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Louis Pasteur.

LOUIS PASTEUR (1822–1895)

French chemist, one of the "Fathers of Bacteriology," a man of extraordinary vision and even greater hope, for he bequeathed to us the herculean job of proving that "it is within the power of man to cause all parasitic diseases to disappear from the world."

MICROBES MILITANT: A CHALLENGE TO MAN

The Story of Modern Preventive Medicine and Control of Infectious Diseases

By

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A REVISION OF "THE MICROBE'S CHALLENGE"

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PREFACE

Man's ever-growing mastery over disease-producing microbes might lead one to suppose that in a relatively short time the control of infectious diseases will no longer be a major problem. The scientific advances we have witnessed in this field are all the more remarkable considering the strong opposition on the part of germs, which defend themselves against man as stoutly as he does against them. Nevertheless, to think for a moment that our conquest has been one-sided is far from the truth and it would be rash to prophesy, as some do, a complete and lasting victory over an invisible enemy. For the time being, our successful control over microbes, like the act of a dog walking on his hind legs, may not be done well, but it is surprising to find it done at all.

Modern microbes are like those of Pasteur's day and of a time long before that, differing only in the way they have compelled a modern point of view regarding their behavior and hidden tendencies. Bacteriology as a science is not, as was once believed, a subject dealing with static or fixed organisms following a uniform pattern of existence. Because of the recognition of this fact many important modern discoveries have resulted. We know now that microbes are capable of changing to meet new conditions that are imposed upon them by a struggle for existence which is as much their problem as ours.

The dominant theme of this book is microbes versus man. In the same sense, it is the challenge of the microbe. From such a point of view, one might gain a clearer perspective of the past and of the many current and future problems that await solution. The purpose intended is to narrate some of the highlights in the history of preventive medicine and to bring together in a logical form the funda-

mental principles upon which the modern bacteriological control of infectious diseases is based.

In a book of this sort, the author finds himself in a difficult position with regard to his audience. The presentation of highly technical subject matter cannot always be simplified beyond a certain point unless one wishes to sacrifice accuracy and truth to popular appeal. This would be as unpardonable as another easy method of "talking down" to the reader in a style hardly less dignified than baby language to a child. Nor can an author addressing himself to an intelligent audience hide his limitations by the wholesale omission of material which is supposed to be "over the heads" of his public.

Wherever possible, attempts have been made to clarify further the meaning of some technical terms by the use of word pictures or analogies which it is hoped will serve their intended purpose. Those who might wish to have a glossary for additional reference will find one at the end of the book. Since this glossary is short, it should be taken as evidence of the author's respect for the intelligence of his readers and not as a tacit assumption on his part that the subject matter needed so little explaining.

Throughout the preparation of this book many original articles as well as some excellent summaries by leaders in their respective fields have been consulted. The listing of a complete bibliography would fall far short of expressing the writer's genuine appreciation of the help given by an army of unnamed collaborators, among whose contributions some have received special mention in the text. A section of the chapter dealing with plague in Manchuria is based upon reports published during 1910–1930 by the Manchurian Plague Prevention Service of Harbin, China. The permission of Doctor Wu Lien Teh, its former Director, to use this material is gratefully acknowledged.

FREDERICK EBERSON

Washington, District of Columbia
September, 1940

PREFACE TO REVISED EDITION

Significant progress in the control and prevention of microbial diseases has necessitated adding to and revising the material presented in the first edition of this book, which was published under the title *The Microbe's Challenge*. The war years intervening have played an important part in bringing about many such changes. These, in turn, have in no small measure contributed to the military successes gained on far-flung battlefields. No less than a war directed toward enemy armed forces, it was, in the tropics as elsewhere, a war against microbes and pestilence.

Aggressive biological warfare that is constantly being waged in the microbial world has reawakened interest in substances of bacterial origin which are capable of destroying disease-producing organisms. These antibacterial substances, known as antibiotics, have in a sense made this the antibiotic age, second only to the atomic era. The subject matter of penicillin, streptomycin, and many other microbial products of self-defense has been condensed in a new chapter.

Fruitful researches in the field of viral and rickettsial diseases have contributed recently to a better understanding of some of the problems concerned with their prevention and control. Not a few unsolved mysteries remain, yet new clues are helping to remove much from the realm of speculation. A discussion of this subject would be incomplete without reference to newly discovered reservoirs of infection and the place of the entomologist in modern public health.

Epidemiology, which does not lend itself to a simple definition, cannot now be treated apart from the startling behavior of micro-organisms, both as related to natural

influences and to the effects produced by drugs and antibiotic therapy. Intimately related to this topic, with all its disturbing implications, is the fundamental problem of microbial change, that which concerns variation and mutation. All these matters have been given due consideration in various chapters, with whatever new trimmings were found necessary.

The theme of this book remains unchanged. It is still the microbe's challenge meeting our newest advances with another kind of resistance. First it was against the sulfa drugs, then toward the action of penicillin, and now against a large class of antibiotics, including streptomycin. Indeed, the problem of bacterial resistance is so closely tied in with the use of antibiotic substances, that it cannot be regarded as less important than antibiotic research itself. In fact, until the chances for survival of intractable organisms are brought under control, we shall be defeating our purpose. Here again is a challenge of the microbe as it turns its natural defenses into a stronger attack upon our borrowed weapons.

To the ever-growing list of unnamed collaborators, known and unknown, whose works and writings have leavened this ferment, the author is most grateful.

FREDERICK EBERSON

Kennedy Hospital
Memphis, Tennessee
August 9, 1948

(*The opinions and views expressed in this book are those of the author and are not to be construed as reflecting the policies of the U. S. Veterans Administration.*)

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MICROBES MILITANT: A CHALLENGE TO MAN

CHAPTER 1

THE SIMPLE LIFE

Microbes Have a Way of Living

We live in the center of a microbial universe. On all sides microscopic organisms surround us and make felt their presence for good or ill. Some are benefactors and philanthropists, without which no life could exist on the earth. Others are agents of disease and death. Because these minute bodies become visible only with the aid of an artificial eye having enormous magnifying power, it is not surprising that century upon century passed into history before scientists were able, following the invention of the microscope, to link such small forms of life with events of importance to man. Before that time things just happened in a delightfully spontaneous way, and thinking about them was considered a dangerous pastime.

Small as they are, microbes are endowed with the ability to perform certain acts which distinguish animate from inanimate things and are therefore living bodies (*micros,* small; *bios,* life). Such acts include growing, eating, reproducing, and dying. In addition, microbes are sensitive and respond in some fashion to the influence of an external agent or stimulus; they can convert into their specific cell structure the food or nutrient material which is absorbed; and they have the power to excrete the products of certain activities.

One of the most arresting facts of microbial existence is an astonishing capacity for adaptation to a succession of changing hosts, including both man and animals. Organisms capable of prolonged existence through countless ages of time are not so simple as they might appear at first glance. Indeed, these tiny bodies contain all the elements

characteristic of higher forms of life, including carbon, oxygen, hydrogen, and nitrogen, seasoned with a little sulphur, phosphorus, and a number of other substances.

The differing life processes that are characteristic of the microbe offer the bacteriologist who studies them a convenient means of classifying them into various groups. He can divide them according to their form, size, shape, and internal structure (morphology); presence or absence of means of locomotion; relation to physical or chemical agents of destruction; behavior in the experimental animal or in the human body; chemical structure of the microbial cell, which probably contains the key to individual properties and behavior (this will be considered in another chapter); and, perhaps most important of all from the human standpoint, their nutritional peculiarities.

In common with all living things, microbes, in order to survive, must have food. How they meet this difficult problem of nutrition is of consequence not only to the micro-organisms themselves, but to the human race. Unlike the higher plants, many bacteria are not capable of manufacturing their food requirements from simple compounds which are present in nature. Instead they must break down more or less complicated substances into simple ones and transform these into still less complex materials suited to their own peculiar needs. Furnished free transportation by way of dirt, food, water, and air, they are brought into the closest possible relationship to human, animal, and vegetable hosts and thus their eternal quest for nutritive substances is satisfied.

The absence among microbes of the structural and functional parts which distinguish more highly developed living things limits a description of bacterial nutrition to certain activities of the organism. However, even among these single-celled organisms certain differences are evident. Some forms of microbial life can use only one type of foodstuff and others many types. Certain bacteria are able to split up the more complex proteins (like egg

albumen and meat products), whereas others cannot do this under any conditions.

Scavengers and Clinging Vines

The ability of bacteria to utilize foodstuffs furnishes a simple means of classifying them into two groups. The members of the first group are known as saprophytes or scavengers because they have become accustomed over a long period of biological time to living exclusively on decomposed or decaying matter. This method of obtaining food offers a wide range of action.

Parasites, which form the second group (*para sitos;* beside food) have, on the other hand, a very limited capacity for the utilization of simple food elements. This type of microbe prefers the environmental conditions present in human and animal tissues, and thus they have become guests whose visible means of support have acquired the flattering but perhaps ironical title of "host." Generally speaking, the parasite flourishes best at the higher temperatures found in the warm-blooded animals, and then only is it able to transform and make available the foodstuffs present in the tissues.

The saprophytic microbe is less squeamish, both as regards the diet and the temperature at which the food happens to be served. But, as might be expected, this separation of types is not hard and fast. In between the strict parasites and the saprophytes are found organisms which partake of the nature of both groups. Such bacteria are more flexible in their adjustment to existing conditions. They are capable of developing abundantly in certain foodstuffs that are peculiarly fitted for the growth and survival of the less fastidious saprophyte. To such a group of in-between parasites belongs the large majority of pathogenic or disease-producing microbes. It is this adaptability to changing conditions which has made the pathogenic organism the formidable enemy it is, as we shall see later.

The temperature range in which the microbe must live has much to do with the rate of food assimilation, growth, excretion of waste materials, and ultimate dissolution of the microbe. That temperature is most favorable which brings about a balance between nutrition and accumulation of waste, so that they keep pace with each other. It is a well-known law of biological behavior that higher temperatures speed up chemical activity and metabolism (transformation of food materials into energy) while cold retards these processes and, as a direct result, hinders the rate of growth of the microbe. Under unfavorable temperature conditions, the organism may lose its vitality and enter a resting stage, or even die.

They Breathe

Important also in the life and growth of bacterial forms is the presence of oxygen. This element in a free state is for many microbes absolutely essential. On the other hand, there is a certain group of organisms for which atmospheric free oxygen is highly inimical. Thus two classes of micro-organisms have come to be recognized according to their oxygen requirements, one aerobic and the other anaerobic. So delicately adjusted are certain microbes to the oxygen pressure most favorable for growth and survival that a number of disease-producing micro-organisms cannot be isolated from their natural setting or maintained in a viable state in the test tube unless this critical oxygen pressure is maintained. But, as in the case of nutritional habits, some bacteria form an intermediate group capable of flourishing in both aerobic and anaerobic environments.

Most of the aerobic microbes obtain their oxygen from the atmosphere like plants, after a fashion, although the liquid medium furnishes the oxygen. Their methods of breathing, however, are very complicated chemical phenomena and conclusions about them which have been drawn from recent experimental studies are still highly speculative. Even more complicated is the manner in

which anaerobic microbes obtain their oxygen supply in an environment where the presence of free oxygen might be fatal. In this instance the cell, endowed with specialized chemical ferments called enzymes, attacks the complex carbohydrates (materials similar to sugars) and the proteins, breaking them down into simpler substances and releasing energy for the use of the organism.

In general, these ferments or enzymes of microbes may either hasten or retard the speed of chemical reactions necessary for their life. The enzymes themselves usually remain unchanged. Perhaps the most remarkable property of many that they have is a two-way action. They are par excellence a stabilizing factor of biologic activities. In their presence chemical reactions will proceed to a certain point and no further. When the balance is overthrown by an excess (or insufficiency) of one or more substances taking part, the enzyme proceeds to restore equilibrium by driving the invisible machinery either forward or backward as required. This vital force, so far as is known, can seldom be imitated in the laboratory.

A crude comparison might be made with a thermostatic control of a furnace in a home. This instrument is connected with an electric heating system and responds to small changes in the temperature of the rooms, so as to open the electric circuit when the desired temperature has been reached and to close the circuit at a slightly lower point. In this manner the source of heat is automatically cut off or turned on, with a more or less uniform temperature as a result. But the thermostat cannot alone adjust itself to the work required of it; it must be set by human hands. The mysterious enzymes in a living cell, however, do have this capacity. An explanation for this behavior is not yet known.

Although the life processes of bacteria are thus among the most complicated mechanisms known to science, in another respect microbes are comparatively simple, and that is in the matter of appearance. We can distinguish

four styles—a berry, a rod, a spiral, and a fine elongated corkscrew. These formations, however, are not very helpful in identifying the organisms, because they do not give any indication of the differences in behavior exhibited by the extraordinary number of varieties of bacteria known at the present time. Morphology, therefore, or form by itself, while furnishing an external resemblance, tells as little about the individual type as would a full beard adorning the features of a Communist and a Socialist.

The bacteriologist, therefore, in order to identify with reasonable accuracy the multitude of forms with which he is brought into contact, must observe their behavior in different environments and see what happens and how. One condition which offers a clue in identifying varieties is that of reproduction.

They Breed

Bacteria are plants, not animals, and reproduce their kind in a very simple fashion. Strange as it may sound, it is true, nevertheless, that when microbes multiply, they divide. The growing organism increases in size up to a certain limit, and at a given time divides, as a rule, at right angles to the long axis. Two organisms now appear where only one was before. This type of simple division or fission (segment) results in the formation of characteristic groupings peculiar to the different species and aids in their identification.

The new daughter-cells, so-called, may separate at once or may stick together. In the latter instance, aggregations of cells are formed during the repeated divisions. Such cell-masses assume a form which depends upon the shape of the microbe and the position in space of the line of division. For example, in the berry-like organism (coccus) the plane of division may be anywhere. In the rod-like (bacillus) or in the spiral forms, the line of cleavage occurs at right angles to the long axis. Hence it is evident why the coccus microbe (micrococcus) gives rise to a greater vari-

ety of cell aggregates than do the other shapes. One sees clusters resembling grapes (staphylococci), chains or strings of beads (streptococci), or groups of four (tetracocci), or cubes or packets resulting from three divisions, of which one occurs at right angles to the other two planes of separation. Certain other bacteria form quite picturesque collections of progeny resembling Chinese characters. This is a characteristic of diphtheria bacilli, which divide in such a manner as to cause the organisms to lie haphazardly at angles to one another.

Of greater importance for identification is the appearance of microbial colonies developed on solid nutrient material (culture media), as a result of the multiplication of individual cells. Such an aggregate of cells is visible to the naked eye and furnishes a method of differentiating species. For this simple and remarkable idea we are indebted to Robert Koch, about whom we shall speak at greater length in another chapter.

Generations of microbes are reckoned by the time it takes for a single organism to divide. The interval between one cleavage and the next may vary with the species and is very definitely influenced by the environmental conditions. From fifteen to twenty minutes, it has been estimated, are required for a simple division. At this rate one cholera germ will yield more than fifteen hundred trillions in twenty-four hours. It is conceivable that the whole world would be overwhelmed by sheer numbers of microbes but for the happy circumstance of a limited food supply and the accumulation of harmful waste products. These factors alone would tend to limit growth and multiplication, apart from any lack of the requisite ideal conditions which do not, as a rule, prevail.

Gipsy Life

Movement from one place to another would certainly seem to be expected of most living organisms, yet the property of independent motion is not universal among

microbes. When it is found, as among the rod-like forms, the spiral types, and rare berry-like forms, movement (motility) sometimes seems to go hand in hand with a parasitic existence. Lack of motility generally characterizes organisms which have become more or less saprophytic, but these have found many easier methods of getting free transportation. However, a vast majority of microbes inhabiting the intestinal tract of man have retained their motility despite a saprophytic existence outside the animal body. They are found commonly in the soil and their frequent appearance in sewage, dust, or in milk and vegetable foods can be readily explained. Such organisms, however, cannot be regarded as strict parasites.

The microbial organs of locomotion, known as flagella (*flagellum;* whip or lash) are thread-like parts attached in various ways to the bacterial cell. The arrangement of these flagella differs for certain species of microbes and helps to identify them to some extent. A single flagellum may be fixed at one end of the organism, or at each end; a tuft of flagella may be attached to one or both ends; yet another structural variety is an arrangement of the flagellar threads all around the bacterial cell.

In considering the movements of microbes, however, it is not enough to regard them as habitually swimming about within the human or animal body in the uncharted seas of secretions, in murky canals of the digestive system, or in lakes the size of a water droplet. Such independent locomotion is secondary to that of borrowed means of conveyance, readily available in such vehicles of transportation as dirt, water, food, and air. "Thumbing a ride" is doubtless a very ancient custom in the microbial world and is indispensable to these tiny forms of life in their migration from host to host.

From the point of view of bacterial activities, they are concerned now, as in the very beginning, with a most important matter—survival. In order to maintain its existence, the microbe seeks a source of food supply and a

favorable opportunity to leave its benefactor for another, should need or fancy dictate the move. The fact that the modes of transportation available to the microbe victimize man and make him vulnerable to infection is really incidental to a simple biological urge, which is characteristic of the living bacterial cell. From the point of view of the human mechanism, however, the urge to survive turns many microbes into a menace.

So much emphasis has been placed of late upon bacteria as bearers of disease that we are prone to overlook another and more cheerful side to the picture. Those who are convinced that microbes cannot ever do good and as bearers of gifts must be suspect do not stop to realize that most of the every-day things we take for granted would be impossible without their assistance. In the vegetable and animal kingdom to which we owe our food supply, there are many examples of community partnership, by which the single-celled microscopic parasite and the many-celled human are united in a common bond. Under such conditions, microbes and host live in a state of mutual service, each giving to the other as well as taking according to individual needs.

An Underground Laboratory

An idyllic existence of this sort is perhaps best exemplified in the soil. To the casual observer, a cultivated field may not give any indication of biological activities going on beneath its surface, but in its depths microbes are enacting a silent drama which makes possible the growth of useful crops. Through their efforts, an essential element, nitrogen, is returned to the soil, from which it is being constantly withdrawn by growing plants.

This essential function of replenishing nitrogen in the soil is performed by bacteria living in its upper layers and capable of abstracting free nitrogen from the air. These microbes were discovered in 1893 by Winogradsky, a bacteriologist, although the way had been pointed shortly be-

fore by a chemist named Berthelot, who had found a definite increase in the quantity of nitrogen in soil specimens allowed to remain undisturbed in the field, whereas no such change occurred in specimens subjected to high temperatures. By means of this simple experiment the presence of some living principle was established.

These nitrogen-bearing bacteria are found in the greatest numbers associated with certain leguminous plants, such as beans, peas, clover, vetch, and alfalfa. On the roots of these plants may be seen small swellings resembling tumors, which are the chemical laboratories where countless millions of bacteria thrive and manufacture synthetic food for the plant by snatching nitrogen from the environment in some mysterious way and converting it into a form suitable for plant growth. When the living plant dies, these microbes cannot survive without its aid and in dying yield up to the soil an accumulated store of nitrogen which is essential to the growth and development of new vegetation. When the roots and the attached nodules are allowed to rot in the soil after each harvesting, they re-infect it with the nitrogen-fixing microbes. Such plants thus enrich the soil instead of exhausting it and are consequently often rotated with other crops. The present-day practice of ploughing under a leguminous crop to prevent serious depletion of soil is based upon this so-called nitrogen cycle.

This capacity of fixing nitrogen of the air in an underground laboratory, which sets in motion a never-ending cycle of activity for the benefit of man, will always remain one of the strangest of biological phenomena. As an example of mutual aid among the lowliest forms of life, it is probably not to be equalled in the animal kingdom for sheer delicacy and precision. The nature of this relationship has been questioned by some, who maintain that it is an infection caused by the microbe, which acts like a parasite and derives all the benefit of the association while the plant gains nothing. Whatever the means may be, the final result, so far as the plant and microbe are concerned, is an

unmistakable blessing for the human and animal kingdom.

These soil bacteria bring us not only our fruits and vegetables but our meat and dairy supplies as well, for cattle and sheep must have grass to live. These animals, together with man, form part of an endless cycle in nature. Cattle eat vegetable matter and man consumes the flesh of the cattle; then the waste materials of both man and animal enter the soil to furnish nourishment for new plants. It is a beautiful chain of events taking place in three environments, beneath the ground, in the atmosphere, and within the animal body.

Good Little Microbes

Before many of these food products reach our table, still other bacteria have played an essential part in their preparation. The delightful flavor of butter and the piquant taste of cheese are caused by microbial activities, which the dairy bacteriologist can regulate to obtain all gradations of flavor and aroma. In the ripening of butter and cheese, for example, a specific kind of microbe determines what shall be the taste, color, and odor of the product. Some microbes develop aromatic alcohols, others generate gases which impart a sharp flavor, and still others produce a delicate acidity. After an organism has been cultivated for many generations in milk and observations have been carefully made of the effect of its growth upon the desired qualities, it can be depended upon to do the things expected of it at all times, provided certain foodstuffs and suitable surroundings are available. The procedure is really not unlike the domestication of animals. In its natural state the microbe can be adapted to artificial conditions and its behavior restricted to suit an immediate purpose.

Another product which owes its flavor to bacteria is wine. Not without the skillful aid of chemist and microbiologist is the sparkling drink brought to perfection. The fermentation of wine results from the activity of certain

favorable micro-organisms and the control of other undesirable ones, which may turn it sour or bitter. Diseases of wine, incidentally, led to studies which culminated finally in the germ theory of infection, as we shall see in our next chapter.

Tobacco, too, owes its fragrance to the microbe which has done the all important job of ripening the tobacco leaf, which in its original state would be unfit for the smoker. The leaf is first dried in air and then heaped up in masses to allow fermentation to proceed. Many varieties of bacteria from the atmosphere play a part in this chemical reaction, during which nicotin is largely destroyed and aromatic end-products are combined to give a variety of flavors and aromas. In the tobacco industry, as in the dairy industry, attempts to regulate flavors by cultivating fixed species of microbes and adding them to the leaf have been successful.

The list of useful products that owe their existence to bacterial processes might be extended indefinitely. The tanning of leather, to add yet another, is essentially a process of putrefaction (rotting). Animal excrement containing certain bacteria is applied to skins for the purpose of loosening their texture and making them more permeable to the chemicals used in later stages of the tanning process. In fact, the study of the activities of "good" microbes merits a book in itself, but it is the purpose of the present volume to deal primarily with the "bad" bacteria which bring disease to the human race.

Microbes and Disease

Infectious disease is due primarily to the existence in human and animal hosts of the microscopic enemies we call parasites. They are the "difficult" children—ill-mannered, destructive, unsocial, and all too clever in getting what they want. There is an enormous difference between such parasites, which demand living tissue for their development, and the type of bacteria known as saprophytes, existing as scavengers on dead or decaying matter.

Whether the easy-going saprophyte gradually developed into the fastidious and highly specialized parasite, or whether the latter gradually degenerated into the saprophytic type, is a matter of speculation. The existence of intermediate types of bacteria, having the characteristics of saprophytes as well as of parasites, suggests that microbes may have gone forward or backward from either one of these stages, but it is also conceivable that both forms may have developed from a more complex organism which was capable of taking one path of development or another. Whatever the method, it is certain that adaptation to new conditions is not a sudden event and that a long-drawn-out process of adjustment must have occurred to provide such an astonishing change of habits. It is equally certain that such evolution in the biological world is still going on, defying man's attempts to define or classify parasites and to fit them into mental pigeonholes.

The parasitic mode of existence is a perfectly normal one in the animal kingdom, but unfortunately what is meat for microbes is sometimes poison for man. When bacteria have gained entrance into the tissues through an infection (*in facere;* to put in or mix in), they bring about certain changes necessary for their life and maintenance. The disturbance set up as a result of such changes often expresses itself as disease (*dis ease;* not at ease, not well-being; hence ill or uncomfortable).

These invading bacteria may reach man through his environment by way of soil, water, food, and air, or, as is more often the case, they may spread from man to man. The lower animals also contribute their share of diseases to the human race, but the problem of controlling them is not so difficult as that of checking our fellow citizens.

There are three main portals of entry into the body of the host, by way of the skin, the digestive system, or the respiratory tract. It is of interest and considerable importance that the avenue of infection chosen by a given microbe will determine in many cases whether or not dis-

ease shall result. Certain organisms cause disease only when taken into the digestive tract by swallowing. Others must enter the body through the skin in order to accomplish their work. In each instance, the microbe adapted to one path of infection is harmless when entering by another route. The cholera germ, for example, does not cause cholera when rubbed into the skin. Neither the dysentery nor the typhoid bacteria are able to cause disease in this manner. These same organisms, however, when swallowed, do infect and may result fatally. On the other hand, certain streptococci may cause serious or fatal infection when rubbed into the skin, yet are harmless when swallowed.

In a healthy and undisturbed condition, the three pathways of infection are well guarded against invasion from without. The skin acts as a mechanical barrier and the digestive, respiratory, and excretory tracts are equipped with protective mucous membranes or "lubricated" coverings. These structures serve partly to check the advance of bacteria in a mechanical way and also to destroy them by chemical means. Even when an infective organism is once well-established within the host, the latter may evolve some sort of resistance to hold it in check throughout most of the host's life.

One of our most important defensive mechanisms against microbe invaders is the skin. The skin is the great insulator, keeping the individual in his own private estate and in a sense isolating the body from its environment. A break in the outer covering of the body consequently may be as disastrous as a hole in a dam. That we are not infected more frequently through numerous injuries inflicted on the skin is due to the remarkable healing powers of living tissue. No sooner is the surface of the skin broken than the juices from the blood stream and the broken tissues rush their forces to the place in an attempt to close the breach and repel the invading microbe.

This response to injury is a complicated phenomenon

and not a simple closing of a gap like that of shutting a gate. The damaged area is a battleground and what goes on here will decide whether the parasite or host is to gain the upper hand. Curiously, the microbe helps to destroy itself while utilizing the food materials made available when the tissue is first damaged. As a result of microbial activities, chemical changes are at once set up at the point of invasion, which in turn stimulate the tissue to concentrate a defensive force of specialized cells whose function is to digest foreign bodies. At the same time, the blood serum and tissue juice are mobilizing other protective substances. Such substances are formless, so far as is known, but they are none the less effective in helping to destroy the microbe, together with the damaging products given off by it.

The specialized cells have a more direct action. These white corpuscles or phagocytes (*phagein,* to eat; *kytos,* hollow place or cell) actually engulf and digest the bacteria and clean up all debris left in the wake of the invader. Repair of damaged tissue begins as soon as the irritant is held in check or removed completely from the invaded region. In a remarkably short time, new growth of tissue has occurred and all is quiet once more. Only a scar remains as a monument to mark the spot where a great army of microbes was destroyed.

If a successful defence of this kind does not take place, a simple infection of the skin may penetrate into the blood stream and cause death. The effectiveness of response to a bacterial attack depends upon numerous factors vaguely understood by the terms general resistance or normal vitality. To offset this, the microbe may be more aggressive or more virulent or endowed with greater powers of invasiveness. Some bacteria are even known to enter the system through unbroken skin, as for example the microbe *Pasteurella pestis,* which causes bubonic plague or the "Black Death." Another organism with similar high invasive property is the one responsible for tularemia, or deer-fly

fever, a disease transmitted to the human by the bite of blood-sucking flies, wood ticks, or from an infected rabbit. Only the outcome of a conflict between host and parasite will decide which had the greater or better battalions.

Once a micro-organism eludes the mechanical barriers which are found on the surface of the body, there are yet other obstacles in its path. A simple factor like the location of the organ or tissue might be incompatible with germ life because of the presence or absence of oxygen. Again, the tissue or cavity within an organ or a cavity within the body itself, such as the stomach or intestine, might normally contain chemical substances whose acidity or alkalinity would check reproduction of the microbe or even destroy it at once.

Entrance by way of the digestive tract is a second and more common mode of infection. Here the microbes gain admission with contaminated food, for dirt and disease are twins and living from hand to mouth is a dangerous business. Certain infections can properly be called filth diseases. Cholera, dysentery, and typhoid fever belong to this class and are frank evidence that the victim has swallowed material polluted with human wastes, usually water, milk, or vegetables, all of which are frequently contaminated in different ways. As though possessed of a "homing" instinct, intestinal germs, however they may be scattered far and wide over the earth by human agencies, will always return to their starting place in the intestine.

Free Transportation

Water rates high as a vehicle of disease largely because civilizations have always been led to migrate in quest of it. Rivers and seas have linked peoples together by making possible the transportation of foods and supplies, and rivers and springs have quenched thirst. No one thing in man's environment excepting air itself is more important than water and so necessary for life.

By a strange irony, it has been said, the way of mankind

has always been to return the blessings poured out by nature in its waters by giving back to streams the filth and human wastes which breed disease. Drinking sewage is even today not unknown in the Orient. There cholera and dysentery cause an appalling number of deaths resulting from the gross pollution of waterways and underground sources of water. In other parts of the world, and in this country, particularly, it is only constant vigilance and supervision of drinking waters and the rigid control of food supplies that forestall serious outbreaks of filth infections.

The appearance of water is not a guide to its purity or safety as a drink. This alone tends to make it an ideal carrier of microbes. Crystal-clear and sparkling as a mountain stream, it may contain millions of typhoid, dysentery, or cholera germs; muddy or dirty and discolored by vegetable matter, as natural water frequently is, it can be swallowed in perfect safety when free from sources and material of pollution. Fortunately for us, there are many natural factors, such as sunlight, air, dilution, settling, and antagonistic organisms—all tending to keep down the numbers of microbes in a water supply, which in itself is not a food medium for germs, although civilized man does his best to overburden whatever protective devices a natural environment may furnish.

Unlike water, milk offers bacteria an abundant food supply and in addition furnishes them an easy means of entrance to the intestinal tract, favoring their multiplication and the spread of disease. Beneath a white cloak of purity, milk often shelters dangerous rascals accustomed to hide under a robe of piety and innocence. Having associated with mankind for ages, parasites have learned to take their nourishment as they find it. Food that is fit for man is highly palatable for the microbe. The opposite is not generally true, however, for what has suited bacterial taste sometimes becomes no longer acceptable to us. Apart from spoilage, which may be recognized and serve as a

warning, milk may contain numbers of organisms associated with tuberculosis, diphtheria, scarlet fever, septic sore throat, and any of the diseases that can be spread through animal and human intestinal discharges. Here is an ideal environment for bacterial growth, offering rich and plentiful food and a natural opacity that hides microbial activities.

Instances of foods as vehicles of infection might be multiplied almost indefinitely. The problems arising from microbial transportation in food constitute in themselves an important branch of sanitary science and public health. From environment to host the microbe takes a short cut by way of the gastro-intestinal tract. In this region begins a cycle without which disease could not be perpetuated in man, were it not for his own helping hand. It is well to guard one's mouth at all times, for what goes into it (and what comes out of it!) may affect the health and happiness of the individual and the community.

The busiest highway for microbes is along the respiratory tract, where with each involuntary breath that we take traffic is sucked into blind alleys of the nose, throat, head sinuses, and lungs. Fortunately this route is well guarded and heavily policed, else serious mishaps would be more frequent, although protection against accidents is far from adequate. At the gateway of the system the nostrils are lined with palisades of fine hairs turned downwards and barring entrance to the deeper passages. In the upper regions of the respiratory tract a maze of twisting chambers which lead to the sinuses or cavities in the bones of the skull is protective only to a limited extent. Whether this device is intended for air-conditioning or to confuse microbes with detours, they gain entrance nevertheless and having arrived there, cannot find a way out. Farther down, in the throat, the larynx or voice-box and the trachea or main air pipe leading to the bronchial tubes, which form two main divisions entering the lungs, are all lined with specialized cells secreting mucus and equipped

with vibrating hair-like parts known as cilia or lashes. These set up rhythmic currents which direct the microbes downstream towards the exits. Thus an unbroken system of mucous membrane extending from the nose and mouth to the innermost air passages is interposed between microbes and their hosts.

Everything is designed ostensibly to discourage access to the tissues, whether by simple mechanical hindrances, such as hairs and sticky secretions that serve the purpose of "tanglefoot" or by more complicated devices which expel microbes through involuntary acts of sneezing, coughing, and expectoration brought on by irritation of the delicate mucous membranes lining the passages of nose and throat. As an added safeguard, the natural secretions of the nose destroy bacteria by means of a chemical substance in the same way tears kill organisms entering the eye.

Notwithstanding all these protective devices, microbial invasion of the respiratory system is the most frequent cause of disabling infections. Common "colds," produced by a large variety of organisms, head the list. Pneumonia, tuberculosis, influenza, diphtheria, meningitis, measles, mumps, whooping cough, scarlet fever, and many other diseases are spread from person to person through discharges from nose and throat. Although some of the microbes have not yet been seen and others not identified with certainty, they are as a group disseminated in a way best adapted to insure survival in desirable hosts. Infection is transmitted generally in advance of characteristic signs by which the disease can be recognized, so that innocent victims and their contacts both remain blissfully ignorant of danger until it is too late for preventive measures.

To make matters yet more difficult, certain microbes, as in diphtheria, pneumonia, meningitis, and scarlet fever, lie dormant in the nose and throat of unsuspecting persons, who for various reasons are not always susceptible to these diseases, yet can pass them on to other persons who are.

On frequent occasions, however, these "carriers" of infection fall a prey to the parasites which they have unknowingly sheltered and fed and transported from place to place. The way of microbial transgressors may be hard but man often smooths the road.

Bacteria Defend Themselves

The battleground of microbe and man is not restricted to the different regions of the human body. Not less important for the survival of bacteria is their capacity to offer resistance against the external environment. Under cover of a simple appearance they conceal an amazing versatility. Evolution has developed some remarkable protective devices which enable the bacterial cell to meet the many and varied physical stresses imposed upon it. Unfavorable factors, like shortage of food, accumulation of waste products, lack of moisture, or unsuitable temperatures, may lead not to the death of the microbe but to the development of a resting stage.

Under such conditions, the bacterial cell produces within itself a spore (*spora;* a seed). The spore is a structure capable of resisting heat, cold, and drought and may be compared in some respects to the seed of higher plants, although it is not always connected with reproduction of the microbe. From the point of view of food preservation and the prevention of a number of serious diseases spread by food products, this peculiarity of bacteria introduces an important problem. The tetanus microbe, which causes "lockjaw" or tetanus, can resist the temperature of boiling water for one and one-half hours or longer. The botulinus germ responsible for "botulism" (*botulus;* a sausage), once popularly known as sausage poisoning although not limited to this food product alone, can be kept at boiling temperatures for more than five hours without impairing its efficiency.

Freezing temperatures similarly offer no difficulties to hardy microbes. Intestinal bacteria, such as cause cholera

and typhoid, have been exposed to temperatures as low as minus 180 degrees Centigrade (−356° Fahrenheit) in liquid air for twenty hours without loss of power to reproduce or to behave in all other respects like the undisturbed originals.

An instance of unusual toughness against drought is found in the anthrax bacillus that causes anthrax or "wool-sorters' disease." Dried completely on silk threads for more than twenty years, the spores have been recovered alive and in full possession of normal faculties belonging to the typical organism. These same anthrax bacilli also demonstrate the ability of the microbe in spore form to withstand not only physical but also chemical agents in such an effective manner as to compel wholesome respect for such a worthy foe. Whereas strong disinfectants like carbolic acid in certain strengths will destroy the ordinary forms of anthrax bacilli in less than half an hour, a five-fold concentration of the same chemicals is well tolerated by the spores. It suffices only to bring them again into a favorable environment to have them revert, as if by some magic, to the original microbial form.

As an example of protective adaptation by bacteria, it is interesting that progressively heat-resisting spores can be developed in the laboratory. By selecting the hardiest strains and "breeding" from them a number of generations, it is possible to produce spore-formers which can withstand boiling for much longer periods than any of the preceding strains. In fact, hell hath no terrors for these untamable species. An experiment like this illustrates one of the natural methods of defence peculiar to microbes, whose resistance towards environmental hazards is expressed in a manner designed to meet any emergency.

Devices of bacteria for self-preservation are not limited in extent to their internal structure alone. There are times when they must take advantage of chance external occurrences in order to gain entrance into the host. When, for example, the microbe causing lockjaw or tetanus, which is

anaerobic and cannot survive in the presence of oxygen, finds itself in a simple surface wound with free access of air, it takes advantage of a strange and innocent microbial partnership. A dirty wound on the skin usually contains, in addition to the tetanus germ when that happens to be present, a mixed population of microbes, harmless messmates which must use up oxygen in order to live, thereby preparing the stage for a dramatic entrance of the villain of the piece.

Regardless of the disease, the general principle behind bacterial activity remains the same. For every type of infection, a specific microbial agent has to meet an individual problem created by immediate contact with an environment, whereby there come into play unique sets of habits and peculiarities which give identity to different species of microbes. Their workings in the human host and all these happenings are part of a primitive host-parasite relationship. When the balance in this association shifts to the disadvantage of the host, the resulting damage and its train of events are recognized as pathological or abnormal—that is, from our point of view. From what has already been discussed, it may be inferred that the student of disease and of abnormalities incidental to it sees only one aspect of microbial behavior.

Armed Truce

Another side of this activity in the human body has received relatively little attention, perhaps because it appears to be less dramatic. Less obvious though it may be, it is not lacking in drama. In the well person or in one who is resistant to infection, there is a host-parasite equilibrium or balance. This is a state of armed truce with the frontier armies ready to invade enemy territory as soon as one or the other makes the first sign of aggression. The science of bacteriology and immunology offers no more alluring field for investigation than this borderland where a balance has been struck between microbe and human or animal host.

In a quiet way, without so much as an outward sign, many things have happened to effect such a change in the character of body cells and tissues. What these changes are and how they have come about are unsolved problems, despite much information which has been gained from a study of the individual cell and tissues outside the animal body. However, the investigation of such material in artificial surroundings is not quite the same as observing the behavior of living cells in their natural environment. Both methods of study have disadvantages in that the latter tells us little and conceals much, while the former reveals more and tells us even less.

Parasitism is a biological fact. For the study of living things the point of view of the biologist will be more adaptable than that of the pathologist. Pathology has ridden on the crest of an enormous wave for over half a century. One cannot begin to estimate the immeasurable value of discoveries made in this field of science for the welfare and benefit of mankind. Yet who will say that this knowledge has added ever so little to what can be understood as the *normal?*

In recent years chemistry and physics have captured the imagination with numerous conquests in the world of matter and have helped incidentally to solve many problems related to pathology and disease. As we survey the trend of pure science, it seems more and more evident that chemistry tends toward physics and physics becomes metaphysics. The pure mathematician, however great his mind, falls into error when he tries to simplify phenomena. In the plan of nature these may appear simple, but reasoning about things as they happen leads often into the pitfall of generalization. To generalize is perhaps a simple method of straddling philosophy and science.

Life cannot be reduced to a formula and biology cannot be summed up in an equation. One may abide safely with the belief expressed by Gilbert N. Lewis in *The Anatomy of Science:*

"We see no limit to the interesting and useful results

that will inevitably come from a further application of the methods of physics and chemistry to the physiology of animals and plants. Yet the belief that even an infinite succession of such investigations would ultimately lead to a comprehensive understanding of vital phenomena seems to be one of those illusions, like the *ignis fatuus* of the mechanistic philosophers, which blind our eyes to many interesting trails that should tempt the scientific explorer."

The trail of the microbe has reached the edge of a jungle. There are no sign posts and the remainder of our journey must be hacked through a dense thicket of ignorance, superstition, and conflicting opinions.

Microbes existed and had a way of living long before the microscope made them visible to the eye of man. Even after the invention of the microscope, a large gap had yet to be filled in our knowledge of living things. This was to be bridged later; first by a new manner of thinking and then by technical discoveries.

CHAPTER 2

THE TIMES CHANGE

Seeing Is Not Believing

The length of a nose, it has been said, changed the course of history. True as this might be of ancient Rome or even present-day Germany, it was a long-nosed gentleman, Antony van Leeuwenhoek, who made history when he made the first simple microscope. For the development of his character, Leeuwenhoek's long nose was an ideal affliction, because he wanted to see beyond it and he had to see far.

In the middle of the seventeenth century, Leeuwenhoek, then a young man in his early twenties, quit his job as a draper's assistant and became "kamerbewaarder der kamer von Heeren Schepenen van Delft." In other words, he was custodian or janitor of Mister Schepenen's rooms. The work was less arduous than speaking its title and Leeuwenhoek found ample time to amuse himself with a new hobby—that of lens grinding.

When he had finished, he had made by his own hands more than five hundred microscopes of silver, brass, and gold. Human nature being what it is, he should not have been surprised when, full of enthusiasm and excitement over the revelations of his microscope, he could not prevail upon others to look through the instrument and see for themselves. For in this period, too, there were critics who are like certain flies which deposit eggs in the nostrils of the finest horses without retarding their speed.

Fortunately, Leeuwenhoek had an admirer, Regnier de Graaf, an anatomist, who, appreciating the value of the lens maker's discoveries, prevailed upon him to write a report on some of his work. Shortly afterward, De Graaf

read this report before the Royal Society of England, in May, 1675, in the form of a paper describing observations made with a microscope and entitled: "Concerning Mould upon the Skin, Flesh, Etc." In the following year, the shy man from Delft himself told the solemn scientific body of the Royal Society how he had seen animalcules in water collected from wells and other sources.

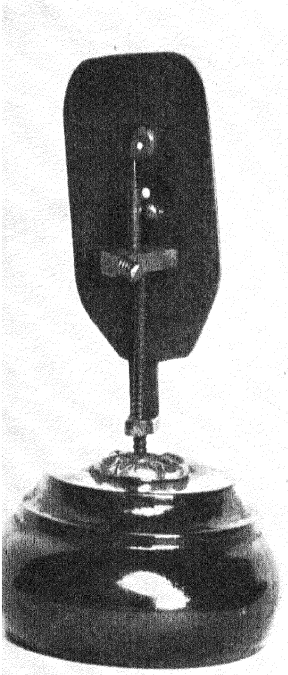
From all sides he was assailed by verbal brickbats. In an earlier era of enlightenment he would have been burned at the stake. No, shouted the learned men of science, there weren't any such animalcules! Since these did not exist, they could not be seen, and to settle the point once and for all, there was no sense in looking. Why put any trust in this man who was so illiterate, so lacking in polish, and horrible beyond words, not connected with an academy or a college.

At this time there also began the great pepper controversy, originating in Leeuwenhoek's discovery of microscopic living things in water in which pepper berries or ginger had been soaked. To disprove his findings, two leading representatives of the Society, Robert Hooke and Nehemiah Grew, were appointed to investigate this ridiculous and unheard of thing. For almost six months these learned gentlemen bungled the technic of a master observer and came to the conclusion that pepper was pepper and the animalcules were only pepper particles, but finally Hooke succeeded in confirming the claims of Leeuwenhoek. Three years later, in 1680, the inventor of the bacteriological microscope was considered worthy of membership in the Royal Society and a gold medal was thrown in for full measure. Now this made everything quite proper.

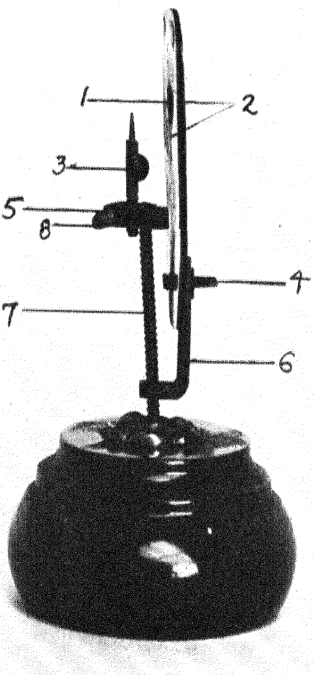
During the next forty odd years, Leeuwenhoek made more than two hundred contributions to this scientific body. What an extraordinary amount and variety of studies poured from this simple "janitor." He helped to put on a sound basis Harvey's discovery of the circulation of the blood by describing what took place in the tiny blood

ANTHONY VAN LEEUWENHOEK (1632–1723)

The first to describe microbes is seen under the microscope which he invented. (U. S. Army Medical Museum negative 32708.)



FIRST MICROSCOPE



SIDE VIEW

THE FIRST MICROSCOPE (1675)

Leeuwenhoek usually referred to this remarkable instrument as a "magnifying glass." The microscope consisted of one double convex lens, which was not, as in our modern instruments, focused upon a stationary object that rested on a fixed platform or stage. Instead, the lens was fixed, and the object to be magnified was moved up or down in order to bring it into sharp focus. The lens (1) was mounted between two thin plates of brass or gold (2).

