BACTERIA

In another chapter we shall look at some of these organisms individually, but for the moment must consider the first principles of identification. Nature has, fortunately, been kind to us in giving many germs distinctive characteristics, so that bacteriologists are able to recognise them at once. There are, however, some so closely related that elaborate tests are needed to identify them.

Of these characteristics the first and most easily noted by the inexperienced is the colour of the germ or, rather, of the colonies. The most common colonies are white, but some are transparent or translucent, while others produce yellow, golden, red, violet, or green pigments. Thus the *Staphylococcus aureus* produces colonies with a beautiful "old-gold" tint (hence the name *aureus*). This germ causes a variety of diseases—the most common being the ordinary boil. It has, however, a "cousin" that is almost always harmless, the *Staphylococcus albus*, which produces a pure white colony.

In addition to the question of colour there is the interesting point whether the pigment produced remains in the bodies of the bacteria or passes out of them into their surroundings. The latter process occurs with *Bacillus pyocyaneus*, which produces rapidly and in large quantity a bright green pigment which at once penetrates the medium in which the bacillus is growing.

Then there are differences in the sizes of the colonies and also in the rates at which colonies grow. The staphylococcus mentioned above produces colonies  $\frac{1}{4}$ th of an inch in diameter, but the streptococcus, a germ usually dangerous to mankind, seldom gives a colony larger than  $\frac{1}{16}$ th of an inch. In addition, the colonies vary in appearance, being smooth, shiny, wrinkled, dry or tending to spread outwards, according to the class of organism.

It is necessary also in this connection to note that most bacteria are very particular about their food and will not grow unless they are given what they want. The plain

agar-broth medium is suitable for some of the commoner germs, which on this medium give a heavy growth in twenty-four hours; others will grow only if blood is added to the medium. Thus the germ called *Bacillus influenzae* (though it is not certain that it does cause Influenza) requires the presence of blood. The bacillus which causes tuberculosis is even more difficult to satisfy, but it is well enough pleased with a mixture of egg and glycerin, on which nevertheless it takes many weeks to produce colonies of appreciable size.

Even more interesting is the classification of bacteria into "aerobes," which must have oxygen before they will grow, and "anaerobes" which only grow if all oxygen is excluded. The exclusion has to be secured by chemical means; usually the oxygen of ordinary air is replaced by nitrogen or hydrogen. There are a few bacteria which can exist either in the presence or absence of oxygen. Further, there is the interesting group which can use and "fix" in their own bodies the nitrogen found in the air.

Apart from whether they will grow in the presence or absence of certain substances, there is also the question of how the germs deal with those substances they do utilise. The most common bacterial phenomenon is that of fermentation, in which a carbohydrate is broken down into lower forms with the liberation of gas and the formation of other chemical products. It has been discovered—and is now a distinguishing test—that bacteria do not all utilise the same carbohydrates. On this fact is based the method of separating into individuals a large group of bacilli, including those of the diseases Typhoid Fever and Dysentery.

Another factor affecting bacteria is the temperature of their surroundings. For the most part the organisms associated with man grow best at body temperature, 37.5° C., and are almost completely prevented from growing by freezing. Freezing does not cause death, and even

## 10 GROWTH AND IDENTIFICATION OF BACTERIA

intense cold may be resisted, but at such low temperatures the germs do not reproduce. Conversely, most organisms are adversely affected by high temperatures; above 45° C. few continue to grow. There are, however, a few germs, the thermophilic bacteria, which grow in manure or in hot springs where the temperature is 55° C. or higher.

All the characteristics so far mentioned relate to bacteria "in the mass." When we come down to individuals as seen under the microscope there are other possible subdivisions and other distinguishing characteristics. To see germs properly under the microscope it is necessary to stain them. They are in themselves practically colourless, but when they have been killed by drying they readily take up certain dyes and so are rendered visible. Some common colours, such as methylene blue, stain practically all bacteria and are therefore capable of showing the size and shape of germs. But even in their reaction to dyes the bacteria vary, and by using something more than a simple stain a further distinguishing factor becomes available. The method of staining generally used is that discovered in 1884 by Gram, a German bacteriologist. It requires the use of two dyes, a solution of crystal violet and one of a contrasting red stain, such as basic fuchsine. Bacteria stained by this method, if they stain at all, either take up and retain the violet—that is to say, are "Gram positive"—or are unable to retain the violet and so stain red, or "Gram negative."

To stain bacteria in this way it is necessary to prepare them by placing them on a glass slide about 3" × 1". With the platinum loop some of the bacteria are spread out thinly on the surface of the glass (using a little water, if necessary) and allowed to dry. The slide is quickly passed through a flame to complete the drying and to cause the germs to adhere; the staining is then carried out. The film is again dried, after which it is ready for examination.

The lens used on the examining microscope is of such high magnification that it is necessary to place between it and the slide a drop of a substance like cedar-wood oil, which prevents loss of light between the film and the slide and the minute lens of the oil immersion objective. The Gram stain is suitable for most organisms, but, as we shall see, some, notably the tubercle bacillus, require a special method of staining.

Lastly, we may require to distinguish between organisms according to their effects on man or other animals. Within the group that injure mankind there are some which are themselves poisonous and some which produce poisons that pass out of their bodies and attack man. Some have a selective action, being more dangerous to one animal than to another.

I have said enough to show that there are many wonderful differences between the classes of bacteria, and that if Nature has made life more difficult for man by the disease-producing germs, she has also provided clues whereby these germs can be recognised. Recognition has already been followed in many cases by a satisfactory solution to the problem of prevention of the microbic disease.

## CHAPTER III

### The Germ Theory of Disease

**A**T the conclusion of the previous chapter it was taken for granted that germs do cause disease. This fact, however, is one that has been accepted only in comparatively recent times. The first bacteriologists, as I have pointed out, did no more than observe microbes, and it was not until about 1870 that any definite evidence was obtained that germs actually cause disease.

To no one person can full credit for this momentous discovery be given, but Lister in this country was probably the first to realise its importance to man. He had been keenly interested in the work of Pasteur and began to elaborate his own technique for "antiseptic" operations before evidence had accumulated to show how right he was in trying to exclude germs from surgical wounds. In Germany E. Klebs, now best known for his work on Diphtheria, came near to proving the theory, but it was really Robert Koch who finally settled the point. His earliest work was published in 1878 and he was the first bacteriologist to realise and to demonstrate that one disease is always caused by one and the same organism. Koch's work, as will be emphasised later, is still the foundation of many routine practices in a bacteriological laboratory, but his proof that a microbe of one kind or another is the cause of many diseases was of enormous importance.

Bacteria are present almost universally in nature. It follows that if we make cultures from any part of the human body we shall obtain a growth of organisms. It is therefore necessary as a first step to be able to make a

“ pure ” culture of organisms, *i.e.* to make a culture from a single germ so that all the germs in it and in sub-cultivations will be true to type. The methods for doing this are manifold, but the most common is a variation of one devised by Koch in which, with the platinum loop, the germs are spread out on a large surface, or plate, of medium so that single organisms become separated from their

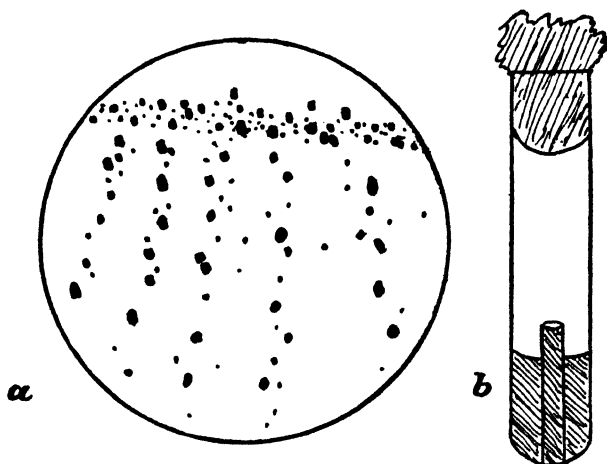


FIG. 2.—(a) A CULTURE PLATE SHOWING SEPARATION OF COLONIES BY SPREADING OVER THE SURFACE.