The object to be viewed was held in front of the lens on the point of a short rod, the other end of which was screwed into a small block or stage (5) made of brass and riveted somewhat loosely on the end of a long coarse-threaded screw (7). This screw acted through a socket angle-piece (6) attached behind the lower end of the plates by means of a small thumb-screw (4).

The long screw adjusted the object under the lens in a vertical direction, while turning of the angle-piece (6) on its thumb-screw (4) allowed motion from side to side. A delicate adjustment of the focus was made possible by turning the object carrier by means of the metal knob (3), and by manipulating the thumb-screw that passed through the stage at one end (8). Pressure was thus exerted vertically against the plates, which caused the stage to tilt upwards at that end.

Parts of the text are taken from Dobell's "Leeuwenhoek and His Little Animals," published by Harcourt, Brace, and Company, New York. The illustrations are reproduced from U. S. Army Medical Museum negative 41932.

vessels of the web of the frog's foot, in the tails of eels and tadpoles, in rabbits' ears, and in the wings of bats. Malpighi, twenty-five years earlier, had already discovered the circulation of blood in the minute blood vessels of the frog's lung. It was also Leeuwenhoek, the "ignoramus," who saw and described accurately the generative principle in the spermatic fluid of fishes, birds, and man. His observations on the anatomy of the nervous system, although not so accurate as his other studies, dispelled much foggy thinking indulged in by his contemporaries. There were yet other masterful bits of work which concerned the corpuscles of the blood, the structure of teeth, bacteria of the mouth, and even fermentation.

There can be little doubt that Leeuwenhoek was among the first to see and explain the meaning of living microbes. He was also the first to suggest a method of demonstrating their living nature, thus preparing the ground for the illustrious names that followed in the path marked by him. Reporting some observations made with rain water taken from a barrel, he noted that animalcules were present in waters exposed to the air and that *after boiling and hermetically sealing the water in a vessel, the animalcules could not be found.* And at this time, Koch, Pasteur, and Lister were yet two centuries away. So it came to be that from these "poor little creatures fast clustered together" in rain water or melted snow or pepper soup, a new world of wonders unseen was put before the unbelieving eyes of men.

Some harsh critics have occasionally insisted that Leeuwenhoek was a mere dilettante, uncultured, and in fact a most mediocre sort of person. The appraisal of the work of pioneer scientists depends a great deal upon those who presume to assess its value. It is rather to the man's everlasting credit that he accomplished his work without any of the advantages that come through an association with an institution of learning. His equipment could not be bought and every part of it, made by his own hand, be-

came the most delicate tool of his eye and mind. Only two hundred years ahead of his time, like the prophets before him and those to follow, he was maligned and at best only tolerated by priggish inferiors.

Germs of the Germ Theory

Let us leap a gap of one hundred and sixty odd years to Berlin, where Jacob Henle, a pathologist, had just published his volume of researches. In this collection was included an essay on "Miasms and Contagia," in which was stated definitely the connection between infectious diseases and specific microbes as their living agents. These, surviving outside the animal body, must account for propagation of disease, Henle said, and directness of contact had much to do with the spread of infection. In this connection, however, the really remarkable work of the Jesuit Kircher, at a much earlier date (1680) must not be overlooked.

Henle, although devoted to the study of minutely detailed structures in organs of the body, had the type of mind that sees beyond isolated facts. Not only did he formulate general principles but he constructed the foundation for a satisfactory test of their validity. The causative agent of an infection, he was convinced, could be proved only when cause and effect were clearly shown to be related. He went so far as to say that the organisms were invisible because they resembled the tissue in which they lived and not because of their incredibly small forms. This was the prophecy. Forty years later, a pupil of Henle named Robert Koch brought the proof and the method, the staining of microbes with aniline dyes to make them visible.

The period from Henle's "Miasms and Contagia" to Koch's first scientific memoir, published thirty-six years later, marked the beginning of bacteriology as a science. During this time there was accumulated a considerable body of evidence pointing to a causative microbe in the disease which was variously known as malignant pustule, spleen fever, anthrax, and woolsorters' disease. A certain

Pollender had seen the organisms in infected blood in 1849. As early as 1863 and 1864, Davaine, a Frenchman, had transmitted the infection to sheep, cattle, and small animals by inoculating them with blood obtained from diseased animals. When the identical organism was found by him in a human patient suffering with the so-called "malignant pustule," there was no longer doubt as to the causative agent. But scientific thinking demanded something more than this to clinch the argument, and Koch undertook the task of furnishing the evidence. His eyes were keen and did not miss within the microbe cell a peculiar little form, which made a clump of organisms look like bamboo chains.

The brain magic that was Koch's throughout his life is evidenced in the way he set to work on this problem. In the course of his study on anthrax in domestic animals, he was led to believe that the bacillus might at times assume a different form, capable of surviving under rigorous conditions. Thereupon he put very small pieces of infected organ tissue, such as the spleen, into blood serum and other nutritive fluids suited to the growth of the microbe. He sat patiently for hours at a time, watching and waiting and observing carefully what was happening under the microscope lens. There before his eyes the microbes grew into long threads and as they did so, small granular bodies appeared with them! Soon these bodies became sharper in outline and he realized that these were independent structures, related nevertheless to the bacillus itself.

What conditions were necessary, he pondered, for this stage to develop? He answered the question by improvising a small chamber in which the organism might grow at different temperatures. There was indeed a temperature most favorable for the phenomenon, just as there was for satisfactory growth and multiplication. But wait—might there not be other interloping organisms present capable of forming spores like these? Why not reverse the procedure and see if the spores will change into anthrax rods?

Although he soon found that such was the case, Koch's

scepticism knew no limit. Not satisfied with the proofs obtained thus far, he decided to investigate solutions containing putrefactive microbes similar in appearance and in their spore stage to anthrax. With these microbes he was unable to produce the disease. Turning again to the anthrax rods, he passed these through a series of mice and succeeded in each instance in producing the typical infection and the characteristic changes in the tissues. The chain of evidence was now complete. The anthrax microbe and no other was the specific agent which alone could cause the disease.

From these observations, Koch deduced the complete life history of the infective agent and its manner of spreading the disease. In the animal body the spores failed to develop, whereas in material discharged on the ground they soon did, but only in the presence of air. These facts had an important bearing upon the future control and eradication of the disease. Pasteur, as we shall see later, made the mistake of ascribing the spread of infection to buried carcasses and to contamination of the ground by earthworms.

In 1876, Koch, then thirty-three years old, published his first scientific memoir, relating how the anthrax bacillus could be made to grow outside the animal body, how the disease might be reproduced by injecting the microbes and nothing else into animals, and how these microbes could later be recovered from the animals after death. These criteria of specificity became known as "Koch's postulates." For the first time definite proof was given of the specific behavior of disease germs. There was no guesswork here, for Koch, a practicing country doctor, carried over into bacteriology his splendid powers of observation and an unerring technical skill.

A Science Is Born

The ingenuity of Koch and his direct attack upon a problem were not lacking in the development of further technics, without which there would have been no science

of bacteriology. First came staining of microbes by means of aniline dyes and a few years later, in 1881, the method of growing bacteria in pure culture on artificial food substances to be known hereafter as solid culture media. With these two discoveries, which took the scientific world by storm, the barrier to accurate observation and recognition of microbes was removed. A third contribution, which had equally far-reaching consequences, was Koch's technic of sterilization. He realized the importance of excluding all accidental microbe invaders during the course of his bacteriological studies. There resulted from these painstaking precautionary measures the types of sterilizers and autoclaves in use today, which gave surgery and preventive medicine their most potent weapons of offense in combatting infections.

Koch's technic for staining microbes had as a background the classic studies of Carl Weigert, who was at the time performing magic with dyestuffs in his laboratory at Breslau. Henle's prophecy, made forty or more years earlier, was now being fulfilled. Weigert, engaged in the study of tissues and cells, found it necessary to make them visible in order to reveal their microscopic structure. Beginning with simple vegetable dyes, he passed on to the use of coal-tar preparations and aniline stuffs. Koch, with infinite patience and careful testing, tried them singly and in various combinations until there was evolved a method of staining the bacterial cell.

The anthrax microbe became the first subject of this astonishing new technic. A minute drop of fluid containing the bacillus was spread and dried in a film on a clean thin plate of glass and a few drops of a solution of methyl violet were poured over the surface. The dye stuff was allowed to penetrate for varying lengths of time and then gently washed off. When Koch examined the preparation under the microscope, the anthrax bacilli were now clearly visible in what must have appeared to him as regal splendor, for they were indeed the tint of royal purple.

Doubters and scoffers, if any remained after such tri-

umphs as these, withdrew wagging heads into their shells when this most persistent young man brought forth another device. In 1877, with the generous help of German optical firms, he introduced a technic for photographing microscopic preparations, thus furnishing a means of visualizing in permanent records the development of bacteria, their internal structure, and the changes they wrought in diseased tissues. The microscope stage was now set for many important dramas never before witnessed. Following Koch came Paul Ehrlich, a scientific giant if ever there was one, who brought to perfection the art of staining tissues and organisms and from there went on to uncover the secrets of chemical therapy. As newer developments in the technic of staining gathered impetus from these masters, no privacy was left for microbes and their innermost secrets stood shamelessly revealed.

Koch Turns Cook

One obstacle remained for Koch to clear away. How was the theory of specific germs for specific diseases to be made an impregnable fact? He thought things out, just as he was accustomed to build, on a firm foundation. Until microbes could be segregated and grown independently of one another, there would be errors in explaining the cause of a given disease. The separation of one organism from another by means of staining properties was helpful, but not refined enough to give decisive proof. Certain of his ground, Koch proceeded to find an answer to this all-important question. He set himself the task of improving the crude and inadequate technic then available for growing microbes in a fluid culture medium.

Of the methods at his disposal, none was satisfactory, whether he used Pasteur's original nutrient material containing ammonium tartrate, water, and candy sugar, or simple broth. In fluids the microbes had favorable conditions for growth, but there was nothing to prevent many different varieties of bacteria from multiplying at the same

time. To separate one type of organism from another was impossible. Such methods were guesswork and threw out a challenge to his orderly mind.

The culture medium devised by Koch was simple in principle, as might have been expected in the work of true genius. The problem was to furnish an environment favorable for the growth of certain bacteria and for making them visible to the naked eye, while setting them apart from any other varieties with which they might be associated. An acceptable technic should also provide a means of transferring indefinitely any one type of microbe to a new environment, under conditions that would prevent the mixing of different organisms. These requirements, he thought, might be met in a culture medium which would solidify. Accordingly, he developed the idea of using sterilized gelatin, to which was added a small amount of fluid containing the organisms.

Koch's procedure was to dilute the fluid in order to "thin out" the bacteria and then add some of this material to the gelatin while it was in a melted state, but not hot enough to damage the microbes. From two to five per cent of gelatin provided a solid, transparent medium upon cooling. In the test tube or on a glass plate or in a shallow covered dish, separate masses or "colonies" developed, representing the growth of individual microbes. These single colonies were next "fished" out by means of sterilized wire points and transplanted to new series of tubes or plates. There was no limit to the number of times a single colony might thus be transferred and, in so doing, "purified."

The year 1881, when Koch made this discovery, was the beginning of a new era in bacteriology and the study of the causes of disease. Before a meeting of the International Medical Congress at King's College in London that year, Koch demonstrated his methods. Among those assembled in this distinguished gathering were two men who later became identified with him in a great triumvirate—Pasteur and Lister. Here was a formidable "Triple Entente" with

a single purpose—to smash the existing boundaries of scientific thought in order to extend knowledge.

Now came in rapid succession one discovery after another of the microbial causes of disease. Within one year after announcing the method of preparing cultures on solid media, Koch isolated and described the bacillus of tuberculosis. Nothing else mattered, now that the great white plague had given up its secret. Little wonder that the entire world was in a frenzy at a time when the disease took its death toll of one out of every seven, and among young adults alone, one of every three. The classic paper, which was announced on the twenty-fourth of March and published on April 10, 1882, will remain for all time an inspiring example of cold logic, clear thinking, and simple expression of a great truth.

But Koch's contributions to the science of bacteriology were by no means complete. In 1883 the fearful microbe responsible for cholera also yielded to his attack. This disease had been ravaging India and Egypt from remote times and from these cradles of antiquity it spread into Europe and America, where countless graves marked its inroads. Nearly 200,000 deaths occurred in three European centers in the single year of 1854. In the second quarter of the nineteenth century, cholera had also swept many of the larger cities in the United States, entering the seaports of New York and New Orleans and then joining the gold-maddened caravan to cut across the continent into California.

Heading a commission appointed by the German government, Koch travelled to Egypt and India in 1883 to study this dreadful disease, which was so swift and so merciless in its attack. From the intestinal contents of a number of the sick and of a larger group among those that had died, he isolated a small comma-shaped microbe. Then he found the identical organism in tanks of foul drinking water used by the natives. Careful study of materials taken from intestinal diseases other than cholera did not

reveal the newly christened *Vibrio cholerae* at any time. Koch had thus found the cause of this disease and proved that it was transmitted by water.

Koch's discovery was not fully appreciated by a number of die-hards, who dragged from the musty recesses of their closeted brains some of the ancient theories of disease. These reactionaries might have recognized the painstaking nature of the man they challenged. Did they not know how he had guarded against every possible chance of error creeping into this work, just as he had in his brilliant discoveries in anthrax and tuberculosis? What he thought of his opponents is well known, as he expressed himself quite vigorously in regard to them, but they were always around in due season, like the grubs that are shaken from old and dusty garments hung out for an airing.

Among his objectors were some whose efforts to disprove Koch's conclusions might have been amusing but for their near tragic ending. Two silly gentlemen, Von Pettenkofer, an old man who should have known better, and his young assistant, Emmerich, deliberately drank some water containing a small amount of culture of cholera germs. They intended to prove with this experiment how utterly absurd it was to link cholera with the organisms, which to them had the appearance of question marks and not commas under the microscope. The elderly doctor had only a mild attack of diarrhea, but the younger subject all but suffered a fruitless martyrdom. Koch knew as we do today that such experiments have no value. When they result negatively, they prove nothing because the conditions necessary for an infection to occur are not always known. If the test turns out positively, as it did in this instance, the volunteer experimental subjects remain unconvinced because they do not die.

Ten years later, Pfeiffer, one of Koch's illustrious pupils, described a simple test known as the *Pfeiffer phenomenon*, which confirmed the teacher's conclusions. The cholera microbe, it was found, behaved like no other similar type

when injected into the abdominal cavity of a guinea pig previously made immune to cholera. Samples of the fluid withdrawn at different times from the cavity showed that the cholera vibrios lost their motility, soon swelled, and finally dissolved. All other organisms, however closely they resembled Koch's type, failed to act in this way. Developing the idea further, Pfeiffer next combined cholera immune serum with the specific microbe and with other similar forms, as controls. These mixtures were then injected into a number of healthy guinea pigs, which were not immune. Again the dissolution of the cholera vibrio was observed and again the other organisms were not affected. There was no doubt whatever that the reaction was specific and established the causal relationship of the cholera microbe to the disease.

From the study of cholera, Koch went on to still other diseases and, with the aid of a body of students and assistants who flocked to his laboratory, his methods and the fruitful results of their application led to a succession of fundamental discoveries. In different parts of the world, other investigators, influenced by his teachings, added to these contributions, until at the close of the nineteenth century no less than thirty specific microbial agents had been described for as many important human and animal diseases. Bacteriology was in the ascendant.

However busy Koch might have been discovering, he still found time to do considerable exploring. Sleeping sickness and relapsing fever in the tropics, plague in India, diseases of cattle in Africa, tuberculosis in Europe and America—every corner of the globe drew upon his fertile imagination and sober judgment. It was from a study of East African tick fever that he rediscovered another new factor in the transmission of disease, as had been previously described by Pasteur in silkworms and by Theobald Smith in Texas fever. A species of tick was shown to be a carrier of the infective agent, which was a spiral-shaped organism. When these ticks laid their eggs, the disease was passed on

to the next generation. Thus the fact of "hereditary" transmission of certain infections was established. The microbe responsible for the type of relapsing fever described by him was duly honored with the title *Treponema kochi.*

The last two decades of Koch's life were devoted to the prevention and treatment of major diseases. To do justice to a small part of these achievements would merit a separate volume. Before drawing the curtain on his work, brief mention might be made of his outstanding contributions to the control of tuberculosis. The introduction of tuberculin, prepared from cultures of the tubercle bacilli, in 1890 signalized a number of attempts to treat the disease in a rational manner. As by-products of this pioneer work, which was attended with extraordinary difficulties and heartbreaking disappointments, there resulted many fundamental concepts related to diagnosis of the disease and its immunity phenomena.

We owe to Koch the present-day understanding of the different forms assumed by tuberculous infections in the human and animal body. In particular, the *Koch phenomenon,* as it is known, has clarified our knowledge of the defenses against the bacillus of tuberculosis. By means of ingenious experiments with living tubercle organisms, he demonstrated in the guinea pig the difference between a first infection in a normal body and an infection in an animal that had already been made tuberculous. In the first instance, the initial changes were slow to develop but progressed to a fatal termination; in the second, a rapid local reaction after one or two days was followed by a mild breaking down of the tissue, which healed rapidly thereafter and did not end in a widespread general infection. It was plain that the normal animal did not respond to the bacillus of tuberculosis like the previously infected one. When killed microbes or various preparations of tuberculin (extracts of the bacilli) were used instead of living organisms, the experiments turned out exactly the same. The

infected animal appeared to be at the same time not only more sensitive to the specific microbe but also more resistant. This seemingly paradoxical reaction opened up a new field of investigation, which helped to disclose the secret of the relation between sensitivity and resistance or immunity to microbial diseases. Some of these disclosures will be discussed later.

Toward the end of a glorious and full life, an unfortunate incident, for which one of his students was responsible, marred Koch's happiness. A premature announcement in connection with an alleged cure for tuberculosis threw a dark shadow over his career, although he lived to see his idea vindicated at least in part, for the treatment of tuberculosis by means of tuberculin did find a place in medicine. This episode does not detract in any way from his record as one of the most gifted investigators of all time and the man who perhaps more than any other started medicine on the road to becoming a science.

Crystal Gazing

Twenty-one years before Koch, Louis Pasteur was born. In 1847 this young chemist of twenty-five began to read the future in crystals. His was not the customary glistening sphere, over which a formula was intoned to the accompaniment of weaving hands, although astrology, phrenology, and animal magnetism were then household words like the vitamines of today. A chemist was less calculated to attract attention than a robed magician practising the arts of divination, yet here was a crystal gazer whose prophecies, while equally fantastic, seemed less credible.

The story had its beginning in the bottom of a wine cask. As far back as 1770, Scheele, a Swedish chemist then twenty-eight years old, discovered in the sediment of wine barrels a thick deposit known as tartar, a name taken from the Arabic derivation "durd" for dregs or lees. More than seventy years later, Mitscherlich, a Berlin chemist who was interested in the structure of crystals, wrote on the nature

of two similar forms of crystals obtained from tartaric acid and which were called tartrate and paratartrate. These two kinds of crystals were particularly interesting to the chemists of Pasteur's day because, although seemingly identical in content and appearance, they showed certain unexplainable differences in optical behavior when a solution of each was prepared.

Pasteur's interest in crystallography was awakened by an unexpected invitation to assist Professor Auguste Laurent with some experiments at the University of Bordeaux. In the course of these studies, Laurent demonstrated to him a crystalline salt which was in a pure state, yet composed of two different kinds of crystals. When Pasteur examined these under the microscope, he found that they had tiny facets which covered only half of the surface but always in a reversed position to that of another set of crystals present in the mixture. They were, so to speak, "right-handed" and "left-handed" facets.

In a sudden flash, it occurred to Pasteur that this lack of symmetry might go with differences in the internal structure and explain the different properties of the dissolved substances. Laboriously he separated the two sets of crystals and proceeded to make solutions of each, hoping to show that if one form produced a certain optical effect and the other one its opposite, then a mixture of an equal number of each in a solution ought to become optically inactive and do neither. The experiment turned out exactly as planned and a new law of chemistry was discovered. In effect, Pasteur's observations showed that there were substances in nature having the same constituents, as represented by a chemical formula having also the same "constitution" or arrangement of the equal number of their parts, but differing as do the members of a pair of gloves. A *right-handed* glove differs from a left-handed glove, but if you look at the *image* of a right-handed glove in a mirror, the mirrored image looks exactly like a *left-handed* glove.

Long Arm of Coincidence

At this point another of those many strange coincidences which were to shape Pasteur's career brought him to the richest industrial center in all France. He became professor in the Faculty of Science of Lille. Surrounded by distilleries and wineries, Pasteur found everything here that was needed to advance from the study of crystals to that of fermentations and their underlying causes. In the meantime, ideas had been fermenting and crystallizing in his mind.

Somewhat earlier, he had been struck by the similarity between the "growth" of crystals and the healing of wounds. The broken surface of a fragment, he observed, was speedily regenerated in the mother-liquor, restoring to the crystal its original form within a relatively short time. Claude Bernard, the physiologist and philosopher-biologist, who, more than any other scientist, contributed to the knowledge of living matter, had also recognized the analogy. He said, in part, that the restoration of crystalline structure could be compared with what happened in more or less deep wounds in living beings; as in the animal, so in the crystal the injured part healed and gradually returned to its original shape. In each instance the replacement of tissue was far more rapid in the affected area than occurred under ordinary conditions.

A second and equally important observation brought Pasteur closer to the nature of "ferments" and disease. At the time, he was very much alarmed because he would have "no important achievement to record by the end of next year." While studying the paratartrate crystals under ordinary conditions of fermentation, he found that only one kind of crystal, the "right-handed" form, fermented, leaving the other "left-handed" ones unchanged. His explanation was that the "ferments" which attack the inactive form of tartaric acid broke down one type of crystal more readily than the other. A fortunate circumstance

clinched this observation and led incidentally to one of the present-day methods of separating the two forms of tartaric acid. While keeping alive a common mold in an improvised culture medium containing paratartaric acid and ashes, Pasteur was astonished to find "left-handed" crystals remaining. The anticipated relation between chemical structure and biologic activities thus found definite expression in a simple fermentative process caused by a mold-fungus.

From "Ferments" to Disease

It was quite apparent by this time that fermentation must be something specific and due to a living principle. Had it not been for powerful opposition on the part of the chemist Liebig, the germ theory of fermentation would have found general acceptance long before Pasteur extended it to diseases of wine and beer. Twenty years prior to this work, in 1836, Cagniard de Latour, a physicist, had already studied yeast "ferment" in beer and described the living cells which reproduced by budding and attacked the sugar in the process of their growth. At about the same time Doctor Theodor Schwann, physician, physiologist, and anatomist, also established the organic nature of yeast. He was no mean scientist in his own right, having discovered pepsin in the digestive juice of the stomach and founded the cellular theory of plants and animals. All three of these contributions were made between the years 1836 and 1839. Such was the heritage handed down to the professor at Lille.

Fermentations of every sort held Pasteur's attention during the years 1857 to 1863. This was a period of consolidation of his observations, which led ultimately to recognition of the germ theory of disease. For each type of fermentation there was a specific organism. Diseases of beer and wine could be traced to undesirable microorganisms, which set up fermentation of their own and interrupted the activities of the yeast. Spoilage of vinegar,

he also found, was due to uncontrolled growth of a specific organism. The difficulty was overcome by sterilization at a temperature of about 130 degrees Fahrenheit, stopping its further development. Thus "pasteurization" was invented.

A strange cycle of events that began in a wine barrel and ended in the same place led Pasteur from crystals of tartaric acid to specific causes of disease. Through the pattern of thought runs the thread of an idea coloring the entire fabric. We have seen how tartrates were inseparably associated with the natural tartaric acid of the grape. This led to the study of fermentation and next came a transition to specific ferments. In the course of these observations arose problems due to undesirable fermentations or diseases of wine and beer. The invaders were obviously germs of diseases and by analogy might be responsible for similar abnormal changes in man and animals.

This idea was illustrated some years ago by Sedgwick, pioneer New England biologist and sanitarian, in a comparison of the simple fermentation of apple-juice or cider with the well-known infectious disease of smallpox. Apple-juice, when exposed to the activity of wild yeast in dust or air, underwent a slow change, during which the "ferment" multiplied. In the smallpox patient, exposure to the infection was followed by a similar period of incubation before active disease commenced. Then the eruption appeared and along with it fever and general disturbance of bodily functions. Corresponding to this, the fermentation of apple-juice proceeded to active "working," with generation of gas bubbles, rise of temperature, and conversion of sugar into alcohol. The next step was the gradual stopping of fermentation and when this had ceased, further alcoholic fermentation could not be induced. Parallelling these phenomena, the smallpox patient was now convalescent (or dead) and immune to a second infection with the disease.

The Naked Gymnast

Prepared as he was by the study of fermentations and having a sure technic at his command, Pasteur's thoughts returned continually to the subject of contagious diseases. An opportunity of testing his evolving theory of disease soon came. A mysterious plague was ravaging silkworms and threatening the life of an important French industry. It was necessary for Pasteur to leave the laboratory and go into the fields. Characteristic of the man, he entered the arena like a naked gymnast to do battle. What did it matter that he knew nothing at all about silkworms? He heard something rattle in the cocoon Henri Fabre gave him and was amazed to learn that there was a chrysalis or mummy inside the silken case. There were caterpillars and their strange metamorphoses too, facts in the life history of the silkworm known to every schoolboy in France. Yet the serene assurance of this man who was to be the deliverer of his people brushed aside these details. "If you only knew how little difference that makes to me," Pasteur once replied to a skilled microscopist who pointed out to him an error in mistaking the form of a microbe seen under the microscope. Perhaps ignorance had its advantages in leaving the mind free to soar and preserving independence of ideas. If reflective intellect might have been lacking in Pasteur, he made good use of that extraordinary domination of his will to probe the bottom of things.

Pasteur was still in the primary school at Arbois when Bassi, in the year 1837, discovered muscardine disease of silkworms. The infection was shown by him to be due to a fungus which was carried from sick to healthy silkworms. Bassi's method of proof, incidentally, established all the facts needed for linking microbes with disease. Had Pasteur known of this work almost thirty years later, his task might have been simplified and the relation between cause and effect discovered with more speed and less drama. He might also have derived much needed support for the germ

theory of disease from what was already known of the silkworm infections he was about to investigate.

Filippi, an Italian naturalist, had discovered in the blood of silkworms affected by this disease a great number of minute spherical bodies or "corpuscles." They were found in every part of the body and passed into the undeveloped eggs of the female moth. From this and similar observations, nobody had drawn any conclusions other than that the bodies were there and whether the relation to the disease was one of cause or effect was not considered. Another Italian, Osimo, had suggested that all eggs showing the "corpuscles" on microscopic examination should be destroyed, but this idea was not taken seriously because other investigators had obtained infected moths from eggs which were free from "corpuscles."

Pasteur was thrown off the track at the very beginning of his observations, when a healthy brood of silkworms was found infested with "corpuscles" while a sickly lot of grubs showed very few or none at all. He concluded that the "corpuscles" were not the cause but the result of the disease. It was not until two years later, when he finally obtained broods of silkworms free from "corpuscles" and was able to infect them artificially by injuring their bodies or feeding the grubs contaminated food, that he reversed his opinion and became certain that the "corpuscle" caused the disease. In the meantime, however, he had been successful in treating the malady by the method formerly proposed by Osimo.

Pasteur was due for another shock, however, when many grubs died without showing these "corpuscles" and a number of healthy eggs developed into diseased worms! The only answer to this riddle, he thought, was to be found in the supposition that there were two diseases instead of one. Such was actually the case, for he found one infection, "flacherie," caused by an organism which spread by way of the intestinal tract like human cholera. The second disease, "pebrine," so-called because of the peppered

appearance of the infected worms, was carried into the egg and, unlike "flacherie," was inherited.

Notwithstanding all the difficulties and obstacles encountered in the problem, Pasteur carried his silkworm studies to a successful conclusion. With the aid of a microscope, he settled the question of contagion in the manner of the Italian investigators who had preceded him. In the chrysalis he found the source of "pebrine" infection, which was first transmitted to the egg and the disease propagated from one generation to the next. All that was necessary to stamp out the scourge was to select sound eggs taken from non-infected chrysalises or moths and to protect the caterpillars from contamination during their development. The method of doing this, as it turned out, was quite simple. At the time of laying, the female moths were separated and placed on small pieces of linen for receiving the eggs. When these had been deposited, the moths were fixed to the corresponding piece of linen for identification purposes, allowed to dry, and a small amount of material prepared from the macerated bodies was then examined for the presence of the parasite under the microscope. Finally, eggs from all moths found to be infected were destroyed by burning.

The "flacherie" disease yielded to the simple procedure of rearing the silkworms in clean surroundings to prevent the spread of infection from dust and dirt. In this causative organism, Pasteur observed for the first time resistant bodies or "spores," which survived the rigors of heat and dryness and perpetuated the disease outside the body of its host. As a result of this brilliant epidemiological work, the silkworm disease was terminated and methods of prevention were established on a scientific basis.

The Great Opportunist

During the years Pasteur devoted to studying silkworm diseases, he stored in his mind ideas which led him eventually into the field of human pathology and preventive

medicine. He was sure that susceptibility to microbial infection was a law of nature and subject to hereditary factors in those who might be so predisposed. He was impressed also with the effect of environmental conditions on the spread of disease and with the difference in invasive power of different forms of the same microbe. Whether in wine, vinegar, beer, or silkworms, each morbid change had to be sought in a microbial cause. To prove this idea, Pasteur brought all the passion and fervor of a blind single-mindedness until he accomplished his purpose. A burning zeal and relentless drive towards practical discoveries dominated his life, and he was never so happy as when the results of his observations were put to immediate use.

Another epidemic of disease was needed at this time to test Pasteur's theories and break down the idea that "ferments" were born spontaneously. As though made to order, splenic fever or anthrax in sheep spread through the agricultural provinces of France in 1877. The cause of the disease had already been identified, the organism grown in pure culture, and the method of dissemination accurately described by Robert Koch in the preceding year. A number of oratorical papers now made their appearance in French medical circles, intending to show that anthrax could be produced without the germ. It sufficed merely to subject blood from diseased animals to the influence of compressed oxygen and the bacterial rods would disappear without depriving the blood of its infective power. These investigators had either forgotten or did not know what Koch had proved regarding the resistant spore stage of the anthrax organism, which developed only in the presence of oxygen. Hence a successful infection with oxygenated material ought not to have been surprising.

Pasteur's opponents in this instance drew wrong conclusions from faulty observations with the inevitable refrain—"spontaneous generation." Nothing could arouse Pasteur more quickly to a fighting mood than those two words, unless it was to have his work impugned. Promptly

he seeded a drop of blood from an animal dead of anthrax into a flask containing a culture medium. From this vessel, after growth had become visible, he took a drop, transferred it to a second flask, and continued the procedure until forty such dilutions had been made. From the fortieth vessel he took a single drop and with it inoculated a rabbit or a guinea pig under the skin. The animals promptly developed anthrax and died. This experiment proved that the infective agent had multiplied and was the same as had existed in the drop of diseased blood. In the course of these observations Pasteur also noticed the development of resting spores, which by themselves gave rise to the typical rod-shaped anthrax bacilli, as Koch had previously shown.

The net result of these controversial studies was a confirmation of all that Koch had said on the subject of anthrax. To these facts Pasteur brought additional support with sheep as test animals. In a region where the disease was prevalent, such an experiment with its distinctive dramatic touch should not have failed to convince even the most skeptical of his opponents. Oddly enough, Pasteur missed completely the significance of the biological behavior of the anthrax germ, or he would not have attributed the spread of infection to the carcasses buried in the grazing field. Under these conditions, the microbe did not assume a spore stage and hence was not disseminated by dust or spicules of grain. What actually happened was that the dried anthrax spores remained alive on the surface of the ground and became potential sources of reinfection of cattle and sheep. Such spores were later found by Pasteur to have retained their infective power more than twelve years after having been deposited on the soil.

These anthrax experiments also led to one of Pasteur's greatest contributions to bacteriologic science—surgical asepsis and antisepsis. Blood from animals dead of anthrax, Pasteur found, often contained another organism which he characterized as putrefactive or septic and which invaded

the blood stream from without. It was this microbe and not the anthrax organism, as his opponents believed, that caused the death of animals inoculated with blood. It occurred to Pasteur that the septic invader did not thrive in the presence of oxygen, whereas the anthrax bacillus, as Koch had shown, did do so. Under conditions where these two organisms were associated, it sufficed merely to exclude air in order to obtain a pure culture of the septic germ. This was accomplished in an atmosphere of carbonic acid gas or in a vacuum. Conversely, the access of oxygen permitted only the anthrax microbe to grow and destroyed the other. Pasteur once again established by this method the fact of two separate classes of micro-organisms; one anaerobic, living in the absence of oxygen, and the other aerobic, requiring oxygen. At a much earlier date in his studies with beer and wine he had discovered that "fermentation is life without air."

In this simple device for segregating the two types of microbes, Pasteur saw a wider application of his theory of preventing blood-poisoning or sepsis following infection of the blood stream. The spores of septic germs, like those he now saw developing in a culture medium, gained entrance in dust and dirt through the air. If it were possible to exclude such contaminating microbes from wounds and from the fields of surgical operation, the dreaded mortality from such operations and from childbed fever might be avoided. Indeed, twenty years before, Trousseau, the great practitioner of medicine, had already recognized the mode of conveying infection in hospital wards from one patient to another. He had even mentioned the possibility of a "ferment" as the cause.

A Generous Ally

In this field Pasteur found a powerful ally in Joseph, Lord Lister. As a practical surgeon, this eminent British doctor was not satisfied with the current theory of atmospheric oxygen as a cause of putrefaction in wounds. He

knew that slight injuries to the skin might result in morbid changes and that sizeable wounds might escape infection. When the work of Pasteur came to his attention, he was quick to appreciate the significance of the causes of fermentations and putrefactions. In confirming this work, he was the first to prove that such changes were due to microbes entering the tissues. He was thoroughly convinced of the processes set up by bacteria in sterilized sugar and protein solutions and accepted Pasteur's interpretation of the facts.

At this time, Lister was unaware of similar unpublished studies by Pasteur and used sterilized urine as a culture medium to demonstrate how decomposition in one sample, resulting from exposure to the air, might be transferred to a clean sterile specimen. He interpreted this experiment as evidence of air-borne bacterial infection and grasped the analogy of a similar process in the putrefaction of wounds. Accordingly he devised a technic of destroying the organisms in the wound itself by the use of *antiseptic* solutions of carbolic acid and of preventing the entrance of micro-organisms by means of *aseptic* dressings soaked in these solutions. Lister was thus the originator of *disinfection* of wounds. From this time he became the most ardent supporter of Pasteur's doctrine and was, perhaps more than any other person, responsible for the acceptance of the germ theory.

Lister's great works, although destined to be overshadowed by those of his more sensational contemporary, supplied the stimulus and practical application necessary for an intelligent understanding of the principles of bacterial infection, as he joined hands with Pasteur in showing the entire world how the cooperative efforts of science might be directed into humanitarian channels. Hospitals which were formerly charnel-houses became models of cleanliness, as Lister's principles of antisepsis and asepsis won general acceptance. The novelty of seeing mothers survive their newborn infants soon wore off and became

commonplace in the maternity wards. The germ theory of disease was no longer a theory—it was now a fact. Pasteur had at last reached the goal which he had set for himself twenty years before.

Accidents and Discoveries

Pasteur himself, meanwhile, had turned his attention to another disease, that of chicken cholera. In the Veterinary School at Toulouse, Professor Toussaint, examining under the microscope some blood taken from chickens that had died of a form of cholera, saw very small organisms, which, he concluded, were the cause of the disease. The culture media he had been using, however, did not prove satisfactory for indefinite growth of the microbe. Pasteur now set to work on the problem and from a specimen of infected fowl muscle sent to him by Toussaint succeeded in growing the organism in flasks of sterilized chicken broth. This microbe was deadly and swift, a minute drop of young culture causing a fatal infection when fed to fowls or injected into their tissues. The extraordinary mortality of barnyard epidemics became clear to Pasteur as he unfolded the life history of the germ and its dissemination through infected excreta.

But the chicken cholera organisms showed a number of surprising irregularities that completely baffled Pasteur. When they were inoculated into guinea pigs, death occurred rarely, but at the place of inoculation the microbes could always be found in the small sore that developed. Now and then some of the chickens also survived an injection for weeks or months. While these problems were still worrying Pasteur, he went away from the laboratory on a much needed vacation.

When studies in chicken cholera were resumed after several weeks, a flask containing a culture of the deadly microbes, which had been put away and forgotten, was brought out and some chickens were inoculated in the usual manner. Nothing happened. Again they were in-

jected and still they did not sicken. Repeated trials gave the same result and Pasteur finally decided to inoculate the same chickens with a fresh virulent culture. To his great surprise, the result was again negative, but when the same culture was injected into a new lot of chickens brought from the market they sickened and died as usual.

Thus through this strange chance Pasteur came upon the secret of artificial immunity, the greatest discovery of his career. The vitality of the chicken cholera microbes which had been put aside for several weeks had been reduced through prolonged exposure to the air, whereas fresh cultures had maintained their virulence. It was now a simple matter to grow the organisms for variable lengths of time in order to lessen their potency in the susceptible fowls. Chickens inoculated with these weakened cultures rarely died or became ill or, if so, showed only slight fever, depending upon the degree of "attenuation" or weakening of the culture. These fowls, having recovered from the initial injection, then resisted large doses of virulent organisms which were regularly fatal in extremely small amounts for untreated animals.

"In the fields of observation, chance favors only the mind which is prepared." Thus spoke Pasteur, the poet of science.

First Principles

The last of Pasteur's three great discoveries was now to be applied to the prevention of microbial diseases. It was established from his work with chicken cholera that "the microbe of an infectious disease, cultivated under certain detrimental conditions, is attenuated in its pathogenic activity; it has become a vaccine." In order to gain this summit of achievement he travelled for thirty years along a rough trail with only two sign posts to keep him away from treacherous detours. Of these, the first was "Each fermentation is produced by the development of a special microbe" and the second, "Each infectious disease is pro-

duced by the development within the organism of a special microbe."

In the field of vaccination and artificial immunization against disease, Pasteur was constantly haunted by the idea of developing a means for resisting the microbes of disease which could be cultivated in the laboratory. This possibility seemed all the more reasonable to him in view of the fact that he knew that Edward Jenner, an English country doctor, had succeeded in this with the virus of smallpox and of cowpox, the nature of which was unknown.

Jenner was a first-rate scientist, whose methods of experimentation and interpretation of the observed facts of vaccination might well be taken today as models for accurate and careful laboratory procedures. Although due recognition has been given his great contribution from the practical side, the usual tendency to consider this work as empirical (based merely on experience) has perhaps not done full justice to the fundamental principles which Jenner, the pathfinder, established. True, he said nothing definite about the possible attenuating effect resulting from the passage of virus from one type of host to another, although he referred to the different animal reservoirs of "pox." He was fully aware, however, of the fact that the virus could be modified by unusual avenues of infection, as, in this instance, the skin or other parts of the body. It is interesting to read what Jenner said in his classic "Inquiry into the Causes and Effects of the Variolae Vaccinae," published in 1798: "Is it not possible that *different parts of the human body may* prepare or *modify the virus differently?* I am induced to conceive that *each of these parts is capable of producing some variation in the qualities of the matter* previous to its affecting the constitution. What else can constitute the *difference between the Smallpox when communicated casually* or in what has been termed *the natural way,* or when *brought on artificially* through the medium of the skin?" This basic principle of artificial immunization was to lead to the second great advance in the theory of preventive vaccination.

EDWARD JENNER.

From the statue by Monteverde.

EDWARD JENNER (1749–1823)

The first human being to be vaccinated by Jenner was his own eighteen-month-old son, as evidence of implicit faith in his own discovery. It was in the same year, 1796, that this young country doctor transferred the cowpox material from the hands of a dairymaid to an eight-year-old boy, who, when subsequently inoculated with virulent material from a smallpox pustule, remained well.

More than eighty years had elapsed since this discovery before Pasteur's observations on chicken cholera realized the attenuation of microbes and its application to the prevention of infectious diseases. The next step in advance was the preparation of a vaccine for anthrax. Pasteur accomplished this by growing the organisms for from eight to ten days at a temperature of forty-two or forty-three degrees Centigrade (107.5–109.5 degrees Fahrenheit). At this temperature, while growth occurred abundantly, the highly infectious resistant spores did not develop. When such cultures were allowed to develop in an incubator at thirty to forty degrees Centigrade, spores were again produced, but these were of lessened virulence, corresponding to that of the bacilli. In order to obtain a graded series of progressively weakened microbes, it was necessary merely to transplant from the original culture at various times. Attenuation, according to Pasteur, was transmitted through successive generations and hereditary. It was found that sheep could be protected against a fatal dose of anthrax bacilli by giving two vaccinations twelve days apart, using a weak culture for the first inoculation and a stronger one for the second.

We have it on good authority that Pasteur remarked: "Nothing would have consoled me if this discovery, which my collaborators and I have made, had not been a French discovery." Only an intense nationalism, such as Pasteur had, might have prompted those cryptic words, and then again it might have been something less superficial than that. Touissant, whom we have met before at the Toulouse Veterinary School, had already succeeded in vaccinating sheep against anthrax by other methods. First he exposed anthrax-infected blood for ten minutes to a temperature of fifty-five degrees Centigrade (131 degrees Fahrenheit). A series of inoculations with a small amount of this vaccine protected the animals against a subsequent inoculation with untreated virulent blood. Pasteur heard of this work through one of his assistants and immediately had twenty

sheep lined up for an experimental test of this method. Sixteen of them survived the first inoculation of vaccine.

In the meantime, Toussaint had resorted to a second method of attenuating the anthrax blood by allowing carbolic acid to exert a graduated action upon the microbes present. With this procedure he succeeded likewise in building up a tolerance toward the virulent germs. There was intense excitement in Pasteur's camp. Doctor Bouley, the field adjutant who was with Chamberland, another assistant, wrote in all haste to his chief. Toussaint, Bouley told Pasteur, was not vaccinating with material devoid of organisms. Indeed the liquid contained anthrax bacilli which were "reduced in power by the diminished number and the attenuated activity." The report went on to state further that the vaccine might be treacherous "in that its power might be recuperated with time."

In retrospect, analysis of Toussaint's experiments revealed clearly that he had discovered two acceptable methods for attenuating microbes and preparing a vaccine, one by exposure to heat and the other by means of chemical action. Both procedures, incidentally, have proved satisfactory throughout the years as standard methods of artificial attenuation. Pasteur's controversy with Toussaint's followers might be dignified as an expression of his insistence upon decisive experimental technic. Notwithstanding, one cannot escape the feeling that his attitude was lacking perhaps in generosity and tolerance toward another scientific competitor.

Pasteur's vaccine was soon to be given a trial on a farm at Pouilly-le-Fort. Nothing less than a public demonstration would satisfy the opponents of this microbe fighter. Pasteur, who had never been known to turn down an opportunity to engage in battle, accepted the challenge. Twenty-five sheep were to be vaccinated and then inoculated, two days later, with virulent anthrax germs at the same time as another group of twenty-five sheep which had not been vaccinated. He said the first lot would be pro-

tected and the second would die. The prediction proved to be correct, for on the day specified, June 2, 1881, all the vaccinated sheep were found gamboling happily in the enclosure and the unvaccinated herd lay dead.

Charming Little Pigs

From this great triumph, Pasteur went on to the study of swine fever or erysipelas, which had been destroying thousands of animals in certain French provinces. In the United States, too, over a million swine had died of this infection a few years before. The organism responsible for the disease was soon isolated by Pasteur, who found also that its virulence could be modified by successive passage from rabbit to rabbit. This new method of attenuation—passage of a microbe through a non-susceptible animal—produced a vaccine for the disease. Preventive inoculations proved to be as practical and successful for swine erysipelas as for anthrax.

As the year was drawing to its close, Pasteur sent a communication reporting the progress of his work to the French Academy. It was his hope that vaccination with the attenuated swine fever microbe would be "the salvation of pigsties." Could anything have been more naive and tender than his closing reference to some little pigs that were being brought to Paris for further experiments: "Pigs, young and old," he wrote, "are very sensitive to cold and will be wrapped up in straw. They are very young and quite charming; one cannot help growing fond of them."

One more crowning achievement and Pasteur's work was done. Hydrophobia, or rabies, despite its rarity and the small number of its victims, excited terror as did no other disease. There was a horror associated with the manner in which it was conveyed by man's most trusted pet, the dog. For Pasteur the disease meant a challenge to solve the behavior of this most mysterious of unseen agents of infection.

Some years before Pasteur's interest was awakened in the study of rabies, its mode of transmission through the saliva and localization of the virus in the nervous system had already been recognized. Such evidence, nevertheless, did not carry conviction. Moreover, the virus had never been cultivated or made visible and above all else, there was no method known for transmitting the disease experimentally. It was most important to find out how to produce rabies artificially in a susceptible animal. Rabbits were known to develop the disease following inoculation with saliva from rabid animals, but the successes were often irregular and only obtained several months after the test. This method was complicated further by the presence in the saliva of many varieties of other microbes, which frequently caused the death of the rabbits. Inoculations made under the skin with material taken from infected brain and spinal cord gave more reliable results than the saliva, but this procedure had some of the same drawbacks.

In the laboratory, Pasteur and his assistants steeped themselves in their work and, as so often happens when an experimenter puts himself in a receptive rather than a reflective mood, a brilliant idea suggested itself. The rabic virus, he decided, ought to be placed into immediate contact with the brain tissue of a dog and in this natural environment infection might occur more promptly. But Pasteur dreaded the ordeal of having to drill through the skull of a dog under chloroform. He had been known to wince even at the sight of a simple inoculation under the skin. As it happened, the experiment was performed during Pasteur's absence one day by Doctor Emile Roux, his associate. Two weeks later the dog developed rabies. Repeated trials with chloroformed animals gave the same results. A method was now available for giving the disease to a susceptible animal.

Unsuccessful in his attempts to cultivate the virus outside the animal body, Pasteur now resorted to a living cul-

ture medium, the brain of rabbits. Inoculation of these animals by this new method was uniformly successful and the disease could be transmitted from rabbit to rabbit. In the course of these successive transfers of rabic material a strange thing happened. The virus seemed to act more quickly and the interval between the inoculation and onset of the disease became progressively shorter, until the incubation period was reduced to six days. At this point the virulence appeared to be fixed, for no matter how many more transfers might be made from rabbit to rabbit, the disease did not manifest itself before six days had elapsed. The outcome of an inoculation could now be predicted to a day.

This occurrence impressed Pasteur deeply, as he recollected what happened in rabbits when they were inoculated with the germ of swine erysipelas. Then, instead of becoming more virulent with animal passage, the organisms were deprived of infective power. He now recognized another general law of microbial disease. The successive passage of germs through non-susceptible animals resulted in attenuation of the microbes while passage through susceptible animals brought about an increased virulence.

The work was not yet finished for Pasteur. He was looking for a means of attenuating the virus, which had now acquired a constant infective power. Recalling the observations on attenuation with chicken cholera, he exposed the rabic spinal cords of rabbits to air in small sterilized bottles deprived of moisture by means of pieces of caustic potash on the floor of the containers. These were kept free of atmospheric dust and dirt by inserting a plug of sterilized cotton wool into the mouth of the bottles. Drying was allowed to proceed at a regulated room temperature and day by day the virulence diminished, until at the end of fourteen days the pieces of spinal cord were found to be harmless when injected into dogs.

Inoculations were then made with this material in the following manner. On the first day the animal received a

small amount of the rabic material which had been dried fourteen days, then the next day some thirteen-day-old cord, and so on until after fifteen days the dog received fresh tissue from the rabbit. Immunization was now complete and the animal did not contract rabies even when subjected to the bites of rabid dogs placed in the same cage or to an inoculation of fully virulent virus directly into the brain.

The problem of protective vaccination against hydrophobia was solved at this point by taking advantage of the long incubation period of the disease. Persons bitten by mad dogs did not usually contract rabies until one month or more after the bite. During this time a state of resistance could be developed by means of a series of inoculations with rabic material of graduated virulence.

An opportunity of testing this discovery soon came to Pasteur. The story of little Joseph Meister, a nine-year-old boy from Alsace, is too well known for repetition. He was the subject for the first human experiment on vaccination against hydrophobia and he lived as a result of Pasteur's great triumph. Shortly afterward, a second boy, a fifteen-year-old shepherd, Jupille, was bitten and treated in the same way, and he likewise recovered. Prevention of rabies became an established procedure and out of this final achievement grew the Pasteur Institute, erected and dedicated by France to the great master in 1888.

The Pasteur treatment for rabies is still in use today, although improved methods have been developed in preparing the virus for inoculation against this disease which, once symptoms have appeared, is uniformly fatal. Since Pasteur's day much has been learned regarding vaccination and the intricacies of modified virulence among microbes. Improvements, however, are easier to discover than principles, and the contributions of Pasteur's genius will always be recognized not by their outer dress but by their inner thought.

Rags and Dirt, Mice and Men

When Louis Pasteur finished his last great work, he had brought about a revolution in human thought with the aid of microbes. A decade of uninterrupted progress from studies on anthrax to chicken cholera and virus attenuation, to swine erysipelas and finally rabies had laid the ghost of spontaneous generation, although his work was never free from attack on the part of those who held to this ancient theory. The history of this phase of the conflict between superstition and science, however fascinating it might be, would lead too far afield. Some of the landmarks along the road merit brief mention here because they pointed the way to experimental answers for empty philosophical debate.

More than one hundred and fifty years before Pasteur was born, Robert Boyle, an English physicist, was convinced that the phenomena of disease were due to living agents and that an explanation of the nature of ferments would solve the problem of disease. Shortly afterward, in 1668, Francesco Redi published experiments on the development of insects and disproved spontaneous generation. This work was doubtless the first to state that living matter came from pre-existing living matter. Maggots, he showed, did not develop in dead flesh, but sprang from eggs deposited by blow-flies.

The simplicity of this experiment was no less astonishing than the great truth which it established. By means of a piece of gauze tied over a jar containing meat, the flies were kept away from the putrescent food, and out of the eggs deposited on the outside of the gauze maggots were hatched exactly as before. This model experiment has served as a basis for all subsequent work done on the subject of spontaneous generation, including those of Pasteur. For, as Redi had said, life was not produced from dead matter but from living germs (seeds) introduced into dead matter. This mode of thinking could not but arouse great antagonism at a time when Van Helmont's formula

for creating mice out of dirty rags and a bit of cheese or grains of wheat placed in a dish was taken seriously.

Toward the close of that century, as we have already seen, Leeuwenhoek and his microscope in 1675 changed the complexion of thought by making germs visible. He anticipated with facts the wild speculations of Needham and Buffon in the middle of the eighteenth century concerning spontaneous generation of animalcules. Leeuwenhoek was the first to show how microbes grew in all waters exposed to the air and how these *living forms could be kept out by boiling and hermetically sealing the water in a vessel.* Spallanzani also did this in 1765, ninety years after Leeuwenhoek, and refuted Buffon and Needham. Pasteur repeated the identical experiments in the middle of the following century. It is an interesting fact that two scientists almost one hundred years apart anticipated one of the most celebrated of Pasteur's experiments by nearly another century, with results which would have surely convinced the careful French observer.

The battle had to be fought, however, over and over again. There was, for example, Helmholtz, who as a very young man, in 1843, separated, by means of a membrane, a fermenting or putrefying liquid from one that was fermentable and showed conclusively that the latter solution remained unchanged. In effect, he proved that the barrier held back organisms which were present in the fermenting material, and that the cause of their development resided in something which could not pass through the membrane.

Using another device, Schroeder and Dusch, in 1854, found that a fermentable or putrefiable infusion when boiled was not altered by exposure to air which had first been filtered through cotton wool. Obviously, very minute particles had been stopped in their progress, but the nature of these remained a mystery. Tyndall afterward proved the existence of these solid particles by demonstrating the destructive action of heat upon them to render the air pure and clean.

All these experiments had a familiar ring as Pasteur

resumed where his precursors had left off and through them he was spurred on to develop the most ingenious experiments calculated to dispel doubt and uncertainty. Far in advance of his immediate predecessors, he introduced a wealth of refined detail into the studies of air contamination by microbes. He also strained air through cotton wool and learned, as they did, that such air was without effect upon fluids capable of supporting life. But Pasteur went further and examined under the microscope the particles held back by the cotton wool. He found microbes there and showed that they developed into living forms when placed in a suitable nutrient solution. Finally, he demonstrated that the cotton wool had no mysterious effect on atmospheric air and did not change the elements composing it, by doing without the cotton wool and using a flask provided with a neck drawn out into a slender tube and bent downwards. He carefully boiled the fluid in the vessel and heated the bent tube in order to kill organisms which might be drawn in with air entering the flask as the solution cooled. The fluid in such vessels remained perfectly clear indefinitely. Organisms could not enter through the tube, although it communicated freely with the air, because the direction of the tube prevented their passage. However, when the neck of this flask was broken off and ready access allowed between the air and the contents of the vessel, the solution which had remained clear up to that time became turbid with developing germs.

Thus fantasy yielded to experiment and a heritage of great thoughts and accomplishments, crystallized in Pasteur's mind, finally gave the answer to a riddle as ancient as history itself.

The Great Miracle

Pasteur's career and devotion to the study of microbial life raise a philosophic question as to the relation of a man of science to the time in which he happened to live. If economic determinism makes men a product of their time,

then it cannot be doubted that history made Pasteur and not Pasteur history. Here was a Frenchman, devout and intensely patriotic, practical and utilitarian, brought face to face with problems upon the solution of which depended the life of his country. The diseases of wine and beer and vinegar, the control of silkworm disease, of anthrax, of chicken cholera, and finally of rabies—all these problems were actually not unrelated in any sense. What strikes one as most interesting in these diseases from the standpoint of the bacteriologist is the remarkable step by step advance in knowledge required for the solution of these successive problems. In themselves they constituted virtually a perfect laboratory experiment ordered and planned by a natural course of events.

Now it so happened that the order of their appearance was purely accidental. Pasteur had no control over that phase of his work. In certain instances we have seen accidents where nature solved problems for him. In other cases, when he was not merely an observer, he was called upon to do passive experiments. This method of approach is vastly different from observing occurrences which would not have arisen naturally and hence require interference on the part of the experimenter. That Pasteur bridged the gap between the different diseases was a notable achievement and would be such in any place or time. Yet the fact remains that it was not required of him to foresee and plan all the intricacies of the problem as a laboratory scientist must, if he is to advance an experiment round by round to its logical conclusion.

Pasteur was one of those unique happenings that are generally associated with the idea of destiny. Perhaps at no time in history before him or since has any one man been given such a golden opportunity for the exercise of his gifts. One is forcibly impelled to recognize the strange and fortunate sequence with which new problems arose at a time when they were most needed to carry forward the ideas and knowledge gained from their predecessors. From

the study of crystals of tartaric acid and the fermentations of wine and beer to vaccination against rabies was an enormous leap in thought and technical application. There lay before Pasteur a beautiful experiment nicely planned in every detail for him to complete as he progressed from one problem to the next in point of time. This will always remain as a miracle of science.

In contrast to Koch, the stolid German, Pasteur had the instinct of showmanship and could dramatize the smallest incident. Patriotic fervor and the national problems of Pasteur's time also lent themselves readily to publicity and acclaim, so that he did not hesitate to make good use of the press. It can be understood quite readily how the French peasantry came to regard him as a worker of miracles. His was the practical side of science and for these people his conquests or failures spelled life or death.

Is it not true of science as in all history that a great event is perhaps only a series of small happenings? Pasteur was admittedly a genius. Gifted with imagination and a capacity for taking infinite pains, he helped found the science of immunology and its methods, whereby infectious agents could be controlled. But it should not be necessary to create a myth in order to appraise his great works. In the evolution of science generally, experimental achievements have resulted from the combined activities of many investigators and observers. Pasteur had a share in these contributions to the human race which cannot be measured in words. Fundamental as his contributions were, it must be recognized that the progress of science owed as much, if not more, to his generalizations.

The Microbe Goes Modern

As times change, problems change. The new is old and the old, more often than not, becomes new. The great pioneers in bacteriology emphasized a fundamental point in the pursuit of causes of infectious diseases and their control—the idea of specificity. For each disease there was a

specific cause, a microbe in the shape of a rod, a spiral, or a berry. Eventually there were ultra-microscopic forms to be known as viruses, capable of passing through the finest porcelain filters, unseen, yet exerting real powers for ill.

Arriving now at the early part of the twentieth century, one finds the idea of microbial specificity deeply rooted in the thought of every bacteriologist. It is nothing short of heresy to suggest the possibility of a hair's breadth deviation from the accepted behavior of a given microbe. But there is no halting the progress of microscopic parasites. They too have been meeting new conditions and changing with the times. Perhaps the gracious hosts did not become aware of this, or if they did, refused to recognize the fact.

CHAPTER 3

THE WAY OF A PARASITE

Origins

Parasites are generally thought of as microscopic in size or as visible little things that crawl, burrow, or bite. The best known, yet least suspected among the larger forms, is the human. Although one cannot even speculate on the prenatal history of microbes, there is much to be said regarding the origin of human parasitism. For we are not only predatory from the time of birth, but we have developed as strict internal parasites long before coming into this world.

In truth, the technic of parasitism is being perfected to the highest degree from the moment a fertilized human egg becomes attached to the inside lining of its maternal organ. This is a food repository, pure and simple, for the period of gestation, which lasts approximately ten lunar months. During this time a collection of miscellaneous cells destined to become a human life develops at the expense of another's blood and vital tissue. From a detached biological point of view, a human embryo differs slightly, if at all, from a tapeworm or any blood-sucking insect.

After birth come the helpless stages of infancy when food and comforts are provided in a new outer environment. Now we find unmistakable evidence of external parasitism. This is merely to say that parasitism, as a perfectly natural occurrence, is not necessarily a vicious trait, but rather an incident of life common to all its forms. Such moral implications as might be suggested by these guileless vital forces need not concern us here. To make a confession, scientific truths do at times become very disconcerting and embarrassing.

Perhaps our instinct of self-preservation is responsible for the idea that parasitism is an abnormal relationship in the animal kingdom. In reality it is a perfectly normal mode of living, whereby microbes try to obtain food or protection or both. The question now arises as to how these human or animal hosts have fared against the predatory habits of their parasites. Fortunately, some sort of partnership has been evolved to hold them in check throughout most of the host's life. On second thought, it would be more accurate to view such a relationship as a superior air of tolerance on the part of the host.

It is in the very nature of parasites to feed fat upon living things larger than themselves. Consequently the struggle to find new providers brings into play many complicated responses towards and from each environment. These result in various adjustments which will determine whether or not the invaders shall survive or die. Having once entered the body of a host, they must now find a way out in order to guard against its possible death. This getting in and out of tight places is so tied up with the origin and development of ingenious devices for outwitting the host that one must let the facts of evolution explain, if they can, how such changes have come about.

Among the lowly forms of life, as in human society, we find the scavengers, plunderers, workers, and the parasites. A more fascinating subject for study could scarcely be imagined than the transitions from one social class to another, or the curious interplay of forces which made now one group and now another powerful enough to mark a trend in its evolution. Unfortunately there is not a great deal of information available on the possible origin of parasitism.

The biologist, however, can point to certain small marine animals, known as crustaceans because of their crust-like or hard covering, which offer a recognizable example of a gradation from the free-living or scavenger type to the genuine parasitic type that lives on and in other

organisms. Many of these crustaceans exist as free swimming forms of the scavenger type in the sea or in fresh water, feeding on all kinds of debris. Others attach themselves to the surface of the bodies of fishes and feed on their slime. Here is a step towards a sort of exploitation of labor, whereby the fish provides transportation as well as simple food. A third form, attached to the gills, gets food from the blood circulating through them. This mode of existence provides living on a somewhat higher plane to the extent that the fishes prepare the food and make it readily available, if not even more palatable. Yet another species of crustacean is found between the fish scales and deeply embedded in the flesh. This form obtains its food from the juices prepared by the host and is thus amply assured of a more permanent home, as well as a richer supply of rations. We see, therefore, in one group of living organisms a gradual development in the direction of a true internal parasite from that of a free swimming scavenger. The second and third examples represent in-between stages of development, in which the one behaves like an undecided sort of external parasite while the other has definitely developed into such.

Roundabout Journeys

If ancient wisdom could not fathom the way of an eagle in the air or of a serpent on a rock, what can modern science do but marvel at the way of a parasite with its host? According to a generally accepted meaning, parasitism is supposed to be an escape from the struggle for existence. Such a notion would soon be dispelled by a true sporting instinct that recognizes a worthy competitor. Although microbes or other invaders of the animal body may be, from our point of view, a source of annoyance, their way of living hardly suggests an escape from the struggle for existence. It would be more accurate, perhaps, to say that the escape of parasites from their hosts *is* a struggle for existence. A description of the life cycles of

some well-known agents of disease may make this point clear.

Regardless of their size—only a small minority is visible to the naked eye—all parasites must meet certain definite requirements if they are to survive. They must first of all find a way into the body of the host and then multiply within it. Next, they are obliged to escape from their temporary prison in order to insure a continued supply of food. Finally, they must move themselves actively to another guardian or enter by indirect means into this new host. Escape is perhaps the most critical period in the life of a parasite because only in this way can it continue existence and maintain an uninterrupted transfer of disease.

The methods of exit are often exceedingly complex and equally ingenious. Much depends, to be sure, upon the place where the parasite originally lodged in the body, and as much again upon the peculiarities of the infective agent. It might be the kind of microbe, for example, that limits its activity to the mouth or throat. Simple and direct is the transfer from host to host under these circumstances. There are other parasites, however, gifted with almost human perversity in their choice of unexpected places for doing mischief. When these cannot escape through their own efforts or by means of mechanical devices such as the intestinal tract, accomplices help to set them free. These are mosquitoes, flies, ticks, and other blood-sucking vermin, all of which have played a part in the history of certain diseases where the microbes live in the circulating blood, by providing the parasite with direct transportation from one person to the next. This method also has its refinements on occasions when the very life of an organism depends upon passing through stages of its development in a specialized portion of the insect body, where the parasite becomes sexually mature. Such development occurs in malaria and tick fever, which will be discussed later.

Another device for transmitting disease from man to man without the aid of insects makes use of larger animals

which serve as reservoirs for the infective agent. In its simplest form, these so-called intermediate hosts merely keep the parasite in its accustomed surroundings, from whence it disdainfully makes excursions to man's kingdom and returns to its own kind. Thus the beef tapeworm passes from man to cattle and back again, the pork tapeworm from man to swine to man, and the fish tapeworm, similarly, from man to fishes and back to man. In the midst of all this bedevilment, we, as the honored or primary host, invite the parasite to our table by the generous act of eating the intermediate host. How wrong was Charles Lamb, when he said, in his palate-tempting essay entitled "A Dissertation on Roast Pig": "Pig—let me speak his praise—unlike man's mixed characters, he is good throughout. No part of him is worse than another—he is all neighbors' fare."

The ingenuity of the lowly forms of microbe life is shown in their choice, whether by accident or design, of several different hosts, so that death or extermination of one or more of them does not interrupt the continuity of parasitic existence. An example of this type of parasite is found in a disease of swine known as trichinosis. In the United States about half a million persons according to reliable estimates, are afflicted annually with this disease. It is caused by a microscopic worm which lives in the rat and thrives equally as well in the pig. The infection is passed from one animal to the other and back again as a result of mutual cannibalism. Man plays host to this parasite by eating insufficiently cooked pork or bear meat and it is probable that he helps to maintain the disease in rats and swine through pollution of the soil with intestinal waste. Were the parasite limited to hogs and man only, trichinosis would soon disappear, but the rat is a natural reservoir, admirably suited to the spread of the disease because of its feeding habits in the wake of swine.

When trichinae-infested flesh is eaten by man, rat, or hog, the imprisoned worm, which is tightly and remark-

ably coiled within a capsule in the muscle tissue, is set free by the dissolving action of the digestive juice in the stomach. From here the trichinae pass into the small intestine where, after a few days, they become sexually mature and fully developed within a week. As many as five hundred young may now be produced by one female worm. Penetrating the wall of the intestine, the myriad parasites find their way into the lymph channels, thence through the veins and, carried along in the blood stream, reach the muscles. Individual muscle fibres eventually become the lodging place where, after several weeks, a full-grown parasite enters upon its stage of encystment, forming a thick protective capsule containing deposits of lime salts. In man these changes may occur during a period of five months and thereafter the trichinae have nothing more to do until awakened from a Rip Van Winkle sleep lasting sometimes as long as twenty-five years. They have been found alive and capable of development after such a period of inactivity in the host.

Trichinella spiralis was first discovered in the muscle tissue by a freshman medical student at Saint Bartholomew's Hospital, London, over one hundred years ago. In 1834, James Paget, who was then twenty years old, ran, flushed with excitement, to tell his professor of the finding. The senior professor, Richard Owen, had been appointed that year to the chair of comparative anatomy. He wrote a paper describing and naming the worm which he regarded as a medical curiosity of no importance. The student, although denied the glory of a great discovery made in early youth, won lasting fame as Sir James Paget through his studies in pathology. He will be remembered for one thing at least—a deformity of the bones which bears the name of Paget's disease.

Round Trips to Strange Ports

Returning from some of the journeys we have just made under escort, we should be able to appreciate the im-

portance of a round trip in the parasite's struggle for existence. Everything that is involved in this struggle has to do with disease as it concerns us. The way microbes get into and out of the human and animal hosts stamps each disease with an individuality which is often helpful in recognizing and controlling the infective agents. In the attempt to trace the source of some of these maladies and their manner of spread, investigators have met with unbelievable surprises.

Take, for example, a strange infection known as sleeping sickness found in the western and central parts of Africa. The scientific name is in itself terrifying—trypanosomiasis—and it tells in words of more than one syllable that the disease is due to a microscopic animal called a *trypanosome* or "*borer-body.*" This organism led its hunters a merry chase in a series of events which began in 1880 when Doctor Evans, working in India, first showed a fatal disease in horses to be connected with the presence in their blood of a trypanosome. Up to this time, however, there was not a single clue which pointed towards a possible source or distributor of the disease. Suddenly came the announcement of an astonishing find. It was Bruce who, in 1895, reported that a certain fly, one of seven or more varieties known as the tsetse fly found in South Africa, carried the infecting organism among domesticated animals. "Fly disease," "nagana," was so deadly that horses and cattle were completely exterminated in many districts of Africa, which made Bruce wonder how the tsetse fly continued to carry trypanosomes when there were no longer any domestic animals left to supply them.

He set to work studying the habits of the insect and soon made two very important observations. The fly, he noticed, was never found in open country or at any great distance from water, but always confined itself to a narrow strip of land along the banks of streams and lakes. To these shady watering places came wild game, such as the antelope, the blood of which Bruce discovered contained

a natural reservoir of the parasites which caused sleeping sickness, although the antelopes themselves were resistant to the disease and did not sicken and die. In this manner they furnished a continuous supply of trypanosomes for the biting insect, which, in turn, carried the infection to domestic animals and man.

Five years afterward a Colonial surgeon, Doctor Forde, made the discovery of the same parasite in the blood of a native living in the River Gambia Colony of West Africa, although its recognition as such fell to Doctor Dutton, upon whom Forde had called for assistance. In the following year, 1902, the same trypanosome was found by Doctor Castellani, not in the blood stream but in the nervous system of a person suffering with sleeping sickness. Thus was it gradually proved that this disease began first as an invasion of the blood stream and later affected the brain and nerves. Only then did the disease express itself outwardly in a manner suggesting the name given to it. A year later (1903), Bruce confirmed these observations and revealed how another kind of tsetse fly spread the disease from man to man.

The history of this disease illustrates how complicated can be a relationship of host and parasite and the agent responsible for its spread to another susceptible host. But there is still another delicate mechanism involved. Mention previously has been made of the part played by an insect's body in preparing a parasite, during a sort of "ripening" period, before setting it free. In this particular case, the trypanosomes of sleeping sickness do not become infective until they have developed first in the intestinal tract of the tsetse fly and later in its mouth parts which manufacture saliva. If conditions are favorable, from twenty to thirty days are required before the fly becomes infective, after which time it remains so for life—approximately six months.

African sleeping sickness lost some of its terrors from the time the habits of the tsetse fly became known. This

discovery and the added fact that wild game were the natural reservoirs of the parasite gave to medical science the weapons it needed for fighting a tricky enemy. The fascination of the search for this knowledge has been woven into a story written by John Masefield under the title "Multitude and Solitude."

Night Flyers and a Double Life

Insects also play an important part in another group of diseases carried by mosquitoes. These are the fly-by-night specialists, biting man late in the day and at night. The feeding habits of these greedy insects make them very efficient in spreading infection from the sick to the well. Even when engorged with blood, they continue to bite new hosts and inject their victims while in the act of feeding on them.

The principal diseases carried by mosquitoes are yellow fever, dengue ("dandy") fever, and malaria, the microbes of which must go through a cycle of development in the bodies of a certain kind of mosquito before becoming infective for a new host. In these cases the insect is a foster mother to the parasites, and it is understandable why a definite time is required for their rearing and nourishment before they are turned loose. When a female mosquito limited to the practice of yellow fever bites a person having this disease, at least twelve days elapse before the insect can pass it on to another person. What is more, this transfer will succeed only when the mosquito has fed on a patient during the first three or four days of the disease.

In like manner, the spread of malaria from man to man is brought about with the help of a specialized female mosquito, in which the parasite spends from ten to fourteen days before reaching the salivary glands of the insect. During this time the male and female elements of the parasite contrive to produce a matured body capable of infecting another person through the bite of the mosquito. In such a case, the length of time man remains infective

for the feeding mosquito is limited to the number of days the parasites are found in the blood. In untreated patients this may last for months.

It would seem from these accounts that the parasite succeeds in human society because of cunning strategy in leading a double life. A part of its existence is spent in man for the purpose of multiplication and continued residence in this host. Its other life is passed in the mosquito, chiefly to hand on the parasite from person to person. This partnership is ideal for an efficient ugly business. Mosquitoes furnish the needed talents of nocturnal prowling and stabbing and their tenants, the parasites, supply the art of double-dealing. Thus, without implying specific intent on the part of the parasite to injure man, its sexual or "rejuvenation" phase of existence is most important for its survival.

Night and Day Shift

When we look further into the struggle for existence among certain agents of disease, we find examples of behavior that appear almost too fantastic for belief. In this class belong the migratory habits of the microbes found in a curious disease known as *filariasis*, the parasites of which seem to regulate their strange activities by the clock. They are found in the blood stream only at night, being almost entirely absent from eight or nine o'clock in the morning until about six or seven in the evening. Then they begin to appear, gradually increasing in numbers up to about midnight and gradually decreasing until about seven or eight in the morning, when, like good union laborers, they knock off work right on the dot.

These strange facts about filariasis, which takes its name from the Latin word "*filum*," meaning thread or filament, because the parasites resemble fine threads or hairs in appearance, were discovered by Patrick Manson, an English physician. Working in China in 1878, he showed that the disease was caused by tiny worms completely surrounded

by a loosely fitting sac, which effectively locked them within the vessels of the circulatory system. The baby worms or embryo filariae are harmless and may be present in a state of arrested development in the blood of persons for years without causing outward signs of illness. The adult or parent forms of the organism, on the other hand, clog certain channels in the blood circulation system and produce enormous enlargement of the limbs or other parts of the body. For this reason a popular name for the affliction, which describes it quite accurately, is elephantiasis or elephantoid disease.

Since the tiny worms cannot escape from their prison in the blood stream by their own efforts because of the sac which envelops them, Manson reasoned that some outside agent, perhaps a biting insect, must make this possible. Further experiments proved this agent to be the mosquito, a discovery which brought about a revolution in medical research by demonstrating for the first time that a disease could be carried by means of insects. It was Manson's work that later led Ronald Ross, a young officer of the Indian Medical Service, to discover the malarial mosquito and the life cycle of its parasite.

Let us look through the microscope at what Manson saw when he made his discovery. Tropical mosquitoes, gorged with blood after feeding on a patient with filarial disease, were kept in containers, from which one or more were taken out daily for study. They were next carefully cut up and examined part by part to locate the parasite. In the mosquito's stomach the filaria escapes from its imprisoning sac or envelope by smashing through the front end with the aid of its armored head. Quickly piercing the stomach wall of the mosquito, the parasite now bores its way and in a few hours reaches the muscles of the thorax. Here it rests for two weeks or more while going through a number of developmental changes. At the end of three weeks it is on its road to the head of the mosquito, and from here travels down to the piercing mouth parts. When the

insect next feeds on human blood, the filariae escape through the puncture wound or even the intact pores of the skin and find themselves back again in the human host. Here the parasites go through a sexual cycle with the result that many baby filariae are born. Now these find their way into the blood stream and wait for the mosquitoes to carry them off to their nursery. From this point the story repeats itself again and again.

Having solved the problem of the transmission of filariasis, Manson once more turned to the mystery of the disappearing parasites. Where did they go after working hours and what became of them? The answer to this riddle was found through a freak of circumstance.

At a fortunate hour for science, one of Dr. Manson's patients suffering from the disease committed suicide one morning about eight o'clock by swallowing a bottleful of prussic acid. The blood from surface vessels was examined after death and, as when the patient was alive, no parasites could be found. But in blood taken from the vessels of the heart, in the heart itself, and particularly in the lungs, the worms were present in enormous numbers. Evidently they hid away during the daytime in the lungs and larger blood vessels. If an acid test was needed to prove Manson's point, this one came at an opportune time.

The hideaway of the parasites was thus uncovered but the reason for their strange behavior has never been satisfactorily explained. One theory advanced was that an increase in the size of the blood vessels during sleep allows the parasite to pass. There is an interesting fact, related by Manson, to support this theory. If a patient with filarial disease sleeps during the day and stays awake during the night, the times of appearance and disappearance of the filariae are reversed. Instead of leaving the blood stream about eight o'clock in the morning, they begin to arrive and increase in numbers until towards six in the evening, when they gradually diminish up to midnight. But the theory falls down when applied to another type of filarial

disease caused by a parasite which resembles the other so closely as to be mistaken for it. In this disease the worm retires during the night and is found in the blood only in the daytime, and changing the patient's hours of waking and sleeping does not reverse its schedule. Such would certainly be the case if a change in bore of the blood vessels had anything to do with its singular behavior.

Curious habits like these, when thought of merely as isolated facts, can excite wonder without reference to a purpose in nature. Yet here we see almost identical parasites so beautifully adapted to their insect hosts that transmission to the human cannot fail. Where the filaria follows a nocturnal schedule, it is taken up by the night-biting common house mosquito. In localities where the parasite happens to be the daytime variety, it is carried from person to person by the day-biting insect. The mosquito is further insured against failure by a protective arrangement found in the young filaria. It will be recollected that the embryo worm, while in the blood of a human host, is enclosed in a sac. This envelope prevents escape of the parasite through the walls of blood vessels into the surrounding tissues, where obviously the mosquito could not reach it.

Unfortunately for the mosquito, this parasite, unlike that of malaria, frequently causes a serious infection in the insect itself. Its greedy feeding habits, while serving a useful purpose from the standpoint of the parasite, may cause the death of the mosquito when too many embryo filariae are crowded into its stomach. What a blessing to man if this form of unintentional suicide were more common among winged parasite carriers. But overindulgence at mealtime is taken care of by nature's excesses, which provide large families to make up for such accidental losses. This plan has worked out successfully in the insect world, for it is the female, exclusively, as a rule, that bites and obtains the blood needed for development of the eggs. A generative urge that compels mosquitoes to bite furiously and repeatedly until sated with human blood is certain to

scatter parasites far and wide. In transmitting disease, the insect merely performs a duty to itself and to the successful partnership of host and parasite.

Inherited Mischief and Texas Cattle Fever

Thus far we have been travelling along avenues and riding in vehicles used by parasites to move from one host to another. We have seen how insects can carry disease and make it continuous by some method of transfer. There is yet one more device for distributing and perpetuating parasites. Of certain ticks, it might well be said that "the evil they do lives after them," for they can pass infection through the egg to future generations of their kind. This discovery, made during the last decade of the nineteenth century, was startling enough to invite boisterous laughter in foreign laboratories and to be ridiculed in this country as unadulterated fantasy. Indeed it was a fairy tale, but Doctor Theobald Smith, who wrote the story, lived to see it acknowledged as one of the most important gifts to medical science. In the years that followed, other insect-borne diseases were conquered as a direct result of this laborious piece of work.

Smith had just graduated as Doctor of Medicine from Albany Medical College in 1883 when he received an appointment as assistant in the Bureau of Animal Industry in the United States Department of Agriculture. He was immediately put to work upon a mysterious sickness among cattle in the southern states, causing ravages and despair reminiscent of the days when Louis Pasteur in France toiled and sweated over anthrax. This disease, known as Texas fever, attacked the animals in a business-like manner. They suddenly sickened with a burning fever, their blood turned to water, and they died. What puzzled everybody was the way southern cattle regularly infected herds from the north, while themselves remaining outwardly healthy.

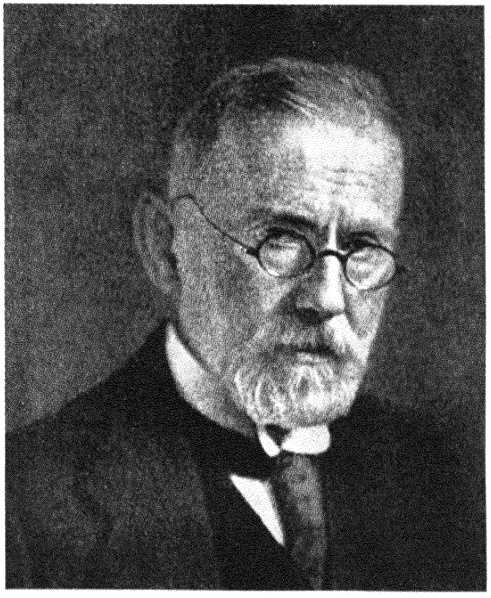
Down in Texas, Doctor Smith and his able helper, Doctor Kilborne, found that the cattlemen had a theory that

Texas fever was tied up in some way with the presence of cattle ticks. They had noted, among other things, that the disease was most prevalent in the summer time when the ticks were most numerous. About one month or longer was required for the infection to pass from southern to northern cattle and it did not appear to be transmitted directly from one to the other. What an opportunity this might have been for some contemplative herdsmen to test their current theory that ticks must have something to do with cattle fever! Nevertheless it cannot be held against them that they found more enjoyment in chewing tobacco and spinning circles with a lasso.

Kilborne decided to try out the popular theory of the cattlemen, who insisted on blaming the ticks while stubbornly refusing to be curious about them. Accordingly he placed in one pen a number of southern cattle from which the ticks had been carefully removed, and in another, a herd infected with them. Into these enclosures healthy cattle were run and the result proved the cattlemen were right; ticks did have some relation to the Texas fever. In the first pen, none of the healthy cattle became infected, while in the second enclosure all the healthy animals sickened and died. For several years these field observations were repeated again and again by Smith and Kilborne, until they were able to say with the ranchers: "No ticks—no Texas fever."

Smith now went to work like a youngster taking a watch apart, to see what the ticks did. His first experiment started him on the wrong track. What would happen, he mused, if some northern cattle were allowed to graze on a fenced-in plot of ground strewn with ticks taken from the deadly southern pastures? In due time this question was answered. These experimental animals died just as though they had been placed in pens with southern cattle. From this, Smith concluded that the cattle had contracted the disease by feeding on the blood-laden ticks.

He was on the very verge of announcing his discovery



PAUL EHRLICH (1854–1915)

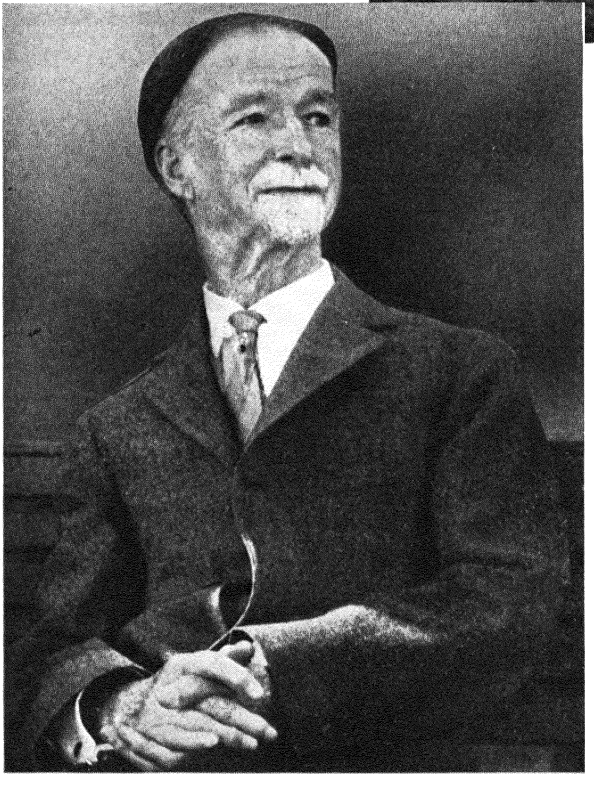
German physician remembered best for his discovery, among other medical triumphs, of "606," an arsenic compound that destroys the germ of syphilis. In 1908 he shared with Metchnikoff the Nobel Prize for medicine.

JOSEPH LISTER (1827–1912)

Pasteur's best publicity man

HOWARD RICKETTS
(1870–1910)

A truly great investigator whose researches in Rocky Mountain spotted fever and typhus opened up new horizons His name was added to the roll of martyrs to science when death from typhus fever cut short a brilliant and promising career at the age of forty



THEOBALD SMITH (1859–1934)

The scientist who glorified parasites and then destroyed them This photograph by Doctor Carl Ten Broeck of the Rockefeller Institute at Princeton, New Jersey, was taken on the occasion of Doctor Smith's seventy-fifth birthday anniversary a few months before he died

when he paused for a further experiment. He wanted to know just how those rebel ticks outwitted smart Yankees. His thoughts returned again to the queer pear-shaped bodies he had previously noticed in the blood corpuscles of sick cows. He was not sure whether these punched-out holes in the cell were due to loss of color in the corpuscles or were actually the microbes of Texas fever. Hundreds of times he had found them under the microscope and now he must try to re-enact the scene.

An idea occurred to him to hatch out in the laboratory fresh baby ticks and let them eat their fill to bursting on a calf. Since these ticks had never come into contact with infected cattle, any changes they might make in the calf's blood, Smith believed, would be due to their feeding habits alone. This experiment, he hoped, would show if an army of ticks could withdraw sufficient blood to cause changes in the corpuscles like those he had seen before. He would examine the blood each day and watch for them.

Did not Pasteur make an historic remark about accidents and a mind prepared? Theobald Smith owned to the accident at least, for one day the little calf sickened and burned with fever. The blood sample taken from a nick in the skin was a pale ooze, watery and brown. The blood had turned to water—this must be Texas fever! When he examined the specimen under the microscope, the punched-out pear-shaped bodies were there inside the corpuscles.

And now, as a result of an unforeseen accident, he had everything straightened out. The parasite must have been planted by the baby ticks hatched from eggs laid by old ticks bred down south. Then it was not feeding on the microbes dropped from crushed blood-laden old ticks that killed cattle. All those weary days and nights spent on that false track had been to no purpose.

The mystery of Texas fever was solved. It seemed so ridiculously simple, looking back to the drawling wisdom of those cowhands. They knew it was fatal to northern cattle to be kept longer than three weeks in the same pens

or fields with herds from the south. This fact could now be readily explained. A wedded mother tick, Smith learned by observation, had to moult for the last time and fill up with blood before dropping to the ground. Here, after several days, eggs were deposited, some two thousand or more, needing at least twenty days to hatch. The baby ticks also took time seeking out new cattle to bite, so that in all about thirty days were required for their development. The pear-shaped parasite in the meantime spent from two to four weeks in the cattle host making ready for another victim.

One more thing remained to puzzle Smith and that was why southern cattle did not die of Texas fever, yet were an unfailing source of infection. He found the solution to this riddle in his own northern calves, that would develop one or more mild attacks of this disease and then become resistant to it later on. In the south, it seemed, this immunity was developing all the time because Texas fever parasites were everywhere fortifying the young animals against this scourge, insuring their old age while making them reservoirs of infection. The trails of insect-carrier and parasite are often long and roundabout, but somehow they manage to catch up with each other and with their host.

Disease Kingdoms and Hereditary Succession

The facts assembled by Smith and Kilborne during the years 1889 to 1893 had a more far-reaching result than doing away with Texas fever of cattle. Actually they had discovered the general law of hereditary transmission of disease in insects, which in turn could carry the same infection to animals. It was almost a decade or more before this revolutionary idea was applied to the study of some human diseases. The first of these was Rocky Mountain spotted fever.

Springtime in the Bitter Root River country of Montana and in parts of Idaho, Nevada, and Wyoming was

always the open season for "spotted fever," so-called because of the purplish spots which appeared on the skin at the height of the illness. Two doctors, who were studying the disease in Montana in the year 1902, felt reasonably sure that certain ticks carried it because every one of the twenty-three infected persons examined by them had been bitten by these insects. Shortly afterwards, Doctor McCalla, a practising physician in Idaho, gave the first decisive proof when he and his collaborator, Brereton, experimentally infected two persons by the bite of a tick which had been found on one of their patients.

During the next few years, 1906–1908, a young pathologist, Doctor Howard Ricketts, who was to write his name large in the history of preventive medicine, was studying the problem in his laboratory at the University of Chicago. From experiments with guinea pigs, he learned how "spotted fever" was transmitted from the sick to the healthy animal by the bite of ticks and how these, when infected, handed down the disease through the eggs to their offspring. Tracing the source of the parasite, he found a number of wild rodents susceptible to the disease. In the chipmunk, ground squirrel, and groundhog, the tick had an unlimited supply of infected blood maintained by naturally infected insects. New generations of "spotted fever" ticks carried the organisms to their hosts, while these furnished transportation and food. Again we meet with the charmed circle in which parasites move to assure an unbroken succession in the kingdom of disease.

While Ricketts and his associates in Chicago were exploring the new fields discovered by Theobald Smith, others were toiling in jungles on the Dark Continent, similarly occupied with another disease known as relapsing fever or African tick fever. Robert Koch in East Africa and two English doctors on the West Coast found a variety of blood-sucking ticks that could also transmit disease by hereditary succession through infected eggs to the human. In principle the developmental cycle and maintenance of all these

diseases was the same as in Texas fever. They differed only in an added feature of transference from animal reservoirs to human hosts.

This fact was a step in advance of Manson's and, after him, of Smith's discovery of the part played by insects in transmitting disease. Ricketts went even further than any of his contemporary scientists in attempting to study the parasite. Within the eggs of infectious ticks, he demonstrated exceedingly small bodies which he believed to be the cause of spotted fever. Although unable to make them grow artificially outside the animal body, he had other evidence sufficient to connect them with the disease. When, a few years later, Ricketts found these same dot-like bodies in the blood of typhus fever patients and in lice infected with this disease, he made a discovery which turned the science of bacteriology upside down. He set in motion a new star, the light of which after twenty-five years has only just begun to reach us and dissipate some of the darkness. Doctor Ricketts did not have time enough to carry on a great work. In Mexico City, on the third of May, 1910, when he was thirty-nine years old, he died of typhus fever, a martyr to medical science. The name Rickettsia has since been given, as a tribute to the man, to those mysterious bodies found in certain insect-borne diseases which they are supposed to cause.

Up to recent years Rickettsia bodies have been associated with five recognized diseases. Rocky Mountain spotted fever has its *Rickettsia dermacentroxenus*,* named after a tick carrying the infection, and typhus fever the *Rickettsia prowazeki* in honor of Doctor von Prowazek, who died while studying the disease. Trench fever has been named variously: according to the place where the disease was first discovered in Wolhynia during the World War on the eastern front, it is known as *Rickettsia wolhynica;* as *Rickettsia quintana* it describes the five day fever of the trenches; and as a memento to the body louse

* Renamed *Rickettsia rickettsii.*

harboring the disease, it carries the name *Rickettsia pediculi.* Tsutsugamushi ("dangerous bug"), known as Japanese river fever or flood fever, which has been prevalent in the Orient for the past thirteen centuries, is now attributed to a Rickettsia-carrying mite. The disease is known as *Rickettsia nipponica* to indicate its geographical distribution. Lastly there is heartwater disease of ruminant animals on the South African veldt, ascribed to a tick found on cattle, goats, and sheep, and appropriately called *Rickettsia ruminantium* by Doctor Cowdry, its discoverer.