(b) A TEST-TUBE OF FLUID MEDIUM WITH SMALL TUBE FOR TESTING FOR GAS-FORMATION.

fellows. Each then gives rise to a colony from which a pure culture may be made. If any culture shows more than one type of colony, then all the types must be tested.

How are they to be tested is the obvious question, and the answer, if not obvious, is at least definite. Before we can say that a disease is caused by a particular germ we must fulfil three conditions. These conditions, usually called “ Koch’s Postulates,” demand “ that before a germ can be said to cause a disease it must

- (a) be constantly found in the diseased parts of the body;
- (b) be capable of growing outside the body; and
- (c) be capable of exactly reproducing the disease if introduced into the bodies of animals or man."

For the moment let us take for granted the suggestion that germs cause diseases in the human body, and let us consider what happens. The germs must first of all gain entrance into the tissues. To do this they may have to pass from one person to another: therefore they must be capable of existing for some time outside the body—and this is possible with most of them, though their vitality varies enormously. Many organisms, unless absolutely dried or treated with heat or antiseptics, can live for a very long time in a state of "suspended animation." Others form "spores" which are, as it were, seeds from which a new germ can arise, and such spores are very resistant to bad conditions. In one form or another, however, germs do pass from one person to another and enter the body. This they may do through the skin itself, though the skin is very resistant to the entrance of germs, and in most cases it is only when there is a cut, scratch, or abrasion that microbes are able to get through. Or the organism may enter by a more vulnerable route, the respiratory tract—the nose, the throat, the windpipe, and the lungs—in every part of which the moist warm conditions are favourable to bacteria. There is also the alimentary or digestive tract, where certain germs normally live in large numbers, but where dangerous organisms may enter and cause such conditions as typhoid fever. Finally, there is the genito-urinary tract by which the organisms of the venereal diseases usually enter.

When organisms do enter the body, Nature puts forth other defences, one of the most important being certain cells in the blood stream, the "polymorphonuclear cells,"

described in a later chapter. It is such cells that form "pus," which is a mixture of these cells and of the germs they are resisting. Here, therefore, we have a convenient starting-place for our study of germs in the causation of disease.

A good example of pus formation, and one of the simplest, is the common "boil," which was indeed one of the earliest studied by a bacteriologist: Dr. Ogston of Aberdeen Infirmary first showed that it is caused by a germ which fulfils Koch's postulates. A "boil" is an area in the skin where there has been inflammation and in which pus has been formed. If we prick such a boil with a needle, the pus will escape, and we can take up some with a platinum loop and smear it on a glass slide. Having stained it, using Gram's method, it can be examined under the microscope, which reveals the pus cells and among them little groups of darkly stained dots, spheres about  $1/25,000$ th of an inch in diameter. These occur in characteristic clusters and are called *Staphylococci*, from the Greek word meaning grapes.

Having made sure that germs are present in the pus, we can next try to grow them outside the human body. After carefully sterilising a platinum loop by heat, and allowing it to cool, we take up a small quantity of the pus and smear it over the surface of a tube of ordinary agar. This forms our culture, which is placed in the incubator overnight. Next day the surface is found studded with small round spots, the largest certainly not more than  $\frac{1}{8}$ th of an inch in diameter. These are the colonies of staphylococci, and they all show a rich golden yellow colour which proves them to be the pathogenic *Staphylococcus aureus*. The culture should be "pure," but if necessary we can transfer a single colony to another tube of agar, and in twenty-four hours we will have a heavy growth of the organism.

We have now fulfilled two of the postulates, for the organisms have been seen in the pus coming from the human

body and they have been grown outside the body. To complete the evidence it is necessary to reproduce the disease, and this can be done if a few of the living germs are injected or rubbed into the scratched skin of a healthy person. In such a case an abscess will be formed exactly like the original boil, and from this we could go through the whole process of identification and growth of the staphylococcus.

It is, of course, understood that it is not claimed that all diseases are caused by germs, but only those known as the "infections." For some of these no germ has yet been found, but by analogy it is clear that they must have a bacterial origin. Some of the worst diseases have, however, been definitely connected with a specific organism. In the case of tuberculosis Koch himself demonstrated that the cause lay in the tubercle bacillus. This is found in the lesions; it can be grown outside the body; and, when injected into a susceptible animal like the guinea-pig, it causes the typical disease. In this case, however, special methods are necessary, since the tubercle bacillus presents difficulties that contrast markedly with the easy cultivation of an organism such as the staphylococcus. The tubercle bacillus does not stain readily, owing apparently to a layer of some fatty substance covering it; but once it has taken up a dye it retains it even if placed in acid. Now that we have such a method of identification it is very easy to prove whether tubercle bacilli are present or not. The early investigators were, it will be understood, confronted with formidable difficulties.

In cultures, also, tubercle bacilli are different from the staphylococcus, for they grow at a very slow rate. The staphylococcus gives an obvious growth in a few hours; the tubercle bacillus takes many days to show any appreciable growth. The staphylococcus requires only a simple medium; the tubercle bacillus requires an elaborate preparation. But the third step in the proof that tubercle bacilli

cause disease is easy, since certain animals are very susceptible to tuberculosis. This fact has been of enormous value to the human race, enabling medical men to make an early diagnosis of tuberculosis in certain cases. If matter containing tubercle bacilli is injected into such an animal as a guinea-pig, the animal will become diseased in a short time, even though the bacilli were so few as to escape detection under the microscope. Strict precautions are, of course, taken against the animal suffering any pain, and such an experiment, apart from the proof it gives that tubercle bacilli are the cause of tuberculosis, may enable treatment with some prospect of success to be started for a human being.

Organisms such as have been here described invade the body and spread throughout the tissues, causing death by their actual presence in the body. This is not, however, the only way in which germs cause disease. Certain bacteria produce a poison—or toxin, as it is called—which pours out of the germ body and enters the blood stream, which conveys it to all the tissues. Disease or death is then due to the action of the toxin. In such cases, as in Diphtheria and Tetanus (lockjaw), the organism grows at one part of the body, where it may cause a comparatively small local lesion, while its toxin rapidly passes to other tissues and causes the symptoms of the disease to appear.

Diphtheria is a familiar example of this type of germ disease. The Diphtheria bacillus is a very characteristic organism and was one of the first disease germs to be definitely identified. One of Koch's pupils, named Loeffler, was the first to show definitely that this bacillus was always present in the throats of children who had died from diphtheria. To afford this proof he devised a culture medium (which is still used and goes by his name) on which *B. diphtheriæ* give a typical growth. The bacillus is a slender, slightly curved organism occurring in little groups that somewhat resemble Chinese writing. On Loeffler's

medium—a mixture of broth and blood serum solidified in slopes by heat—the bacillus grows rapidly and, when stained by certain dyes, is found to possess small granules which stain very intensely compared with the body of the bacillus. These granules usually appear at each end, though only one may be visible. In a culture on blood serum the appearance is absolutely characteristic of true diphtheria bacilli.

Loeffler found that this germ was present in all cases of diphtheria. But he was very puzzled by the fact that only the throat is affected and that he could not discover the germ in other parts of the body. He tried to carry out Koch's teaching and sought to reproduce the disease in animals, but he was not entirely successful. Some years later, in 1888, two French workers, Roux and Yersin, were able to show that the disease could be reproduced and also that it was due to a toxin given off by the bacilli. While the disease causes intense discomfort and even death by the local inflammation in the throat, it is most serious in its general manifestations and especially in its effect on the nervous system. These results, Roux showed, are due to the toxin pervading the body. If *B. diphtheriæ* are grown in a fluid broth for some days, the broth gradually becomes saturated with toxin. The broth is then filtered through porcelain so that the bodies of all bacilli are removed, the filtrate being sterile. Such broth injected into man or animals will produce the same result as an injection of living organisms, thus providing a clear proof that the toxin causes the disease.