The rickettsial diseases of man may be divided for convenience into four well-established groups:

Typhus—epidemic and endemic (murine)
Spotted fever—Rocky Mountain, South American, boutonneuse (Marseilles fever), South African tick-bite
Tsutsugamushi—(scrub typhus) (Japanese river fever)
Q fever—Australian "Q" fever, American "Q" fever

Tsutsugamushi disease or Scrub Typhus has been found to prevail in Japan, Malaya, Sumatra, India, Queensland (Australia), and, more recently, New Guinea and the Philippines. The disease resembles the other rickettsial infections, and is caused by an organism, *Rickettsia orientalis.* From the reservoir, field mice and certain wild rats, the disease is transmitted to man by a mite, which is not unlike our "red bug" or chigger, and is named *Trombicula akamusi.* This mite lives in the top layers of the soil and, when seeking food, climbs on blades of grass in search of contact with warm-blooded animals. In its immature state (larva) the mite feeds only once, taking from two to five days for the meal, and then dropping off to the ground where later development takes place. The adult form does not feed on its animal hosts. Accordingly, the rickettsial infection acquired by the mite in its meal of blood must go through a complete cycle of development from the larval

form of one generation to the next, before it can be passed on to man or to another animal.

Q Fever

First described in 1937 by Doctor Burnet in Australia, this disease was shown to be caused by an organism now bearing the name of *Rickettsia burneti.* At about the same time a rickettsial organism isolated from ticks in Montana, U. S. A., and given another name, was found to be identical with the Australian infectious agent. In its native habitat *Rickettsia burneti* has been isolated from ticks and from the bandicoot, a marsupial, both reservoirs of the disease.

Unlike all other known harmful strains of Rickettsiae, the *Rickettsia burneti* is not held back by ordinary bacterial filters, indicating an extremely small particle size. This might explain in part at least its remarkable tendency for producing an explosive outbreak among laboratory workers and animals in buildings over wide areas. By its ready dispersibility in air as well as resistance to certain chemical and physical agents, this organism is set apart from the well-known Rickettsiae. As a significant hazard in the laboratory, it differs sharply from those handled under more or less identical conditions.

Q fever is recognized in two forms: one, presumably transmitted by the tick; and the other, an air-borne disease from infected animals or perhaps from dried intestinal contents of infected ticks. As might be expected, the second differs from the usual variety in having certain characteristics of atypical virus pneumonias associated with respiratory infections.

Rocky Mountain Spotted Fever

This disease, no longer confined to the United States in the originally known area of the Northwest, has been identified since 1930 in the Eastern states, the Middle West, in several provinces of Western Canada, and in South America. In Brazil, the disease has been named exanthe-

matic typhus of São Paulo, and in Colombia, it was first described as Tobia fever.

Although several kinds of ticks are implicated in the spread of Rocky Mountain spotted fever, only three that are known to bite man have been found naturally infected in the United States. Of these, the two best known are the dog tick and a close relative, the wood tick, found in the Northwest. A species of rabbit tick, also naturally infected, while it does not bite man can and usually does maintain and distribute the disease-producing agent in nature. As a natural reservoir host, the wild rabbit similarly plays an important part in the Brazilian form of spotted fever, along with the agouti (a rodent resembling the rabbit), the domestic and wild dog, and the opossum.

Epidemic Typhus

Known as one of the greatest pestilences of history, this disease has travelled in the wake of armies and, no less than any other weapon, has helped to destroy millions of soldiers and civilians. "Camp fever," one of many aliases that include jail fever, ship fever, spotted typhus, and tabardillo, labels typhus as a disease associated with over-crowding, famine, filth, and lousiness (technically known as "pediculosis").

Epidemic typhus has a mild-mannered twin, variously named endemic typhus, murine typhus, or Brill's disease, first observed by Brill in New York and now, chiefly as a result of Zinsser's studies, is regarded as an imported form of the European epidemic typhus. The difference between the two is largely in the method of spread and in the severity of the disease. Epidemic typhus is readily transmitted from person to person by means of the body louse, and possibly head lice, infected with *Rickettsia prowazeki,* while the causative organism of endemic typhus, the *Rickettsia mooseri,* is spread from rat to rat by rat fleas and rat lice. It is thought that this disease is transmitted to man by means of the infected intestinal excreta of rat

fleas. Cases among humans, usually linked with rat harborages, give unmistakable evidence that a reservoir of the infection exists in nature in the common rat and other rodents.

Louse-borne typhus is not transmitted through the egg to the offspring of the louse. Only by feeding on blood from typhus-infected persons does a louse become infective after eight to ten days, and it remains so for the rest of its life. If deprived of blood meals, an adult louse will die in nine or ten days, whereas the uninfected louse enjoys a much longer, perhaps less eventful, life span of about thirty days.

Trench fever died out with the close of World War I and, having reappeared in Europe during the recent war, is now being studied more carefully to clarify its relationship to the rickettsial infections.

As in many other transmissible infections, it is fortunate that certain helpful laboratory tests make it possible to identify and label the different forms of rickettsial diseases. One of these tests, the Weil-Felix reaction, makes use of several different strains of *Bacillus proteus* (*Proteus vulgaris*), an organism which was originally isolated from a patient with typhus fever. As will be related later on, this organism, while not the cause of typhus or other rickettsial infections, reacts with the blood serums obtained from such patients. Indeed, with the exception of Q fever, this group of diseases produces certain substances (agglutinins) in the blood serum which, if added to a prepared suspension of the organism during the second week of the illness, will cause a clumping known as agglutination. The mechanism of the Weil-Felix reaction is not altogether clear and is probably not specific. Nevertheless, it is by virtue of the composition of the bacterial cell substances found in the different strains, known as *Proteus* OX-19, OX-2, and OX-K, that this diagnostic test has been of great assistance in distinguishing one rickettsial group from another. To illustrate: In typhus fever the characteristic reaction is

strong with OX-19, weak with OX-2, and absent with OX-K; in tsutsugamushi disease, OX-K alone gives a strong reaction; in Q fever no strain of *Proteus* is agglutinated. The Weil-Felix reaction gives no help in differentiating spotted fever from typhus fever, although OX-2 is clumped more often in serums from patients with spotted fever than with typhus. This deficiency is overcome by using instead the characteristic rickettsial organisms that are associated with each disease. In the same way it is also possible to separate epidemic typhus from the endemic or murine type. Recently this invaluable aid has also been extended to tsutsugamushi disease by researchers at the National Institute of Health where the *Rickettsia orientalis* (nipponica) has been propagated successfully in the chick embryo and prepared for use in serum tests.

Prevention and Control of Rickettsial Diseases

Successful military operations in the far-flung corners of the world during the recent war emphasized, as never before in history, the importance of defensive scientific weapons that are usually associated with preventive medicine and the peaceful activities of a quiet laboratory. In the tropics as elsewhere, it was a war against microbes and pestilence as much as it was a war directed toward superior ammunition and fire power. Victory over a human enemy needed a miracle of organized preventive and control measures to insure victory over disease in unexplored terrain, where vast armies of strange parasites and unheard-of germs lay concealed from the eye of unsuspecting invasion forces. That the challenge was met with the degree of success achieved in rickettsial diseases is a well-deserved tribute to the mobilized skills of laboratory scientists and physicians. The newer knowledge in this field has only begun to point the way toward so much unfinished business that lies ahead.

Knowing something about the reservoirs of infection and the vectors or carriers of rickettsial organisms is in

itself a preventive measure. Thus, the control of epidemic typhus rests on suppression of lice, and of endemic typhus on the control of the rat population by poisoning, rat-proofing, and trapping. Spotted fever, however, poses a difficult problem in control directed toward eradication of ticks. Considering the great variety of hosts which nurture these parasites, it is easy to understand the difficulties that have to be met. Reduction of ticks, accomplished partly by destroying rodents, dipping domestic animals, the burning over of tick-infested brush areas, and similar measures, have not been very successful in lessening the disease. Much more effective seem to be the avoidance of known infected areas, and personal care during the tick season.

From lessons learned in the jungles of New Guinea it has been found that prevention of tsutsugamushi disease (scrub typhus or mite-borne typhus) is also a matter of reducing the risk of exposure to mites. This has been accomplished by burning brush, avoiding infested regions, and by scrupulous personal care. Under combat or field conditions, however, where protection becomes more difficult, an insect-repellent fluid for clothing and body can be used effectively for short periods, provided the protective chemical is not washed out by exposure to heavy rains and wading through streams.

The attack on focal points of rickettsial diseases is now directed along two general lines. One, the control and eradication of carriers and reservoirs of disease, is, as we have seen, environmental; the other, internal, is concerned with attempts to develop active individual resistance or immunity to these external dangers, by means of protective inoculation.

Protective inoculation, a method of producing an artificial immunity against typhus fever and other rickettsial diseases, is still in the experimental stage. Pioneer investigations of Doctor Hans Zinsser, whose name looms large in this field, have shown that vaccination with living typhus virus is dangerous to both the individual and to the com-

munity. In principle this confirms the earlier successes of Spencer and Parker of the U. S. Public Health Service, achieved in Rocky Mountain spotted fever by the prophylactic use of killed virus. Such vaccines, prepared with emulsions of ground-up infected ticks, are made harmless by adding a calculated amount of carbolic acid and formaldehyde.

Ingenuity and many years of hard work have been applied to devising better and safer methods of preparing and inoculating rickettsial vaccines. Progress can be measured by going back to the time, almost twenty-five years ago, when a prophylactic was first prepared against the epidemic typhus virus. The idea, as conceived by Weigl, seems quite fantastic in retrospect, yet the method has given good immunity through the years. Unfortunately, the vaccine cannot be produced on any large scale because its preparation is laborious and exacting. The steps involved in this bizarre undertaking are real, though hard to believe. Lice must first be infected by injecting rickettsial organisms through the rectum and into the large intestine where the Rickettsiae come to lodge in the cells lining the intestinal passages. The lice are then allowed to feed for more than one week on persons who are immune to typhus. After this time a sufficient number of Rickettsiae can be harvested from the infected intestines, which are carefully removed and ground up in a carbolized solution. For the vaccination of a single person, material obtained from one to two hundred lice may be required.

Improved technics designed to secure large amounts of typhus Rickettsiae, like those of Zinsser and his collaborators, and of Cox, Plotz, and others at the National Institute of Health, make use of the developing chick embryo. Fertile hen eggs are inoculated by injecting the material directly into the yolk sac, and then treating the abundant harvest of virus with carbolized formaldehyde. In test animals these prophylactic vaccines have given good results, but have not been "tried by fire" in an epidemic of typhus

fever. However, an opportunity for adequate field testing on a military scale was created as soon as it became apparent that our armed forces would be exposed to danger from typhus already existing in many areas outside the United States.

Whether or not an ancient scourge of wars has been reduced to a minor annoyance as a result of the recent world-wide experience with typhus vaccine, only time will tell. Under certain conditions, immunization against epidemic typhus on such an unprecedented scale as was carried out, might be expected to prevent the disease in vaccinated persons. At the same time, the immunizing effect would also tend to lessen the exposure to infection of others not protected in this way. However, where the added precautions taken have included a diligent application of miraculous insecticides and lousicides, credit for part of a victory cannot be given to typhus vaccine alone. These chemical innovations came as a godsend to the natives and combatants in the vermin-infested places. In grateful acknowledgment of assured restful nights, DDT became known as "sleeping powders."

What has been said for epidemic typhus does not necessarily apply to endemic typhus and certain rickettsial diseases in which animals other than man are known to be reservoirs of infection. In such instances vaccination on a grand scale might prevent infection of vaccinated individuals without protecting others from exposure at the source.

The results of more than twenty years of prophylactic vaccination against Rocky Mountain spotted fever have shown that the vaccine has definite protective value as an immunizing agent. Against the usually fatal form of the disease children may be fully protected, while adults only occasionally. As a general rule, those who are vaccinated can expect to maintain sufficient immunity for nearly one year against strains of lesser virulence and invasive power. Accordingly, the only safe practice appears to be annual

vaccination, in conformity with the plan of a "stimulating" dose that is being recommended also in the use of typhus vaccines.

Prompted by the occurrence of tsutsugamushi fever (scrub typhus) in combat areas, the possibilities of developing an effective method of immunization against this disease have been thoroughly explored and tested. Early attempts made with vaccines prepared from rickettsial organisms of louse-borne typhus or of Rocky Mountain spotted fever were abandoned as soon as it was found that these gave no protection against the mite-borne Japanese infection. Until recently, the results of their successful work, a top secret of the U.S.A. Typhus Commission, have been withheld from publication and discussion, for military reasons.

During 1944 and 1945, in a series of confidential reports, it was stated that formalinized vaccines prepared from the organs of white mice and rats experimentally infected with *Rickettsia orientalis* protect mice against scrub typhus disease. Soon afterward, Plotz and his co-workers, following their original method for making typhus vaccine, succeeded in preparing a vaccine from Rickettsiae grown in tissue cultures and yolk sacs of chick embryos. At the present time it can be said that these improved preparations are as effective as those made from the organs of infected rats. Practical application of these methods—the inevitable goal—will have to overcome many technical difficulties, which unfortunately must continue to be part of all so-called "glamorous" research. Although a successful vaccine has not as yet been prepared, it is now possible to make sufficient quantities needed for protection of laboratory personnel exposed to the dangers of working with infectious materials.

Offsetting what might appear to be a discouraging prospect in the biological control of rickettsial diseases is the application of a newly found chemical approach to treatment. This has its basis in some early observations on the failure of certain sulfonamide drugs ("sulfa com-

pounds") to prevent the growth and multiplication of bacteria. In other words, bacteriostatic properties could not be demonstrated. Investigation of many such compounds tested in animals experimentally infected with typhus fever met with repeated failure until para-aminobenzoic acid (PABA) was tried, with mildly encouraging results. Doctor Plotz and his collaborators then succeeded in checking the growth of typhus Rickettsiae inoculated into the yolk sac of the chicken embryo. Similar tests made by others with the organisms of Rocky Mountain spotted fever and tsutsugamushi disease proved conclusively that the drug had a suppressive effect upon their growth. In short order, the curative properties of para-aminobenzoic acid (PABA) were confirmed in animals infected with endemic or murine typhus, Rocky Mountain spotted fever, and tsutsugamushi disease. More recently, during 1945 to 1947, human cases of louse-borne epidemic typhus and the better-known rickettsial infections have, with the exception of Q fever, responded favorably to treatment with PABA.

How and why so mild a drug can accomplish what many other highly potent chemical agents have failed to do is now explained by an ingenious theory. It is believed that PABA interferes with the growth of rickettsial organisms by substituting itself for a chemically similar, although unknown, food factor necessary to their life. The mechanism responsible for this anti-rickettsial activity is supposed to be like the antagonistic behavior of sulfa drugs toward bacteria, which require PABA (a vitamine of the B complex) for growth and multiplication. In either case, what is meat for one organism may be poison for another.

The nature of these mysterious bodies has not been determined and their relation to certain diseases has stirred up quite a to-do among investigators, some of whom claim that the minute bacteria-like bodies are not living organisms. Early attempts to cultivate Rickettsiae of typhus and Rocky Mountain spotted fever suggested the possibility of multiplication taking place, but gave no evidence that

they could be maintained in successive generations under artificial conditions. These necessary requirements were fulfilled, however, in 1932 by Landsteiner and Nigg, who reported a method of cultivating Rickettsia bodies in typhus fever. In their hands, cultures were successfully carried on over a period of many months in a medium containing fragments of living tissue and serum, without any reduction either in the number of organisms or their virulence. This method of "tissue culture," which is becoming increasingly important in studying diseases of unknown origin, will be discussed more fully in a later chapter.

Many problems have arisen in connection with Rickettsia bodies. Nothing is known about their relationship to bacteria or how an infection actually develops following the bite of an insect. Equally obscure is the mechanism of immunity which, in certain diseases like typhus, Rocky Mountain spotted fever, and heartwater, prevents a second attack, while in tsutsugamushi disease and trench fever it does not. Most perplexing has been the discovery of Rickettsiae in the intestinal canal of a large number of insect hosts that are, for the time being at least, not known to be carriers of any disease. Among such crawling, burrowing, and biting vermin are certain species of flies, mosquitoes, fleas, lice, mites, ticks, bedbugs, spiders, and beetles associated with the human, animal, and vegetable kingdoms. The significance of these disturbing observations cannot be appreciated until something is learned about a possible life cycle of Rickettsiae found in these insects. Perhaps new diseases will make their appearance at some future time to help solve the riddle, as the following incident suggests.

Rickettsialpox

In a suburban area of greater New York, during the summer of 1946, there was a localized outbreak of what was thought by some physicians to be an unusual form of chickenpox and by others a hitherto unrecognized disease.

The mysterious epidemic occurred in a housing development with a resident population of nearly 2000 occupants. An undetermined number of resident mice also shared the premises, according to tenant eyewitnesses. It was quite natural, therefore, to pin suspicion on these mice and their well-known insect companion travellers.

Trapping rodents suspected of spreading disease by means of insect vectors or carriers, while routine for the epidemiologist detective, is only the first step in a search for necessary clues. Sometimes these are found in the right place, at the proper time, by mere chance; more often, however, through the coordinated studies of various specialists who write a skillful continuity for the full length dramatic piece. The first clue, found on the basement walls in one of the buildings, was a small mite identified by experts at the U. S. Department of Agriculture as a blood-sucking ectoparasite (parasite living on the outside of the body) of house mice. The big name of this extremely small pest is *Allodermanyssus sanguineus,* which should explain at least why it is a "blood relative." This type of insect, along with the mosquito and the tick, is also referred to as being hematophagous, meaning that it eats or thrives on blood. One can readily understand now why we must have entomologists who, by definition, deal with the study of insects. Of the entomologists' well-deserved place, however, in modern epidemiologic science, more will be said later.

At about the time when *Allodermanyssus sanguineus* was discovered, Doctor Armstrong and his co-workers at the National Institute of Health isolated from the blood of two patients a rickettsial organism known as *Rickettsia akari.* This was identified by means of laboratory tests as the cause of the disease to which was then given the name rickettsialpox. Lest there be some misunderstanding, this form of "pox" is not to be confused with smallpox or chickenpox.

The connection between suspected mites and rickettsial-

Accidental factors in one form or another have surely played an important part in stimulating microbes into activity. Too common, unfortunately, is the sudden explosion of tuberculosis following physical strain or acute respiratory infections. In the human patient as well as in animals, a latent disease is often awakened by such an added burden. One of the most striking illustrations of this fact, well known to laboratory workers, is the frequent death of mice from mouse typhoid, following an inoculation with pneumonia organisms. This small rodent is a common carrier of mouse typhoid in a latent form and the mere introduction of a depressing influence from without, in this instance a pneumococcus, causes a fatal infection, not with pneumonia as one might expect, but with the hidden parasite. Experimenters in the laboratory have had other experiences of this kind, much to their surprise and embarrassment. Not always does the test animal give back what has been put into it. Yet accidents in this category have on some occasions brought fame to more than one astonished investigator, when an unsuspected microbe popped out of nowhere to shunt them off the wrong track straight into a useful discovery. Be it luck or chance, a skilled observer is prepared for such events.

The wanderings of parasitic organisms from place to place through man, animals, or insect and back to man have told us in no uncertain terms that migration to new hosts is the main concern of microbes. In the course of their travels a hint has been given of some startling changes which are likely to follow a prolonged residence in different hosts. There is nothing man can offer in his defence that is quite equal to the adaptability of parasites as an offensive weapon. Surrounded as we are by so many animal and insect species, the problem of overcoming disease begins to look almost hopeless. Microbes, changing their ways to meet new conditions, take on strange disguises and behave in outrageously unorthodox fashion. The immediate and long range effects of this behavior lead to a

new chapter in the conflict between host and parasite. From this point forward, we shall see an inevitable change in opinion regarding certain ideas of microbial behavior, which were once considered wildly speculative and fantastic.

CHAPTER 4

CHANGEABLE MICROBES

Heresies

In the early days of bacteriology it was believed that microbes behaved according to precise and fixed patterns. These rules of conduct and living had already been set forth by Robert Koch in his famous "postulates," the fundamental conditions according to which bacteria never varied.

Koch's laws of specificity, however, were actually intended to make an end of loose thinking and slovenly technic in studying microbes of disease. He wished to discourage slipshod methods of work which frequently led many investigators to mistake impure breeds of germs for a transformation of one type of germ into another. As a result of the unquestioning acceptance of Koch's teaching, the pendulum had swung definitely towards a new point of view, with emphasis on the model behavior of parasites. Henceforth they must not be suspected of changing their grooved pattern of existence.

Now the pendulum has swung back, if not to the old extreme, at least in the direction of recognizing in microbes the capacity to change. Intent as the early great pioneers were on the study of microbial behavior, they reckoned but lightly with the obscure effects the host might have upon an invading parasite. As the science of bacteriology developed, it should have become more apparent than it did that rules of procedure cannot be hard and fast because it is in the nature of living organisms to undergo change. It was soon discovered, for example, that a number of definite diseases of the animal kingdom could not be reproduced experimentally. To make matters yet more difficult for the

investigator, there were many infections in which no discernible microbe could be found. However vigorous and painstaking were the studies designed to improve all known methods of artificial cultivation of disease germs, such infections refused to yield up the hidden cause.

In the midst of glorious successes, advocates of the germ theory of disease began to have anxious moments. There were oddities that required some explanation. The "antis" found something to chuckle over when Loeffler discovered the germ of diphtheria. He must be mad, they thought, to try to convince anybody that this microbe could cause a disease and yet exist in the nose and throat of persons who were perfectly well! The answer to this riddle was much simpler than they imagined. Most assuredly microbes caused disease, but nothing had been said to imply that the same microbes must always give rise to disease. Some years before, in 1880, Pasteur himself found in the mouth of a healthy child an organism which later proved to be the germ of pneumonia. The "cause" of a disease was there, yet it was powerless to generate the infection. These two observations were naturally interpreted in an unfavorable light by opponents of the germ theory. The mysterious hidden factors which at times made a microbe behave variously were not yet understood.

On the other hand, it was not so easy to satisfy those who demanded visible proof of the germs which were held responsible for different diseases. Nevertheless the germ theory could not be overthrown. It was merely another instance of a prodigy being born fifty years too soon. Bacteriologists did not relinquish their idea so long as transmission of disease could be accomplished, even when a causative agent remained hidden. As for the other peculiarities shown by microbes, time alone would tell. There were undoubtedly within the animal body many complicating factors subject to a fickle environment. Some day the laboratory would hurdle this barrier. And the laboratory did do that.

Microbes had been evolving new characters long before bacteriologists dared to talk about such things above a whisper. As is the case with all living organisms, a long time is needed to modify bacteria, so that what might be taken for a sudden change or variation is perhaps only the shock experienced by a surprised observer. The "sudden" appearance of new characters or unusual behavior among bacteria was given the name "mutation," a term borrowed from the plant world. For those who regarded the temporarily fixed state of microbes as one of changelessness, this was nothing short of heresy. The thought of abrupt change leading to a new species was abhorrent to the majority of bacteriologists.

Pasteur himself was perhaps chiefly responsible for the idea of hereditary fixed characters, notwithstanding an extraordinary amount of evidence he had brought out to contradict this belief. When he discovered accidentally that a stale culture of chicken cholera germs was harmless for hens, he concluded that the property of lessened virulence was hereditary. What were the facts upon which this conclusion was based? An attenuated or weakened culture of the microbes, it was found, had given rise in the test tube to a number of generations having a similarly reduced virulence. Today we know that such an observation can be explained by the limitations of artificial environments in which germs are placed.

Only during the past thirty years, particularly in the last decade, have noticeable concessions been made to a newer school of microbe lore. The newness here is perhaps common to all old things when seen through the eyes of observers now grown thoroughly accustomed to the dazzle of modern times. More recently they have begun to perceive the importance of complex changes in form and the occurrence of life cycles in the growth and development of organisms hitherto believed to be stable and simple.

These observations reaffirmed pioneer studies, some of which go back to the seventies when bacteriology was a

nursling. Forty years later, Löhnis, a noted botanist, reported evidence of transformation in a species of microbe found in the soil and having the remarkable property of plucking nitrogen gas from the atmosphere. His contributions did not cause a ripple of excitement at the time, but he came out of hiding in 1921 and 1923 with further reports upon the life cycle of bacteria. The year 1927 saw Philip Hadley, a courageous scientist at Ann Arbor, Michigan, daring to support the cause of Löhnis, and at the same time backing up his belief with forceful laboratory evidence.

Soon afterwards a multitude of confirmatory reports coming from various research centers stimulated a lively interest in an important though forgotten subject. Static bacteriology stopped sputtering and became dynamic and eloquent. There was excited talk of new and unusual forms of microbes differing from their parent or "normal" ancestors in certain characters, such as colony formation and the power to infect or develop protective substances in the human or animal body. These "variants" are often so radically different in general behavior and distinctive properties as to suggest analogy with biological "sports." They have become "dissociated" or split off from the parent type and this sort of variation is now known as "dissociation."

When this phenomenon is influenced by factors which can account for the resemblances and differences of microorganisms related by descent, dissociation is said to be genetically controlled. Nevertheless, it can be modified by changes in the environment, especially those which affect the rate of growth and viability. Inherent growth rates play an important part in the control of the amount of dissociation that takes place in a culture of organisms. This can be shown by differences observed in rate of growth in lines of descent from different single organisms, starting from a single ancestor and reproducing a line of descendants having like hereditary constitution. When first observed,

these differences were thought to be due to a different rate of appearance of variants in the original strain. Now, however, there is evidence that differences in amount of dissociation can be profoundly influenced by rate of growth and survival of organisms, even though the new variants appear at equal rates.

Under natural conditions of population growth, dissociation can be recognized as the spontaneous appearance of variants in a microbial population that is subject to the control of factors present within the organisms and also in the environment. It should be remembered that a small number of variants (mutants) always appear when active multiplication of bacteria takes place in a culture medium. Regardless of the amount of material inoculated, growth of the viable cells reaches a peak, although the total population continues to increase at a steady rate. When the total number of bacterial cells exceeds that of the viable number, population pressure forces the development of spontaneous variants. If, as these most recent studies indicate, the rate of growth and viability of a variant affects its chances of survival in a competitive population, then changes in growth rate or in viability can bring about differences in dissociation.

As a case in point, during the early days of sulfonamide treatment for bacterial infections, it was recognized that micro-organisms became resistant to the action of these drugs. While studying this unusual effect, several investigators noticed the strange behavior of antagonism shown by para-aminobenzoic acid toward the antibacterial action of sulfa compounds. Para-aminobenzoic acid, a substance now referred to as PABA, is found in yeast and liver, and is one of the vitamines of the B complex. It is a chemically close relative of the sulfa drugs, as well as a factor vitally necessary for the growth of bacteria. In other words, PABA is essential to the metabolism of micro-organisms. By inference, it is also believed that sulfonamides do not act directly upon the bacteria in question, but interfere

with their use of PABA. In short, these two chemically similar drugs compete with each other in one or more of the enzymic activities of microbial growth. As an illustration of what occurs, the PABA normally present in the enzyme may be blocked and then replaced by a sulfa drug having a similar chemical architecture. Under these competitive conditions, the outcome of such a contest for survival will be influenced by the relative amounts of the antagonistic substances which can exchange places in the chemical reaction. The significance of these observations will take on added meaning when we consider what happens to organisms that are exposed to the activity of penicillin, streptomycin, and other antibiotic materials, in the human and animal body or in the test tube.

How are attenuation, increase of virulence or development of resistance to antibiotics or chemicals to be interpreted? The possibility of bacterial cultures developing in some particular direction can be changed, or their behavior might so be regulated as to encourage or suppress the development of certain individuals which breed true to type (biotypes). In many instances, however, it is impossible to know the nature of single bacterial cells from which cultures are grown. Moreover, an apparent increase in virulence of micro-organisms resulting from the influence of the human or animal body is sometimes due to the maintenance of certain biotypes and the elimination of others. Therefore, such changes that are said to be caused by increased or diminished virulence and adaptation may be the result merely of a selection or shuffling among the members of a mixed population. Of this, more will be said in another place.

Variable Characters

The tendency of bacteria to vary was first observed as a difference in their ability to invade the animal body and cause disease. Pasteur's work, as we have seen, showed clearly that virulence or invasive power of microbes was a

variable character. A change might occur in one direction or another, leading either to loss of virulence or to a remarkable increase. In each case the infective power could be influenced correspondingly by passing disease germs through non-susceptible or susceptible animals. This method became the starting point for all future work, which led to the control of infectious disease by the use of protective serums and vaccines. It was not so strange that in the excitement of the chase the deeper meaning of variations in virulence was not appreciated more seriously.

What is at the bottom of this curious behavior of microbes? It is quite simple by the application of heat to weaken any germ gradually, the anthrax germ, for instance, so that it first loses the power to kill cattle, then sheep, then rabbits, and lastly guinea pigs. In order to restore the virulence for sheep, it becomes necessary to pass the organism first through guinea pigs, using microbes that are deadly for these animals only, and then successively through rabbits and sheep. This shows that an increase in the power to infect one kind of animal host is accompanied by a like increase for other animals. Strong microbes develop at the expense of the weaker, which fall by the wayside as the virulent ones multiply more rapidly during their successive passages through a suitable host. In the end this weeding out process leaves only the virulent microbes, because they are more abundant and can be recovered in greater numbers during each passage from one animal to the next. An old and familiar story—adaptation of bacteria to their environment—comes at once to mind. In the language of biology, it is Darwin's law of selection applied to the microbe kingdom, where the weak must also give in to the strong.

During all the years that history was being made in the bacteriological world, nothing was said about the mechanism of variability, although its occurrence was seen on every hand. This is a curious fact, yet not surprising in view of the peculiar zigzag path taken by scientific dis-

coveries. Until recently, it was thought, only vaguely to be sure, that any microbial population was made up of organisms having more or less the same virulence. Their power to invade and cause disease was supposed to vary slightly from a certain average which belonged to the group as a whole. Environmental or any other factors which might bring selection into play were regarded in this sense as increasing or decreasing virulence in a narrow range. The net result of such continual selection would be a race of microbes having infective power coming near to the highest attainable limit of that race.

Variation taking place in this manner might be likened to changes in depth of a mixed color resulting from the addition of one or another of the colors composing it. The quality of a deep green, for example, can be intensified up to a certain value depending upon the amount of yellow or blue added to the original. In this instance, greenness as a whole, like microbial virulence, becomes a varying character in which the *changed content of blue or yellow* represents an increased or a decreased intensity of green approaching the limits set by such a mixture. The color, however, remains green.

What are the facts as to the mechanism underlying bacterial variation? It is known that the separate organisms in any microbial population are not, as has been supposed, more or less equal in their capacity to infect. Changes in virulence, moreover, do not mean a fractional loss or gain in this property by each individual microbe. That is to say, the virulence of the microbes as a whole does not increase or diminish because of a similar variation in virulence of the organisms making up the population. A gradual selection of less virulent or more virulent offspring, it also appears, is not responsible for the change. To illustrate, when microbes are exposed to unfavorable conditions causing reduced virulence, this is not due to a loss of invasive power in formerly virulent germs, the change taking place uniformly and all at once; actually what occurs is a decrease in the number of fully virulent bacteria

at a time when a new type of non-virulent ones appears suddenly and increases in the microbial population.

Lessening of virulence, therefore, is not the result of a fractional loss of this power but a thinning out of the bacteria present originally by a number of harmless or less virulent organisms. If we pursue our color analogy further for the sake of illustration and again represent virulence by the quality of greenness as a whole, this character will now be seen to vary in the direction of yellow or blue resulting from a *shift in the internal arrangement of the mixture* because of chemical substitution. In this case the color might change from green to yellow or to blue in the same manner as virulence might shift towards the weak or the strong side.

New Era in Microbial Variation

A few concrete examples of bacterial variation will explain the nature of some of these changes. Whooping cough organisms, when freshly isolated from their source in a patient, require the addition of human or animal blood to the artificial foodstuffs in order to multiply, but after several transplants on this rich material will grow quite readily on ordinary medium in the test tube. Something has happened to the microbes in the meantime. If animals are inoculated with these two varieties of the same germ, they differ remarkably in their capacity to produce protective substances in the blood stream.

Another quite common form of variation, first observed by Jules Bordet of the Brussels Pasteur Institute in connection with typhoid germs cultivated in the laboratory, is the peculiarity of behavior associated with changes in their visible growth. The relation between virulence and the type of growth in anthrax germs is one of the earliest discoveries of a similar nature and the forerunner of comparatively recent developments showing how microbes split off into two distinct types of visible growth, one yielding a virulent and the other a non-virulent strain.

From the historical standpoint, the question as to who

was the first to place bacterial variation on a firm basis cannot be easily decided. Priority in this as in other fields of bacteriology is often a matter of opinion and not necessarily related to the actual content of an author's work. Complications ensue because the use of certain terms and the expressed meaning of published results can be interpreted in various ways by different investigators. Notwithstanding the earlier contributions of a few pioneers already mentioned, Baerthlein's, made in 1918, has been acknowledged as the first to clearly show the importance of microbial instability. He analyzed in great detail the variations in colony growth of certain kinds of bacteria and demonstrated a strict relationship between a number of recognized characteristics and different degrees of virulence.

To Arkwright in England belongs the credit of having first identified in 1921 two distinct types of microbial colonies in the group of organisms responsible for typhoid fever and dysentery. These colonies, one described as rough, granular, and irregular in outline, and the other as smooth and round, he found associated with differences in character of growth and immunity reactions. As a general rule, the smooth form was the normal and the rough an abnormal type. This work marked a new era in the evolution of researches in microbial variation. The symbols R and S, for rough and smooth, invented by Arkwright to represent a newly discovered phenomenon, have now become a part of bacteriological language. Up to this time little attention had been given to the possibility of connecting these changes in character of growth with infective power.

The same year as Arkwright's discovery, Paul de Kruif, working at the Rockefeller Institute in New York, had an annoying experience known to many laboratory workers who are obliged to use rabbits in certain experiments. Sudden outbreaks of "snuffles" would occur among healthy or experimental stock at most inopportune times. This epidemic disease, which resembles plague, often swept

through an animal population with lightning speed, causing death from a septicemia or blood infection associated with a kind of pneumonia. De Kruif was the sort of young man who could generally make annoyances pay good dividends. Studying the disease more closely than any of his predecessors, he made the problem his own. As a result, we now have the first clear indication of what variation in bacterial growth really means and how it is related to differences in microbial virulence. Specifically, the organisms causing "snuffles" in rabbits are made up of two distinct types that can be recognized by their manner of growth in suitable culture mediums. One of these types is virulent and the other is not.

This can be shown in a very simple way by planting some of the offending germs in test tubes containing bouillon and allowing growth to proceed in the incubator. After a time the contents of the tubes become evenly clouded and a granular sediment settles out at the bottom. From the sediment and its overlying fluid, separate portions are next transferred to the same kind of bouillon and also to a solidified agar jelly containing suitable food materials. The organisms obtained from the deposit will now grow as granular colonies in the solid medium, while those taken from the upper layers of cloudy fluid will grow evenly as before and give rise to opaque smooth colonies. Upon testing these two types further for virulence in rabbits, it is found that the granular or *rough* type does not infect, whereas the non-granular or *smooth* form is highly virulent and infective. As the microbe is forced to grow under unfavorable conditions a remarkable change has occurred.

At last here is definite proof of the relation between virulence and variation in character of growth. With this information at hand, De Kruif made the "snuffles" germ perform to his liking. At only one point did his observations apparently fail to confirm his findings and for a time it appeared that this experiment might upset the apple

cart. For the rough, non-virulent germs, which are known to be infective for rabbits when inoculated in large amounts, seemed to develop a greater virulence after successive passages through young animals. But an examination of the rabbits' blood showed that this virulence was due, not to the rough, non-virulent germs, but to the presence of a small number of microbes of the smooth virulent type. Such cultures, however, retain intact all other characteristics of the rough strain, although a mixture of the virulent and avirulent types may be present at the same time.

A change from rough to smooth form has also been accomplished in a few other instances, notably with pneumonia and typhoid germs. The manner in which such reversion occurs under natural and experimental conditions is not understood at present and, curiously, it is generally believed that the rough variants of microbes are always more or less fixed and not easily reversible into their normal smooth virulent forms. Were this mechanism better known, we should be in a strategic position to combat plague, which is caused by an organism similar in many respects to the one that is responsible for "snuffles." The fact remains, however, that bacterial virulence is associated with type of growth; smoothness goes hand in hand with virulence and roughness with avirulence. One might be tempted to compare this microbial trait with its counterpart in human society, where dangerous cunning is more often than not concealed beneath a smooth exterior.

Dual Nature of Microbial Cell Substance

If we want to understand the fundamental nature of variation in the microbial universe, we must turn to the ordinary events that happen in it. One of the most interesting examples of variation is the loss of motility on the part of certain microbes. At different times some observers have encountered non-motile forms of the typhoid germ and regarded them simply as freakish varieties. As

it happens, the loss in power of locomotion is only superficial evidence of an invisible change which has occurred within the microbial cell following a mysterious disappearance of its "legs." Perhaps this phenomenon should have been taken more seriously, yet the fact remains that an entirely different organism, a rank outsider, revealed the true nature of what had happened.

In the eastern part of Galicia in 1916, Weil and Felix were studying typhus fever. From the urine of a patient they isolated a peculiar microbe belonging to a group known to bacteriologists as *Proteus,* so named after a many-formed Greek deity by a Professor Hauser, who had christened the germ many years before. Weil and Felix called their bacillus *Proteus X19.* It is in some strange manner related to typhus fever, although not even remotely connected with typhoid disease. Reference has already been made to the practical application of this idea, which has led to the use of certain strains of *Proteus X* as valuable adjuncts in the laboratory diagnosis of the groups of rickettsial diseases. This test became known as the Weil-Felix reaction. *Proteus X19,* ordinarily motile, grows in a freely spreading layer on the surface of a hardened jelly food substance as the microbes swarm from the edge of parent colonies and produce a hazy type of growth. The non-motile variant grows in the form of separate colonies wholly unlike the other.

Although the contrast in appearance of colonial growth is obvious at a glance, this particular change is only part of a more profound change affecting the internal structure of the microbe. As the normal bacterial cells lose their flagella and become non-motile, there is a simultaneous loss of some substance in the cell. As a result, germs of this type do not behave like ordinary members of their group.

The precise nature of the change can be detected by an ingenious experiment which reveals the dual nature of motile bacteria belonging to the Proteus family. The

method is to compare the behavior of heated and unheated organisms exposed to the action of a specific serum prepared against them. Similar tests are made upon microbes which have been extracted previously with alcohol. In this manner advantage was taken of the familiar reaction of agglutination or clumping that occurs when organisms are suspended in blood serum taken from an animal inoculated with them to the point where it can tolerate ordinarily fatal doses. Microbes or any other substances used in this way to build up an immunity in the animal body are known technically as *antigens* because they stimulate the tissues of the body to generate *anti*substances or *antibodies* in the tissues and blood stream. Such antibodies can be demonstrated by means of various happenings in the test tube when an immune serum is combined with the antigen used in its manufacture. One of these reactions, already referred to, is the visible agglutination of bacteria.

From it we learn that the flagella contain a substance which is destroyed readily by extraction with alcohol or by heating to a temperature of 150° F. This flagellar material can be identified in the test tube by a rapid formation of large loose clumps. The substance of the bacterial cell, contrariwise, resists the action of boiling temperature, is not affected by alcohol, and is agglutinated slowly in the form of fine clumps. In a general way, this indicates that the microbial cell may be made of highly specialized parts, each having a different chemical constitution which accounts for specific antigenic properties.

To be more explicit, other tests show us two fundamental things; first, the antigen specific for an individual strain resides in the cell body, and second, the flagellar substance contains the antigen common to the entire Proteus group. This can be proved by testing a number of different breeds with a serum prepared against the microbes whose flagella have been destroyed. The resulting antiserum agglutinates only the organism that has been used

in the production of serum and none other. If, however, the motile, flagellated type is used in preparing a serum, the resulting antiserum clumps not only the Proteus X strains but also a wide variety of organisms belonging to this group. But this does not tell the whole story.

Physical Constitution and Immunity Reactions

Turning now to a study of the microbes comprising the typhoid and paratyphoid fever group, it is surprising to find that in each of these species the flagella carry the specific factor while the common group antigen is identified with the cell body of the germs. These results flatly contradict the conclusions drawn from the Proteus experiments, but help to explain the nature of variation as it occurs in ordinary normal strains of microbes. It is understood, of course, that the smooth normal or motile form is made up of both antigens, whereas the non-motile form is not. The motile organisms, as we have seen, carry the flagella, which contain a specific antigen that is sensitive to both heat and strong alcohol. The cell body, on the other hand, contains a specific heat-resistant substance. When the normal smooth type gives rise to a rough variant, the change in antigenic power does not affect the flagella, but only the cell substance. This portion of the microbe, therefore, is replaced by another kind of heat-resistant antigen which will be found only in the rough variant.

These changes make identification of individual species of organisms difficult, as we shall see later. Because of the dual nature of the specific antigenic substances, microbes can be identified more readily when in the "smooth" state than in the "rough." In the first case, the organism contains a flagellar antigen characteristic of the species. The new variant form, on the other hand, is a mongrel of a sort, having within its cell a specific factor as well as another that is common to all members of the group.

Much of the subject matter dealing with this phase of the newer bacteriology is yet in a state of flux and, like the

bacterial variations which it treats, is also going through a continuous succession of changes. The dual nature of the substance composing the microbial cell gives an inadequate picture of its complex inner workings. Independently of the "cell" materials, but similar in effect, the flagella also play a double role, as Andrewes in England discovered from a study of certain bacteria belonging to a helter-skelter group known as "paratyphoid." This has always served as a kind of waste basket for bacteriologists who are unable to decide what to do with a variety of organisms that have confusing resemblances and cannot be segregated for lack of pointed differences. Here are at least eighteen microbial dialects spoken at one time by as many races.

To make a long and involved story very brief, the flagellar substance or antigen, like the bacterial cell itself, may be composed of two distinct parts. One of these gives to the organism a characteristic common to the species, while the other ingredient is peculiar to the race as a whole. That is to say, species specificity is not the same thing as group specificity. An army of microbes might be compared, for purposes of illustration, with a similar collection of soldiers. Infantry, artillery, or cavalry have specific duties to perform and can be recognized by individual uniforms or special equipment. As a group, however, they possess a characteristic common to all the members comprising it, namely the function of soldiering offensively or defensively. Such an army has its regiments, battalions, companies, and lesser subdivisions, all requiring adroit manipulation in segregating their activities.

By means of delicate agglutination tests, we can find law and order prevailing in what seems to be a disorganized mob of organisms. Provided a sufficient number of bacterial colonies are selected in making a test, it is now possible to classify the organisms accurately by means of artificially prepared serum which represents the entire group. With this method different races of microbes belonging to the same species can be identified as easily as one might

grade fruit according to size and quality. Perhaps this is taking a somewhat optimistic view of the ease of identifying bacteria, yet present-day advances in laboratory methods seem to point in this direction.

The significant discovery made by Weil and Felix in 1916 and elaborated by Andrewes five years later marked a turning point in the history of bacteriology. This advance foreshadowed another radical change in our ideas of specificity, to be developed from a study of bacterial structure. Strangely enough, the foundation for this work had been laid at least thirteen years before by Theobald Smith, whose modesty sometimes prevented full appreciation of his rare gift of discovering fundamental laws. As early as 1903, Smith described differences between motile and non-motile strains of hog-cholera bacteria belonging to the "paratyphoid" group. In an article published at that time, he concluded that the serum prepared from non-motile organisms contained agglutinating substances for the bodies of the bacilli, and not for their flagella; further, that the mechanism of clumping as it affected the bacillary bodies differed in kind and not merely in quantity from that of the flagella.

Practical Applications

Out of these discoveries have come facts leading to newer and better methods of identifying disease-producing microbes. In attempting to establish the nature of an infection, the only certain means at our disposal is to isolate the causative germ. Evidence of this kind, which is based on the new order of bacteriology, cannot be obtained as heretofore from simple routine tests made in the laboratory. Finding the microbe means nothing without precise knowledge of the place it occupies in a group or subdivision to which it belongs. For checking up on these criminals with modern methods, something like a Bertillon system is required. Identification of closely related organisms causing diseases of the intestinal tract therefore

becomes a research problem and while diagnostic procedures have lost in simplicity they have gained in accuracy.

One of the difficulties in diagnosing typhoid fever and related diseases has been overcome by taking advantage of some of these newly discovered facts. The usual procedure was to test some blood serum from the suspected patient for power to agglutinate a suspension of typhoid and paratyphoid germs. A positive reaction, evidenced by clumping together of the microbes in contact with such serum in certain dilutions, furnished proof of an infection. This test was most useful during the early stages of the disease when bacteriological examination was valueless because the germs were not yet being discharged in the intestinal excreta. But the method was faulty in many other respects, among which was its failure to detect a false positive reaction which might be due to previous inoculation against the disease. Moreover, it was not possible to recognize the presence of different microbial constituents arising from the species, race, or particular type of infecting organism that prevailed at the moment.

As the test was performed ordinarily, the prepared bacterial suspension used as an indicator for the patient's serum in question contained only the flagellar substances. Now it would have to be assumed in any given case that a patient happened to be producing antibodies corresponding to these. To complicate matters, many patients have been found to produce agglutinating substances affecting only the microbial cell, that is to say, exclusive of its flagellar portion. Hence it has become necessary, on the basis of present knowledge, to arrange a comprehensive series of tests with a patient's serum, so as to detect both the flagellar and cell substances in all strains of bacteria that are likely to cause enteric fever (typhoid). Proceeding along these lines, "indicator" cultures of bacteria are treated with formaldehyde solution in order to destroy the cell substance while leaving the flagellar portion intact. To a corresponding series of microbial cultures strong alcohol is added,

thus destroying the active ingredient residing in the flagella, while that of the cell is not affected.

Such cultures furnish accurate means of identifying the organism responsible for the patient's disease. In the inoculated or vaccinated person, the serum taken some months later rarely contains agglutinating substances against the cell ingredient in significant amounts. That is to say, a sample of serum, when diluted one hundred or more times by volume, will fail to detect this microbial substance. The serum of an actively infected patient, however, will clump the properly chosen bacterial suspension quite regularly. It is now possible, therefore, to distinguish an inoculated person having an enteric disease from one similarly vaccinated, yet suffering from some other infection.

A test of this sort begins to make sense even in face of the fact that the labor is both arduous and costly. The careful control of typhoid fever and related enteric infections in any civilized community is surely worthy of the best possible laboratory service. Missed cases and carriers are responsible for the perpetual headaches of conscientious health officers. Nevertheless antiquated laboratory routine is permitted to flourish on a grand scale in many enlightened communities.

Environment and Microbic Variation

Now that microbic variation is taken for granted, how important are the test tube observations when applied to disease in the infected human host? A few beginnings which have been made in this direction only emphasize the fact that little is known about a problem now awaiting thorough investigation. Its solution will undoubtedly revolutionize the prevention and treatment of communicable diseases.

In speaking of recovery from an infection, it is customary to attribute a happy outcome to such vague influences as "strong bodily resistance" or "mild disease."

We are beginning to think that bacterial variation inside the body of a victim may have something to do with this, and may be taking place on a scale even greater than in the laboratory. As the matter stands today, recognition of these changes would be difficult enough without the added hindrance contributed by neglect of such an important field in applied bacteriology.

It is not surprising that diseases of the respiratory and intestinal tracts of man furnish the bulk of experimental material, for here the germ population is extremely varied and subject to change. Such work as has been accomplished already in this direction suggests the importance of microbic variation in the infected host. It should be mentioned in passing, however, that artificial laboratory conditions necessary for detecting changes of this kind may affect the accuracy of these observations. Diphtheria and dysentery will serve as concrete examples.

From the laboratory has come no satisfactory evidence that diphtheria bacilli have lost caste by changing into any other type with which, aside from appearance, they would have nothing in common. Frequently an interesting variation is encountered among many strains of diphtheria which differ from the genuine in not producing a powerful poison or toxin. These are looked upon as avirulent diphtheria organisms whose relationship to the well known toxin-producers is not understood, other than that it represents a weakening of originally potent breeds.

A great mass of evidence on this subject does not show any convincing proof that these strains can become virulent, even though in many instances apparently "harmless" types are derived from some of the most extraordinary generators of toxin known. These offspring are typical diphtheria germs, lacking only the power to cause the disease. Their origin can be traced by means of an ingenious device that enables one to pick up a single microbe and transplant it to a suitable environment for further study. Starting with a single virulent diphtheria germ, it is possi-

ble to identify in this way the harmless varieties that split off from their parent cells. Unusual interest attaches to this observation because the occurrence of mild cases of diphtheria during epidemics is frequently associated with abnormal types of diphtheria germs which are regarded as variants of the well-known strains.

There is another line of evidence bearing on this question of microbial transformation in the human body. Dysentery, a common intestinal disease in this country, is caused by a germ named after Doctor Flexner, who first discovered it in the Philippine Islands. Early in the history of this infection, Flexner found that over half of the cases of infantile diarrhea were due to the organism. It is a notorious fact, however, that in any large epidemic of dysentery, the bacterial population of the intestinal tract is made up of several types, all appearing at one time. Frequently the bacteriologist who studies them encounters dysentery-like organisms which cannot be classified satisfactorily and today there are so many different yet related forms known that the task of separating them is almost hopeless. Associated in certain instances with frank intestinal disease and in others with none whatever, it is hard to believe that these microbes are not variants of but a few distinct types. Of course this is only a guess, although some available evidence seems to favor such a theory. During epidemics of bacillary dysentery, for instance, the intensity of infection varies among different persons, despite the fact that a single type of microbe may be responsible for the particular outbreak of disease.

In such an outbreak of Flexner type bacillary dysentery, the characteristic organism is regularly obtained from fatal cases or others who are desperately ill. Among those who are less severely infected and recover, a Flexner type organism is also found, yet upon closer study this microbe differs sharply from the other in the form, texture, and character of its growth in colonies. According to these criteria, the dysentery bacillus has given off a variant progeny differing

from the parent germ not only in colonial appearance but also in a loss of power to infect. This is not a hasty conclusion, because the original microbial colonies which develop from material taken from a number of fatal cases show two distinct types of organisms growing side by side. Here the genuine Flexner bacillus predominates generally but is never quite free from its variant colonies, the weaker sisters.

From another quarter, more evidence confirms this radical behavior of dysentery germs. Turning now to a near relative of the Flexner group of organisms, let us watch patiently while the microbe goes through its act of transformation under the microscope. If we use cultures from two to five months old, we can observe the occurrence of variant colonies appearing infrequently and without any regularity. Out of every hundred normal colonies, only one may show pronounced differences in size and general appearance. The microbial populations in such atypical colonies have a set of habits quite foreign to the ancestral type. They reproduce their kind at a much slower rate, taking from three to nine times as long as the normal cell for the simple act of division. They differ also in their lessened ability to utilize certain foodstuffs and in the products given off during life.

All these unusual characters disappear and normal behavior is restored as soon as the variant colonies revert to their original state. To bring about this magic transformation it suffices only to change the environment of the growing microbes. By providing a richer food supply and making more frequent transplants from one test tube generation to the next, it is possible to alter the whole picture. Thus several independent observations furnish evidence of similarity between the microbic variations seen in the test tube and those occurring within the body of a human host. Recognition of this phenomenon has led to a drastic change in methods of attacking disease-germs, notably in the use of antibiotic substances.

Corkscrew Microbes

Such direct methods of studying bacterial transformation cannot be applied to all groups of organisms. In some the changes that have taken place during slow evolution have resulted in an extraordinary number of varieties. There is no yardstick for this sort of variation, which is best exemplified perhaps by the spirochete microbes. They belong neither to the animal nor the plant kingdom and while more closely related to bacteria, they have something in common with the lowest form of animal life, the protozoa.

One hundred years ago Christian Gottfried Ehrenberg, a German naturalist and a student of theology who later became professor of medicine in Berlin, found a peculiar corkscrew-shaped microbe in a swamp near Berlin. He named the organism a "spirochete" which, according to Greek derivation, means "coiled hair" and in a general way describes its appearance. It was looked upon as a free-living, harmless curiosity. When in 1905 Fritz Schaudinn saw a remarkable pale and ghost-like spiral organism in material taken from a patient with syphilis, he called this microbe, which was the cause of the disease, a "treponema" pallidum or "turned thread" pale in appearance. Ehrenberg, who had long since passed away, might have relished the drawn out dispute on classification and the war of words which followed. He would have been reminded of the ancient scholars who argued about "homoiousian" and "homoousian," being unable to decide whether it was of like or of the same substance. Now the systematic bacteriologists of the twentieth century are splitting hairs with as great vehemence as those men who quarreled long ago over a diphthong.

The question of classification might be disregarded as irrelevant but for the fact that it emphasizes the broad variety of forms found in the group of spirochetes. These range from free-living species to highly organized para-

sites that are responsible for various diseases in animals and man. Eighteen or more known species inhabit different varieties of shellfish, including oysters and fresh water mussels. Some have a special preference for the blood stream and cause relapsing or tick fever in birds, mammals, and man. Of these fourteen types of spirochete, at least seven are known to attack man. In the harmless group are found seven other strains occurring in the blood of rats, mice, bats, otters, birds, reptiles, and fish. Other varieties have been isolated from ulcerated areas of the skin, mouth, throat, or lungs in man. To these eight species can be added thirteen more, common invaders of the mouth and teeth in man and animals. Even mosquitoes, mites, and dog fleas do not escape, for they too have their special varieties. The pale spirochete (pallidum) of syphilis has a close relative which causes a similar disease known as yaws, occurring in the tropics and affecting chiefly young negroes. Adding to these sixty-five organisms the spirochetes responsible for infectious jaundice (Weil's disease), "seven-day fever" of Japan, caused by a very similar microbe, and that of rat-bite fever, the list of sixty-eight members of a numerous family is still far from complete. Perhaps thirty others can be accounted for.

It is interesting to speculate on what happened during the evolution and development of this group of microbes with characteristics that might aptly be described as amphibian. Their history somehow suggests an aquatic origin and an ancient tendency to inhabit a fluid environment, to which they are readily adaptable. A structure made up of delicate coils has given them a flexibility of movement, unlike that of any ordinary microbes, and a freedom which has made them equally flexible in the varied adjustments necessitated by different environments. One of the most recently discovered pathogenic spirochetes, which causes Weil's disease, shows how essential these conditions are for disseminating the organism and planting the disease produced by it. The striking fact of this particular

example is the manner in which the infection spreads through animal excretions discharged into moist or wet surroundings.

"Weil's disease," or infectious jaundice, was first reported as a distinct disease in 1886 by the German physician A. Weil, who described an infection characterized by a sudden onset of high fever, chills, muscular pains, inflammation of the kidneys, bleeding into the skin, and marked yellowness. A similar disease had been known in Japan for many years, taking its toll of thousands of victims annually among miners, farmers, and laborers, with a mortality ranging from thirty to forty per cent. The cause of this disease was not known until 1914, when five doctors in Japan—Inada, Ido, Hoki, Kaneko, and Ito—succeeded in transmitting the infection to guinea pigs and discovered the organism. They named it Spirocheta icterohemorrhagiae, thus identifying it as a spirochete causing jaundice and hemorrhage. Shortly afterwards, the same microbe was found responsible for extensive outbreaks of jaundice among soldiers in the trenches of Flanders.

How the disease was communicated to man remained a mystery for some time. While it assumed almost mildly epidemic proportions at times, there was no evidence of its being carried from one person to another. The Japanese doctors noticed that infections were most common in mines where the workers went barefooted and came into contact with wet soil. Rats were very numerous there and were naturally suspected. It was found later that almost half of the rodents caught were carriers of the spirochete, which they discharged in the urine and thus contaminated the wet ground. In this situation the organisms remained alive and virulent for months.

It soon became known that rats were implicated in spreading infectious jaundice, regardless of locality. Halfway around the globe another Japanese doctor, Hideyo Noguchi, in 1917 found the identical spirochete in wild rats caught in and about New York City, and other doctors

reported the same disease in Tennessee rats. All these strains of spirochetes, including those isolated from wild rodents on the European battlefields, were in every respect like the type discovered in Japan. Noguchi, shortly afterwards, in a characteristic piece of work, beautifully detailed, pointed out that an error had been made in naming the causative organism Spirocheta. He substituted for it the correct term Leptospira, meaning "thin coil," and Leptospira it has remained ever since.

Successful control of Weil's disease would at first glance appear to depend upon efficient rat catchers. Under natural conditions, Leptospira icterohemorrhagiae is short-lived in human feces, being rapidly destroyed in the intestinal tract by common bacterial inhabitants found there. Drinking water, artificially contaminated with massive doses of spirochetes, will remain infectious for not longer than one week. Were this fact of great moment in spreading the disease, infections would certainly be more common. Available evidence, therefore, indicates that direct contact with recently discharged organisms is necessary. Hence, frequent association of rats with places where infectious jaundice has occurred makes the rodent suspect until proved innocent.

Opposed to the practical side of the problem, the question has been raised as to the actual part played by rats. The common finding of Leptospira in ponds and fresh waters has introduced complications. In recent years on the eastern coast of the United States, these organisms were found in well over half of all samples of potable waters, including nearly ninety per cent of municipal drinking waters in many widely scattered cities. A number of instances has been reported of the harmless species of Leptospira changing into the disease-producing type following passage through guinea pigs. Complicating the problem still further is the observation that some strains of Leptospira occurring in water are definitely infectious for certain animal hosts. In view of these findings, it has been sug-

gested that the supposed harmless spirochete (known as Leptospira biflexa) of water may be a variant of the icterohemorrhagiae type. The facts of microbial dissociation should awaken some interest in this idea and stimulate further study. Enough has been said already of the spirochete to warn us of its changeable nature. One cannot tell which way it will turn.

We have had a glimpse perhaps of the way bacteria adapt themselves to shifting and troublesome environments by a method of change and variation. The rapid progress now being made in the study of microbial diseases and the behavior of parasites in general raises the question as to whether man will conquer his germs through a better understanding of their surroundings, or by learning how to tolerate an evil in the best possible manner. Taking the long view of the question, it is likely that environmental control will be the deciding factor in the conflict between man and microbe. From the facts at our disposal emerges an inescapable conclusion: the environment of bacteria determines how and how much they can vary in their power to cause disease. Prolonged residence in *different* surroundings is the most probable cause of changes in virulence. Sufficient evidence has been presented, it is believed, to show why differences in power to infect are less likely to develop in microbes that prey upon animals of the same species. Where similarity of environment exists, as in this instance, there is no need of drastic change in habits or adaptation of the parasite to meet the given situation.

The problem of how pathogenic microbes vary or mutate is mainly one of accounting for the differences and resemblances of organisms that are related by descent. To put it another way, the study of genetics of micro-organisms is the problem itself. That they do vary is certain, and such variation is extremely important in any discussion of principles underlying preventive vaccine and serum treatment.

Unless one knows something about the purity of the

original culture used in propagating successive generations, conclusions regarding the nature of variation in micro-organisms cannot be justified. It is all very well to say that a group of individual organisms has exactly the same hereditary constitution; proving this assumed identity is quite another matter. In test tube cultivation, for example, single individuals have been isolated, or more often, "pure cultures" are obtained by repeated dilutions from the original, which represents many individuals. Accordingly, what then is known of the genetic constitution of a single organism?

If it is supposed that cultures stemming from single pure lines of the micro-organism can be obtained and propagated, there is no certainty of maintaining this purity because mutation of organisms is a common occurrence. Cultures of originally single pure lines are known to yield great numbers of such well-marked heritable variations in a short time.

CHAPTER 5

JEKYLL AND HYDE TYPES

Travelling Intestinal Bacteria

When microbes first moved into the intestinal tract of man from an outside environment which had always been shared in common by man and animal, they changed their way of living from that of free rovers to an easier sheltered existence. It is tempting to think of this migration as an extraordinary leap in evolution from saprophytism to parasitism, but the developmental history of the human intestinal system suggests that the change might have come about quite easily. For the outer covering of the embryo, at a certain stage in its growth, folds in upon itself to produce a simple tube that will form a future mouth, stomach, and intestine. Actually the skin of man has become an inside covering for the digestive tube, evidence of which can yet be seen in the kind of cells lining the intestinal tract throughout its length.

It is therefore likely that the habits of bacteria found there can be traced to the shifting of a familiar environment to a strange place. From this point of view, the close relationship exhibited by these specialized microbes can be explained more readily and it is also easy to understand why germs living in this environment were not compelled to make any drastic adjustment in their former habits. As saprophytes in nature they had existed in various forms close to man and had probably adapted themselves to a more advantageous mode of life in an easily accessible host. Within the human or animal body these same microbes did not really sacrifice their old ways, for in the new environment the intestinal tract of man could not be considered farther removed from the outside world than

his protective skin covering. In such surroundings, bacteria found most favorable opportunities not only for development within the host but also for easy dispersal over a wide area insuring a safe and prompt return to food and shelter.

This group of intestinal organisms, commonly referred to as "colon bacilli," tells an interesting story of evolution through a stage of saprophytic existence to one of varying degrees of parasitism. At one end of the scale is the harmless *Bacterium coli,* which may cause infection only under exceptional circumstances, whereas at the other end its more dangerous relatives make up a family of typhoid, paratyphoid, and dysentery germs, all of which are strictly pathogenic. The best known representative of all colonic inhabitants is the *Bacterium coli* (*communis*), which, as its name implies, is a common resident of the intestine. Its closest competitor, a variation of this type, which was thought by its discoverer to be more abundant in the human and animal than even *coli* (*communis*), is known as *Bacterium coli* (*communior*). The typhoid-paratyphoid group, which includes organisms responsible for food poisoning, and the members of the dysentery group also have resemblances among themselves.

Recognition of individual breeds among such microbes is thus exceedingly difficult, owing to their similar characteristics. In order to distinguish one from another, the bacteriologist must observe the biological activities of each organism in the hope of detecting certain differences in behavior. Among such activities, one of the most important is the fermentation of sugars or carbohydrates necessary for bacterial growth, whereby the carbon that is locked up within these complex foodstuffs is set free. In test tubes serving as a miniature laboratory, we can see what happens when typhoid, paratyphoid, or coli germs are given a meal of certain sugars dissolved separately and sterilized in a nourishing bouillon. To take a simple illustration, these tubes will each contain one of three well-

known sugar solutions, glucose (grape sugar), lactose (milk sugar), and saccharose (cane sugar), all having a trace of chemical substance which turns red in the presence of acid. Into one set of these tubes some typhoid germs are inoculated, while a second set receives paratyphoid and a third the coli organisms. After one or more days in an incubator maintained at body temperature, the effects of microbial activity will have become visible. The tubes containing *Bacterium coli* (*communis*) show large amounts of carbon dioxide gas and acid in both glucose and lactose, and the saccharose solution will be affected similarly if the communior type happens to be present. Paratyphoid germs develop acid and gas from the glucose alone, while typhoid organisms attack this sugar to form acid only.

These differences in behavior are striking enough for purposes of identification, provided microbes can be depended upon to do the same thing under like conditions at all times. Generally speaking, this might be true, but it is an error to assume that the carbohydrate-splitting power of bacteria can never vary through successive generations. Formerly, any such deviations from expected results were regarded as unimportant and it must have come as a shock to conservative bacteriologists when in 1907 Doctor Twort in England reported an unusual trait in a typhoid strain he had been studying. Its fermentative activity in lactose solutions more nearly resembled that of the common garden variety *Bacterium coli;* never before had a typhoid germ been known to split milk sugar. This peculiarity, it was found later, was lost when the organism was restored to its accustomed surroundings. Nevertheless, as an example of variation, however temporary, it indicated a lack of stability in what had always been regarded as a well defined characteristic of typhoid bacteria.

Selective Breeding

The earliest undisputed record of this type of variation was described by Massini in 1907 from some experiments

with a microbe resembling the *Bacterium coli.* When this organism was first obtained from the intestinal tract, it failed to ferment milk sugar like the ancestral strain and the colonies which grew out in the agar jelly medium containing lactose and a dye did not turn red, but remained white because no acid was produced without breaking down of sugar. After a few days, however, the white colonies began to develop little reddish knobs or bud-like growths and transplants made from these gave rise to typical red colonies only. The descendants of the microbes forming the red buds had thus inherited the property of fermenting milk sugar. Repetition of the transfers from one generation to the next showed that this power was retained.

Going back to the original white colonies and planting material taken from the white area only, Massini now found that the white colonies would develop as before with the red, bud-like growths again putting in their appearance after several days. The same sequence of events could be repeated with successive generations of white colonies. Massini had discovered a new and permanent variant or "mutation" in an organism which he named, appropriately, *Bacterium coli mutabile.* This microbe differed from its original type in not fermenting lactose, yet when grown in an environment containing this sugar, threw off variants capable of utilizing it as a food.

These observations brought out an undreamed-of fact. Bacteria lacking a certain property or character could be "trained" to develop that missing quality in their descendants. Whatever the mechanism underlying these changes may be, it suggests the familiar one of adaptation to environment. Hence the question of whether or not these induced changes are lasting is of considerable importance to us as possible victims of disease germs.

In so far as highly specialized forms of bacteria are subject to change, the conclusion might be drawn that an organism causing a certain disease can readily be transformed

into something totally different. While no convincing evidence has been found to support such a theory, there is good reason to believe that a number of types which have been regarded as separate breeds in this sense are in fact variations of an original microbial strain. Such changes do occur among different members belonging to a fixed type, like the pneumonia germ or some of the other organisms already mentioned. They represent the natural tendency of living organisms to adjust their needs to a changing environment, in which the ability to survive depends largely upon flexibility of habits. In no sense is this a transformation of one type into another; it is rather a modified behavior of different races within a group. The phenomenon is not restricted to any one place. Irrespective of locality, microbes tend to develop fixed types and in the course of this transition give to epidemic diseases many of their peculiar characteristics.

Interesting evidence of such changes is found in a variety of pneumonia germs encountered among natives working in mines on the Rand in South Africa. Pneumonia is virtually unknown in the tropical regions from which the natives have come, and they are therefore unusually susceptible to infection. As soon as they make contact with the more or less resistant white population, among whom the disease has been smouldering, pneumonia at once becomes epidemic. Five main types of pneumococcus organisms have been identified here, of which three correspond with those found on the eastern seaboard of the United States and in Germany. The other two races of microbes are unusual and seem to be peculiar to South Africa.

From this illustrative experience, we see how it is possible for a new group of disease-producing organisms to develop when a type that is weakly invasive for a more or less immune white race gains entrance to a highly susceptible black population and establishes itself as a fixed parasite. With each outbreak of pneumonia, fewer infections occur

among the smaller number of remaining susceptible persons, as a result of the gradually developing racial immunity which prevents further evolution of new pathogenic types.

Radical as some of the changes appear to be, there is no likelihood of bacterial species running wild to the extent of losing their identity. Variation always takes place within limits and our immediate interest is in the bearing it may have upon human and animal diseases and in its effect upon the defences mobilized against them. Today we are just beginning to realize how important a part microbial variations play in the causation of disease and the protective reactions incited in the host. Bacteriology in the awkward stage of adolescence had no satisfactory explanation for many unusual changes taking place under artificial conditions in the test tube or in the experimental animal. It was always a safe guess to assume that variation came about while the organisms were resisting the defensive onslaughts of the body and the hardiest microbes were selected in the course of adapting themselves to unfavorable surroundings.

Recent experimental evidence has proved this idea to be correct, although not wholly in the direction assumed. Some bacteria can step up their invasive powers in certain surroundings, while others under the same conditions are transformed into harmless varieties. A most important factor causing such changes is found in the blood or serum from an animal which has been inoculated with the microbes in question. The typhoid germ, when grown in the presence of anti-typhoid serum, undergoes a change resulting in the selection of a type that cannot be harmed by the action of the serum. Such variants are found to be more virulent than the original parent microbes. Acting in the opposite direction, the pneumonia organism (pneumococcus) becomes less aggressive in the presence of anti-pneumonia serum. Here the virulent form passes over into the non-virulent type, while in the preceding case the change is from lesser to greater virulence. This difference in behavior, which has been associated with specific bacterial substances, will be considered in the next chapter.

Jekyll and Hyde

How does one guess which way a cat will jump? It is equally difficult to foretell the behavior of disease-producing microbes which exhibit Jekyll and Hyde changes. Researchers have been quite busy lately cooking up schemes to outwit these dual characters. They suspect that the human and animal host combats parasites by forcing the virulent ones into the background in order to encourage growth of the harmless types. If it were possible to make this happen in an infected body, protection against disease would be far more effective than it has been.

One of the ever-present difficulties, unfortunately, is to apply successfully in the human body the results of animal or test tube experiments. Particularly is this true when dealing with generally fatal bacterial infections of the blood stream, caused by streptococci, the microbes that grow in beautiful chains or bead-like arrangements. They make a pretty picture for the classroom when seen under the microscope in a drop of blood from a patient, but the sight of them strikes terror in the heart of the physician, for only rarely is it possible to halt the killing power of the germ, which dissolves the life-giving red corpuscles under the influence of a toxic substance.

For years all efforts to develop some sort of curative serum against various kinds of streptococci have not met with success. The outlook now appears to be changing as investigators have begun to make certain Jekyll and Hyde microbes stage a performance for the benefit of their victims. Taking advantage of the fact that deadly streptococci in the blood stream of untreated infected persons throw off non-virulent forms, these organisms have been put to work manufacturing a serum in the horse. For ten years investigators have carefully observed what happened when patients were treated with this serum. In over one hundred cases of generalized streptococcal infection, almost ninety per cent recovered, the greatest mortality occurring in those patients whose blood stream had already been over-

whelmed by an explosive type of disease. These results are, to say the least, very promising and are further to be commended for the note of caution expressed that this work is still in an experimental stage. It is believed and hoped that the serum will transform virulent streptococci into their non-virulent forms in the blood stream of those who are seriously infected with the germs. These changes actually took place in drops of blood that had been removed from these patients.

Perhaps this is no idle dream, because we know from an earlier discovery that pneumonia germs behave in the same way when exposed to a serum prepared against them. In fact, whatever the mechanism may be, once a microbe is made vulnerable to the natural defensive reactions of the human body, the infecting agent is treated in exactly the same manner as any lifeless foreign material that happens to be floating around in the blood stream. Phagocytes recognize neither rank nor station. They engulf whatever comes their way, be it in the blood or in the tissues elsewhere in the body. It is upon this basic fact that modern treatment with serums plans its future wars against modern microbes.

In passing, it is interesting to recall a surprising discovery made by Doctor Carrol Bull at the Rockefeller Institute more than twenty years ago. Streptococci and pneumococci, he found, disappeared as if by magic within a few minutes from the blood stream of rabbits following an injection into their veins of enormous doses of these organisms. He examined film preparations made from the blood at close intervals and saw the microbes huddled together before going to their slaughter by the devouring phagocytes. The clumping of organisms and the speed with which they disappeared seemed to depend upon their virulence or what was recognized as such at the time. This study, from our present point of view, was perhaps the forerunner of many recent developments in the knowledge of immunity and recovery from microbial infections. The

history of bacteriology is replete with instances such as this where important observations made far in advance of the times have fallen like seed on stony ground. So, too, has it fared in recent years with the revived discovery of sulfonamide drugs, whose extraordinary effects in streptococcal infections must not be minimized.

Two-faced Tubercle Microbes

Revolution in bacterial society has penetrated the most exclusive circles. Even an aristocrat like the tuberculosis germ has not been preserved from the fate of common herd microbes and has become unsettled in its ways. According to rule, tubercle bacilli are supposed to be slender rod-like forms which can be tinted a bright red with certain dyes and they have been distinguished from other microbes similarly stained by their ability to hold fast to this color even when washed in strong acid. This property of acid-fastness inevitably came to be regarded as royal armor and a defensive weapon.

But in 1907, Doctor Much in Germany began to suspect that the tuberculosis germ led a double life and often took the form of exceedingly small non-acid-fast granules which were actually dwarfed bacilli. He found them regularly in the organs of animals suffering with tuberculosis where, much to his surprise, none of the ordinary acid-fast bacilli could be seen. Because these granules also gave rise to typical disease after animal inoculation, it was reasonable to suppose they were tubercle germs. But Much's light struck the blind-spot of his fellow scientists, who showed little interest in the work. To them a tubercle bacillus meant only an acid-fast, rod-shaped organism, and for practical purposes its recognition as such depended upon that rigid standard. Much's granules were laid to rest, talked about from time to time, and then forgotten.

In a distant laboratory several years later, another researcher made an observation quite unrelated to Much's work but destined to revive the subject and reawaken in-

terest in it. Professor Fontes discovered a peculiar kind of tuberculosis in animals resulting from inoculation with tubercle bacilli which had been previously ground up and then passed through extremely fine porcelain or clay filters. The same thing happened when, instead of using minced germs, he strained some thinned-out cheesy material taken from affected organs or glands. Tuberculosis produced by these methods violated all the known laws of the disease. Guinea pigs, instead of dying within two to three weeks, lived on indefinitely or succumbed after two to four months. In these animals, which showed only slight abnormal changes, no acid-fast bacilli could be demonstrated under the microscope.

Was Fontes dealing with tuberculosis germs and if so, what had become of them? The next step in his experiment answered that question. He took diseased material from the first group of animals for inoculation into a second set and sacrificed the latter five months afterwards. Now a careful search of small damaged areas in the lungs or glands revealed a few of the familiar-looking acid-fast microbes. Apparently tubercle bacilli were not always virulent nor were they stubbornly acid-fast. Short of technical boggles, such a conclusion was permissible from experimental facts, which seemed to confirm Much's earlier discovery by a different method of approach.

Like a prima donna in retirement, the tubercle germ was lured back for a reappearance in an old rôle after another fourteen years, when one of those strange mass outbursts of research activity was being witnessed among numerous investigators who were independently attacking the same problem in Europe and in America. As a result of all these labors, Much's forgotten granules and Fontes filterable tubercle microbes were rediscovered and fitted into the newer scheme of bacterial variability, which had been coming to the fore and was now being discussed as a likely explanation for the peculiar changes exhibited by tuberculosis organisms. Calmette, venerable French philosopher scientist, saw in filterable tubercle bacilli a menace stalking

unborn infants through the mother's blood. One of his fellow countrymen, by transferring the germs to potato broth, forced the growth of a filterable and non-acid-fast granular type which became acid-fast when fed another kind of food material. Opinions were divided as to the existence of a filterable form of the tubercle bacillus. While the French investigators found mutual support among themselves, reports from Germany were contrary minded and in the United States a number of careful technicians tempered their conclusions with diplomacy.

In the midst of confusion, one piece of work stands out as a praiseworthy effort to bare the private life of this highly publicized microbe. At Cornell Medical College, New York, Professor Morton Kahn trapped tubercle bacilli in a finely drawn glass tubing with an inside diameter of about one ten-thousandth of an inch. In a drop of the contained fluid measuring approximately one thousandth part of an inch across, he was able to isolate one germ at a time, seal it air-tight under the microscope, and watch developments day by day. From his observations he learned that tubercle bacilli, unlike most disease-producing organisms, apparently do not multiply by splitting into two equal parts from which descendants spring by a similar method. Instead, the rod-shaped microbe divides into three or more oval or egg-shaped bodies, which become smaller and smaller until they are reduced to minute granules. From these come yet smaller particles, which continue to divide until the limits of visibility are reached. At this stage, small rod-shaped forms not yet acid-fast begin to develop, then grow larger and thicken until they take the shape and size of the tubercle bacillus. These organisms now have become acid-fast and capable of producing tuberculosis in the guinea pig. But even those particles so small as to be almost invisible fail to pass through fine porous filters, thus disappointing many observers who thought tuberculosis germs had a filterable stage in their life cycle.

The story does not end here. We learn that tuberculo-

sis germs carry within themselves the ability to change simultaneously their form and their power to cause disease. Colonies numbering thousands of individuals, when developed from a single germ, may change from a type that is smooth, glistening, and moist to one that is rough, dry, and irregular in outline. Of two kinds of descendants, one is exactly like the parent microbe and the other totally different. The first variety will cause tuberculosis, but the second will not.

Interesting as these newly discovered facts may be, they have not simplified the problem of how to meet the added threat of subtle changes in microbial behavior. A familiar type of germ suddenly disappears from the body and all seems to be well. When least suspected, one or more of its variant forms may let loose the full force of destruction. This truth has been witnessed so often as to occasion little surprise now that variation is generally recognized as a property of microbes.

Lawless Plague Germs

The tubercle bacillus has an equally ancient and historic fellow in crime, the bacillus (*Pasteurella*) *pestis* or germ of plague—the Black Death. Attempts to find a cure for this most dreaded of all diseases have been less fruitful than for many other bacterial scourges of mankind and until quite recently, the veil of darkness covering this fearful microbe had been raised only sufficiently to reveal a fleeting glimpse of form and general features. But the plague germ can now be added to that ever-increasing company of two-faced organisms, for it is an out and out nonconformist.

Plague is strictly a disease of rodents and only because of their close association with human environments has man fallen a prey to it. But so far as is known, it has never adapted itself fully to its rodent host. Such an event would have resulted in rather general occurrence of chronic plague infection among rats or other rodents, and while

instances of this are reported, they can properly be regarded as a form of animal disease limited in extent which, because of its mildness, leads to recovery and immunity.

Obviously, this is not the way plague is spread among rodent populations; rather, in order to keep the disease alive, a severe generalized infection is needed. Thus germ-laden blood, which is indispensable for transmission by fleas, is put within easy reach of the biting insect. It might appear self-contradictory that this disease can maintain itself under such conditions and not result in extermination of important animal hosts, but an explanation of this anomaly can be found in the fact that the amount of plague is influenced in turn by the extent of infection among the rodent and flea populations. Further, plague "carriers," as understood in the usual sense, are not known and, if they do exist, are unimportant sources of infection. So far as the *Pasteurella pestis* is concerned, a balance between host and parasite, rather than a state of equilibrium within the animal body, determines the frequency and spread of epidemic disease.

With the discovery of the pestis microbe in 1894, an international controversy began as to whom major credit should be given for this achievement. The squabble has revolved about two doctors—Yersin, a Frenchman, and Kitasato, a Japanese. Before discussing the merits of the case, it is interesting to observe that the French have won a moral victory, at least as concerns the naming of the germ. It is officially known as *Pasteurella pestis* in honor of Louis Pasteur, who discovered the chicken cholera microbe, which the plague organism resembles in form and in its tendency to invade the blood stream.

In so far as accuracy of observation and correct interpretation of what has been seen must always be the basis for accepting something as fact, then to Yersin should go the palms of victory and to Kitasato honorable mention. These two doctors arrived at Hongkong, China, only two days apart, Kitasato on the twelfth day of June, 1894, and

Yersin on the fourteenth. Plague had been raging in the city since May. During the first week after his arrival, Yersin, who was denied permission to do autopsies, made the best of a difficult situation and, under trying conditions, studied such specimens as he was able to obtain from corpses. The inflamed glands or buboes furnished him with materials which he used in animal experiments and microscopic studies of direct smear preparations and bacterial cultures.

There is no mistaking the fact that Yersin saw the microbe in splendid isolation, unaccompanied by the mixed brood found by Kitasato in an autopsied patient. The description of the organism, as given by the two men, working independently, differed in very important details. It is upon these reports and subsequent observations that Yersin's claim to the discovery should be recognized. Even as late as 1898, Kitasato regarded his microbe as different from the one described by Yersin on the grounds that, contrary to the latter's findings, it was motile and when stained with certain dyes, resisted the decolorizing action of alcohol. Without laboring the point, both investigators had doubtless seen the same microbe, although Yersin alone had described it accurately in all details and, what is especially significant, recognized its presence in the carcasses of rats which, as his diary tells us, were plentiful throughout the city. This observation was of the greatest importance, although the fact was not given the recognition it deserved.

Pasteurella pestis is a very small oval-shaped non-motile organism which stains lightly with coal-tar dyes. Under the microscope it appears to be darker at each end or "pole" and is therefore characterized as "bipolar." It has been customary in describing this microbe to comment on the extraordinary variations of form and growth exhibited under laboratory conditions. Present day knowledge of changeable germ characteristics will account for much of this instability, yet a failure to adapt itself to its host or environment can be seen on every hand.

The plague germ reminds one of the mercurial qualities of a delayed adolescence. Old in history as the disease may be, the microbe has not yet found itself. We can judge better of this by the company it keeps and its unpredictable conduct. Lest the reader gain a false impression from the last remark, this is not to be construed in the sense that a pestis germ will cause plague on one occasion and a different disease at another time. Rather, the striking similarity of its behavior in a wide variety of animal species suggests a common microbial origin which as yet has not taken a definite direction. *Pasteurella pestis*, as we have seen, is a disease of rodents, transmissible to man, but the *Pasteurella pseudotuberculosis*, while almost indistinguishable from it in every respect, does not infect man. *Pasteurella tularensis*, on the other hand, causes tularemia in man and rodents. Another near relative, the aviseptica, affects fowls and domestic and wild animals. This organism, discovered by Pasteur in 1880, is the familiar one of "chicken cholera." Curiously, it is shown by serum tests to be related to both the pestis and pseudotuberculosis microbes. In close companionship we find types with suitably Latinized names occurring in sheep, hogs, cattle, calves, rabbits, mice, and guinea pigs. Extraordinary as is the virulence of all these germs, it is none the less subject to considerable variation.

As for the *Pasteurella pestis*, there is accumulated evidence, not to be brushed aside lightly, to remind us of its modern way of doing the unusual. The plague microbe, as might be expected of a killer of the first rank, draws our attention to the important question of virulence under natural and experimental conditions. To say that infection will occur by simply rubbing the organisms into an unbroken skin does not adequately describe their destructive power in ordinary surroundings. It is difficult to make comparisons when dealing with the multitude of complicating factors that prevail in epidemics, and impossible to evaluate observations made in different parts of the world upon isolated examples of human or animal

material. Consequently experimental studies, under artificial conditions which can be controlled in the laboratory, offer the best means of arriving at certain conclusions.

Early encounters with the fluctuating virulence of *Pasteurella pestis* were only incidental to serious attempts at producing weakened cultures for "vaccination" against plague. The relative merits of these different methods and the results of their practical application will be discussed in a later chapter. It seems unlikely that this problem will be solved until the behavior of the microbe is better known than at present and there is great doubt that a dependable vaccine can be developed. From available statistics in India, where most of this work has been done, the results are hardly encouraging. The question still remains as to what can or should be done with living plague cultures, provided these could be shown to be permanently avirulent. To argue the point may seem superfluous but for the fact that vaccination with live organisms, while theoretically the best known method for making animals immune, is dangerous on general principles and perhaps foolhardy in a disease like plague.

When are plague germs harmless and how far can they be trusted? Not without reason have they inspired awe and the urge to keep them at a safe distance. A single organism is enough to cause the death of guinea pigs weighing one pound or less, and in proportion to body weight only a few hundred would be needed surely to infect a human adult. Moreover, the pestis is remarkably long-lived under conditions which ordinarily do not favor other microbes. It will remain alive and fully virulent for ten years or longer at a temperature below seventy degrees Fahrenheit. In a natural environment, such as the organs and tissues of infected animals, the plague bacillus is amply protected against external agents that might shorten its life.

While it is true that virulence of plague microbes may be lost under certain conditions, the loss appears to be only

temporary. A test tube culture of non-virulent germs has been known to regain its original killing power after several months storage at ice-chest temperatures. This is only one of many environmental factors frequently causing such a mysterious change.

It is not surprising, therefore, to find analogous influences operating similarly in the animal body. An early experience of this type befell the present writer with startling suddenness more than twenty years ago. In the laboratory of the Plague Prevention Service at Harbin, Manchuria, he inherited from Doctor Wu Lien Teh an old culture of pestis germs, obtained from a patient during an outbreak of plague in Shanghai seven years earlier. With this strain of microbes, which had been under artificial cultivation for eighteen months prior to 1916, it was impossible, using enormous doses of the culture for inoculation, to kill a guinea pig. Massive growths in bouillon or other suitable fluid and solid media were injected by every conceivable route into the animals. They continued to grow fat and huge, for they never missed a meal. When the pigs were painlessly destroyed by means of an overdose of ether, not a trace of plague germs could be found anywhere at autopsy. This culture of pestis germs was unquestionably avirulent and had behaved in exactly the same way on numerous other occasions.

An accidental discovery was to play a part in correcting the false impression that these plague organisms were harmless. Later, in the course of certain experiments, it was necessary to incubate for several hours at body temperature test tube mixtures of these live bacilli and fresh serum from the guinea pig. The material was whirled rapidly in a centrifuge and small amounts of the clear overlying fluid were then injected into healthy guinea pigs. The animals died suddenly a few days after this experiment, showing an overwhelming plague infection. Microscopic examination of the material used for injection revealed rare bacilli which had not been removed by centrifugalizing.

Incredible as it seemed, a few microbes did cause a fatal infection, whereas massive inoculations repeatedly had failed to do so.

An experiment was next devised to test the possibility that an increased virulence had resulted from the contact of these plague microbes with the animal serum. Materials were prepared in every detail as before, but now the sedimented organisms obtained after prolonged whirling in the centrifuge were suspended in salt solution and known quantities inoculated into a series of guinea pigs. Actually the plague culture was diluted ten times and the equivalent of one hundredth of a cubic centimeter, or less than one-sixth of a small drop, was injected. All the animals died within two to three days from acute plague. Here was conclusive proof of enhanced virulence following contact of *Pasteurella pestis* with serum from a plague-susceptible rodent. Had the term "microbial dissociation" then been current, it would have properly described these observations in 1916.

One of the obvious deductions made from these experiments at the time was that vaccination against human plague with so-called avirulent live bacilli was hazardous because their fate after injection into the body could not be foretold. Our observations suggested what might happen under conditions which in a general way imitated those occurring in the human body. Enthusiastic workers were then proclaiming the benefits to be derived from vaccination with live harmless plague germs. Doctor Richard Strong in 1907, following in the path of the German Plague Commission, also advocated the use of this method and tried it on nine hundred persons in Manila without an accident, according to a report. The value of this demonstration could not be determined, however, since there was no plague in Manila at the time. More recently, in 1934, mass vaccinations with live plague germs have been practiced in Java with cultures that are said to have become totally harmless after six months in the laboratory.

It is too early to draw other than guarded conclusions from the observations made thus far.

Although the problem of antiplague vaccination should be viewed in its entirety with an open mind, there are fundamental reasons why conservatism may be the better part of valor. In a situation where the burden of proof falls upon the experimenter, it is important to recognize, first, that microbial variation is an established fact, and second, that this is virtually unexplored terrain. In the Java studies, incidentally, the harmless parent strain showed a tendency to split off two kinds of organisms, of which the virulent type was far more effective than the avirulent for immunizing experimental animals. This is merely another way of saying that virulent organisms make the best vaccines—if they remain harmless! Similar experiences have been reported by a number of investigators, from whose work it can be concluded that the plague germ does not remain stable once it has been modified. On the other hand, if ever a totally avirulent strain could be developed in the laboratory, there is nothing to hinder it from degenerating into a type that is too weak to manufacture protective antibodies against virulent pestis infection. In any case the vaccine would be useless.

The *Pasteurella pestis,* as we now know, has days when it can be either virulent or avirulent or both, and each variety can be identified by serum tests. Characteristically, the avirulent strains show a feeble reaction in serum prepared from typical virulent organisms, while these in turn are only slightly affected by a serum manufactured with the weak culture. These tests give the impression that a permanently harmless variety has been developed, yet after storage in the ice-chest, the microbe makes a sudden about face, resumes its original normal form, and becomes fully virulent.

During the past ten years there have been more definite attempts to coax plague germs into showing the better side, if any, of their behavior. They have been inoculated into

and recovered from the bodies of immune rats, resulting in an avirulent strain of pestis. Unfortunately, a short time later it was found that this "reformed" pet had broken parole and was the same old killer. A trio of Russian experimenters, working with a peculiar breed of plague germs that grew in colored colonies and were harmless for animals, were able to restore the original state of virulence and form to these microbes after one or two transplantations in the usual bacterial foodstuffs. In South Africa, Pirie (1929) made a successful attempt to dissociate pestis organisms into an avirulent type by allowing them to grow in bouillon, to which were added gradually increasing amounts of alcohol. This method, originally described by a member of the German Plague Commission in 1904, had also been tried by Doctor Strong in 1907, and they were among the first to use live plague bacilli as preventive "vaccines."