This result is of enormous importance, as will be seen when we discuss the defences of the body against germs. Meantime, enough has been said to show that many of man's worst diseases are caused by pathogenic micro-organisms or by the poisons they produce.

## CHAPTER IV

### The Principal Organisms and their Effects

THE classification of bacteria presents difficulties comparable with those that confront the biologist who sets out to arrange all known animals in their respective species. A century ago bacteria were quite frankly regarded as unclassifiable, and even to-day there is no universally accepted classification; none, certainly, simple enough to be given here. It will be enough for us to consider bacteria according to their shape and to note the most important members of each group.

The principal division is that between germs which are spherical and those that are cylindrical, the latter group being further subdivided. The spherical organisms are known as cocci, though a few members of this group are not strictly spherical but show a slight departure from this shape. The cocci are distinguished from each other by their behaviour with Gram's stain, by the arrangement of the cocci in relation to each other, by the size and style of colony produced on ordinary media, and by their behaviour when cultured under different conditions. There is also the fact that we now know what diseases are caused by certain cocci, so that the source of the germ is in itself an indication of the type of organism.

Cocci that are Gram positive (*i.e.* stain dark by this method) form our first group, comprising staphylococci, streptococci, and pneumococci. Staphylococci we have already discussed as the cause of boils, noting that they form little "clusters," give fairly large colonies on ordinary

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media, and can be subdivided by the colour of their colonies. Streptococci, on the other hand, produce small colonies and microscopically are found to arrange themselves in chains like a string of beads. The length of the chains depends on the exact type of streptococcus and on the type of medium used for their growth. The *Streptococcus brevis*, which is often not harmful to man, grows on ordinary agar and gives very short chains of 4 to 6 cocci. The *Streptococcus longus*, which is usually very pathogenic, only grows well in the presence of blood in the media; when grown in broth containing the sugar glucose it produces very



FIG. 3.—VARIOUS TYPES AND SHAPES OF COCCI.

long chains. Some varieties of these streptococci will not grow in the presence of oxygen. Certain strains, all important, have the power of "hæmolyzing" or breaking down blood. They cause many serious diseases in man, such as endocarditis (inflammation of the heart-valves) and scarlet fever.

The *Pneumococcus* is clearly related to the streptococcus and grows only in the presence of blood. It occurs as a "diplococcus," i.e. as two cocci lying together, and these cocci are a trifle longer on one axis than on the other, giving a lanceolate appearance. Under certain conditions each pair of cocci is found to be enclosed in a capsule, a coating

of some undifferentiated substance which requires special methods to render it visible with stain.

It is, of course, the *Pneumococcus* that is found in the lungs in cases of pneumonia. It is usually very virulent towards man. Where there is doubt as to its exact identification, its intense virulence towards white mice leaves no doubt as to its nature.

Cocci which do not retain Gram's stain form an interesting group of four principal members. *Micrococcus catarrhalis* is the most easily grown, giving a good growth of white



FIG. 4.—PUS CELLS CONTAINING GONOCOCCI.

The large black masses are the cell nuclei.

colonies on ordinary media, and having no particular microscopic appearance. It is to be found in the throat of almost everyone, and it is doubtful if it ever causes harm to man. It can usually be separated from its companions in this group—a very necessary thing, as all these cause definite diseases.

One causes the disease known as Malta Fever. The remaining two, the *Meningococcus* and the *Gonococcus*, both occur as diplococci, and although they differ slightly in size and shape, they cannot be distinguished with certainty by the microscope alone. Here we have a strong, indeed an almost infallible, indication as to which is which by ascertaining their source. The *meningococcus* causes Cerebro-

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spinal Fever, and it is therefore found in the cerebro-spinal fluid which is withdrawn by needle from the spinal canal. The *gonococcus* causes Gonorrhœa, one of the venereal diseases, and it is found in the pus which comes from the lesions. The *gonococcus* and the *meningococcus* both show a slight departure from the sphere, being bean-shaped, the flatter surfaces facing each other in each diplococcus. Both these organisms are found "inside" pus cells, the *gonococcus* more usually so and in large numbers. Pus, we have noted, is one of Nature's defences against bacteria, and this finding of the gonococci inside the pus cells is an example of phagocytosis, or the taking up by pus cells of the organisms they seek to destroy. Both the *gonococcus* and the *meningococcus* need media containing blood, the former being particularly delicate and difficult to cultivate.

The bacilli present a greater problem than the cocci. We shall not consider in any detail those bacteria which, instead of forming a simple cylinder, are found as "spirilla" or "spirochætæ," i.e. organisms curving or having a wavy outline. The most important is the *Spirochæta pallida*, a very delicate organism only about 0.25  $\mu$  in breadth although 4 to 14  $\mu$  long, occurring in a corkscrew form with 8 to 12 windings. This organism, which moves actively when examined in a fluid, is the cause of syphilis and has been grown a few times only in artificial cultures in a very specialised medium.

Bacilli may, like the cocci, be divided into those that retain Gram's stain and those that do not, but one group—known as the "acid-fast" bacilli—lies outside this classification. These do not stain by Gram's method. The most important member is the tubercle bacillus, but enough has already been said about this and we need only note that the germ causing leprosy is very similar in shape, size, and staining. Other members of the group do not affect man.

The bacilli that are Gram positive form such an important

group that we could do justice to them only in a large text-book. The *Bacillus diphthericæ* has already been noted, and it has been pointed out that its identification depends chiefly on its staining powers with certain other stains. If there is any doubt about a bacillus being a true diphtheria bacillus, it can be tested by injection into a guinea-pig; when the characteristic of producing death without spreading in the body will be discovered.

This characteristic it shares with other Gram positive bacilli such as *B. tetani*, which possess a feature not already noted—that of spore-bearing. Under certain conditions there is formed in the body of the bacillus a “spore” which appears at one end so that the bacillus looks like a drum-stick. Other, and perfectly harmless bacilli, have this appearance also; consequently a virulence test (*i.e.* injection into an animal) may be the only means of distinguishing between them. Another Gram positive and also spore-bearing bacillus which may be identified only by a virulence test is *B. anthracis*, the cause of anthrax in sheep and cattle and also in man. It was with *B. anthracis* that Robert Koch first made bacteriological experiments proving both that the germ causes the disease and that it forms highly resistant spores.

The Gram negative bacilli are the largest group of organisms. Nearly all of them cause disease in man. They vary in size over a wide range (microscopically speaking); they may be motile or non-motile; they may grow rapidly and easily, or slowly and only in special media. They may produce pigment like *B. prodigiosus*, which is red and was, when growing on bread, the cause of the supposed miracle of the “bleeding Host.” So wide are the varieties in this group that many subdivisions are necessary.

One of these subdivisions includes very tiny Gram negative bacilli which will grow only on media containing blood. The *Bacillus influenzae* is the principal example of this type. Although found in so many cases of influenza,

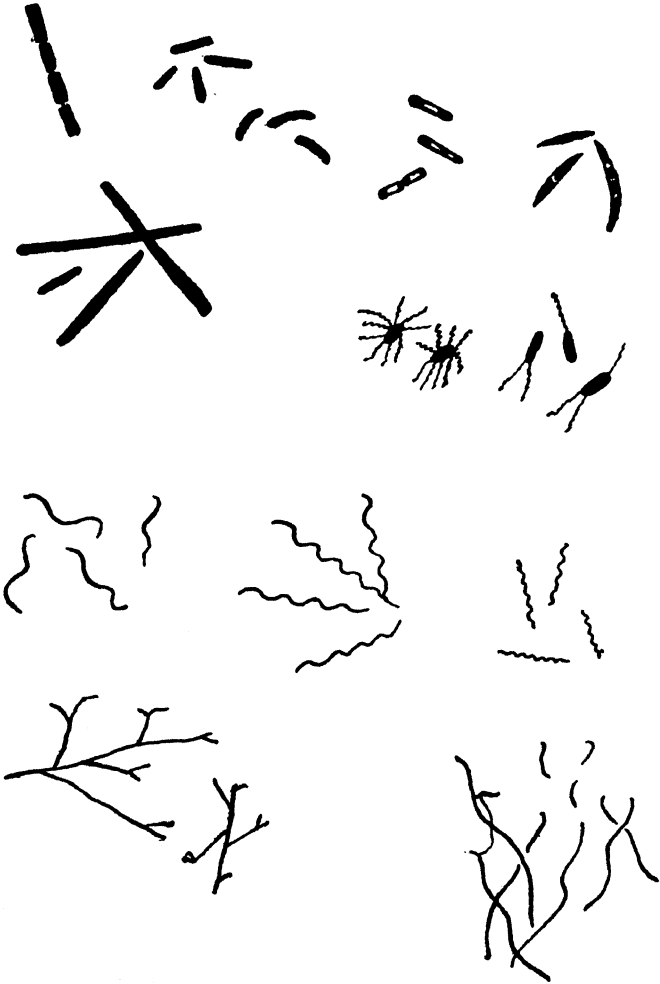


FIG. 5.—VARIOUS TYPES AND SHAPES OF BACILLI AND SPIROCHETES.

particularly in the throat and lungs of fatal cases, this organism is not definitely accepted as the cause of influenza. It is none the less an interesting germ. On appropriate media it gives a growth of small transparent colonies. Microscopically it is a very small bacillus, and it exhibits another feature called "Pleomorphism." This term means variety of shape, and the *B. influenzae*, although almost always small, is also found to grow into larger and longer forms, especially as the culture grows older.

The main group of Gram negative bacilli may be spoken of as the colon bacilli. Microscopically these are practically identical. They are fairly straight rods which vary considerably in size according to the culture media used. Some are actively motile and possess flagella which drive them through fluid media. On ordinary agar they give a heavy greyish growth. To separate them from each other more elaborate methods than have yet been described are necessary.

The first of these is biochemical. It is based on the marked capacity of these colon bacilli for fermenting sugars, with the production of acid and often gas. To demonstrate this effect, special media are prepared in which the sugars are incorporated. To these media is added a dye such as litmus or neutral red, which changes colour when the fluid changes from alkali or neutral to acid. Such a medium is placed in ordinary test-tubes with a very small test-tube placed mouth downwards in the medium. During sterilisation the heat drives all the air out of the small tube, which thus becomes filled with the fluid. Now, if an organism growing in this medium produces acid, the colour will change; if it produces gas, the small tube will be filled with bubbles. Thus the commonest colon bacillus, *B. coli*, will produce both acid and gas in media containing glucose, lactose, and other fermentable substances. But, for some unexplained reason, the colon bacilli can be sharply divided by their behaviour in this test towards lactose. *B. typhosus*,

the cause of typhoid fever, does not ferment lactose. Further, it produces acid but no gas in glucose.

*B. paratyphosus* also does not ferment lactose, but it does produce both acid and gas in glucose. It is different from some of the bacilli causing Dysentery, which may attack glucose but not the substance mannite, which *B. paratyphosus* does ferment. Other sugars are, of course, necessary to separate all the organisms in this group, but we need not discuss them here.

The complete identification of the germs of this group—except for *B. coli* itself, which is usually clear enough—depends on a serological test, a modification of the Widal reaction discussed in a later chapter.

*Bacillus coli* exists as a normal inhabitant of the human digestive tract, which is also the seat of the disease in typhoid and in dysentery. In a case thought to be either of the latter diseases, the preliminary test for the organisms must be such as will separate them from the almost identical looking colonies of *B. coli*. A solid medium, providing the fermentation of sugar test described above, is used. Such a medium contains lactose and a dye, and when the organisms are planted on it so as to give separate colonies, the *B. coli* in fermenting the lactose changes the colour of the medium, while the *B. typhosus* or *B. dysenteriae* does not. Pure cultures can thus be obtained and further tests performed.

It is also interesting to note that while *B. coli* is normally found in the intestine and does not cause any harm, yet in other parts of the body it becomes very dangerous. Thus, if the stomach or intestine is ruptured by disease and *B. coli* escape into the abdominal cavity, they cause a dangerous peritonitis. They may also invade the urinary tract and cause a severe inflammation.

There are many other Gram negative bacilli, but they can best be described in a different connection. For the moment let us look at another fact that is best illustrated

by this group of bacteria. Although they cause so much harm to man they have enabled us to discover how man's body can resist their activity, and from this discovery to devise methods for preventing and curing the diseases they cause.

## CHAPTER V

### Man's Defences

**S**URROUNDED as he is by germs, and coming into contact with them in everything he touches and in the air he breathes, man has need of many defences against the pathogenic micro-organisms. Surprise is often expressed at the number and variety of microbic diseases and at the number of people who are affected, but it is at least equally surprising that so many people escape these diseases and that so many microbes cause no harm. The reason for both these phenomena is that man has developed during evolution a strong natural resistance to these invaders; and in modern times he has added many methods of preventing the attack of some of the most virulent.

To cause its appropriate disease, an organism must first enter the body. The two chief routes of entry are, as we have seen, the respiratory tract and the digestive system. Germs present in the air, in dust, or in globules of moisture are sucked into the nose, throat, and lungs with every breath. Food and water carry organisms, normally destroyed by the digestive juices, to the digestive tract. Fortunately these organs do not fall a victim to every germ; they deal effectively with most of them and allow infection to take place only when some circumstance such as cold or exposure has lowered their natural resistance. Once these barriers are passed the germs come into contact with two weapons provided for the body by the blood stream; weapons which man now uses in his scientific battle against disease.

The blood in animals and man flows through the arteries into every part of the body, carrying food-stuffs and oxygen to the tissues and carrying away waste products through the veins. Blood consists not only of fluid but of cells—the red and white corpuscles. The white corpuscles assume different shapes and most of them have a nucleus made up of a number of lobes, from which these cells take the name “polymorphonuclears.” These all play an important part when the body is attacked by bacteria, for they have the power of forcing their way out of the blood vessels into the tissues and of “eating up” the germs. In so doing they behave like the simplest form of animal life, the unicellular amœba. The amœba consists of a single microscopic globule of protoplasm, and it moves by pushing out this protoplasm in finger-like projections. When any substance suitable for food is found, the amœba simply “flows” around it, taking up the particles direct into its body and digesting them. Metchnikoff, the Russian biologist, studied the action of certain lowly forms of life towards food, and from these observations he went on to study the polymorphonuclears in man and discovered that these cells, like their prototypes in lower forms, could engulf and digest bacteria. If, then, as we saw in the case of a “boil,” bacteria have entered the body, the polymorphonuclear cells of the blood become phagocytic, pass out into the tissues and begin destroying the bacteria. So “pus” is formed, and in many cases the phagocytes are strong enough to destroy all the disease germs, enabling the tissues to recover their normal condition.

Metchnikoff and many others thought that this was a sufficient explanation of how man got rid of disease germs of all kinds. But it was soon found that while, for example, the pyogenic cocci are dealt with in this way, other germs are not “phagocytosed.” When phagocytosis is going on in any part of the body, large numbers of polymorphonuclears are needed, since many are destroyed in the

struggle with the bacteria. The blood stream therefore becomes filled with them from the seat of their origin in the bone-marrow. In this way the number may be increased to five or ten times the normal figure, so long as the disease goes on and the patient is able to resist it. But in some diseases, such as tuberculosis, the number may not be increased; in some—for example, typhoid fever—it may actually be decreased. Under such conditions the poison produced by the invading bacteria calls forth no response on the part of the polymorphonuclear cells.

Yet man is able to recover from such diseases and destroy all the disease germs by the production of “anti-bodies”—substances which render the germs inactive and kill them. This is very clearly shown in such a disease as Typhoid Fever, where the Typhoid bacillus infects the alimentary system and causes ulceration of the intestines. The general effect on the patient is profound and the illness lasts some weeks. During this time the body is actively preparing an anti-body to the typhoid bacilli, and the detection of this in the blood, by what is known as the Widal reaction, forms one of the most reliable tests in diagnosing the condition. Some of the serum (clear fluid) from the patient's blood is diluted and then mixed with some of the typhoid bacilli suspended in fluid. This mixture of serum and organisms is kept at a temperature of 58° C. for one hour. During this time a change takes place. At the beginning the bacilli are suspended in the fluid so as to give a uniformly turbid appearance with no deposit. If the Widal reaction is positive the bacteria will be found, when the hour has elapsed, at the bottom of the tube “agglutinated” or formed into small clumps.