To Pirie we are indebted for convincing proof of dissociation of *Pasteurella pestis* into the recognized and now conventional forms—a virulent type and its avirulent twin. His conclusions have been questioned by a number of Russian bacteriologists, who are of the opinion that the virulent form is harmless. All agree, however, in emphasizing the ease with which the harmless variant reverts to the deadly type. In the somewhat confused state of this knowledge at present, it is wise to withhold judgment until we can be sure that these variants are unquestionably "pure" types.

There is, of course, always a strong probability of mixed and intermediate types being present under conditions that bring about dissociation. Confusion might be avoided if observers would agree on an important point, namely, that the degree of virulence in *Pasteurella pestis* cannot be explained by the appearance of the growing colony. Differences based on form alone will only complicate things all the more, since the behavior of pestis microbes under laboratory conditions depends largely upon the kind of culture medium used.

In control of disease-producing micro-organisms, whether by vaccine therapy or the use of serums and drugs, we are concerned mainly with eliminating or checking the development of the infectious agent. How well these attempts succeed will depend upon how much is known about the limits of variability of the organism. As in other diseases controlled by immunization, the situation in regard to plague is complicated no less because the strains of *Pasteurella pestis* may themselves vary in their characteristics or else new strains may appear.

It is clear that the degree of mutation must be known before it is safe to conclude that pestis organisms "*never become virulent again*" and that "*we must recognize them as completely stable dissociants*" (substrains which differ from the parent strain).

The point at issue here is whether an organism changed by the environment can be subjected to man-made conditions so imposed, or if certain surroundings will bring about heritable changes that are poorly or better suited to the new environment.

Aside from these uncertainties, the method of immunization using living avirulent *Pasteurella pestis* as vaccines has definite limitations: The immunity appears to be short-lived; death from plague has not been prevented even after repeated vaccinations; protection against air-borne infection with pneumonic plague cannot be obtained by ordinary methods of inoculation.

During World War II plague vaccines were given a thorough trial in the Armed Forces. It was found that protective substances developed in the blood serum of vaccinated individuals gave evidence of some antibody response in two-thirds of those who were inoculated. The statistics are not highly impressive and at the same time it is emphasized that the vaccines prepared from killed plague organisms are not inferior, if not superior, to avirulent living vaccines.

If this story points a moral, it is to show that stability is not a necessary virtue for the success of *Pasteurella pestis.*

Taking advantage of this peculiarity may offer some hope of controlling the activities of the plague microbe. Should present trends in bacteriologic research be any indication, more definite gains are likely to result from a study of less elusive characters connected with the organism. These are inherent in the microbe itself and determine which parts are active in attacking the host and which of these can be used successfully in manufacturing protective substances beneficial to the victim. Chemistry has now joined forces with anatomy in dissecting, analyzing, and reconstructing the bacterial cell. The microbe as seen from the inside leads to a new chapter in the modern warfare against disease.

CHAPTER 6

CHEMISTRY AND BEHAVIOR

Since the highest powers of magnification have failed to reveal what makes germs behave as they do, we must let chemical analysis replace the microscope if we wish to learn more of their private lives. By looking at microbes through the eyes of a bacteriological chemist, we may learn something of their chemical behavior and how to control it.

There was a chemist turned bacteriologist who long ago had an idea based on the structure of crystals and their specialized chemical reactions. From such a beginning, he went on to investigate specific fermentations and finally arrived at their origin in microbes. This crystal gazer, Louis Pasteur, told us all that we needed to know about chemical constitution and behavior. Yet it is one of the strangest facts in the history of a science that this study was dropped where it might have begun. Only now are Pasteur's earliest theories being realized, for in living organisms, as in crystals, chemical differences run parallel to structural differences.

The cause of this unprecedented lag in pursuing a brilliant idea is perhaps not hard to find. It can be ascribed to a strong belief that the blood-relationship of breeds determined their capacity to react only with corresponding tissues obtained from human and animal sources. That is to say, identification of these materials by means of a certain test depended upon their biological origin. This thought had been carried over from observations made upon the reacting substances or antibodies developed in the blood serum of animals by inoculating them with blood taken from different animal species. When the blood-serum of animals so treated was combined in a test

tube with the material which had been injected, a sediment resulted from this union, indicating the presence of an *antibody* capable of reacting with the *antigen,* namely the substance that produced it. This test distinguished easily between serums of unrelated animal species but became less and less definite as the relationship grew closer. Whereas an antiserum for the blood of the horse did not react with that of the dog, in more closely related species, such as apes and man, fox and dog, or rabbit and hare, the antiserum for the blood of apes or man reacted almost as sharply with one or the other; similarly, in the case of dog and fox or hare and rabbit, the differences in reaction were very slight.

With these facts alone, it was quite natural to emphasize the importance of biological relationship, the more so as the serum proteins might be chemically similar even though derived from unrelated breeds of animals. On this basis, the properties of protein substances did not appear to depend upon chemical differences, but, as other methods became available for studying the problems, the idea was shown to be erroneous.

In the early part of the century, attention was being drawn to the proteins found within the tissues of animals and plants. With the discovery that these proteins could be extracted in a pure state and distinguished by chemical methods, a new science of immunology and bacteriology was created. A significant fact was the finding in any one breed of plant or animal of several different proteins which had more distinctive properties than many chemically similar substances from biologically unrelated sources. From a study of specific reactions by means of serum tests, it was also evident that chemical composition rather than biological relationship determined specificity. Wheat and barley, for example, yielded pure antigens which, despite different origins, produced similar antibodies; on the other hand, the crystalline albumin isolated from eggs of the duck and hen could not easily be differentiated by means of immunological tests; finally, a pure protein from a given

source might react in the presence of a serum developed with other pure proteins, provided these had chemical resemblances to the original.

These discoveries fitted very neatly into the pattern of some earlier experiments made with the protein of the crystalline lens of the eye. Lens substance, when injected into experimental animals, produced antibodies in their blood serum which reacted not only with the original lens protein but also with that taken from different species of animals. The obvious reason for this was the chemical similarity of the materials used in the test. However, these lens antibodies did not react at all with the natural proteins found in the blood of animal species which furnished the lens substance. In this particular instance, two chemically unlike protein substances originating in one animal species differed sharply in their immunological response.

Chemical Warfare

Such clear-cut distinction between tissues from the same animal body should have settled the question as to whether or not chemical structure determined specific immunity reactions, but the importance of this fact was not appreciated until later. We are quite sure now that the behavior of a protein is more a matter of chemical than of family relationship. This brings us a step nearer to the modern ideas of the structure of bacterial proteins and leads directly to the chemical causes of microbial behavior. With this important connection established between chemical structure of proteins and their specific properties, bacteriologists commenced shooting with rifles instead of shotguns.

Control of infectious diseases in the future will depend in larger measure than before upon knowledge gained from the chemical composition of bacteria. The individual differences that can be detected by analytical methods explain some of the mysteries of parasitism in man and animals,

the modes of infection, and the defensive agents of the host. As we have learned in an earlier chapter, accurate recognition of certain diseases is almost impossible, because of difficulty in distinguishing the various types of organisms that are implicated. To name only a few, there are the microbes of typhoid fever and their near relatives, which affect man alone, and similar species that normally attack rodents while causing severe gastro-intestinal upsets in the human; other bacteria, found naturally in cattle or swine, have cousins or second cousins which can and often do cause a typhoid-like disease in man. These overlapping characteristics are accounted for by a certain part of the structural material, which is common to all these microbes. For practical purposes, however, we should like to find out which part can be used in manufacturing a protective immunity.

Some important advances have been made in this direction. Complete dissection of microbes is being accomplished by chemical analysis and now the bacteriologist can proceed on a surer footing, knowing that he is working only with active ingredients towards the goal of immunity. This problem, because of its complexity, had to be worked out piece by piece like a jigsaw puzzle with some of its parts taking shape long before the rest had begun to make sense. The early studies of Theobald Smith and of Weil and Felix on microbial constitution fitted in with later observations on the remarkable changes which bacteria might exhibit in their form and behavior. But key pieces in the puzzle were still missing until chemical analysis of the microbial cell made possible a separation of its various parts.

Two engrossing questions now awaited an answer. What was the nature of specificity in immunity reactions, or in other words, what gave them their distinctive character? What fraction or fractions of bacteria were concerned in developing these specific immunity responses? The gist of the problem was to determine whether or not inoculation with these materials incited the formation of

protective substances or antibodies. If this happened, the materials were antigens. Their chemical nature was then discovered by observing the results of their union with antibodies, a method reminding one somewhat of the tail wagging the dog, but in this instance useful in finding a cause by observing its effect.

Underlying all the earlier studies on antibody reactions was a principle upon which most investigators were in agreement. The antigen or substance that gave rise to the specific antibody and was capable of uniting with it must be a protein. Since everything known on the subject of specificity at this time revolved about the structure of proteins, the possibility of antibody reactions resulting from substances that were not proteins seemed remote. Nor did it appear at all probable that antibodies could unite with materials which in themselves lacked the power to produce immunity in experimental animals. In short, specific immune reactions were part and parcel of chemical constitution of proteins.

Against all this, there is evidence of another kind, which is contradictory and so must be discussed in some detail even though it is rather technical and deals with the most difficult parts of the theory of immunity to infectious diseases. In 1902, Professor Pick, a chemist at Vienna, found in cultures of typhoid germs a substance which had none of the characteristics associated with proteins. It reacted, nevertheless, with antityphoid serum to form a precipitate in the test tube, but lacked the power of stimulating specific antibodies in the tissues of animals. This freakish substance, unlike ordinary antigens, failed to produce antibodies in the usual manner; like a protein, however, it could be identified by a specific precipitation test used for detecting the presence of antigens. These discordant facts were harmonized by assuming that bacterial antigens did not contain proteins exclusively but were made up partly of certain important non-protein material in an effective combination.

After a lapse of more than twenty years, this discovery

began to bear fruit as other investigators revived interest in the subject by repeating these experiments with typhoid, pneumonia, tuberculosis, and influenza organisms. At the Rockefeller Institute, Heidelberger and Avery developed exact methods of isolating from bacteria a number of different substances which were responsible for the characteristic antibody reactions. As soon as these immunologically specific properties became known, the chemical attacks of microbes were met with a new line of defence. The way was first cleared for a successful advance by a prolonged siege under the guidance of Doctor Karl Landsteiner, who became the leader of a new science of chemical immunology.

A Chemical Master Key

Professor Pick had laid down the first general principles of chemical specificity of proteins and the laws underlying their behavior. To Landsteiner we owe the precise facts regarding the chemical nature of specificity, particularly as it affects the structure of bacterial antigens. This work, begun while he was professor of pathology in Vienna during the years 1910 to 1920 and continued at the Rockefeller Institute in New York a few years later, showed how the characteristics of antigens were modified by remarkably slight changes in their chemical structure. These experiments penetrated the most obscure regions of immunology and simplified its language, to the great joy of an army of investigators.

Landsteiner took immunity phenomena from the land of Babel where stood the tall tower erected by Paul Ehrlich at the turn of the last century. Ehrlich unquestionably had laid a sound foundation and marked out the correct path even before Pick and others who followed him, but the superstructure lacked cohesion and fell before modern methods of chemical warfare. A single new term was invented by Landsteiner in 1921 to describe an extremely complicated series of events. It was a word of two

syllables, "haptene," derived from the Greek root meaning "to join," and summed up the basic facts of specific antibody reactions.

The haptene is that part of a class of antigens which gives to the whole substance its specific character, that is to say, it is an active chemical portion joined to a silent partner and making up what is known as the complete or combined antigen. Haptenes react specifically in the test tube with the corresponding antibody that is developed in animal serum by injecting a complete antigen, which in itself appears to be always a protein material. However, when the chemical portion is separated from the portion to which it is joined, the power of stimulating immune substances in the animal body is lost.

From these and related facts, Landsteiner concludes that the protein invests the chemical compound with its antigenic properties, while these in turn owe their specific qualities to the chemical compound alone. It is possible, for instance, to observe this effect by uniting a particular chemical substance with a variety of protein materials having marked differences in their own chemical structure. As a result of such a combination, the various modified proteins acquire strikingly similar antigenic power and behave very much alike in their manner of inciting antibodies. When they are injected into animals, antibodies are produced in the blood, which will now react with any and all sorts of proteins whether or not they are related in origin, provided they have been subjected to the same chemical treatment.

As an illustration, horse serum, gliadin from wheat, casein from milk, and rabbit serum—all proteins from different sources—are first united with a certain non-protein substance. Doctor Landsteiner used a chemical having the name metanilic acid or meta-amino-benzole-sulfonic acid. With the serum from animals immunized by repeated injections of any one of these compound proteins, it is then possible to detect all the others in suitable mix-

tures prepared in the test tube. Although the non-protein material itself is not an antigen, it does confer specific properties upon substances with which it has been combined.

Generally speaking, even the slightest change in architecture of the added chemical is enough to cause differences in the structure of antigens and the antibody reactions they evoke. Taking advantage of this fact, Landsteiner has prepared numerous compounds having a known chemical structure and containing specific reacting components which can be detected by serological methods. Perhaps by means of an old analogy of lock and key we can form some sort of picture of these structural changes as they occur with a change in location of the chemical groups that make up the compound. As one can imagine, many combinations of this kind are possible, depending upon the place where a hook-up occurs. A chemist knows how to modify the characteristic properties of a compound by changing the relative position of its parts with respect to one another. In the same way the arrangement of various parts in a lock can be shifted so that the original key will not fit. Yet it is possible to have many different locks which can be opened with a single master key.

In short, the experiments prove that antibodies are specific when the chemical groups are identically placed in the compounds. For example, a serum produced by injection of an artificially prepared antigen reacts with all other combined antigens showing a similar arrangement in the chemical structure, but not with the unaltered original protein. The deciding factor appears to be the location of certain chemical groups without regard to other constituents. Most striking is the fact that serum reactions will identify the compound antigen and at the same time pick out other proteins which are linked to identical chemical substances or to chemically related ones. This is analogous to a master key that opens a number of different locks.

For an illustration, let us suppose a chemical compound, A, is joined to different proteins such as X, Y, and Z to form combinations resulting in AX, AY, and AZ. When these are injected separately into experimental animals, corresponding antibodies are produced in the serums, namely ax, ay, and az. Each serum is now found suitable for identifying any one of the combined antigens AX, AY, or AZ. Irrespective of the nature or origin of proteins X, Y, and Z, the chemical compound A alone gives to each a common characteristic as a partial antigen or haptene. Similarly, if another chemical substance such as D, different from A although structurally related to it, is used in parallel tests, the combinations DX, DY, or DZ behave like the A series because of a similar chemical constitution. Not only are they precipitated in the test tube by serums ax, ay, and az, but the corresponding set of serums dx, dy, and dz will also react with the antigens, AX, AY, and AZ. No matter how many varied combinations are tried, the specific reactions depend always upon the chemical compound rather than upon the protein substance with which it happens to be joined.

The work of Doctor Landsteiner with his haptenes had as a natural sequel the isolation of specific substances from bacterial cells. Considerable evidence had been accumulated showing that certain organisms contained a number of substances which were recognized by differences in their immunizing and chemical properties. The next step was to study microbial architecture and find out which part of the structure was ornamental and which useful and what kind of material went into its framework.

Lethal Pneumonia Germs

These questions were uppermost in the minds of investigators who tried for many years to learn how pneumonia germs behaved in the human body. Little progress was made in this direction for almost three decades after the first definite proof in 1886 that the disease was caused

by a microbe—the pneumococcus. To be sure, important work had been done in the meantime, but it failed to reach the main objective, which was to find a curative serum for the disease. And even now pneumonia, which takes about one hundred thousand lives a year in the United States alone, ranks third among all causes of death and is the most destructive of the respiratory diseases.

A golden decade in medical science was begun in 1913 at the hospital of the Rockefeller Institute, where Dochez and his associates were engaged in a thorough investigation of the activities of pneumococci in mice and men. Their brilliant research, which culminated in one of the most important bacteriological discoveries of recent times by Heidelberger and Avery in 1923, had its roots in a series of scattered observations made in Europe twenty years before and in this country shortly afterwards. These investigations showed that, contrary to the previous belief, all pneumococci were not alike and differed materially in their power to manufacture immune substances in the body. Testing the protective action of antipneumococcal serums in mice, it was found that a certain serum would prevent the death of animals inoculated with one strain of pneumonia germs but would fail to protect against another. On the basis of these experiments, it was concluded that a majority of pneumococci belong to one of several types of organisms.

These types and the finer characteristics by which they might be identified were described by Dochez and Gillespie in 1913 and by Dochez and Avery two years later. Pneumococci obtained from patients with lobar pneumonia, they found, could be divided into three general groups, which represented about four-fifths, and a miscellaneous group one-fifth, of all strains encountered in this disease. These became known as Types I, II, III, and the remaining types classed together as group IV and were remarkably well defined in their immunological reactions. A serum prepared by animal injections with Type I, for example,

did not affect Type II organisms and similarly, Type II antiserum failed to react with Type I pneumococci. Three of the groups appeared to be universally distributed, for in a place as remote as South Africa types of pneumococci were found identical with those of the United States and Germany.

These differences in antigenic properties made possible a more accurate identification of pneumococcal varieties and laid the foundation for controlling infection by means of serums which were specific for any one of the particular types. Although an appreciable reduction in mortality from pneumonia resulted from the improved method, there were still many disappointing failures with this new form of therapy. It is only recently that these failures are known to be due to the fact that the miscellaneous group IV contains twenty-nine distinct types, against each of which only one particular serum is effective. Until this discovery, no one suspected the existence of so many different varieties of pneumonia germs.

Inasmuch as the effectiveness of serum treatment depends upon its prompt application, no time must be lost in determining the type of pneumonia germ responsible for the infection. The method now in use is a rediscovery of a test described by Doctor Neufeld about thirty-five years ago, but which was until recently a purely scientific curiosity. Sputum collected from a patient is mixed in small quantities on a thin glass plate with the various types of antipneumonia serum, a few drops of a blue dye solution added, and then examined under the microscope. In mixtures where the type of serum matches exactly, the capsule of the pneumococcus appears greatly swollen and sharply outlined, whereas no reaction occurs with other types of serum. Thus, if the patient has Type I pneumonia, the organisms in his sputum will react only to Type I serum and to no other types, enabling one to make a rapid selection of the proper serum for treatment.

A careful study of various groups of patients brought

out the fact that the probable outcome of the disease depended to a large extent upon the type of infecting organism. Because fatal infections occurred more frequently with certain types of pneumococci and the severity of the disease appeared to be related to harmful substances given off by the microbe, Dochez and Avery began a search for these substances in the body.

This investigation differed from earlier work of a similar nature in one particular, which enabled Dochez and Avery to succeed where others had failed. Studying the harmful effects produced by pneumococci in lobar pneumonia, they suspected that the cause of toxemia or "poisoning" might be found early in the disease, since bacteriological studies of the blood had already given definite indication of an early invasion of the blood stream. Accordingly experiments were planned with the germs under conditions resembling a natural infection in the human body. A flask of bouillon was seeded with a young and actively growing culture of pneumococci and from time to time during their growth small amounts of the fluid were withdrawn and filtered to remove all bacteria. To the clear material, antipneumococcus serum was added and tested for the presence of a precipitable microbial substance. This could be identified by the sediment which formed when antiserums of Types I, II, and III were combined with filtered cultures of the corresponding pneumococcal types.

The substance discovered by Dochez and Avery was unusual in that it represented soluble material liberated in a fluid environment from living bacterial cells during early stages of their growth and was not an ordinary product resulting from the death and consequent breaking up of old bacterial cells. Whether it would be possible to find it both in artificially induced and natural infections was the next inquiry. A search for this substance in experimentally infected rabbits showed that it was present in the circulating blood and urine during the first few hours

after inoculation of pneumococci. For practical purposes the results thus far checked perfectly. The organisms formed a specific soluble substance in the animal body as well as in an artificial culture medium. When a crucial test was now made with the blood serum of patients suffering with lobar pneumonia, the same reacting substance was found to have been set free in the circulating blood during the course of the disease and then eliminated through the kidneys.

The presence in the urine of considerable amounts of this substance thus became the basis for a specific diagnostic test which made prompt recognition of the disease possible in its early stages. When a sample of urine was combined with antipneumococcus serum of the type that matched the infecting organisms, a precipitate was formed as a product of the reaction between the specific soluble substance and the serum. Occasionally, in order to detect very small quantities of the precipitable material, it was found necessary to concentrate the urine by boiling and refining with alcohol. Even this rigorous treatment did not impair the specificity of the soluble substance, a fact which hinted at its extraordinary chemical composition.

Aside from this guess there was not much else to be said about the nature of the pneumococcus substance. However, as evidence accumulated regarding its behavior, certain incidental facts shed new light upon the mystery. It had been noticed, for example, that the severity of the disease generally varied with the type of pneumococcus and that the precipitable substance in the urine was more abundant in severe than in mild infections. From the standpoint of severity, Type III pneumonia ranked first, Type II next, and Type I last, accounting respectively for a mortality of forty-five, thirty-two, and twenty-three per cent of the cases in general. Moreover, the largest amount of specific substance was formed by Type III pneumococcus, somewhat less by Type II, and the least quantity by Type I. These facts taken together suggested

an important relationship between virulence of the organism and the characteristic substance it produced.

This relationship proved to be especially significant when added to other evidence that pertained to the chemical constitution of this substance. Pneumonia germs are peculiar in having an outside covering or gelatinous envelope known as the capsule, which has always been associated with the power to invade the tissues and cause disease, since it is a well-known fact that certain microbes, if deprived of this surrounding material, are quickly gathered up and made harmless by the scavenger cells or phagocytes of the body. Doctor Avery and Doctor Heidelberger, the latter a chemist, therefore directed their attention to the structure of the pneumococcus, in an attempt to relate its chemical composition to its disease-producing activities. They set to work on a large scale to segregate the outer and inner portions of pneumonia germs by literally stripping off the capsules. Chemical analysis of this purified material showed that it was composed of a complex sugar-like substance or carbohydrate manufactured by the organism. Each type of pneumococcus had its own specific substance and yielded upon further chemical treatment certain peculiar sugars, with properties so widely different that it was almost impossible to believe they belonged to three such closely related and fixed types of pneumococcus.

As a first step in understanding the nature of these constituents, we can profitably consider some of their immunological properties. In a purified state, each substance, if combined with the corresponding type of serum in a test tube, will cause a specific precipitation, which is visible even with dilutions as high as one part in five to six million. The delicacy of this reaction may be surmised from the fact that it is possible to detect as little as one one-hundredth part of a milligram of pneumococcus Type III substance. (Thirty thousand milligrams are equivalent approximately to one ounce.) When injected into

animals, however, these materials have no power to stimulate antibodies. Hence the specific carbohydrate behaves in all respects like a haptene or partial antigen in that it gives individuality to the microbe, conferring type-specificity on it and identifying the germ as a *particular kind of pneumococcus*. In sharp contrast, the inner portion of pneumococci is composed principally of protein, which is common to all three types and reacts with serums prepared against any one of the three. Therefore the cell body contains only material that is characteristic of the breed but not the special type of organism, thus merely *identifying it as a pneumococcus* but not telling us what kind of pneumonia germ it is.

The correctness of this line of reasoning is illustrated further by means of a neat experiment, in which we can compare the immunological behavior of various compounds prepared with carbohydrate substance and protein. Two different kinds of carbohydrate united with the same kind of protein, when injected into animals and later combined with their blood serum, react like unrelated and distinct compounds. On the other hand, when one kind of carbohydrate substance is joined with two different proteins, the compounds lose their specificity, so that it is impossible to distinguish one from another by means of the serum test. The role of these unique substances in the routine life of bacteria is perhaps only another example of their dual nature. Like "split personalities" found in human society, one part of the microbes, the carbohydrate, tends to make them "queer" and the other, the protein, to give them the appearance and behavior of the common herd.

Antidote for Sugar-coated Poison

The pneumonia germ is certainly aggressive and formidable because of its sugar-coating. Phagocytes in the blood find these pills too hard to swallow because there is something in the sugar that acts like a poison and at

the same time unites with protective antibodies in the blood stream to prevent their mobilizing against the infection. There are definite reasons why the capsular portion of pneumococci is a most powerful offensive and defensive weapon. In its situation on the outside of the organism, it makes the first contact with natural or artificially developed antibodies circulating in the blood. Furthermore, as Heidelberger and Avery discovered, the human body does not manufacture a ferment or enzyme capable of dissolving the capsular material or disrupting the complex sugar contained within it. In fact they learned from experience how difficult it is to decompose this carbohydrate by known chemical methods.

With these observations as a guide, it seemed highly probable that pneumococci might be attacked successfully by means of some enzyme capable of destroying the protective covering of the germs and thus making them digestible morsels for scavenger cells in the infected body. Where to look for the mysterious agent was a problem indeed. Doctor Avery had with him at this time a young assistant, René Dubos, who had received preliminary training at the Institute of Agronomy in Paris and later at the New Jersey Experiment Station under Professor Lipman, the elder of two renowned brothers. Doctor Dubos, who was accordingly thoroughly grounded in microbiology of the soil, knew where to search for the enzyme.

To begin with, these investigators had a definite idea in mind as to the chemical similarity between the specific pneumococcus capsule and certain products found in the decomposed framework of most plant structures or their cells. Here the basic substance is cellulose (found also in linen and cotton wool), a complex carbohydrate which is broken down by chemical disintegration into molecules of grape-sugar. It would be necessary, therefore, to select from the teeming population of microbes found in the soil a species that is known to attack cellulose or the substances entering into its composition. A limited number of

bacteria having this unusual property had already been described in 1899 by a Russian bacteriologist.

How many acres of soil were dug and examined bacteriologically is not known, but after much painstaking work the long and arduous quest of Dubos and Avery was finally rewarded. In 1930 they succeeded in isolating from the peat bogs of New Jersey a breed of micro-organism belonging to a family of cellulose-splitting bacteria. This microbe produced a chemical ferment or enzyme which attacked the specific carbohydrate substance of pneumococcus Type III, so that a trace added to a vigorous culture of pneumonia germs in the test tube prevented the usual development of capsules. Mice and rabbits experimentally infected with pneumonia recovered after an injection of the enzyme, which, it was also found, made healthy mice so immune that they could withstand enough deadly pneumonia germs to kill a million normal animals.

Spurred on by these discoveries, other investigators joined in the pursuit. Three years later, from Doctor Wadsworth's laboratory in the New York State Department of Health, came the announcement that soil bacteria had been found which could produce an enzyme to decompose the specific carbohydrates of pneumococcus Types I and II.

The difficulties involved in isolating this enzyme for practical use brought out some interesting facts regarding the manner in which the peat bog bacillus manufactures its specific ferment. Secretion of this active substance is affected by conditions prevailing in the culture medium, where the living organism has to compete with its own enzyme for the available food supply. An indispensable ingredient of the culture medium is the Type III pneumococcus carbohydrate, for without this capsular substance or a product derived from it, no specific enzyme can be obtained. It is inevitable that at certain times the free enzyme and undecomposed sugar will be present together. When this occurs, growth of the organisms is

interfered with as their carbohydrate food is progressively used up and more enzyme in turn is liberated from cells that are rapidly dying of starvation. Ultimately a vicious circle results and all activity ceases.

To meet this difficult situation, a culture medium is provided containing a small amount of yeast extract and the cultures are incubated in the presence of an increased air supply. Under these conditions, growth of the microbes is rapid enough to insure complete decomposition of the capsular sugar and at the same time to check the supply of enzyme released from dissolved microbial cells. This illustrates a curious biological phenomenon suggesting an emergency reaction to a situation in which the bacterial cell will attack a less favorable source of energy when no other food supply is available.

There has been much speculation as to the probable connection between specific carbohydrate substance and virulence of micro-organisms. While its significance in the actual mechanism of infection and resistance is not precisely understood, certain evidence links the capsular material with invasive properties. We find, for instance, that mice will succumb to a fatal infection when inoculated with a harmless pneumococcus culture that has been mixed with a small amount of carbohydrate substance. Its apparent effect is to give to avirulent organisms an infective power associated ordinarily with capsulated strains. These changes can be shown in another way by means of ingenious experiments devised by Doctor Griffith in England. If we allow the virulent capsulated pneumonia germs to grow in specific antiserum prepared by first injecting them into animals, the virulent organisms are transformed into an avirulent strain. This new form in the process of change loses its surrounding capsule and simultaneously its usual property of clumping in the corresponding antiserum. Chemical analysis of the avirulent organism will show that it still retains the common protein which is characteristic of all pneumococcal types, but not

the carbohydrate (capsule) substance which gives identity to the type from which it has been derived. Loss of microbial virulence by means of induced variation therefore involves the loss of a chemical structure responsible for type specificity. This is another biological demonstration of the fundamental laws of chemical immunology investigated by Landsteiner.

Now the question naturally arises whether one specific type of pneumococcus can be converted into another under the influence of substances present in the latter. A second exploit by Griffith in 1928 is even more striking than his earlier one in showing for the first time a transmutation of this kind. If mice are inoculated with living avirulent organisms of Type I, together with *killed cultures* of virulent Type II, we can recover from the animals deadly germs of the second type! In similar fashion a harmless Type II can be converted into a fully virulent Type I, and avirulent Types I and II likewise are changed into active Type III. Each of the newly developed types acquires capsules in the process and will now react specifically with the corresponding type antiserum.

The significance of this discovery goes much beyond a simple relation between capsular material and virulence. Out of it there has come a new concept of bacterial variation and its possible bearing upon infectious disease in the human body. In substance the facts indicate that specific bacterial types are not invariably constant and can under certain conditions be transmuted into other types. Although the essential nature of this transformation is not clearly understood, it implies the starting up of inheritable changes in microbes, with the result that successive generations reproduce the cause of this change along with the specific carbohydrate material which is originally absent. It is not yet possible to estimate the danger to man of this offensive weapon, but there can be no doubt that it has given to pathogenic organisms a decided advantage over their hosts.

Fighting Fire with Fire

By unearthing a most unique method of fighting germs of disease with harmless varieties found in the soil, Avery and his associates introduced a new kind of biochemical therapy. That it was destined to find important practical application in the treatment of pneumonia and of infectious diseases in general has now been realized. Today there is hardly a group of living micro-organisms that does not boast of several members capable of producing substances of microbial origin which destroy disease-producing organisms. These substances are known as *antibiotics*. In 1939, when Dubos assembled his observations and studies made over a period of ten years, *Bacterium brevis* or *Bacillus brevis* was being highlighted in the news as a source of "germ-killing" substance especially active against staphylococci, streptococci, and pneumococci. These organisms, it will be remembered, have a peculiar selective staining reaction to certain bacterial dyestuffs, identified with the name of Gram, who first described the method, and are therefore known to bacteriologists as "Gram-positive." In memory of Gram, and because of its high antibacterial activity against Gram-positive microbes, this new antibiotic was first named "gramicidin." Later studies showed that this substance contained a mixture of antibiotic substances, one of which, called "tyrocidine," was active against Gram-negative and Gram-positive micro-organisms. Renamed "tyrothricin," the antibiotic is now being widely used in controlling wound infections of skin and bone, certain diseases of the eye, ear, nose, throat, and nasal sinuses, and infected body cavities. Unfortunately, its use is limited to local applications by various methods and is dangerous when brought into the circulation either directly or indirectly through an area communicating with the blood stream. For tyrothricin, while highly active against bacteria in such an exceedingly minute amount as one thousandth of a milligram, is also capable of destroying red blood corpuscles.

To Be Taken Internally

As a further development of this new line of investigation, attention has been directed in a prominent way to other sugar-coated villains found in cholera, plague, and tuberculosis. Nothing was known of the chemical composition of the cholera germ until Landsteiner and Levine in 1927 investigated its specific carbohydrate substance and isolated from it a complex sugar-like material chemically similar to that found in pneumonia germs.

The scene now shifts to India, where cholera, flourishing since remote antiquity, has been accepted as casually as many other blessings bestowed by the river Ganges. At Calcutta for a number of years the All-India Institute of Hygiene and Public Health has been making a detailed study of the chemical constituents of cholera germs to learn what effect these substances may have upon virulence and variation of strains. One of the first things to be discovered was a chemical difference in the specific substance present in pathogenic and in non-pathogenic cholera-like vibrios that are found in water. In the former there are two carbohydrates; one, known as galactose, which can be derived from ordinary milk-sugar (lactose) by chemical decomposition, occurs regularly; another, found infrequently, is a peculiar gum-sugar closely allied to gum arabic, and known as arabinose. In the non-pathogenic strains arabinose alone is present, a fact of unusual interest in pointing out that a close relationship exists between cholera germs and similar non-pathogenic types found in waters in regions where the disease is always present.

A number of other surprises developed in the course of this work, as chemical studies gradually disclosed certain peculiarities in the make-up of these cholera vibrios. Daughter colonies arising from a parent strain frequently contain protein and carbohydrate constituents that differ from those of their ancestors. In the course of this transformation, an avirulent strain develops a new kind of protein material that is found ordinarily in harmless water

vibrios and, in addition to this change, a remarkable type of specific carbohydrate, presumably glucose alone, makes its appearance. So we find that cholera germs are indeed far from the stable types Japanese investigators have thought them to be. Not only does their polysaccharide content vary, but they change from one chemical type to another with disconcerting irregularity. A breed of organisms containing galactose may be replaced by one containing glucose, and after six or more weeks of growth in test tube cultures the new variety will return to its original state.

Largely through some work done in Japan in 1913, it was quite generally believed that an "epidemic" or virulent breed of microbes was associated with violent outbreaks whereas an avirulent variety was present in commonplace scattered infections. More recently, at the Shanghai Science Institute, an unusual opportunity arose to test the validity of this theory in the course of a cholera epidemic in Shanghai in 1932, when more than four thousand cases occurred during the spring and summer months. From abundant material several kinds of organisms were isolated and classified on the basis of their ability to agglutinate or clump in serums prepared with authentic types of cholera vibrios. By this method the Japanese investigators identified two distinct varieties, one conforming to the virulent or "epidemic" type and another corresponding to the avirulent or "variant." Curiously, the virulent type was seldom encountered in this outbreak, for it was found that most of the strains during the early period and even a higher percentage of strains during the middle and final periods belonged to the "variant" group. From these observations, it was inferred that "there is apparently no possibility of the transformation of one type into another"—a surprising deduction in view of the immediate facts which suggested that the occurrence of two dissimilar varieties of an organism in a widespread epidemic could reasonably be due to microbial variation.

Changed virulence and variation of cholera vibrios seem to depend, as in pneumococci, upon the nature and type of sugar present. Its role in cholera, while more problematical than in pneumonia, has been regarded similarly as one of interference with defences of the body in the intestinal tract. In this situation where the disease is localized, the germs would ordinarily be dissolved unless protected in some manner from destructive agents mobilized against them. A strong case appears to have been made out for the carbohydrate substances in cholera germs by analogy, perhaps, but not without good reason for suspecting their guilt.

Meanwhile the Calcutta scientists are engaged in extracting in a pure state all the different polysaccharides thus far identified in these microbial cells. Working with known substances, they hope to learn eventually how these influence the course and development of cholera in the human body. Whether or not preventive vaccines and curative serums are likely to result from these studies will depend largely upon a successful application of modern bacteriological methods, which have on other occasions conclusively shown that, so far as microbes are concerned, the part can be more important than the whole.

The typhoid organism, like the cholera vibrio, has also yielded a specific "sugar," which Landsteiner and Fürth in 1928 found was the most important of three distinct antibody-producing portions contained in the bacterial cell, endowing it with highly characteristic properties. At some future day, perhaps, travellers on their way to places where cholera and typhoid germs lurk to trap the unwary may be found leisurely sipping vaccines instead of having them injected under the skin. The idea of vaccination by mouth against intestinal bacteria is of course not new, having been suggested in typhoid fever as a logical and quick approach to the site where the microbe thrives. This method, however, did not always work out according to plan because the juices in the stomach generally weakened

or destroyed the vaccine before it could do any good. A strictly modern vaccine would contain nothing but the purest active principle in solution, flavored to taste and sweetened by its own specific carbohydrate substance—to be taken internally before meals.

A microbe as notoriously virulent as the *Pasteurella pestis* of plague might have been the first to reveal the secret of bacterial capsules. In a paper read before the Bombay Medical Society in April, 1907, an English doctor gave an interesting account of some physical and chemical properties of pestis germs that had been grown at high and low temperatures with a remarkable effect upon the character of their growth. Above 100 degrees Fahrenheit, the stickiness of the organisms contrasted sharply with the type of growth below 80 degrees. When seen under a microscope, the organisms grown at high temperatures were virtually all capsule and little or no body substance, while those cultivated at low temperatures had hardly any capsule. In the whole collection of plague cultures, the virulent strains alone exhibited this wealth of glutinous material at higher temperatures, whereas the avirulent showed none at all.

Having a particular bearing upon this point was the fact that a certain culture, which had been extraordinarily virulent for animals six months before, had now lost its infective power and at the same time its capsular material. All the evidence suggested that this substance developing from the bacilli and causing their stickiness was also an indicator of their virulence. These considerations led the doctor to test the efficacy of plague vaccines prepared from sticky cultures by "salting out" the glutinous material with strong solutions of ordinary table salt. When thus freed of bacterial residue, a "purified" capsular substance protected vaccinated guinea pigs against doses of plague which were surely fatal for untreated control animals.

It is odd that a few early contributors to this subject anticipated present-day applications of their discoveries

without making much progress in their own particular field. Whatever the reasons may be for the lag, our knowledge of the chemical composition of pestis organisms has not kept pace with recent developments in bacteriological control of other diseases, and with passing years all these observations have not lost their newness. Loss of capsule and of virulence are in a general sense still interchangeable terms and the capsular material also holds its ground as the essential part of bacterial armor.

Pasteurella pestis, however, has been modernized with the rest of the microbes. It is now recognized as having two antibody-producing substances—a cell body portion and another contained in the gelatinous envelope. This capsular part is the essence of an efficient vaccine and gives to the organism its specific character. By chemically separating the envelope or capsule from the bacillary framework, it has been possible to prepare antiserums with each part, so that capsulated pestis organisms are attacked only by serum from animals immunized with capsular substance and are not affected by the antiserum prepared with cell substance alone. In other words, the invasive power of the plague germs, which depends upon an outer coating of specific substance, can be checked only by attacking that highly specialized portion which acts independently of the remainder. A specific carbohydrate is doubtless "just around the corner."

A Parthian Weapon

Perhaps it is truer for tuberculosis than for any other germ disease that to seek something new is to exhume buried remains and clothe them with new raiment. Not only have modern methods of treating the disease shown a tendency to reincarnate old ideas, but a similar trend has also appeared in experimental work on the nature of the tubercle bacillus and its products. Constructive archaeology—for such it might be called—often serves a useful purpose in digging up the good out of the past and showing

how science can make a step forward by going backwards.

The organism responsible for tuberculosis has continued for years to keep well ahead of its pursuers, who have been weighted down with "cures." Equipped with a tough constitution, the microbe can resist environmental forces which ordinarily make short shrift of other bacteria. A waxy coat of armor, enveloping its body, gives to the tubercle bacillus an astounding power of resistance and imperviousness towards all known destructive agents. In part at least, this peculiarity has accounted for many unsuccessful attempts to study immunological phenomena.

More recent developments have been pointing the way to biological methods of attacking this problem with the aid of reinforcements from chemistry. But before materials could be supplied upon which to base future studies, it was necessary to put the microbe to work for its own destruction. Koch, as we have seen, made a good beginning in this direction with tuberculin, which is a concentrated mixture of by-products, consisting of bacterial cells and their contents and substances given off during several weeks of growth. These products may be toxic materials derived from broken-down cells or the result of bacterial action upon the nutritive substances in the culture medium furnishing the food supply, usually a kind of bouillon prepared from beef or veal extract containing a variable quantity of glycerine.

What happens in a flask of culture medium is only part of a more complicated series of events taking place in a person suffering with tuberculosis. As tuberculin is released into the blood stream of one who is infected, all the tissues become charged with the substance and are made hypersensitive to it. The abnormal changes set up in the body can be recognized by an inflammatory reaction of the skin when minute amounts of tuberculin are brought into contact with it either by injection or mere rubbing. Skin tests have therefore been very helpful in detecting the disease during its early stages and in studying the workings of susceptibility and resistance.

In these respects tuberculosis is quite unique in its outward expression of complicated reactions taking place within the body. The physician is made aware of them as he observes the varied forms which the disease takes in different persons and its changeable course in an individual case. When the tubercle bacillus has finally overwhelmed all bodily defences, the tuberculin test evokes no further response from exhausted tissues. For with this disease to react is to live and to live is to react, and with truth it might be said, in the words of a modern writer, that "to be absolutely still and unresponsive is a virtue only of the dead."

There were obvious shortcomings in a method that depended upon the use of a material as complex as tuberculin. Since the object of the skin test was to determine the presence of a specific infection on the basis of an inflammatory reaction or reddening of the injected area, it was important to exclude all possible causes of false reactions. In the mixture representing products of tubercle bacilli, there was much stuff which irritated the skin and modified its capacity to react, often producing local changes that had nothing to do with a similar effect evoked by genuine tuberculosis. In order to avoid misleading reactions of this kind, it was necessary to devise some method of obtaining a purified tuberculin substance for the test. By analogy with bacterial composition, certain portions of tuberculin were expected to have important properties which were not exhibited by the material as a whole. Stating this again in another way as a well known simple chemical law, the characteristics of individual parts isolated from a compound may be strikingly different from a mixture of these. Water, for example, composed of hydrogen and oxygen, will quench fire, yet hydrogen burns to form water in the presence of oxygen which kindles the flame.

Many fruitful investigations had been devoted to the chemical composition of tubercle bacilli long before similar studies were made of culture mediums in which they had been grown. Separation in a pure form of active tubercu-

lin components obviously was impossible unless the products of bacterial growth could be obtained free from foreign proteins and other substances unrelated to the tubercle bacillus itself. Since it was known from past experience that the organism flourished in nutrient solutions containing simple mineral salts, attention was now directed to the manufacture of tuberculins from a variety of artificial culture mediums. Some of these recipes dated back as far as 1894 and modifications which appeared in print during the ensuing thirty years differed principally in a somewhat altered content of citric, phosphoric, potassium, sodium, calcium, or ammonium salts. From such food mediums with a known and fixed combination of ingredients, the products of actively growing tubercle bacilli were readily isolated by chemical means and then purified. These preparations were remarkably complex and could be further subdivided into a number of unique parts or "fractions" having a definite chemical composition and specific properties.

Knowledge of the mechanism and meaning of tuberculin reactions was nevertheless in a somewhat chaotic state. On every hand experiments with fractions of tuberculin and tubercle bacilli raised many controversial issues. It had been thought for many years that a positive tuberculin test did not occur unless there was present a state of exaggerated sensitiveness ("allergy") which had developed from an existing infection. This point was brought into question in 1926 by one investigator at the University of California Medical School, who succeeded in producing typical skin reactions in non-tuberculous animals by repeated injections of tuberculin substances manufactured in a non-protein synthetic culture medium. In these experiments it was shown for the first time that a condition of hypersusceptibility ("allergy") could be developed artificially and demonstrated by positive tuberculin tests with the fractions as well as with ordinary preparations of tuberculin. Furthermore, normal animals which had be-

come "allergic" by this method, responded to an inoculation of tubercle bacilli in the identical manner as animals that had been previously infected and then inoculated with the germs a second time.

These skin reactions were in every respect characteristic of a reinfected tuberculized tissue; that is to say, a prompt local inflammation was followed by early formation of the tubercle, which came rapidly to a standstill and later healed completely. On the basis of differences observed in these experiments with the use of various chemical preparations of tuberculin, it was suggested that certain fractions of tuberculin contained active ingredients which might prove valuable in the diagnosis and treatment of tuberculosis. Similar substances had been described a few years earlier by a number of European and American workers, although they left unsolved the fundamental question as to whether sensitization can occur without previous tubercle bacillus infection. This fact was established in 1926 and confirmed by Doctor Seibert at the University of Chicago five years later.

Tuberculosis research has made greater advances during the past decades than for any other comparable period, perhaps because the magnitude of many unsolved problems indicated the need of a national program. To meet the situation, the National Tuberculosis Association inaugurated a cooperative research plan, which in scope and thoroughness has set exemplary standards for scientific investigations on an unprecedented scale. With all the available resources and personnel of some of the largest educational institutions and commercial laboratories in the United States at its command, it was inevitable that this type of organization must succeed where isolated discoveries, however brilliant, might fall short of their desired aims. Professors Johnson, Anderson, and their associates at Yale University, Long and Seibert, formerly at the University of Chicago and later at the Phipps Institute in Philadelphia, a Rockefeller Institute group including Doctors

Sabin and Doan—and many others too numerous to mention—have made noteworthy contributions.