It has been found that if some dead typhoid bacilli are injected into an animal or man the blood will show, by the Widal reaction, that anti-bodies have been formed. To such an extent are anti-bodies formed that immunity (*i.e.* complete resistance to the disease) is created. During the

war the value of this procedure was very clearly shown. Such an injection (or a series of injections is commonly given) is called a vaccine, the name being derived from the fact that smallpox (*vaccinia*) was the first disease to which immunity was artificially produced. But in smallpox it is not an emulsion of dead germs that is used, the patient being given a mild attack of the analogous disease cowpox.

Another variation of the process is effective in rabies. This disease, which attacks the nervous system, can be prevented by injections of brain matter from animals infected by it.

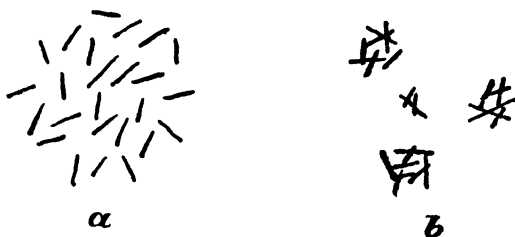


FIG. 6.—AGGLUTINATION AS SEEN WITH THE MICROSCOPE.  
(a) EMULSION OF BACILLI. (b) CLUMPING OF BACILLI.

These are all examples of “active” immunity. The material injected or otherwise introduced into the tissues causes the body to produce its own anti-bodies, which are then demonstrably circulating in the blood stream. It follows, therefore, that if such blood were taken and injected into another patient suffering from the disease, the anti-bodies thus introduced would aid the patient in his fight against the disease. If enough anti-body is injected to cure the disease, the patient has been given a “passive” immunity—*i.e.* from outside his own body.

The importance of this method of treatment is best illustrated in those diseases which are caused, not by the invasion of the tissues by the germ itself but by a poison

produced by it, for example, in Diphtheria. The toxin produced by such an organism circulates in the blood stream and may poison an organ, such as the nervous system, far away from the spot where the germs are acting. When such bacteria are grown in fluid media they produce their toxin just as they do in the body, and if the toxin is injected into an animal it produces the disease. When injected in quantities too small to cause serious injury, it stimulates the animal to produce anti-bodies, and if repeated toxin injections are given, the amount of anti-toxin becomes very great.

Such a serum from an animal can be used to protect human beings; in the case of Diphtheria, anti-toxin is now the recognised treatment. Such anti-toxin is produced from horses. Another animal serum so produced affords protection against Tetanus. This is also a disease caused by a toxin produced by germs that do not invade the body to any extent. The anti-serum is very potent and neutralises large amounts of the poison. Given at the proper time it can prevent the symptoms of the disease from developing.

Anti-body production in the blood is of great interest, and substances which cause it are called antigens. As a rule the body produces only the anti-body to the specific substance injected. Thus an injection of *B. typhosus* will cause the production of anti-bodies to *B. typhosus* but not to the closely related *B. paratyphosus*. Occasionally, however, the anti-body is not thus specific against the infecting organism. In Typhus Fever, for example, the blood reacts with a *B. proteus* (Weil-Felix reaction), although that organism is not now believed to be the cause of the disease. In syphilis the Wassermann test used to diagnose the disease depends on the blood reacting with a solution of the lipid bodies in heart-muscle. These lipid bodies are present in all muscle and are obtained by treating the heart-muscle with ether and alcohol. So far no explanation

can be given for this peculiar reaction with a substance from normal tissues instead of with the infecting agent.

When either active or passive immunity is produced, the body will for a time be completely able to avoid infection. Unfortunately this immunity may not last for any long period. It may, as in smallpox, last for years, or it may last for a few days only. There are indeed some diseases in which an attack appears to have the opposite effect and to render the victim more susceptible to the disease. There are also some diseases in which so far no effect from the injection of the causative organism has been obtained, but this is probably due to some peculiarity in the chemistry of these germs and may yet be overcome.

## CHAPTER VI

### The Allies of Bacteria

**G**ERMS, it has been shown above, are found everywhere and may cause a variety of ill effects in man. Yet they have to overcome many barriers before they can invade the human tissues, and it is of more than mere academic interest to discover that in their attack on the human body they have many assistants. To call these assistants "allies" is really wrong, for there is no active joining of forces. The assistants of the bacteria are usually either quite passive or they suffer equally with the human being attacked through them. Bacteria cause disease in many of the lower animals—in birds, in fish, in insects, and even in plants. It is therefore clear that as man comes in contact with all these natural objects, he must at times come in contact with the bacteria causing disease in these other forms of life. Where the germ is not noxious to man there is no harm; but in many cases man is even more susceptible than the lower forms.

Man, as in many of his activities, is in this direction his own chief enemy to some extent. Many risks of infection arise from his insanitary habits. In a country like our own this risk, together with many of its attendant diseases, has almost disappeared, but in a country like India epidemic diseases have every chance to spread. Water, whether for drinking or washing, is one of the most dangerous vehicles of disease. Unless every danger of contamination with sewage or human excretion is avoided, the water supply of a district may convey the Cholera

bacillus or *B. typhosus* and its related germs to the entire population. The Cholera bacillus is a Gram negative bacillus, usually spoken of as a "vibrio," as it is a curved or comma-shaped organism. It is actively motile in fluid and grows on ordinary media. It is present in enormous numbers in the excreta of patients suffering from Cholera, and it may live for some time in water.

Man's food may also convey to him disease germs or their poisons. There is, indeed, a special "food-poisoning" group of organisms, all Gram negative bacilli, closely related to the Paratyphoid family. Their final identification depends on the phenomenon of agglutination already described. The Widal reaction, as has been stated, consists of mixing the blood serum of a patient with a recognised strain of the suspected bacilli; should the blood contain anti-bodies the bacilli will be agglutinated. The same phenomenon is utilised in the reverse direction, the bacillus thought to be, for example, *B. paratyphosus* being mixed with a serum from an animal previously injected with a recognised *B. paratyphosus*; should agglutination occur, then the identification of the bacillus is complete.

The chief of the food-poisoning group is *B. enteritidis* which causes disease in cattle. It may be found in the flesh of animals sold for food. If eaten it grows in the intestine, causing an acute gastro-enteritis and affecting the whole body by its toxin.

In addition to this Gram negative group of bacilli there is a Gram positive bacillus which infects food and causes severe illness and often death in man. This is the *Bacillus botulinus*, which produces an exceedingly powerful toxin and need only be present in very small amounts to affect the whole nervous system. It is a spore-bearer and may therefore survive the process of cooking. Sometimes it is found in preserved meats or vegetables.

The tubercle bacillus may also be conveyed by meat, but the chief carrier is milk. Modern regulations and

hygienic methods of handling have diminished the number of samples of milk containing tubercle bacilli; nevertheless the proportion is still very high. Another method by which tubercle bacilli reach the human body is through the expectorations of those suffering from pulmonary tuberculosis. The bacilli may be present in moisture coughed out of the mouth, or they may be present in dust, where they can exist for a long time even when dry. It has been found that very often the bacilli present in milk are different from those present in human sputum, and various tests have shown that there are at least two types—bovine and human—infecting human beings. There is also an avian strain, affecting fowls but not of importance to man.

Man's use of other animal products brings him into contact with various diseases. In the island of Malta, where goat's milk is extensively used, a febrile disturbance frequently occurs. This disease, usually called Malta Fever, is due to a Gram negative coccus, somewhat similar to the *meningococcus*, which is found in the milk of goats and is thus spread to man.