Tuberculins by the barrel and tubercle germs by the ton are now being subjected to endless chemical treatment in the hope of prying loose the secret of this microbe's stalwart defence against the best that science can offer. More than ten years of work had led to the discovery in tubercle germs of a number of compounds with peculiar properties. One of the most interesting substances occurs in the waxy coat and makes up one-third or more of the weight of dried organisms. From ten pounds of tuberculosis germs extracted with a liberal mixture of ether and alcohol, one can obtain twenty ounces of wax, ten ounces of fat, and ten ounces of fat-like material containing phosphoric acid and sugar. To this last compound, perhaps the most extraordinary of the numerous and varied substances conjured out of tubercle bacilli, the name phosphosucride or phosphatide has been given.

All these portions of the microbe have been turned over to Doctor Sabin and her associates at the Rockefeller Institute for testing in experimental animals. It is their task to find out what relation may exist between the properties of these substances and the various ways in which tuberculosis expresses itself in the human and animal body. The fatty portion of the phosphosucride, we are told, is responsible for the development of the tubercle, a kind of tissue that is characteristic of tuberculosis, and contrariwise may retard the progress of abnormal changes which the substance itself has incited. The glucose and sugar acid it contains appear to be very poisonous for tuberculous but not for normal animals when injected into the abdominal cavity, acting in this regard like the protein material found in tuberculin and in the bacterial cell. The sugar-like substances, however, do not take any part in forming tubercular tissue, which is stimulated only by certain fatty acids found in tubercle germs.

A picture of the disease can now be graphically recon-

structed piece by piece in a series of events caused by these specific chemical substances. From the wealth of information on all the things that the tuberculosis germ can do and how it contrives to do them, we should be able to devise some method of striking back at the microbe. The phosphosucride, as we have learned, has the capacity both to fight by producing harmful tubercular tissue and to retreat before the antibodies which are developed by itself while in the act of attacking its host. To what extent this Parthian weapon may prove useful in immunity reactions and defences of the body is still an open question.

From present indications, however, it would seem that the microbe might be attacked by controlling the tissue changes which result from its presence in the host. But the problem is complicated by the fact that certain cells that are piled up in the tissues to form tubercles contain living bacilli, whereas other cells take part in walling off these menacing structures. The tubercle, accordingly, has a latent capacity for doing damage in a varying degree, proportional to the kind and number of different cells that are mobilized in an infected area. On this hypothesis, the phosphosucride has been tested for its possible effect upon the numerical relationship of cells concerned with defensive tissue reactions. Actually it happens that resistance to the spread of infection is presumably increased when a certain type of cell becomes more numerous following injections of the phosphosucride directly into the tissues. Such protection is measured experimentally by the length of time animals will survive an infective dose of tubercle germs. A critical analysis of these results, however, has led to the unfortunate conclusion that the disease cannot be controlled entirely in this way.

On theoretical grounds these studies hold out great promise, yet it is clear that immunity towards tuberculosis involves many factors not fully understood. The phosphosucride itself, regarded at one time as the most useful substance for developing antibodies, has given conflicting

results. In a number of instances, preliminary treatment, designed to protect animals from a subsequently induced infection, lowers their resistance to such an extent that they succumb to even more extensive disease than is seen in untreated control animals. It is difficult, however, to assess the protective value of any substance when tested under conditions such that the size of an infecting dose and the virulence of organisms greatly influence the course and probable outcome of the disease.

In view of its peculiar behavior in the animal body, a number of interesting experiments with the microbial phosphosucride suggest themselves. Conceivably this substance might become a more effective antibody-producer when combined with certain specific proteins obtained from the tubercle bacillus, particularly since these products, by first increasing the sensitiveness of tissues, might be expected to make them more resistant according to the accepted laws of immunity. From facts available at present, the phosphosucride alone does not appear to be able to accomplish this. Its nature is probably that of a haptene or partial antigen, as Laidlaw and Dudley in England, and Zinsser in this country, pointed out in 1925. They were among the first, incidentally, to isolate from the tuberculosis germ a similar carbohydrate substance in the form of a non-protein gum, which by itself does not give rise to antibodies when inoculated into animal tissues, but does react in the test tube when combined with the corresponding antiserum.

Shortly after Professor Anderson brought out his purified phosphosucride (phosphatide) in 1927, Doctor Pinner at the Desert Sanatorium in Tucson, Arizona, demonstrated its capacity to develop specific antibodies when injected into the blood stream of animals. If this substance, judged by its behavior, is a genuine Landsteiner haptene, which in the nature of things it appears to be, then as a further hypothesis a chemical combination of some sort might make it effective not only as an immunizing agent but as a chemical remedy.

Seventy-five years ago, an illustrious scientist remarked with his customary gravity and humor that the great tragedy of science was the slaying of a beautiful hypothesis by an ugly fact. What Thomas Huxley said then will always be true, but today we must continue nevertheless to seek beautiful hypotheses with which to slay ugly facts. Tuberculosis and many other plagues still remain to excite the imagination and to remind us of the frailty of thought. Perhaps it would be better to follow the suggestion of a renowned contemporary of Huxley, the great scientist John Hunter, who told his students not to think so much as to *try*.

If epigrams could simplify problems, then the riddle of tuberculosis might well have been solved during all the years of trying and creative thinking since first these words were written.

With the advent of sulfa drugs came renewed hopes that an antibacterial approach to tuberculosis was at hand. Beginning in 1938 a feverish search for some chemical weapon against the tubercle bacillus centered on the sulfanilamide group and its derivatives. One of these, named *Promin,* among the many compounds studied, proved to be unusually effective in guinea pig experiments. The results were so much better by far than all earlier attempts at chemical therapy that, during the next five years of productive research, hundreds of patients with various types of tuberculous disease were being treated in a number of institutions. By all standards of what is regarded as improvement in this disease, the results obtained were considered remarkable. As a "cure," however, Promin did not come up to expectations, for several reasons. A main drawback was the toxic effects of the drug in proportion to the amount that could be used with safety. Another serious difficulty, although not unexpected with sulfa drugs in general, or antibiotic substances, was the tolerance developed by the tubercle bacillus toward the drug during treatment. This well-recognized defence of micro-organisms against destructive chemical agents is thus double-

barreled in its action. As the microbial resistance increases, requiring larger doses, the safety factor is correspondingly reduced and the drug becomes dangerous.

Aside from this, accurate evaluation of tests with Promin could not be made. Experimental trial, as in the case of a new therapeutic agent, demands rigid control of all variable factors that might tend to influence the results of treatment. In tuberculosis, more so than in any other disease, such factors have not been excluded to meet the basic requirements of sound experimentation. A majority of patients often improve or get well by resting in bed and accepting the approved methods of tuberculosis therapy. There are also those who show excellent progress when they are under the impression that a new "wonder drug" is being tried, even though this is being withheld from them as a necessary check on purely imaginary successes. Whatever the reasons, Promin was found to be unpredictable in its action; in some cases progressive disease appeared to be retarded or reversed, while in others, despite intensive therapy, the results were either unfavorable or patients became worse.

At the present time the drug can be regarded not as a substitute for the accepted treatment but as a possible aid. In the animal and human body, Promin does not and cannot behave as a bactericidal drug in tuberculosis.

Two highly important conclusions resulting from these investigations emphasize first, that the disease in its early stages appears most promising for therapy; second, other approved methods of treatment should not be omitted. At this point we have travelled full circle, back to the starting place of the tubercle bacillus.

CHAPTER 7

MICROBIAL CIVIL WARFARE—ANTIBIOTICS

Penicillin—A Brush with Death

A chance observation with untold possibilities often lies buried for many years and forgotten. This is literally true of penicillin, a product of one of the commonest molds found in the soil. As far back as 1824, a German botanist, Link, described such plant-like organisms and gave to them the name Penicillium, from the Latin word *penicillus*—a brush—because of the appearance of the fruiting parts of the mold. Since that time hundreds of varieties of molds have been found in the soil, to a depth of slightly more than twelve inches. In going back to the early history of bacteriology, in Pasteur's time, it is mentioned repeatedly that the soil appears to have some purifying action causing the disappearance of buried organic matter and also of disease-producing germs such as the typhoid, staphylococcus, and similar microbes. The reason for this was not known, although students of bacteriology attributed the action to unfavorable conditions present beneath the soil. However, this phenomenon did not escape the notice of Pasteur who, as early as 1877, observed that air-borne micro-organisms which contaminated his cultures of anthrax bacilli interfered with their growth. He noted also that when the anthrax bacillus, mixed with other micro-organisms which are in themselves inoffensive, is inoculated into animals, anthrax does not develop and they remain well.

For historical purposes it should be remembered that penicillin was not an overnight discovery. It has long been known that microbes have their own private wars in the midst of mutually beneficial relationships designed for

survival. One of the earliest clues along this line was supplied by Emmerich and Loew in 1899 when they announced the isolation of a potent antimicrobial substance, "pyocyanin" (also "pyocyanase") obtained from an organism known as *Bacillus pyocyaneus,* now called *Pseudomonas aeruginosa,* found in association with greenish-colored pus in wounds or infected tissues. However, attempts to use this "pyocyanin" in treatment failed. It is interesting to consider here what might have resulted from a more helpful attitude than was shown toward this work by leading scientists of that day, notably Metchnikoff. He opposed strongly all attempts, however seductive, to attribute to bacterial products a share in the elaboration of antimicrobial substances. It was better, he stated, to confess ignorance of the chemical nature of these substances in general—this despite convincing experimental evidence to the contrary. For, even ten years before Emmerich, a number of other investigators had confirmed Pasteur's observations by means of experiments, which showed that various saprophytic organisms, including *B. pyocyaneus,* as well as their products, were antagonistic toward the anthrax bacillus.

To be sure, there was no malice in all this on Metchnikoff's part. He was simply defending his monumental contribution to the cellular theory of immunity—the role of phagocytes in stimulating resistance to infectious diseases. As he expressed it, direct action may also intervene, as in the case of the pyocyaneus organism, but this is not indispensable in animals whose phagocytes require no added help in arresting infection.

Many years have been devoted to the careful study of products given off by molds. Notable work published in the early nineteen hundred and twenties by Raistrick, in particular, has revealed the important role of the so-called antibiotic (*against life*) substances which play a part in

the defensive machinery of many types of molds. In penicillin, nature has given us one of its own antibacterial agents produced by molds themselves. It is an interesting fact that such a promising line of attack upon microbes had not been seriously thought of, despite the highly suggestive contributions of Doctor Alexander Fleming's predecessors and his contemporaries, for during this period, also, investigators were tackling the same fundamental problem in practical applications of "bacteriophage" and "antivirus" to bacterial infections.

In 1939, while the entire world was clamoring for the newly exploited "wonder-drug," the sulfonamides, Doctor Dubos aroused tremendous interest in antibacterial substances with his announcement of the isolation of gramicidin, obtained from a microbe which he found in the soil. This was the culmination of work which he had started ten years previously in his search for a soil bacterium that would dissolve the lethal capsular substance of the pneumonia germ. Gramicidin was extremely effective against bacteria, but unfortunately proved to be too toxic for the patient when administered by routes most likely to insure success.

It required the urgency of another war to awaken renewed interest in these promising studies. At this time the forgotten work of Fleming came into the picture.

The unfolding of an exciting drama enacted in a bacteriological laboratory was first reported by Doctor Alexander Fleming in 1929. The drama took place in the course of daily routine work at St. Mary's Hospital in London. On the surface of a culture medium prepared for the growth of staphylococci which he was studying, he observed in the midst of the colonies of growing organisms a contaminating mold. This was not at all remarkable because such molds are common nuisances to the bacteriologist in his efforts to maintain pure cultures of microbes. This mold, an accidental contaminant from the air, was not unlike those found on bread, cheese, or other foodstuffs,

and might not have attracted attention but for one very striking thing. In the neighborhood of the mold, the bacterial colonies had become transparent and undergone dissolution. It occurred to Doctor Fleming that the material given off by the mold prevented bacterial growth by flowing into the surrounding area, thus accounting for the clear zone in which no organism could survive. He used this observation in his experimental work in an attempt to obtain pure strains of organisms free from unwanted invaders, such as are frequently encountered in material obtained from patients. By growing the mold in a fluid culture medium prepared from broth, he demonstrated that the mysterious antibacterial substance was easily dissolved in water and that filtered broth cultures contained this active substance.

Fleming gave to the active material the name penicillin, since it was derived from the mold known as Penicillium. He suggested the use of this bactericidal agent for holding down the growth of contaminating organisms and went so far as to say that the material might be useful as an antiseptic in localized or systemic infections caused by organisms which are susceptible to the activity of the penicillin.

It was not until 1940 that the curative possibilities of this remarkable agent were recognized. At the University of Oxford, Chain and Florey and a group of investigators were able to isolate the active principle of penicillin in the form of a crude brown powder easily dissolved in water. The extraordinary facts emerging from this work showed that the material was virtually non-toxic for laboratory animals and had proved to be entirely successful in a limited number of human infections.

In the following year Professor Florey visited the United States under the auspices of the Rockefeller Foundation, and a program was devised for large-scale production and clinical investigation. In this work the National Research Council, the United States Department of Agriculture, and a number of pharmaceutical concerns collaborated. The

monumental work of the United States Office of Scientific Research and Development and of numerous agencies of the National Research Council is an everlasting tribute to co-operative scientific enterprise and allied unity.

Penicillin is now well established as an antibacterial agent with a remarkably wide range of usefulness, including the treatment of venereal diseases such as gonorrhea and syphilis, gas-gangrene, and sundry wartime infections. For most diseases which respond to sulfonamide treatment, penicillin is equally effective, and in some which resist the sulfa drugs it often succeeds when all else fails. It has certain other advantages over the sulfonamides because the activity of penicillin against microbes is not interfered with by pus and broken-down tissue products found in wounds and infected body cavities.

New sources of penicillin are already being discovered and exploited in the world of molds and bacteria. Already antibiotic substances are being tested against arch-enemies like the *Mycobacterium tuberculosis* and other tough microbes, with a certain amount of success on a purely experimental and modest scale. But there is a snag in this finely woven pattern of the microbe's challenge and our war against disease germs. Successful cures with antibacterial substances like penicillin, no less than that of the recently proclaimed sulfonamide "miracle drugs," depend upon more or less intimate contact of the therapeutic agent with the organisms in the body. Another equally important requirement is that the microbes must be susceptible or sensitive to the action of the penicillin substance.

In one instance, we might be dealing with a disease in which organisms are circulating in the blood stream as in blood poisoning (septicemia) or are localized in accessible organs or tissues where the germs are exposed to attack. There are other fatal conditions, however, where the organism, while susceptible to penicillin, cannot be reached by it. To illustrate, there are certain bacterial infections of the heart in which the small valves, although provided with

a rich blood supply and bathed in the blood stream, are not easily accessible to the circulating antibacterial substance. In such cases streptococcal organisms, embedded well beneath the surface of the tissue, break away only now and then in showers into the blood stream. Here their growth is checked by the penicillin, provided it is present in sufficient quantity, and the infective agent is susceptible to the action.

Various laboratory methods have been devised for testing the sensitivity of culpable microbes isolated from infected persons. A simple and reliable procedure imitates the first observations made by Fleming as he studied the behavior of molds growing in company with micro-organisms. Small glass tubes, pressed into the surface of a jelled agar foodstuff in a dish, are filled with various dilutions of penicillin. After suitable incubation periods, the test organisms will grow abundantly except in the resulting clear circular spaces of different diameters surrounding the tubes. Here the penicillin has passed into the agar jelly and prevented bacterial growth. Thus, it is quite simple to measure the antibacterial strength of penicillin, and at the same time determine the degree of resistance or sensitivity on the part of certain microbes to various doses of the test material.

The mystery surrounding penicillin-resistant microbes has not yet been solved, and until this is clarified, the usefulness of antibiotic substances must be limited. Touching upon this problem early in their developmental work with penicillin, the London investigators, Abraham and Chain, first reported the finding in certain intestinal bacteria of an agent which destroys penicillin. This substance they called penicillinase, because it partook of the nature of an enzyme. Unfortunately, the property of nullifying the activity of penicillin was found to reside not only in bacteria known to be resistant, but also in those which were susceptible to penicillin. In the United States, Doctor Kolmer and associates have amplified and extended these studies and shown

that the sensitivity of organisms to penicillin does not depend alone upon inability to produce penicillinase. Similarly, the acquired property of elaborating penicillinase is not necessarily related to the ability of bacteria to develop resistance to penicillin.

There is a long list of microbes yet to be conquered among the many die-hards that resist penicillin therapy. To these must be added diseases caused by viruses and rickettsial organisms. In hopeful contemplation of an unending war against microbes, we cannot ignore the limitations always imposed upon a newly developed weapon of science.

Penicillin is only one of many antibacterial substances known to be produced by microscopic organisms existing in the world of molds, fungi, and bacteria. There will, no doubt, be many more such in the future, and one cannot foretell which antibiotic, whether discovered first or last, will prove to be the best.

Already seventy-five or more antibiotic agents have been isolated, but only a few have been used in treatment because of toxic effects, which outweigh the extraordinary successful results obtained in test tube and animal experiments. Among the more promising antibiotics, aside from penicillin, gramicidin (tyrothricin), and streptomycin, are: *streptothricin,* derived from a fungus, acting on Gram-positive and Gram-negative organisms, and useful in the treatment of typhoid fever and bacillary dysentery; *subtilin,* produced by *Bacillus subtilis,* commonly known as the "hay bacillus," which is found on hay, straw, and in soil and dust, is very active against tubercle bacilli, and checks the growth of Gram-positive organisms including anthrax bacilli, pneumococci (Type III and sulfa-resistant strains), Gram-negative organisms such as the gonococcus, and certain fungi. Metchnikoff, a half century ago, and others before him, observed that cultures of *B. subtilis* inhibited the growth of anthrax and other organisms. Viewed in retrospect, his interpretation of this remarkable event seems

to have postponed the recognition and use of antibiotics for a later generation. Subtilin has aroused more than passing interest because of its lethal effect upon the tubercle bacillus, which was first reported in a French publication in 1926. Recent studies from laboratories in the United States point to this antibiotic as one to be reckoned with among other worthy competitors. In addition to subtilin, the *Bacillus subtilis* group of organisms has yielded several antibiotics. One of these, named *bacillin,* is not only as effective as subtilin against staphylococci but also inhibits the growth of paratyphoid, typhoid, and similar organisms; the other, *bacitracin,* is especially active against many organisms which are not affected by penicillin.

Under experimentation are other antibiotics produced by common soil fungi and molds classified under the names of Actinomyces and Aspergillus. Some of these have antibiotic properties similar to penicillin but are more toxic, others are notably active (in the test tube at least) against tuberculosis.

Of historical interest because of its origin is *penicillic acid,* discovered and reported in 1912 by Doctor Carl Alsberg of the United States Department of Agriculture while studying maize spoilage due to a mold, *Penicillium puberulum.* The material, then described as a new complex organic acid, was obtained from cultures of Penicillium grown in corn meal and was found to be toxic for guinea pigs and white mice. This product, recently named puberulic acid, and now included among the antibiotic substances, has weak antibacterial activity toward Gram-positive organisms.

The search for new antibiotic agents continues in the hope that other and better substances will be found. However attractive those now available may be, they are limited in usefulness because they do not affect certain important disease-producing micro-organisms or, when they do so, cannot be used with safety because severe toxic effects are produced at the same time. Satisfactory antibiotics must combine a high level of antibacterial activity with a mini-

mum of toxicity—necessary criteria for any drug or form of treatment designed to cure a disease without killing the patient.

Already the quest for new sources of antibiotic substances has led to greener fields. In addition to the fungi, including Actinomyces and related forms, and bacteria, the juices of a number of plants have been examined for antibiotic activity. "*Tomatin,*" isolated recently from the common tomato plant, is one of these new antibacterial agents. Its effectiveness against organisms that cause wilt disease of tomatoes, peas and cabbage does not stop with ailments due to plant fungi alone. Most striking is the antibiotic activity of tomatin against certain parasitic fungi responsible for infections in man. These are, among others, "athlete's foot," ringworm of the scalp or skin, eczema, and similar afflictions which do not respond to treatment with sulfa drugs, penicillin, or streptomycin. The possibility of extending the use of this plant antibiotic to human and animal fungus diseases must await further experimentation, which is now in progress.

Streptomycin—Underground Feuds

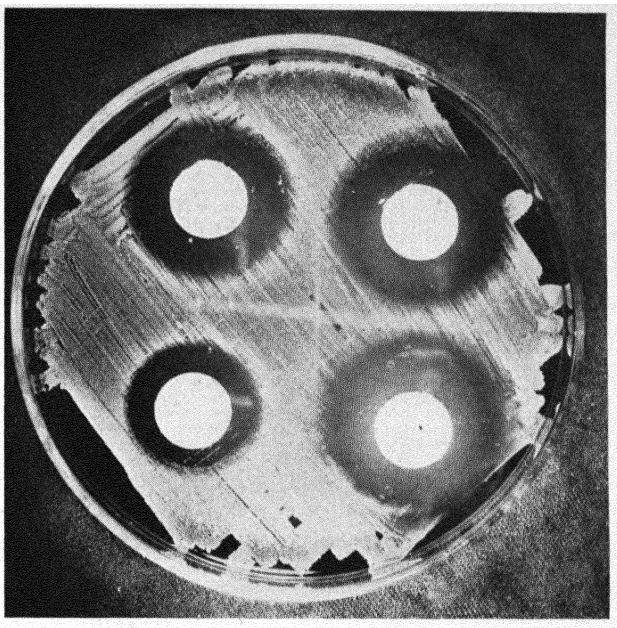
In the search for chemical antibacterial substances that might be suitable for general use, the hope of developing an antituberculosis agent has not been abandoned for one moment. Aside from penicillin, which has a much wider range of activity than most antibiotics, streptothricin alone began where all the others left off, until overshadowed by streptomycin, its highly publicized successor. Both were isolated by Selman A. Waksman from molds found in the soil—streptothricin in 1940, and streptomycin four years later, from a micro-organism called Actinomyces, meaning "ray fungus," which describes its ray-like filaments as seen under the microscope. It is now known as Streptomyces, and the antibiotic substance derived from it, and effective against the tuberculosis organism, has been named streptomycin.

A kinship of a sort exists among these fungi and molds

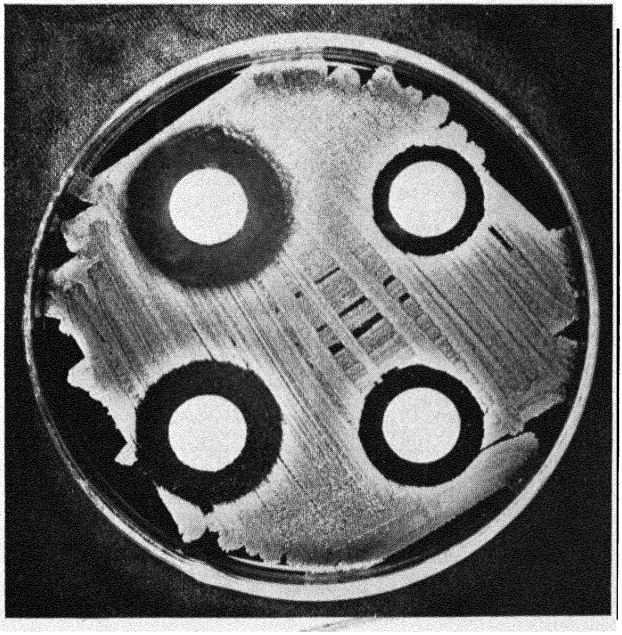
of the soil, for they belong to a group of micro-organisms that are related to the mycobacteria (fungus-bacteria) which include the tubercle bacillus and its rather complicated family tree. It might be interesting if not factual to think of this phenomenon as an illustration of hereditary quarrels between families, with results more beneficial perhaps than sporadic feuds. Was it pure accident to find, as Waksman did in 1915, the first strains of his now well-known streptomycin-producing organisms in heavily manured soil and in a chicken's throat? Or, was Vaudremer merely guessing when, several years before this event, he treated a number of tuberculous patients successfully with extracts of a mold, *Aspergillus fumigatus,* which is also native to the soil, manure, and compost? There would appear to be more than chance in the relation of antibiotic substances to the causative microbial agents attacked, both as to their origin and to the antibacterial effects observed. One need but examine the multitude of tuberculosis strains found in man, cattle, birds, snakes, frogs, and fishes, and the related harmless varieties isolated from timothy grass, hay, or butter, to appreciate the complexity of this problem.

A comparison of the antibacterial activity of streptomycin and streptothricin, closely related as they are, suggests that, with the exception of a few organisms, both antibiotics exhibit identical properties in test tube experiments. In the animal and human body, however, streptomycin seems to have an advantage over streptothricin in being less toxic and in having greater activity against certain micro-organisms. This matter of toxicity is causing much concern and cannot be dismissed lightly from the point of view of practical use in human tuberculosis; the more so as in this disease, unlike acute infections, the drug must be given over a long period of time.

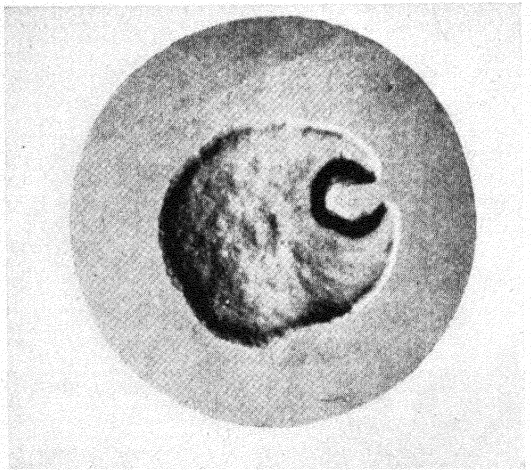
Compared with Promin, streptomycin can be used in doses nearly ten times as large, and like Promin, its value in the treatment of tuberculosis is that of a useful auxiliary.



Action of Streptomycin on Intestinal Organisms—Colon Bacilli. Clear zones show areas where growth is prevented.



Effect of Penicillin on Staphylococcus. Clear zones surrounding paper pads saturated with varying amounts of penicillin indicate absence of microbial growth.



A Colony of Bacteria Partially Dissolved by Bacteriophage (magnified 12 times natural size). The hollowed-out portion within the irregular circle of microbial colony growth represents dissolved bacteria that have been destroyed by the bacteriophage. Material taken from this clear zone will produce similar transparent areas in other colonies of the same breed of germs

It suppresses, with the aid of natural defensive weapons, a considerable proportion of certain forms of the disease, while in other types such as meningitis, striking improvements have been reported. Emphasis is placed upon the fact that the application of known methods of treatment can often be highly effective, even when streptomycin is not. For such is the nature of the disease that destructive changes produced in the organs of the body cannot be reached by drugs. Added to the difficulties there is the bugbear of "drug resistance" or "drug fastness," whereby a strain of tubercle bacilli in the body develops a tolerance toward streptomycin while the patient is being treated. Disquieting reports from research study units indicate that streptomycin-resistant organisms make an appearance in approximately two thirds of patients after a period of from two to three months of treatment. This is a serious threat to the general use of streptomycin, and poses the difficult problem of knowing how often and at what stage of the treatment such resistance appears. Only after a matter of weeks, by growing cultures of the tubercle bacilli isolated from the patient and then testing the organisms for their sensitivity to streptomycin, can this be determined. If they have become resistant to the drug, further treatment is useless in these patients as there are good reasons for believing that resistance has become fixed and the patients made refractory to streptomycin therapy. In a manner of speaking, it is a contest between the antibiotic substance and intractable strains of tubercle bacilli, which multiply and spread beyond control. No less disturbing are certain instances of intractable tuberculosis already known to have been transmitted from such persons by contact infection.

Considering the energy and thought now being devoted to the search for a relatively harmless antituberculosis drug, something should come out of Pandora's hope chest. Perhaps newer and better antibiotics will be found, or a mutually helpful combination of two, such as streptomycin and Promin, will accomplish what neither one can by itself.

In theory and practice, it is a matter of greatest importance that the antibiotic substance be kept at a sufficiently high and safe level during the course of treatment. If this is not done, the originally stubborn micro-organisms, tubercle bacilli in this instance, are left free to multiply and produce unbroken lines of resistant generations. From our past experience with combined penicillin-streptomycin therapy or the use of sulfonamides and penicillin together, it is known that resistance to any one of these drugs does not necessarily influence its sensitivity to the other. On this basis it is possible to stop the multiplication of drug-resistant bacteria by means of smaller and safer doses of each antibiotic when it is combined with another.

Streptomycin, while perhaps being talked about too much as a tuberculosis remedy, has proved its undeniable value in the treatment and control of many bacterial infections. While most of these are not at all influenced by any other known antibiotic substances, some have responded equally well to penicillin and other antibiotics. For the treatment of typhoid, paratyphoid, and dysentery, chronic infections of the urinary system, tularemia, whooping cough, "influenza"—all organisms, incidentally, known as Gram-negative—streptomycin has been found effective to a remarkable degree.

The problem of bacterial resistance is so intertwined with the use of antibiotic substances that it cannot be regarded as less important than antibiotic research itself. In fact, until the chances for survival of intractable organisms are brought under control, we shall be defeating our purpose. Here again is a challenge of the microbe as it turns its natural defenses into a stronger attack upon our borrowed weapons.

CHAPTER 8

THE BORDERLAND—ANIMATE OR LIFELESS

Every science has its borderland where known or visible things merge with the unknown and invisible. In this hazy uncharted realm of speculation objects loom grotesquely large, as in a dense fog. Their importance, nevertheless, cannot be ignored, however much they deceive our senses, for from this borderland startling discoveries occasionally emerge.

Bacteriology during recent years has been exploring the mysterious fields outside the range of microscopic visibility. Here we are not so sure of the actual rôle played by some agents, which up to this time have been implicated in certain infections. In particular, there is a group of diseases caused by the so-called "filterable viruses," which have stirred up a controversy as to whether these are living organisms or lifeless protein substances.

Nobody seems to know what viruses are, although they have been defined and talked about voluminously for nearly fifty years. Certain descriptive terms established by usage have only added to the existing confusion and are now fortunately being discarded. It is not necessary, for instance, to speak of viruses as "filterable," since this property of passing through fine porcelain or clay filters is an arbitrarily chosen standard of comparison with the smallest known bacteria, which are unable to pass through pores of a certain size. There are, moreover, filterable organisms which cannot in any sense be regarded as viruses. In fact, filterability has no natural limit and depends merely upon the construction of the filter and the size of the particles which it is intended either to hold back or allow to pass.

"Ultramicroscopic" and "ultravisible" are other terms that have been commonly associated with viruses to emphasize their minuteness beyond the range of ordinary microscopic visibility. However, these descriptive names are no longer acceptable since improvements in microscopic technic have made it possible to see and even photograph certain viruses. At the National Institute of Medical Research in London, Doctors Barnard and Elford succeeded in photographing in 1931 the virus causing a peculiar disease in mice, known as infectious ectromelia (*"loss of limbs"*), and, in 1933, the viruses of canary-pox and Borna's disease or encephalomyelitis (*inflammation of the brain and spinal cord*) of horses.

The technical innovations which led to this important achievement depend upon the use of invisible ultraviolet light rays from a mercury vapor quartz lamp as a source of illumination and a suitable arrangement of focussing lenses made of quartz, through which material these extremely short light waves can pass. With this method, particles smaller than 1/500,000th part of an inch and well beyond the range of visibility of any known microbial species have been demonstrated. This microscope operates on the principle which is crudely illustrated by a sunbeam piercing a darkened room and creating a brilliant shaft of light resulting from the illumination of myriad dust particles floating in space. Technically this device is known as "dark-field microscopy" and is a familiar example of the "Tyndall phenomenon," named after its discoverer, John Tyndall, who in Pasteur's time proved that microbes, like dust, were present in the atmosphere.

As Big As Life

How "big" is life—or how small? According to popular legend, the bulk of philosophic talk should be inversely related to knowledge of the subject. It is thus not surprising that the study of viruses, which are both the smallest and least known incitants of disease, immediately brought

up the question of whether or not they are living agents and thus transformed staid bacteriologists into philosophers searching for the origin and evolution of life.

While there is no denying that a topic dealing with life itself or with the boundary between inert and living matter has its fascinating side, the time has not yet arrived to permit one to distinguish between poetry and prosaic facts. With these reservations in mind, we shall perhaps obtain a better perspective of viruses from a consideration of their nature and known properties, and of their behavior in the tissues of man, animal, and plant.

Towards the close of the last century, an explanation of the origin of certain diseases was sought in living organisms, which even the best microscopes failed to make visible. The existence of these mysterious viruses was first discovered in connection with a disease of tobacco, in which mottling of the leaves occurred quite characteristically like a mosaic pattern and suggested the name mosaic disease. In 1892, a Russian scientist, Iwanowski, transmitted the disease to healthy plants by inoculating them with fresh juice obtained from sick plants or with this material after it had been filtered through exceedingly fine stone filters. When Beijerinck, a Dutch scientist, repeated the experiments four years later and found that the juice did not lose its infective properties even after passage through dense porcelain filters and agar jelly, he concluded that the disease-producing agent must be a contagious living fluid substance rather than something having definite solid form. This idea was erroneous, of course, since it was found afterwards that agar jelly did not differ from any other type of filter except in the smaller size of its pores.

Before the excitement attending these discoveries had time to subside, there came from Germany in 1898 a report of Loeffler and Frosch's work on hoof-and-mouth disease, which they attributed to an invisible filter-passing virus. It caused a severe blistering afflicting not only cattle, but sheep, goats, and pigs, and less frequently horses, cats, dogs,

and birds, and rarely deer and camels fell a prey to sweeping epidemics. More significant than its confirmation of the observations with mosaic disease was the proof given by this newly discovered infection that an unseen agent, however minute, could be transmitted from sick to healthy animals through ordinary contact. Experimentally, by inoculating the merest trace of virus that had been passed through porcelain filters, it was also possible to infect in susceptible animals an unlimited series from one to the next. For these reasons Loeffler and Frosch felt justified in believing the infective agent to be a living organism capable of reproducing itself in a suitable host.

As time went on, a long list of diseases was added to those which are now recognized as virus infections. More than seventy principal diseases affecting man and lower animals, and an unknown number found in over a thousand breeds and varieties of plants, have been linked with viruses. Even our most important cultivated crops are involved to an extent that has not yet been determined. In the plant kingdom, mosaic diseases represent a large group including, apart from the virus affecting tobacco, mosaic of peach, sugar cane, tomato, potato, cabbage, lettuce, and many other vegetables including spinach.

Among the better known human ailments caused by a virus are smallpox, chickenpox, poliomyelitis (infantile paralysis), rabies (hydrophobia), yellow fever, psittacosis (parrot pneumonia), dengue fever (dandy or breakbone fever), foot-and-mouth disease, epidemic influenza, mumps, the common cold, warts, epidemic encephalitis (sleeping sickness), and even the common garden variety cold sore or fever blister and shingles.

Lower animals get their share of foot-and-mouth disease, hydrophobia, hog cholera, swine influenza, distemper, sundry varieties of pox, louping ill of sheep, which is common in Scotland, Rift Valley fever, also affecting sheep in British East Africa, Borna's disease or encephalomyelitis of horses, encephalitis of foxes (inflammation of brain), sali-

vary gland disease of guinea pigs, and a number of unclassified virus infections occurring in rabbits and mice.

Insects also are not exempt and although some of our worst pests are from time to time subject to sweeping epidemics, this form of natural control falls short of accomplishing a much desired end. The gypsy moth, European moth, and tent caterpillar are susceptible to a peculiar "wilt" caused by a virus resembling a many-sided geometric figure or polyhedron, from which the name polyhedral disease has originated. Unfortunately this organism also attacks silkworms and is responsible for widespread damage in an important industry. Among honeybees likewise is found another kind of devastating virus infection that confines itself to the larvae or worms in a brood and reduces them to a hollow shell or sac. The name sacbrood given to this disease describes adequately what occurs in the brood comb.

In a field of investigation that has been growing more and more complex as knowledge concerning it accumulates, there are nevertheless three characteristics generally accepted as associated with disease-producing viruses which set them apart from all other well-known types of bacterial infection. First, they are much smaller than ordinary microbes. Secondly, they do not appear to be capable of multiplying in laboratory culture media except in the presence of living cells or tissues. And lastly, in certain diseases they produce characteristic changes in the infected cells known as "inclusion bodies," resulting either from the presence of the virus itself or from its peculiar activity.

One need not assume, however, that these three criteria are not subject to change should newer methods of study be developed. In particular, with regard to cultivation of viruses on lifeless media, it is conceivable that a time will come when the essential ingredients of living tissue may be made available in the test tube, just as many finicky bacteria have yielded already to artificial laboratory persuasion. The present viewpoint clings to the idea of a

virus as a strict parasite so intimately related to the living cell that existence apart from it is impossible. This question cannot be settled until we discover just what happens in a cell during the fleeting interval before and after death occurs. We know nothing whatever about this.

A Matter of Life and Death

Whether viruses are animate or inanimate is perhaps the most important issue at the moment; for while the question might appear to be academic, it has a practical bearing upon the modern conception of disease. Some investigators have been leaning towards the idea that certain viruses cannot be living things because their size would hardly permit the carrying on of those functions which are recognized as vital. In this class, to which belong several viruses, is that of foot-and-mouth disease, which according to accurate measurements is approximately ten millimicra in diameter. A millimicron is the millionth part of a millimeter, and one millimeter represents about one twenty-fifth of an inch. The cause of mosaic disease of tobacco is said to be only three times as large. Other viruses, among which might be mentioned poliomyelitis, rabies, fowl-plague, cowpox, and encephalitis, are probably considerably smaller. Such viruses would have to be magnified a thousand or more times in order to make them comparable in size to a germ like the one that causes typhoid fever.

Applying a yardstick to living things might lead somewhere if it could be shown that size had anything to do with the characteristic qualities recognized in what is alive as opposed to what is dead. Who can say how large or how small that "something" really is which causes adaptation to environment, the absorption of food so that it becomes an integral part of the original cell and similar to it in structure—otherwise known as assimilation—and the reproduction or multiplication of its kind? These are the recognized properties of living matter and because disease-

producing viruses are known to behave in all these respects like living things, it is assumed they are alive.

Nevertheless, even these criteria of life have been called into question recently, since it is now claimed that the virus causing mosaic disease of tobacco is a chemical substance capable of transmitting infection to healthy plants. From enormous quantities of crushed tobacco leaves, a crystalline protein has been extracted, which, from a chemist's standpoint, is actually lifeless, yet does all that living things are supposed to do, even reproducing itself in some mysterious fashion. To explain the strange behavior of this inanimate agent it is described as a substance which requires the presence of living cells for its multiplication. In a susceptible plant host, this newly discovered "protein-virus" is regarded as the result of abnormal changes within the "infected" cells, rather than one of reproduction in the manner of microbes.

The infecting agent of tobacco mosaic is assumed to be a chemical compound because no way has yet been found of separating its disease-producing activity from the protein substance with which it is associated. In the living cells of plant tissue the behavior of such a virus substance might be compared with the effect of a spoiled apple upon a barrelful of sound fruit. This would be another way of saying what a distinguished scientist, in referring to the nature of viruses, described as "products of cellular perversion capable of inciting similar perversions in other cells."

It has been implied that many or perhaps all other virus diseases are of this nature and from these studies it might be inferred that the gap between living and dead matter has been closed at last. However, reasoning from chemical analogy is a far cry from an established fact. While there is always the possibility of having mistaken a result of sickness for the cause in tobacco mosaic, it would be straining our credulity to accept a similar explanation in certain virus diseases, where according to this chemical

theory, their cause and effect would have to be the same thing. How a "virus protein" goes about the business of breaking down living cells into an unlimited number of reproducible units like itself has incidentally not been revealed.

The question next arises as to how an "infecting" chemical agent can account for so many virus diseases which differ strikingly from one another, although investigators who are occupied chiefly with the study of certain viruses in the laboratory believe that they have many features in common. Emphasis has been placed, perhaps disproportionately, upon the fact that viruses are peculiarly attracted to the susceptible cells in a host and are invariably closely associated with them. This point of view, while adequately describing certain similarities which cannot be seen with the naked eye, ignores gross differences that do not have to be magnified. Excepting the common factor of a virus as the cause, what resemblance does hydrophobia, for example, bear to yellow fever, or infantile paralysis to influenza; psittacosis (parrot fever) to warts; hog cholera to herpes (cold sores); or smallpox to any of these diseases? What strange chemistry is this that will produce such kaleidoscopic changes following mere contact of a mysterious agent with the susceptible cells of human or animal tissues? How can a lifeless chemical substance do so many different things to living cells and make them permanently resistant to future attacks of the same diseases? We must, however, grant that the viruses have many properties of living things; also that some viruses, at least, have a relatively simple crystalline chemical structure. It would appear that the distinction between the living and the lifeless state is really a meaningless one and depends, perhaps, upon one's definition.

Arguments for and against inanimate chemical viruses can be found to support either side of the debate. The weight of evidence, however, seems to favor those who believe infectious diseases are caused by living agents. If

adaptation alone is considered, it is very difficult indeed to disregard this common characteristic of parasites. In smallpox, for example, a highly contagious disease that is spread from person to person, the virus can be toned down by passing it through calves. As a result, a disease is produced which is quite different from smallpox, yet sufficiently like it to cause an immunity towards smallpox when inoculated into man and animals.

Still another point in favor of viruses as living agents is the fact that in some diseases they must "ripen," so to speak, in the bodies of specific insects before becoming infective for a human host. In this manner yellow fever and dengue fever, for example, are carried only by a species of mosquito. In the plant kingdom, also, while insects may act merely as mechanical carriers, we know that the viruses causing "curly top" of beets, corn "streak," potato "leafroll" and "yellows" of asters go through a period of incubation in an insect. It is a curious fact, not explainable by chemistry, that the leaf-hopper should transmit this last disease rather than the peach aphid, for instance, which is responsible for a large variety of mosaic diseases. Significance must also be attached to the fact that certain virus diseases are carried by insects for long periods of time without losing their infective power for susceptible plants. To make things even more complicated, a virus causing spinach blight is dormant in a species of aphid, in which the disease can be transmitted through the fourth generation.

There is also strong evidence pointing to the existence of carriers among apparently healthy plants. In what is probably the first record of its kind, there was reported in 1925 a "ringspot" or "mottle" virus of the potato capable of infecting related plants and producing streak disease of tomatoes. Quite recently (1937) a hidden virus has been described which is widely distributed among bulb-grown lilies and causes "tulip-breaking," sometimes referred to as the "oldest known plant virus disease," the

earliest record being said to have appeared as far back as the sixteenth century. In the experiments just reported, this virus is shown to be identical with one responsible for removing the color of tulips, a characteristic change associated with "tulip-breaking" disease.

A Cell for Life

Let us for the time being assume that viruses are parasites and think of them as extremely small units of life. Because they do not have the bulk of ordinary microbes, viruses must get along without a chemical laboratory for converting food materials into something that they can use. This need is supplied by the living cells of their host, which in the course of their own activities furnish both board and lodging to the parasite. Having gained entrance into the tissues, a virus sets in motion a series of events which make up the picture of some definite disease. Intimate association with susceptible host cells thus becomes indispensable for its survival and reproduction and hence no unusual explanation is required of this relationship.

In the human body, viruses differ strikingly in their manner of affecting individual cells, thereby accounting for certain abnormalities that serve to distinguish individual diseases. The significance of these changes was first emphasized many years ago by Doctor Thomas Rivers of the Rockefeller Institute, who pointed out their importance in this connection. Stated briefly, the virus parasite may exert its effect upon a cell with explosive rapidity or with leisurely calm, depending upon the kind of cells that are attacked. In certain regions of the body, injury to cells may be followed by an extremely rapid growth of the damaged part, as in the familiar scarring which follows cuts or puncture wounds in the skin. Similarly, a virus disease can also cause overgrowth of tissue cells and form warts, as an expression of abnormal restoration in the affected area. On the other hand, different viruses, like those causing chickenpox and smallpox or foot-and-

mouth disease, can destroy the skin tissue and produce blisters or pustules. In the long run, whether cell growth is stimulated or retarded will depend obviously upon the kind of virus.