More dangerous to man is another disease of sheep and cattle—Anthrax. This is a septicæmic (blood-poisoning) disease; the germ passes into the blood stream and is carried throughout the body, and after death it is found in most of the organs. The *Bacillus anthracis* is a Gram positive organism which is rectangular in shape; under certain conditions it contains a central spore. The spores never form when the organism is present in the body tissues. They appear outside the body in aerobic conditions—that is to say, where oxygen is present. They are very highly resistant and survive for a long time in dust or on the skin and hairs of animals. Owing to the ability of the spores to exist in dust and to leave the spore state and resume the bacillary form when once inside an animal body, the disease is easily spread among animals grazing

in an infected field. Man is infected through the handling of animal skins and by the use of unsterilised bristle shaving-brushes. The spores may also be present on wool, and workers handling this may, by inhaling small particles, acquire "wool-sorters' disease."



FIG. 7.—THREE TYPES OF SPORE-BEARING ORGANISMS.

In all the diseases so far discussed the germ's "ally" has been entirely passive. Those still to be mentioned are

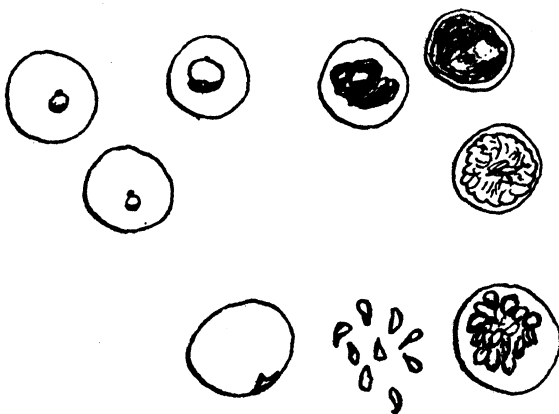


FIG. 8.—THE MALARIA PARASITE INVADES THE RED CORPUSCLES OF THE BLOOD AND GOES THROUGH A CYCLE OF GROWTH, THEREBY DIFFERING FROM BACTERIA.

also passive in the sense that the conveying of the disease germ fulfils no purpose in their own lives. It is a mere accident of their existence, although they actually introduce bacteria directly into the bodies of human beings. Insects,

fleas, ticks, lice, and mosquitoes belong to this group, and the diseases they carry are among the most formidable man has to face. The mosquito, as everyone knows, carries the organism causing Malaria, but as the *Plasmodium*, as this organism is called, is of an entirely different nature from the organisms we are discussing, we need only mention it here.

The body louse conveys the organism of Relapsing Fever of the type found in Europe; a tick plays a similar part in West Africa. Both diseases are caused by almost identical organisms—long, delicate spirochætes which are found swarming in the blood of infected patients. The one found in the European type of disease—*Sp. obermeiri*—was first discovered in 1873, being one of the earliest observed organisms of human disease. The louse bites an infected person and sucks up some of the blood containing the spirochætes, which multiply within its body. If the louse goes to another host and bites him, the organisms enter the blood stream and quickly increase in numbers.

Of all diseases handed on by insects the most deadly—and indeed one of the worst of all infections—is Plague. This is carried by fleas. It is caused by the *Bacillus pestis*, a small round-ended bacillus which stains gram-negatively. When stained with a simple dye like methylene blue it shows a very characteristic bipolar staining. When grown in culture it shows marked pleomorphism or changes in form, some bacilli being long while others become very fat and irregular in shape. Although it shows the bipolar staining it does not form spores.

This organism infects most small rodents and is found particularly in rats. Fleas present on such rats become infected with the bacillus and may leave the rat (especially when it dies from the disease) and pass to a human being. When the flea bites it regurgitates some of the bacilli which have multiplied in its stomach; they thus enter the skin and spread quickly through the body. They

cause two distinct types of disease, the more common being the "bubonic." In this the lymphatic gland near the infected bite becomes inflamed, accumulates large numbers of the bacilli, and gradually breaks down. The other form, known as "Pneumonic Plague," chiefly attacks the lungs. As a result of coughing and expectoration the organism in this type may pass from one individual to another without the intervention of fleas and rats.

The pathogenic bacteria, it will therefore be realised, have many opportunities of reaching man and causing serious illness within his body. So far, however, no one has discovered how organisms first assumed this power. But there are other germs, whose characteristics we will now examine, which not only do not harm man but may produce effects which are necessary or useful to him.

## CHAPTER VII

### Bacteria that Aid Us

CONSIDERING their universal presence and the extraordinary rapidity of their growth, with the consequent utilisation of food products and chemical substances, one might expect bacteria to produce results of economic interest. Their value in this direction has been shown in a great variety of cases; "economic bacteriology," in fact, is now developing into a separate science. It is not concerned, however, entirely with the beneficial effects of this class of germ; there are unfortunately bacterial processes which are economically harmful. These are chiefly in connection with disease in animals, insects, or plants used by man, as in the infections of bees or silkworms, in "mosaic disease" of tobacco, diseases of root vegetables or the blights of fruit trees.

Others which will be mentioned in the course of this chapter illustrate the fact that a useful and a harmful germ may take part in the same economic process. Indeed to this fact we owe to some extent the commencement of bacteriology as a science. One of the most common phenomena in bacterial growth is fermentation, by which sugars are broken down to alcohol or alcohol is used for the production of acids. In the production of alcohol yeasts are the bacteria utilised, and although man has used fermented drinks for centuries it was not until Pasteur took up the question that the rôle of the yeast was proven. Pasteur investigated the subject at the request of an alcohol manufacturer whose vats produced an acid instead

of alcohol. He discovered that, instead of the alcohol producing yeast, the vats contained a lactic-acid producing bacillus. He was able to demonstrate that by taking precautions to have only the proper organism present the acid production would cease and alcohol be formed as before.

The action of yeast is, of course, utilised also in bread-making. Here the yeast again attacks fermentable carbo-

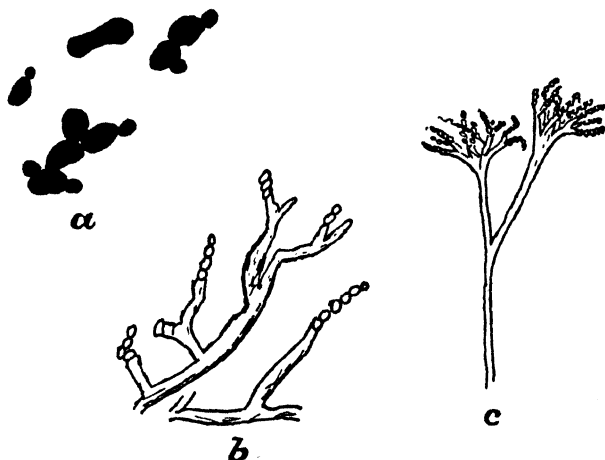


FIG. 9.—FUNGI. (a) COMMON YEASTS. (b) A COMMON INHABITANT OF SOUR MILK. (c) THE USUAL CAUSE OF GREEN CHEESE.

hydrates, producing carbon-dioxide and an alcohol. Its full action, however, has not been explained. When the bread is baked the temperature of the oven should be high enough to kill the yeasts and any other bacteria present. But in the centre of the loaf some spores may escape, and in this case there may arise a "disease" of the bread due to a bacillus, *B. mesentericus*, the effect produced being described as "ropiness."

One of man's first uses of bacteria was in connection with the "retting" of such fibre plants as flax; the process

being designed to free the strong flax fibres from their softer supporting tissues. The discovery that the substances binding the fibres, a pectin material, is destroyed by bacteria belongs to this century, but the general process was utilised by the ancient Egyptians and by man in other regions still earlier. Now that the part played by bacteria is recognised, man is endeavouring to improve and quicken the process by introducing large numbers of organisms recognised as breaking-down pectin, or by changing the conditions of the "retting" so as to favour the growth of bacteria. The danger of these attempts is that the process may go too far and that the bacteria may attack the flax fibres. However, as this is a recognised danger, steps are taken to avoid it.

To some extent similar is the action of bacteria during tanning and the preparation of leather. When the hair is removed by soaking in lime water or when the skins are exposed in an air-tight room, bacteria are busy. In the actual tanning bacteria are also present and aid the penetration of the tannin. Here again care is required to prevent the germs going too far and, by their ability to break down protein, destroying the strength of the skins.

In both retting and tanning the germs concerned are capable of breaking down a variety of substances of highly complex nature. One such substance found in all plants is cellulose, which is not acted on by our digestive juices. Cellulose can, however, be utilised by certain aerobic and anaerobic bacteria, and the bye-products of their action become available as food for man or other germs.

Animals living mainly on grasses depend for their digestion to a large extent on the presence within their intestinal tracts of organisms that break down the cellulose of the plant cells and render the contents assimilable. The process has an important economic extension in the preparation of manure. In ordinary farmyard manure there is a large amount of dry straw, and it is the action on this of

cellulose-destroying bacteria that makes it useful as manure.

The manuring of soil has a double purpose. By the introduction of partly "digested" vegetable matter the soil is given a proper texture, facilitating aeration and enabling moisture to be retained. In addition to thus producing "humus," manure makes nitrogenous products available. The growth of all plants requires carbon, oxygen, and nitrogen, and some other chemicals in small quantities. The carbon and oxygen are obtained chiefly from the air through the action of chlorophyll, the green substance in the leaves of all plants. A direct supply of nitrogen can come only from the soil; it is found there both in conjunction with other inorganic chemicals and as the result of organic decomposition. The great source of nitrogen is, of course, the air, which contains 80% of nitrogen. The nitrogen must, however, be in the form of nitrites before it can be used by plants. One of the most fascinating discoveries in bacteriology is that certain bacteria can form nitrites from ammonia; and even more fascinating is the process by which other organisms can take up the nitrogen of the air and make it available in the soil for plants. These "nitrogen fixers" or Azotobacteria are found living in the soil. The most interesting member of the group is *B. radicicola*, which lives in "symbiosis" with leguminous plants such as peas. (Symbiosis is the condition when two or more living things find it mutually advantageous to live together.) This bacillus was found, in 1886, to be present in nodules that appear on the roots of certain leguminous plants. When a plant becomes thus "infected" it secures a supply of nitrogen which *B. radicicola* takes from the air. So important is this aid from a germ that if Legumens are planted in a field where none have been before it is necessary, in order to secure satisfactory growth, to scatter cultures of *B. radicicola* on the soil.

In another direction bacteria are utilised to break down substances which are obnoxious or dangerous to man. I refer to the "septic tank method" of dealing with sewage. In this process the fluid sewage is allowed to pass very slowly through a closed tank in which there is practically no air and in which, therefore, anaerobic bacteria flourish. These break down cellulose and protein and even attack fats. The sewage is then sprinkled over filter beds of stone so that, as there is a large surface area, the greatest possible exposure to the purifying oxygen of the air is obtained. To kill off the remaining bacteria some antiseptic (preferably one of the chlorine group) may be introduced. The sewage effluent should then produce little or no harmful effect when it passes into a river.

Certain of man's food-stuffs, especially those derived from milk, are produced by the action of bacteria. Milk itself is always contaminated by organisms from the air, from the cow, and from the milker, and if these are allowed to grow the resulting souring of the milk will render it unusable. Nevertheless the process of butter-making depends on the action of germs on the milk. The flavour and quality of the butter depend on the organisms present, and it is only when lactic-acid producing bacteria predominate and by their acid production stifle other germs, that the butter has a pleasant flavour. It follows, now that the process is recognised, that it is better for a manufacturer to pasteurise the milk, killing off all or most of the germs, and then to add a pure culture of lactic-acid producing bacteria. Provided a good and constant "starter," as such a culture is named, is used, the resulting butter will be of good and uniform quality.

The making of cheese also depends on the initial production of lactic acid in milk by bacteria. The process is readily spoiled if certain germs gain entrance. Pasteurisation would ruin the milk for cheese-making, and great care is therefore necessary to avoid contamination. This is

more important in the second or "ripening" stage of cheese-making. The organisms used to acidify the milk remain in the solid cheese and continue to grow so long as conditions are favourable. At this stage the by-products of bacterial growth affect the flavour of the cheese. Germs other than the lactic-acid group play a part in these changes, and the various flavours obtainable in ripe cheese are due to the variety of organisms present.

Many other bacterial processes take place in man's food, but most of them are unfortunately more apt to spoil the value of the food than to enhance it. So precautions have always to be taken against bacterial infection. This is of particular importance to the vast modern industry of "canning," whether applied to fruit, vegetable, or meat. In the process of canning the food is sterilised by heating, but if this action is not efficient, spore-bearing germs may escape and later grow inside the tin. This particularly applies to the anaerobes of which *B. botulinus*, already described, is an important example.

Thus we see that the question of germs in man's food is of vast importance. Some produce results of great value, but even these must be carefully controlled. Others are very harmful and must be excluded. The same remarks apply to the industrial field. A knowledge of bacterial activity is valuable in many manufacturing processes, some of which man has used for centuries without any recognition of the causative agent. There is still ample room for research in this connection, both for the purpose of subduing harmful germs and for making wider and better use of those we can call to our aid.

## CHAPTER VIII

### Germes we Shall Never See

**I**N discussing the germ theory of disease it was shown that before any particular organism could be accepted as the cause of the disease certain conditions must be fulfilled. There have arisen, however, a certain number of cases where these conditions are not fulfilled yet where we can be certain that some bacterial agent is the cause of the disease. In these cases it has been shown that the disease can be transmitted from man to man or from animal to animal in the same way as a disease due to a recognised germ; the agent has, in some cases, been shown to live outside the human body and still be capable of reproducing the disease. Nevertheless the agent has never been seen, and so has not been shown to be constantly present in cases of the disease. Thus two of Koch's postulates have been fulfilled, while the third appears incapable of proof.

This lack of proof arises from the fact that the agent in these diseases is so much smaller than the ordinary germ that not even the most powerful microscope can render it visible. Objects less than  $0.1 \mu$  in size cannot be "seen" by a microscope, as they are shorter than the wave-length of light and so do not affect light. On this account these agents have been termed "ultra-microscopic." The term is, however, not conclusive, for reasons that will be mentioned immediately. These small bodies are now grouped under the heading of "viruses," and they have other characteristics which are in part possessed by a few bacteria that can just be rendered visible by a magnification of about 1500 diameters.

The viruses are also spoken of as being "filter-passing." This is the most interesting fact about them. Filters can be made of paper, of porcelain, or of collodion; and, according to the size of the pores in the filter, particles below a certain size will pass through while particles above a certain size will be held back. Bacteria pass through pores of very small size, but when a porcelain filter (such as those first made by Chamberland) is used, it is found that germs cannot pass. There are of course differences in the sizes of the pores in a Chamberland filter, but the finer pores will not pass any recognisable bacteria. The apparatus can therefore be used to render fluids sterile. In bacteriological work it serves to separate out a toxin produced by an organism while growing in a fluid medium.

But though germs cannot pass through a porcelain filter, a virus can. It is only when the very finest of collodion filters is used that viruses show they really possess "size," as they cannot pass through such a medium. A filtrable agent is not necessarily ultra-microscopic, for if it is very soft—as in the case of certain spirochætes—it may, although easily visible under the microscope, pass through the filter. Others are just on the verge of visibility, as in the case of the virus of pleuro-pneumonia in cattle. It is now suggested that by the use of ultra-violet rays, with a shorter wave-length than light, photographs of viruses may be obtained.

Although the viruses cannot be studied by the ordinary methods of bacteriology, since they do not appear to grow in culture media, they appear to be definitely the cause of diseases in man, in animals, and in plants. In all about one hundred infections are now thought to be due to them. They cannot be cultivated, and they usually produce lasting active immunity in those who recover from the attack. As a class they are highly infective, as we know by the passage of the disease from one person to another, in rabies, in smallpox, and febrile herpes, and by the difficulty of

checking the spread of foot-and-mouth disease in cattle. Influenza appears to be due to a virus, and its amazing infectivity is known to everyone. Distemper in dogs is equally infective, and in experimental work on the cure of the disease ordinary quarantine methods did not entirely prevent the infection spreading. This peculiarity is due to the remarkably small dose of the virus necessary to cause infection.

While one speaks of the viruses as being filterable there are, as has been noted, variations in the size of filter-pores through which they will pass. So with the question of cultivation outside the body. Most viruses live outside the body for a time, but in ordinary culture-media they show no apparent increase. In one case, however—pleuro-pneumonia of cattle—a visible growth is obtained on solid media, although the “individuals” making up this growth cannot be seen by the usual methods. Other viruses are said to increase in fluid media, provided a small piece of living tissue is present; but this is too recent a discovery to be generally acceptable.

We saw that bacteria, when they infect the body, may cause the tissues to develop anti-substances which destroy the invading germs and their poisons, thus producing a lasting resistance to the bacteria. The same principle applies to most viruses. It is illustrated in the case of anti-smallpox vaccinations. It is also the basis of treatment in rabies, by the injection of a preparation of the spinal cords of rabbits that have suffered from the disease. It is of interest to note that Pasteur not only developed this treatment for rabies but suggested that, as his ordinary search for a germ was without result, the infecting agent must be exceedingly small. It is unfortunate that this lasting immunity is not universal in virus disease, for it appears that the common cold belongs to this group.

It has also been suggested that cancer, probably man's most dreaded disease, is caused by an agent of this group.

In work published in December, 1931, Dr. Gye claims to show that malignant disease is due to a virus activated or assisted by other factors within the human body. Confirmation or refutation of this work will take much time and effort, but it opens up a vast possibility.

A rather mysterious agent, which has the power of attacking the larger bacteria, may be a filterable virus. It is known as the Bacteriophage, and was first observed by Twort and d'Herelle working independently. There is no agreement among bacteriologists as to whether it is a living thing or not, but the evidence points to it having most of the characters of a filterable virus. This agent, living or not, is found associated with bacteria either within the body or in cultures. When present in a culture it prevents the normal growth of the organism, and when introduced into a culture it causes the bacteria to liquefy and disappear. This fact at once suggests that the bacteriophage could be used to treat bacterial infections. Attempts are being made along this line.

There is a tremendous amount of work to be done on these filterable viruses. While it is true that they may for ever be invisible to man, there can be no doubt that their exact nature will yet be discovered. When we have achieved this knowledge, the way will be clear for devising methods of preventing and treating the diseases they cause.

## CHAPTER IX

### The Future of Bacteriology

**B**ACTERIOLOGY is one of the youngest of the sciences. Apart from the first immense and sudden gain in knowledge when Pasteur and Koch were laying the foundations of the subject, advances have been in many instances slow and arduous. The number of organisms is so large and their presence so universal that the business of isolation and classification is in itself enormous. When the relationship between organism and disease is investigated the great variations in the resistance of the host and in the virulence of the infecting germ must be accounted for and estimated before accurate results are possible. The presence of the filterable viruses and of the bacteriophage, whose potential place as a therapeutic agent is still undetermined, makes the problem still more difficult. Nevertheless advances are being made, and difficulties are gradually being surmounted in many directions.

From the human point of view the most important advances that Bacteriology can make are those that lead to cure of the bacterial diseases. This must, as a rule, be preceded by discovery and demonstration of the infecting agent, although there are many diseases in which the organism is definitely recognised but in which no equally definite cure has yet been found. There is therefore ample scope for experiment which is now proceeding along two lines.

In the first place we have already discussed how certain organisms produce anti-bodies either in causing disease or

when injected into the body in the dead state. When these can be produced in the body of an animal, a serum can be made from its blood and injected into the blood of a patient, so that the animal anti-bodies may attack the infecting organism or its poison. Recent work shows that if the blood of a patient convalescent from measles is injected into a patient in the early stages of an attack, a more or less complete cure results. The agent of measles, being filterable, is not yet definitely proven, but from this proof of the production of anti-bodies it follows that the cause must be bacterial in nature, and also that if such an anti-body can be produced experimentally we shall acquire an added weapon in combating the disease.

Secondly, a wide prospect is opening up in the discovery of methods of preventing the occurrence of bacteriological diseases. Where the organism is conveyed from patient to patient by an outside agency (as in those diseases discussed in Chapter VI) it is possible to stop the spread of the disease by attacking the carrying mechanism, whether it be passive like milk or active as in the case of insects. It follows, therefore, that as sanitary conditions generally and the health of animals used for human food are improved, bacterial diseases will become less frequent. Welcome as this advance appears to be, it is not entirely beneficial. The constant presence of a disease in a community, even in a small number of cases, gradually produces a racial resistance to that disease. Measles, for example, seldom attacks adults in this country, but when the disease has been introduced by Europeans among the natives of newly-discovered countries it has proved exceedingly fatal.

It is suggested, therefore, that by preventing the spread of disease we may destroy any racial immunity there is to that disease. If, then, the disease is introduced from some other country it may take on a virulent epidemic form and cause a much greater number of deaths than it does at present. To prevent such a catastrophe it is necessary

either to develop some means of artificially immunising the community or some very effective means of treating cases.

On the former lines much has been done. Smallpox vaccination was the first attempt, and there can be no question of its power of protection. Typhoid fevers can be guarded against by the use of a vaccine of the dead germs, injections being given under the skin. The efficacy of this safeguard was proved during the War, when the cases and death-rates among inoculated troops were only a fraction of those recorded among uninoculated troops during the South African War. In France, and to a lesser extent in other countries, experiments have been going on for years in the prevention of tuberculosis among children by means of a vaccine given by mouth. It is always difficult to assess the exact results of such a method, but it holds out a hope for us in the struggle against tuberculosis.

In the cure of disease there is the possibility of treatment by an anti-serum prepared from the blood of animals immunised against the disease. This has already been mentioned as the method of treating Diphtheria. It is now being tried against Pneumonia, a disease of the lungs due to the *Pneumococcus*; also in various streptococcal diseases. It is available against the deadly Tetanus, but here early treatment is necessary. It is also of value, again if used early, in the condition of Gas-gangrene which follows wounds contaminated with dust.

There is, in addition, the possibility of "Chemotherapy," or the destruction of bacteria within the body by chemicals. To Ehrlich, the great German chemist, is due the credit for the idea that it might be possible to discover a drug fatal to an organism but harmless to the tissues of the body. Since both body cells and bacteria are composed of protoplasm, this seems at first sight a rather wild idea, but so vast are the numbers of chemical compounds and so

different are the conditions under which body tissues and organisms exist, that it has been proved to have some foundation. Ehrlich spent years in experiments, using chiefly derivatives of arsenic, a substance which in certain forms is poisonous to all protoplasms. In the end he discovered the "salvarsan" group of arsenical compounds which are very effective against the spirochætal diseases such as syphilis and yaws. These drugs are utilised everywhere to-day and are relatively harmless to human beings. Their action on organisms is so specific that they have practically no effect on any disease except those mentioned. The search for a drug which will destroy other bacteria within the body is therefore still going on. In septicæmic conditions and in tuberculosis it is hoped that some effective chemical compound will yet be found.

The bacteriophage which, it has already been pointed out, attacks and destroys bacteria, is claimed by some as a weapon against disease. At the moment large numbers of people in India are being treated with bacteriophage against cholera; the results will be of great interest. Whether in the human body the bacteriophage acts on bacteria as it does in a culture tube has yet to be proved. If it does so, Nature will have produced her own weapon against her ubiquitous bacteria.

Whatever line future developments may chiefly follow, man is gradually acquiring full knowledge of bacteria. Armed with that knowledge, he is developing methods of dealing both with germs and with their effects on the body. Bacteria, from their size, their extraordinary rapidity of growth and the power of their poisonous products, cause much trouble to mankind, but there seems no reason to doubt that science will, thanks to the patience and skill of an army of workers, in time achieve a full conquest over these insidious enemies.