What happens in the skin is not necessarily true in the nervous system because the cells found there are unable to multiply and regenerate following an injury. When important nerve cells are destroyed, the lines of communication between nerves and muscles are cut as by the blowing out of a fuse, which in this case cannot be replaced. Thus it is evident why in diseases like hydrophobia and infantile paralysis the viruses, because of their location, cause more destructive effects, such as paralysis.

The major part of the knowledge about the behavior of viruses has been acquired in the past by studying them in a susceptible host or experimental animal. From these sources, however, as will be shown later, only general information was gathered. A more efficient device was needed to observe directly what goes on in the cells when they are attacked by a virus. This want was supplied by the development of the method of "tissue culture," which made viruses "visible" by their activities, even though the agents themselves in most cases remained hidden from sight.

By "tissue culture" is understood a method of cultivating or keeping alive outside the animal body a collection of cells or, more specifically, a fragment of living tissues such as nerve fiber, skin, brain, bone marrow, or heart. The idea originated with Professor Ross G. Harrison of Yale University, who in 1906 worked out the simple procedure of placing in a drop of blood plasma (colorless part of blood after the corpuscles have been removed) a bit of nerve fiber and observing its development under the microscope. A few years later, a brilliant young student of his, Doctor Montrose T. Burrows, carried on similar work with individual cells taken from the heart muscle and was able to maintain them alive and beating rhythmically in an apparatus containing a solution of

nutrient food materials. These he renewed continually by ingenious contrivances designed to remove waste products and other substances that were likely to interfere with normal growth of tissues. When still in his early twenties, Burrows became associated for a few years with Doctor Alexis Carrel at the Rockefeller Institute, where the technic of tissue culture was developed as a fine art bordering on a state of perfection. In the hands of Carrel and his colleagues during a period of twenty-five years, this method has led to important advances in surgery and medicine.

Application of tissue cultivation to the study of viruses simplified the problem of bringing them into closer relationship with the living cell, upon which they depended for propagation. Some investigators, with the early crude methods then in use, accomplished this purpose by planting infected tissues in a drop of blood plasma. In 1913, at the Pasteur Institute in Paris, Professor Levaditi showed that infantile paralysis virus contained in a bit of spinal cord retained all its infective power after several weeks and similarly, the virus of hydrophobia in rabbit brain survived more than one month in blood plasma from monkeys. From such observations it was learned that certain viruses could be kept alive and active for a long time.

Vaccine from "Artificial Calves"

Important as these facts were, they shed no light upon the question of whether or not survival was in any way associated with actual multiplication of virus in these tissues. A new practical turn was given to this problem in 1923 by Frederick Parker and Robert Nye, bacteriologists at the Harvard Medical School. They cultivated in plasma bits of rabbit tissue previously inoculated with smallpox vaccine virus and maintained their growth through successive generations. From time to time samples of this material were withdrawn and tested to see if the

contained virus had remained active and multiplied. It was found that the amount of vaccine in the eleventh generation of tissue culture was more than fifty thousand times greater than in the first.

These experiments suggested interesting possibilities of preparing more or less purified virus to be used in vaccinating against smallpox. The time-honored method of preparing such vaccine on the scrupulously clean skin of calves has proved its unquestionable worth for many years, but notwithstanding the extraordinary care and skill exercised in its commercial manufacture, there is a deeply rooted prejudice in the minds of many persons against inoculating animal substance into the human body. On scientific grounds, moreover, certain objections to vaccination need to be overcome by obtaining absolute purity of the virus and freedom from unpleasant incidents, which, though extremely rare, may be avoided by using some other method of preparation.

Search for a safe and practical method of artificial cultivation of the vaccine virus has consequently been undertaken by a number of investigators. As happens frequently, scattered observations had an important practical bearing upon the nature of this problem and its eventual solution. In 1927, Doctor Howard Andervont, a young biologist at the Harvard University School of Public Health, found that inoculations with vaccine virus "took" more easily in newly hatched than in full grown chicks. During the same year, Carrel and Rivers utilized a method based upon a similar principle and in 1930 Rivers modified another procedure, which had originated in England two years earlier, and successfully manufactured vaccine virus in flasks containing a pulp made of various portions of nine to twelve day old chick embryos or the whole embryo. The minced tissue was first suspended in a sterilized solution made by dissolving a quantity of table salt, some phosphates and carbonates, salts of magnesium, potash, lime, and glucose in water, which imitates quite

closely the chemical requirements of growing tissue in the animal body. To the mixture was next added an emulsion of active virus and the flasks incubated at body temperature for five days. Cultures were carried on through successive generations by simply transferring some of the material to flasks of fresh solution and tissue.

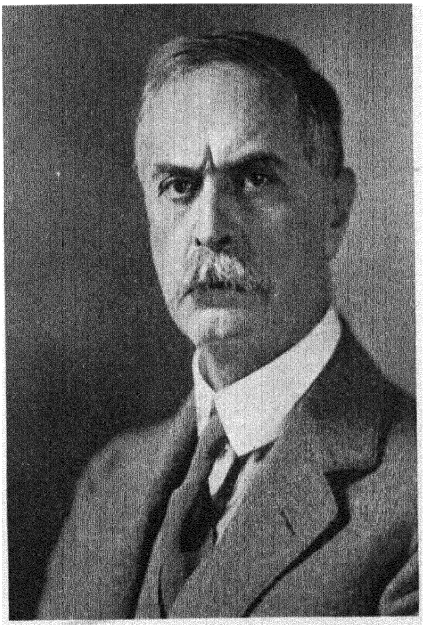
In this manner vaccine virus could be cultivated for an indefinite number of passages and after three years, during which one hundred and thirty successive transfers had been made, it remained active. When placed on the skin, such virus, even though diluted one part in a million, produced a typical vaccination "take." For man this culture vaccine proved to be safe and effective in over one thousand trials, as judged by every known device for testing the protective value of vaccination against smallpox. Now that this has been accomplished, modern bacteriology can point to the invention of an "artificial calf," which is kept in a small glass container and produces as much vaccine as a stall-fed animal. Doctor Rivers, with his customary reserve and extreme caution, let his case rest with the hope that the method might be used more extensively to determine its value.

"*Hatching*" *Viruses*

Rivers' procedure, while it left little to be desired, had several objectionable features. In due course of time, following successive transplants of the virus culture from generation to generation, its vaccinating power became weaker and had to be rejuvenated. This was best accomplished by inoculating rabbits with the vaccine and recovering it from these susceptible animals, but the method was undesirable because rabbits were known to harbor unidentified hidden viruses which might be united in some mysterious way with vaccine through its activity in the nervous system. In fact, Rivers himself, with Tillett in 1923, had described a new virus disease of rabbits causing inflammation of the brain (*encephalitis* or *sleeping sick-*

A Japanese bacteriologist and pupil of Doctor Simon Flexner. The study of spirochetal infections was one of Noguchi's specialties. He contributed many important researches on syphilis and infectious jaundice or Weil's Disease, and during his last years was in Africa working on the problem of yellow fever. He thought he had found the germ of this disease and named the microbe Leptospira icteroides ("slim spiral causing jaundice"). But this was a mistake. The microbe Noguchi saw was not the cause of yellow fever, as he supposed, but of infectious jaundice. He came to a tragic end in Africa, where he contracted yellow fever and died.

HIDEYO NOGUCHI
(1876–1928)

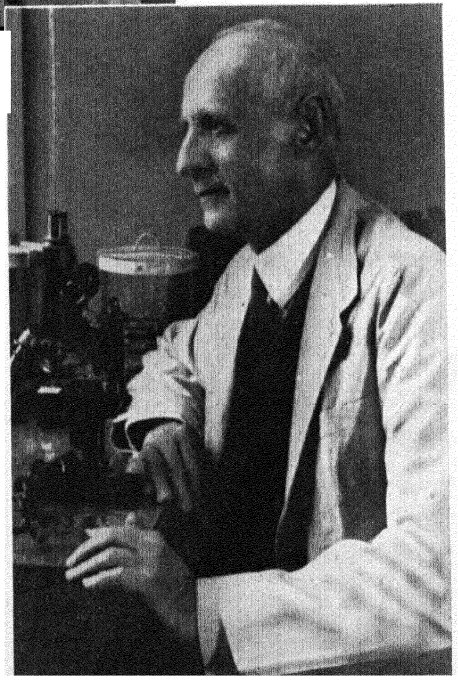


Accomplished the first successful transfer of infantile paralysis to monkeys in 1909. On the basis of Landsteiner's fundamental researches in immunity and his philosophic approach to many difficult problems of this nature, his name can be added to the group including Ehrlich, Metchnikoff, and Bordet. In 1930, Doctor Landsteiner was awarded the Nobel Prize for medicine.

KARL LANDSTEINER
(1868–1943)

THOMAS M. RIVERS
(1888–)

In the field of virus diseases Doctor Rivers is pre-eminent as an investigator and thinker. He is best known for his work in growing viruses in artificial culture of tissues and experiments with vaccines produced by this method.



SIMON FLEXNER
(1863–1946)

Although best known for his discovery of a curative serum in the treatment of epidemic meningitis and many valuable contributions in infantile paralysis research, Doctor Flexner was an outstanding example of the rarest combination in science—the gift of investigative ability coupled with unparalleled administrative capacity. As director of the Rockefeller Institute for Medical Research from 1903 until his recent retirement from active leadership, he set a standard of medical research for the entire world.

ness). This discovery, which was made incidental to attempts at transmitting chickenpox to rabbits, showed clearly how a dormant virus infection might be awakened following an inoculation with some other disease-producing agent. It was soon found that natural carriers of this so-called "virus III" were quite common among rabbits in the United States, England, and Switzerland. The virus is not transmissible to man but it can at times cause in rabbits an encephalitis resembling a disease produced in man by the virus of herpes or fever blisters.

In addition to the dangers attending the passage of vaccine virus cultures through susceptible animals, another obstacle was encountered in maintaining vaccines free from contamination with microbes. Filtering the coarse material through porcelain or earthen filters was difficult and other methods of removing undesirable bacteria, short of destroying the vaccine, proved unsatisfactory.

This problem was solved by two investigators at the Vanderbilt University Medical School in Nashville, Tennessee, who furnished a simple and effective technic for cultivating vaccine virus in a pure state and preserving its virulence without the intervention of passage through animals. Doctors Woodruff and Goodpasture, first working with fowl-pox in 1931, introduced the ingenious method, invented by Professor Levaditi of the Pasteur Institute, of growing a virus in fertile hen-eggs, and later successfully propagated anti-smallpox vaccine on the surface of incubating chick embryos ten to twelve days old. Through a little window flap made by breaking the shell, virus was injected with a hypodermic needle into the exposed area, which could be identified readily by a "blood spot" indicating the future chick enveloped in its transparent covering. This membrane served as a living tissue culture for virus that usually can be inoculated the first time without risk of bacterial contamination. In order to make doubly sure, however, Woodruff and Goodpasture ground up the membrane, filtered it through a porcelain

filter, and in this way obtained a sterile vaccine, which they used for seeding chick embryos in a successive series at intervals of a few days.

During the fifteen months that such virus was carried through eighty-five generations, it remained uniformly active. Tested upon experimental animals, the chick vaccine behaved in essential details like calf vaccine and in man proved just as effective and reliable, yet had none of its alleged disadvantages. Compared with ordinary vaccine, the new preparations, when grown either in tissue cultures or in chick embryos, caused milder reactions and less scarring on the skin.

As the work with vaccine virus progressed, it was obvious at every turn that "tissue culture" methods could be applied more generally to the study of all known viruses. In certain diseases, the virus was gradually adapted to the artificial environment by a preliminary sojourn in animals that were known to be susceptible. Accordingly, within a short time the viruses of encephalitis, poliomyelitis, influenza, the common cold, rabies, and yellow fever were cultivated in tissues outside the body. As this was but a step removed from the method of growing them in living embryos, the new procedure was promptly adopted.

Hatching viruses from a developing hen's egg has become quite popular since Woodruff and Goodpasture showed how it could be accomplished. In England, Wilson Smith (1935), and, in Sydney, F. M. Burnet (1936) both have successfully propagated human influenza virus by this rediscovered method, after passing the virus through ferrets previous to cultivation in chick embryos. More recently in this country Dochez and his associates at Columbia University, New York, trapped the virus of the common cold in a more direct manner, although they had already grown it five years before in the conventional minced embryo tissue.

The latest exploit was staged with the help of two volunteers, who allowed themselves to be inoculated by

experts instead of by careless fellow passengers in the New York subways. The "cold" virus was obtained from the nasal washings of a patient suffering with a full-blown head cold. The material, having been filtered through tightly pressed layers of sterilized fine asbestos, was freed of all bacteria and a few drops promptly deposited on the membrane of a developing chick-embryo twelve days old. In two or three days its transparent covering began to show opaque spots where the virus was "taking root." The membrane was now removed, ground up in a small amount of bouillon, and a few drops of the diluted mixture inoculated into a second set of hen eggs. From these another transfer was made in the same manner to a third set, which supplied the material used for infecting human volunteers.

After three such passages of diluted virus through chick-embryos, it was safely assumed that active multiplication and not mechanical carrying over of the original virus had occurred. The crucial test was made upon two volunteers who had been kept under quarantine for one week in the meantime. Some of the diluted virus culture was inoculated directly into their nasal passages and they returned to a detention ward for careful observation. That same night things began to happen. Their throats became reddened and sore, eyes and nose poured out watery discharges, and they coughed, sneezed, and spluttered. For the next two days things became worse until the fifth day, when all signs of a head cold disappeared and the men recovered. Of such stuff are heroes made. The virus of "common colds" had been cultivated and successfully transmitted to man.

These experiments were the first to utilize the method of growing any virus from a patient directly in the developing hen egg without preliminary cultivation in bottled tissues or passage through a susceptible animal. Within a few months a similar triumph over human influenza virus was announced by Doctors Thomas Francis

and Thomas Magill of the International Health Division in the Rockefeller Foundation. They collected rinsings from the throats of several patients acutely sick with influenza and prepared the material in the particular manner prescribed for cultivating a virus. First it was whirled rapidly for half an hour in a centrifuge (a more elaborate type of cream separator) to throw down heavy particles along with unwanted microbe intruders. Then the top fluid layer was passed through a filter made of collodion ("new skin") with pores averaging in size 1/2,000th of a millimeter (one millimeter equals 1/25th of an inch). With a minute drop of this crystal clear liquid developing chick embryos twelve days old were inoculated and at four day intervals new sets of eggs were seeded with material taken from the preceding ones. After a few such passages from egg to egg, influenza virus had multiplied sufficiently to induce the typical disease in artificially infected ferrets and to a lesser extent in mice.

In these as in other examples already given, adaptation stands out as a characteristic trait of viruses. They cling tenaciously to parasitic habits associated with living things, yet at the same time keep their identity as specific causes of disease. Life in bottled embryonic tissue or in a developing hen egg does not appear to have any noticeable effect upon their ability to attack susceptible animals or human hosts in which these viruses had their origin. This is strikingly illustrated in the behavior of yellow fever virus, already cultivated in the laboratories of the Rockefeller International Health Division. A highly virulent African strain can be passed through as many as ten developing hen eggs and yet cause a speedily fatal yellow fever in inoculated monkeys. In other cases the disease will develop even after injection of a virus which has been carried successively through more than one hundred and fifty artificial tissue cultures and many developing hen eggs.

The Virus at Home

Facts like these might raise a natural question as to the practical value of virus culture in combatting virus diseases. Why go to all the trouble of nurturing microbes like hothouse flowers if in the end they turn out to be tough weeds that retain all their vigor despite cultivation? It is essential to understand the purpose of these experiments and the peculiar difficulty encountered in preserving viruses without impairing their usefulness as preventive or curative agents. Any vaccine, whether it be prepared from ordinary bacteria or these strange viruses, must imitate the disease and yet have none of the hazards attending a natural infection. To combine safety with highest efficiency in a vaccine is an ideal that can only be approximated unless it is desired to lower one of these factors at the expense of the other. In the same way, the usefulness of protective or curative serums also will depend upon the native vigor and undisturbed qualities of the organisms used in their manufacture.

This problem is all the more complicated in handling viruses because they cannot flourish as do other microbes in artificial surroundings, from which the growing population can be removed at pleasure. In the case of viruses, as we have seen, the house and its occupant are inseparable, so that up to the present time it has not been possible to observe all the important details of their existence. Nevertheless, experience with antismallpox vaccination and preventive measures against hydrophobia (rabies) has taught us how to gain practical knowledge by watching the behavior of viruses in an environment which imitates as closely as possible their natural surroundings.

This method of virus cultivation, despite its drawbacks, has helped to explain how the animal body develops an immunity or resistance towards certain diseases. It has enabled us to segregate the offensive and defensive mechanisms operating in a unit of living material represented

by tissue cells. With this information a picture is constructed of what happens during virus infections and how much dependence can be placed upon the ordinary measures now available for combatting them.

Let us examine at closer range some of the things happening inside a cell which has been infected with fever blister (herpes) virus, which will illustrate on a very small scale what happens in a patient suffering with the disease. The virus is added to an emulsion of suitable tissue placed in some blood serum taken from a healthy animal, while for comparison, material similarly inoculated is kept in serum from a rabbit that has recovered from an artificially induced herpes infection and is therefore immune. Each of these combinations thus represents a tissue culture in which two unlike environments act with vastly different effects upon the same virus. Ordinary serum does not hinder its attack upon the cells, where growth of the virus occurs in characteristic manner by producing changes exactly like those observed in living infected animals. These alterations in structure of the cell have already been referred to as "inclusion bodies," which are generally regarded as evidence of virus activity. Serum from an immune animal, however, literally cuts the cells off from contact with the virus, causing it to die out.

In the success of the latter experiment, the element of time plays an important part, for once the virus gets a head start, no amount of serum will save the cell from destruction. The speed with which a virus can attack cells is amazing. A delay of a few minutes in adding immune serum to tissue so inoculated enables the infection to progress beyond control, even though the cells be immersed in serum by that time. Doctor Andrewes, who made these significant observations at the National Institute of Medical Research in London, proved this with a simple test on the living animal. Into the skin of a rabbit he injected a small quantity of immune serum and marked the exact spot. Five minutes later herpes virus was in-

oculated into that same place and nothing happened, but when the procedure was reversed by introducing virus five minutes before the serum, infection resulted at the marked spot.

Independently, Doctor Rivers and his associates in New York have shown the same thing by another method, with vaccine virus growing in the infected tissue taken from animals. Rabbits are inoculated on the transparent outer covering of the eyeball (cornea) and bits of this material are kept in prolonged and close contact with serum from animals that have been immunized against vaccine virus. Even under these circumstances, the protective antibodies contained in the serum are powerless to prevent infection and multiplication of virus within the tissue.

Hidden Reservoirs of Infection

All these experiments offer a reasonable explanation of the failure of serums to treat virus maladies successfully, once they have taken hold in their victims. A virus entering into the body of its destined victims finds a snug harbor in various cells, from which it cannot be dislodged. This has a bearing on many problems connected with the control of virus diseases and particularly their manner of spreading, which is still an unsolved mystery. Whatever may be the ultimate answer to these questions, we do know that the cloistered life of viruses has some connection with the development of resistance towards the diseases which they cause.

It is quite commonplace for parasites to hide away in remote parts of the body and to persist in the tissues for months and years following an infection. Familiar examples of this kind are found in syphilis, where, long after the disease has spent itself, the guilty spirochete can be recovered from various organs and other hiding places. Additional instances of this sort of thing, as in typhoid fever, have been given already in previous chapters describing the wanderings of microbes. Here it is necessary only

to emphasize that some diseases give rise to "carriers," whose resistance to another attack of the same disease depends upon the presence within the body of organisms that have settled down to a state of armed truce. In other bacterial infections, like pneumonia, the immunity is less permanent or entirely absent.

Virus diseases for the most part confer upon fortunate survivors an immunity so solid and lasting as to make this one of the characteristics by which a virus can be distinguished from any other disease-producing agent. To mention only several, there are chickenpox, smallpox, measles, mumps, infantile paralysis, and yellow fever; all, so far as is known, are not "repeaters." Now it is highly probable that such extraordinary immunity can be explained by the survival of viruses in the tissues of previously infected persons. Unfortunately, studies of this nature have suffered from an appalling neglect and indirect evidence outweighs the scant positive knowledge that is available at present concerning the human subject.

In the plant and animal kingdom, however, we find a durable immunity unmistakably associated with persistence of virus in the tissues. It will be recalled that certain potato plants, while themselves immune from mosaic disease, can transmit infection mechanically through their juices to other plants. Even grafting a piece from an immune carrier to a susceptible variety will accomplish the same result.

Similar observations reported early in the summer of 1937 from the Colorado State College may have more fundamental meaning than is implied in the mere demonstration of virus-carrying fruit trees. Peach mosaic has been responsible for heavy losses in a certain district of Colorado, where plant pathologists had reason to suspect the presence of some healthy carrier among other trees found close by. They hit upon the idea of investigating the source of trouble in some plum trees, strange to say, and in the fall season grafted buds from these into young

peach seedling trees. With the coming of spring, the peach trees developed mosaic disease. In another test, roots were cut from the plum trees and grafted on roots of peach seedlings with the same result. These experiments were conducted in a region many miles away from the place where mosaic infection existed and furthermore, since all ungrafted peach seedling trees remained perfectly healthy, there could not have been an element of chance or accident in the way things turned out. Thus we learned that a plum tree without any symptoms of disease was frequently a carrier of virus responsible for mosaic disease in the peach! Who knows but that similar studies with certain viruses affecting man might not reveal undiscovered reservoirs of infection in out of the way places?

Turning to experimental work with animals, there are some striking examples of immunity associated with an entrenched virus. A case in point is the severe infectious anemia of horses, where, as long as seven to fourteen years after recovery from this disease, the blood will be found virulent when injected into other horses. In monkeys the virus of infantile paralysis can be recovered five months after an experimental infection which has made the animals fully immune, since they cannot be inoculated successfully again. Cowpox virus is found in the tissues of rabbits several months after recovering from infection, and similarly foot-and-mouth disease can be isolated from immune cattle and psittacosis from parrakeets and love-birds.

A Modern Jenner

These facts seem to confirm the suspicion that with all this knowledge we are in a hopeless predicament regarding the prevention and cure of virus diseases in man. If a lifelong immunity can be won only by having the good fortune to survive an infection, the situation offers a rather gloomy prospect indeed. Yet the outlook is not without its optimistic side because some practical advances have

been made in this field and others will follow by the simple expedient of turning failures into successes. Laboratory scientists, who are only human after all, have a way of muddling along until certain very obvious facts become insistent enough to ripen into a brilliant discovery. Rare successes merely emphasize how difficult it is to apply to man the promising results obtained in the laboratory.

In 1948 we are still wide of the mark, trying to match the direct hit made by Edward Jenner in 1798 when he went straight to the heart of this problem and developed vaccination for smallpox. Attempts to use a similar method of protection by taming a live virus through prolonged residence in an animal have succeeded in a few virus diseases. Yellow fever is one outstanding example and here the "best laid plans of mice and men" did not go amiss. At the Harvard University Medical School in 1930 Doctor Max Theiler made an interesting observation when he found that young white mice could be infected with yellow fever if the virus and an irritating substance like salt or starch solution were injected into the brain at the same time. (In the preceding year Doctor Zwick and his collaborators in Europe had described this method while studying a virus disease of horses, the so-called Borna's infection or encephalomyelitis.) In all previous work on yellow fever only monkeys had been found susceptible to the disease, but the virus taken from animals that had recovered from an experimental infection was too virulent for vaccination purposes. Doctor Theiler solved this difficulty by passing the virus successively through several hundred mice until the infective power for these animals reached a fixed high level and was at the same time correspondingly reduced for monkeys until they could no longer be infected with yellow fever, if inoculated under the skin.

This discovery came at an opportune time for Doctor Sawyer, who was in charge of the yellow fever studies being conducted by the Rockefeller Foundation at the

laboratories of the Rockefeller Institute in New York. Six members of his staff had already contracted the disease in the course of their work but were fortunate enough to survive. When he called upon Doctor Theiler to join the group of valiant researchers, the stage was set for a dramatic experiment, in which all six recovered patients took part. Since they were now completely immune to the disease, their blood serum contained protective substances that were needed for the test about to be performed with a group of sixteen non-immune members of Doctor Sawyer's laboratory staff. These men were the first to be vaccinated with a virus that had been stabilized in mice and made safe by administering it together with the serum obtained from the persons who had recovered from yellow fever. It was found that vaccination became effective after one to three weeks and produced an immunity of long duration.

In order to detect the presence of protective substances in the blood of these immunized subjects, a test was devised whereby mice were injected with a mixture of yellow fever virus and the serum to be tested. In this particular experiment the animals remained well, proving that these persons were not now susceptible to yellow fever. If the mice had developed the disease, it would have shown that the blood lacked substances necessary to counteract the yellow fever virus.

It is hoped that this artificial immunity will last even longer than is indicated thus far by tests which have been made on these persons during the past few years. It will take a long time to find out whether or not vaccination produces a resistance as effectively as does recovery from a natural infection. After thirty years one of the original volunteers who contracted yellow fever while working with Doctor Walter Reed in Cuba, where the latter discovered the part played by the mosquito in transmitting this disease, was found by the mouse test to be immune.

In the meantime, those who have occasion to visit or

live in the tropical regions of South America and Africa need not fear the deadly yellow peril. Successful immunization on a small scale has been achieved already with material that has been frozen and sealed in tiny glass tubes and shipped to those countries. At some future day all who wish it will obtain this protection merely for the asking and the practice of vaccination against yellow fever should become as commonplace as that for typhoid fever, smallpox, and diphtheria.

For this great blessing we are indebted to Doctor Sawyer and his band of "yellow-jack" fighters, who braved unusual dangers in the field and in the laboratories of remote corners of the world. Casual statements of fact hardly do justice to the living and dead among those who have wrestled with this disease. During the earlier years six deaths occurred among thirty-two men who had contracted yellow fever in the course of their work. When the history of its conquest is finally written, none will be found more worthy of sharing in the glory of its achievement than these members of the West African Yellow Fever Commission. It is a sensational story of brilliantly conceived researches made possible by a scientific team of the International Health Division of the Rockefeller Foundation.

Like so many other fine beginnings, this experiment did not work out according to plan, and with the turn of events came to an unhappy ending. A hidden source of danger that had escaped notice earlier was revealed when a high percentage of persons receiving the vaccine developed jaundice (yellowish skin) within from two to eight weeks after the inoculation. The cause of this was found in the immune blood serum which had been combined with the virus vaccine in order to make the mixture safe. This method, known as "serovaccination," was therefore discontinued when mass vaccination as a means of controlling yellow fever in Brazil was adopted in 1938.

Instead, a vaccine was prepared with material from

chick embryos infected with the yellow fever virus, and more than a quarter of a million persons in one area of Brazil were inoculated. No harm resulted from its use, although well over half of those vaccinated were found to be just as susceptible to the disease after vaccination as before. With the outbreak of war in Europe, it was feared that the danger of yellow fever spreading among native populations could not be removed until a safer and more effective vaccine was made available for immunization on a large scale.

While this problem concerned Brazil for the time being, it was as much the concern of a neighbor across the ocean, in French West Africa. There, at the Pasteur Institute of Dakar, a new method of preparing and applying yellow fever vaccine, devised by M. Peltier and his co-workers, was being used for the first time. The method combined all the necessary requirements of scientific procedure with the added virtue of simplicity. The vaccine, consisting of previously tested mouse brain virus, is inoculated *into* the skin as in smallpox vaccination, and does not require the use of hypodermic needles. A small drop of the vaccine material, placed on the arm, is scarified lightly by means of two short parallel scratches made through the drop. Ingenious, too, was the idea of combining the vaccination against yellow fever and smallpox in a single operation, so as to reach large segments of the population requiring either or both protective inoculations. In principle, the method is based on knowledge that safely modified yellow fever virus can penetrate the skin and produce immunity. Thus, a vaccine prepared from a virus carried through more than 250 passages in mouse brain will not cause yellow fever in the monkey or man, even though virus may be circulating in the blood a few days after vaccination. To test this point further, mass inoculations were given during the yellow fever season, when natural infection of the mosquito was most likely to occur. The results confirmed the belief that the virus could not be

developed or maintained in the mosquito, which under these circumstances could not transmit the disease by biting even the most susceptible of experimental animals, the rhesus monkey.

Over a period of six years, beginning in 1941, almost the entire native and European population of the French West African territories, numbering about fifteen million persons, were vaccinated in this manner. As a result of this campaign, cases of recognized yellow fever have, at last reports, reached the vanishing point. In order to determine the duration and extent of immunity developed against the virus, samples of blood serum were collected at random from native populations, which included persons vaccinated from four to seven years previously. Protection tests against yellow fever virus in susceptible animals showed that the immunity in each group was still present in 86 per cent of the first group and 82 per cent of the second. If these results are compared with a 98 per cent score obtained five weeks after vaccination, confidence in the wide application of the method may be justified. Periodic inoculations every four years have been advocated on this basis.

Bearing in mind the eccentric behavior of virus infections, it remains to be seen whether or not the apparently avirulent mouse passage strain will maintain its benign characteristics.

CHAPTER 9

VIRUS RIDDLES

The Taming of the "Flu"

A ghost of medieval times now steps into our ultra-modern setting. Influenza preserves its ancient flavor in the Italian name for this disease, which implies that it is an "influence" or some mysterious force invisibly exercised upon persons or things. While influenza modernized as a virus disease is regarded quite naturally with less superstitious awe, the infective agent still remains mysterious and invisible and no less awe-inspiring.

In 1892, Doctor Richard Pfeiffer, a noted German bacteriologist, described in great detail a very small microbe which he had found in the material coughed up by influenza patients. He named the organism "bacillus influenzae" and said it was the cause of a disease that had been sweeping over Europe since 1889 and at the same time was spreading rapidly to the American continent, Asia, Africa, and Australia. For a quarter of a century nobody questioned Pfeiffer's statements or the work upon which they had been based, and the influenza bacillus won an unassailable position for itself.

When influenza became epidemic in the United States during 1918 to 1920, the Pfeiffer germ again attracted attention and renewed attempts were made to study it more closely in the numerous army camps, where there was no lack of material among the sick and dead. Much to everybody's surprise, the influenza bacillus was found in only a small percentage of persons afflicted. Its importance as a causative agent was now brought into question, particularly since the reputation of the germ had been based upon its previously undoubted association with this

disease. The validity of Pfeiffer's original interpretation was further impugned when the organism, inoculated into susceptible persons, failed to transmit influenza.

Evidence of another kind gave a decisive blow to the assumed importance of the influenza bacillus during the next two years, when experiments on an international scale reproduced the disease with infectious material that had been freed of all microbes by filtration through porcelain filters. When bacteriologists in France, England, Japan, Germany, and the United States successfully inoculated influenza into human volunteers and monkeys with this material, a filter-passing agent was established as the probable cause of this epidemic disease.

Among all the evidence presented, however, none was so thoroughly convincing as the experiments of a group of Japanese doctors. Taking advantage of the fact that persons who have recovered from influenza and still harbor the bacilli cannot resist another attack, they inoculated the nose and throat of such human volunteers with filtered blood and sputum obtained from influenza patients. After two or three days, these human guinea pigs developed genuine influenza, while a second group of volunteers who had received massive doses of influenza bacilli alone or combined with pneumonia germs and other disease-producing organisms found in the nose and throat, did not.

Another epidemic of influenza came along about ten years later to restimulate interest that had flagged somewhat in the meantime. With this opportunity of retracing old ground, a group of experimenters in Baltimore took a census of the microbial population in many noses that were counted among the sick. Pfeiffer's influenza bacillus was again missing and the microbe sleuths decided to check upon some of the work that had been done by others with filtered secretions from the nasal passages of patients. This time chimpanzees were used in the experiments. A small number of these very costly animals were inoculated through the nose. In a short time they came

down with a disease which corresponded to human influenza and then the case for ordinary germs was lost.

Research work on influenza may be divided into three epochs. The first epoch of twenty-five years, beginning with Pfeiffer's discovery of the "bacillus influenzae" in 1892, was not very fruitful in results. During the second epoch, commencing with the world-wide epidemic of 1918 and ending in 1930, when a lesser outbreak struck this country, experimental work established a virus as the cause of the disease, rather than Pfeiffer's bacillus or any filter-passing microbes which were suggested from various sources. Now we are witnessing a third epoch, which from present indications promises to bring the problem to a successful conclusion.

Actually this recent period, marked by brilliant advances first abroad and later in the United States, had its origin in England in investigations on dog distemper that were conducted at the National Institute for Medical Research in London. Regarding this work in the light of more recent happenings, it furnished the key pieces in the mystery of influenza, although its proper recognition was delayed by a deplorable lack of coordinated effort, which frequently characterizes scientific research.

These investigations led to the discovery in 1926 that dog distemper could be reproduced experimentally in a highly susceptible animal, the ferret. A further observation of unusual interest was the fact that ferrets became solidly immune to dog distemper after inoculation with the disease, a fact that gave definite direction to future studies of vaccination against influenza.

Although dog distemper resembles human influenza more closely than any known virus disease of animals, it was not until seven years later that the vital importance to influenza research of these British discoveries was realized and the disreputable ferret, suddenly glorified, became man's strongest ally in his war against influenza. Again exciting news came from London in 1933 with the an-

nouncement by Doctors Smith, Andrewes, and Laidlaw that ferrets could easily be infected with influenza by nasal inoculation with filtered nose and throat washings from human cases. The disease could be passed from animal to animal in the same manner and by natural contact. Moreover, as they recovered from an experimental infection, they became immune to further attacks and developed protective substances in the blood serum, so that when it was mixed with virus and injected into healthy ferrets these animals remained well.

At this time the English experimenters had an opportunity to study the virus of swine influenza, which attracted considerable attention in the United States because it resembled the human disease. Little was known about the animal infection, apart from the fact that it assumed epidemic proportions among swine and was first recognized in this country during the year 1918. In 1931, Doctors Lewis and Shope of the Rockefeller Institute at Princeton, New Jersey, found that a virus was responsible for this disease, and they succeeded in transmitting it from infected to healthy pigs by means of nasal inoculations. Swine influenza, according to the newest idea, is caused by a virus in combination with a microbe that resembles the "influenza bacillus," both acting in partnership. The germ alone is harmless and the filtered infectious material from pigs causes a barely recognizable disease, while a mixture of the two always produces typical swine influenza when inoculated into the animal.

With this virus, Doctor Laidlaw and his associates produced in ferrets a disease exactly like that caused by human influenza. The following year these results were confirmed in the United States by Doctor Shope, who repeated the experiments and in so doing found he could develop a more severe type of infection similar to pneumonia. It sufficed merely to put ferrets lightly under ether before instilling the virus into their nasal passages. The British researchers now returned the compliment by repeating this

work successfully and discovered at the same time that influenzal pneumonia could be imitated also by injecting the virus directly into the lungs through the chest wall. What was more important, they infected white mice just as easily in this manner and by the nasal route, making it possible to study influenza in small animals, which are more readily available for such work than the ferret and react somewhat differently to the disease under certain experimental conditions.

These investigations brought out evidence of a definite relationship existing between the viruses of human and swine influenza, based on the following facts. Both viruses produce the same general disease and give rise to abnormal changes in the lung, comparable with influenzal pneumonia. Each virus passed in turn through ferrets or mice retains its infective power for either animal and inoculation with one kind of virus results in a protective immunity towards a later inoculation with the other. Similarly, the blood serum taken from these resistant animals also counteracts the selfsame virus which causes the disease and to a certain extent the other virus. A like protection is exerted by serums prepared artificially in horses and ferrets by repeated inoculations with either type of influenza. To test the protective effect of serums, they are first combined with infected mouse lung and a small amount of the mixture is dropped into the nasal passages of ferrets or mice. The extent of pulmonary disease which results will indicate the efficiency of each serum and its relation to the virus in question.

While making a more detailed study of this point, it was observed that a serum manufactured with human influenza virus is highly effective against swine influenza. This overlapping activity, however, is much less conspicuous in the case of swine influenza serum towards human influenza virus. These facts naturally suggest a close relationship between the viruses responsible for the two diseases without necessarily indicating identical causes,

for in that event there would be no difference in the range of protective action with the two serums. Unusually interesting in this connection, nevertheless, is the observation that in animals repeated exposures to the virus tend to obliterate differences whereby one strain may be distinguished from another. From such experimental evidence alone, it follows that a specific substance, common to both viruses, is present in larger amount in the human influenza type.

It is only a guess, of course, but this difference in composition might explain a peculiar characteristic of swine influenza virus, which was first observed while studying its mode of infection. The virus, it will be remembered, produces disease in swine only when accompanied by another influenza-like organism, with which it acts on some sort of cooperative basis. On the other hand, in mice and ferrets alike such a partnership is not necessary, the virus alone being sufficient to cause a severe and sometimes fatal infection.

A riddle like this one is not solved easily. The swine virus by itself, when inoculated into animals, will make them resistant to further attacks of the disease, whereas the influenza-like organism, without which the virus cannot produce the disease in swine, does not confer immunity. Complicating the problem further is the possibility that a filterable stage of the influenza bacillus might be related in some way to the virus. For example, during experimental infections with the virus of the common cold the influenza organism appears suddenly in greater numbers than before and changes from a harmless into an aggressive, virulent type. What this means is not clear, but it hints at strange transformations which, as we have learned, occur frequently among microbes and may be true for some viruses. Who knows?

Perhaps in the future chemical analysis of viruses will succeed, as with microbes, in unravelling some of these knotty questions. The discovery late in 1938 at the

Rockefeller Institute that different influenza strains are often responsible for different epidemics and that more than one kind of influenza virus may be present during a single outbreak offers a likely explanation of its caprices.

Outbreaks of the disease in man have been accompanied frequently by similar epidemics among animals, as in the memorable year of 1918, and it has been suggested, therefore, that infection is spread from man to animal and back to man. Despite strong analogies between the two diseases, as already emphasized, conclusive proof of their identity has not yet been given. It is a striking fact, nevertheless, that a disease resembling human influenza can and does affect highly domesticated animals like the dog and swine. As time goes on, perhaps many other new hosts will be discovered. Only quite recently, hedgehogs and rats have also been found susceptible to inoculation with the virus. There is no conclusive evidence, however, that swine influenza virus has ever transferred back to man during all the years that this disease has been studied.

In order to explain how outbreaks of swine influenza appear in many widely scattered places at or about the same time, it is necessary to account for the whereabouts of the causative agent during variable periods of time between epidemics. If the disease-producing agent does not spring up anew each time, then it is reasonable to assume that it lies dormant somewhere between outbreaks. Thus it could survive, without causing recognizable disease, in its own natural host, or it might be carried in an intermediate host, which serves as the reservoir. Such an intermediate host was discovered by R. E. Shope who found in the lungs of swine a parasitic roundworm (threadworm), which carries the virus of swine influenza and is capable of passing it from animal to animal. But this is not a simple process. Although the swine lungworm as an intermediate host is the virus carrier, it cannot complete the job of transmitting the disease without the aid of a second intermediate host, the common earthworm! Within the body of this worm, the

lungworm passes through three separate developmental stages before becoming infectious for the pig.

A description of the life cycle of the lungworm will help explain this remarkable epidemiologic discovery. The eggs, laid by an adult female lungworm in the bronchial tubes of the swine, are coughed up and swallowed, then discharged in the intestinal contents to reach the soil. What happens next depends upon ingestion of the eggs by earthworms. The lungworm eggs hatch within the earthworm, and develop into larvae which are now in an infective stage and remain so until the earthworm is eaten by a pig. In the swine the lungworms complete two more developmental cycles before reaching their final destination in the respiratory tract, to become adults. The elapsed time required for the entire life cycle will vary from slightly over one month, if conditions are most favorable, to a period of more than two and somewhat under three years.

These facts illustrate more than a casual connection between seasonal epidemics of swine influenza and the pre-epidemic seeding of a virus implicated in this disease. The close similarity of the epidemiologic pattern to that of human influenza invites a comparison of the two diseases, even though analogous intermediate hosts have not as yet been discovered for man. This concept might even be applied profitably to the unknown vectors and possible intermediate host reservoirs implicated in a virus disease such as poliomyelitis.

Laboratory studies, in the meantime, have gone ahead with more tangible things, all of which promise to bring influenza under control. From present indications, hope for a preventive vaccine and a curative serum need not be long deferred if a recent discovery can be realized in practice. At the Rockefeller Institute Hospital human influenza virus from various sources has been grown in an artificial environment by the already familiar tissue culture method, employing minced chick embryos contained in a nutrient fluid. It makes no difference what part of the country this virus comes from, for in each case, after

multiplication in the test tube, it exhibits the same power to cause disease in mice and ferrets if inoculated into the nasal passages. The capacity to infect animals is apparently not changed even though the virus culture is transferred successively from one generation to the next at frequent intervals over a long period of time. A strain of virus can be kept alive and active in this way for many months. Mice and ferrets inoculated under the skin with virus thus propagated outside the animal body resist further attempts to reinfect them with influenza virus, which ordinarily proves fatal in extremely small doses for these susceptible animals. This accomplishment of Doctors Magill and Francis verified and extended the work of English experimenters, who had also vaccinated ferrets and mice successfully with ordinary influenza virus injected under the skin or into the abdominal cavity.

These carefully planned tests gave every indication that an attempt at vaccinating the human subject was a venture worth while and might succeed. The use of a virus which had been grown in an artificial environment rather than in animals had many advantages to recommend it and there were volunteers both ready and willing to have the experiment tried upon themselves. Three men were first chosen by Doctors Francis and Magill for a preliminary trial to determine the immediate effect of inoculating the live culture virus beneath the skin. As no unfavorable or remarkable reactions occurred, the method was considered safe.

A larger group of volunteers was next selected for the purpose of determining whether systematic vaccination with this artificially cultivated virus would protect them against the disease. Before the first injection, a sample of blood serum was obtained from each person, who then received three inoculations of virus, which were given singly in increasing doses one week apart and followed two or three weeks later by another injection. After a lapse of about ten days, a sample of blood serum was again obtained from each subject in order to find out if protective substances had developed during the treatment.

The protective power of a serum was measured by combining it with influenza virus obtained from experimentally infected mice and injecting some of the mixture into healthy mice by the nasal route. If the serum contained abundant immune substances, the test animal survived and showed no evidence of disease. In a general way, the degree of protection afforded in each case depended upon the amount of protective substances that were present in the serum. Such tests would mean nothing, of course, unless the alleged properties of the serum were compared with those found in samples taken from the same persons before they were vaccinated. The net results of the comparison showed quite conclusively that vaccination with an artificially cultivated human influenza virus caused remarkable changes in the blood serum of individuals so treated. The immediate effects were to increase whatever protective substances existed naturally in the blood and to stimulate definitely their production when none could be detected by the most delicate methods.

As far as immunity responses on the part of the human body are concerned, these experiments prove that the blood has acquired certain properties which appear to be effective towards the virus. How long these artificially manufactured antibodies remain in the blood stream is not yet known and how useful they will be in warding off an attack of the natural disease, particularly during epidemics, cannot be foretold. It remains to be seen whether or not simple vaccination alone will furnish the best defensive weapon against influenza. The recent discovery that there are many different breeds of influenza virus and infection with one kind does not make a person immune towards any of the others explains why the disease may be contracted more than once and why epidemics strike the same community from time to time in populations that were thought to be protected by a previous attack.

From what has been said already, it is evident that a vaccination program for influenza cannot be effective un-

less repeated visitations of epidemic disease in the same locality are prevented. For a long time, the epidemiological significance of this has been recognized and the nubbin of the problem found in the multiplicity of virus strains that are responsible for separate outbreaks. Influenza type A, for instance, can result from more than one particular breed of virus, and the same is true for type B. The causes back of all this are now known to reside in the composition of the individual virus substance itself, the selfsame differences that exist in the cellular make-up of bacterial strains. Immunity or resistance to infection with one kind of virus, either naturally acquired or artificially induced, does not guarantee protection against another type, even though these may be related in kind. What does make an important difference is the power of any given strain to stimulate the production of substances antagonistic toward the same or related virus.

An experimental approach to this problem has brought out significant facts, which have a bearing upon reinfection with influenza A virus and the present status of vaccination against the disease. In the ferret, an animal especially suited to the purpose, artificial infection with a certain strain of virus makes the animal resistant toward this particular strain alone. An immunity so developed does not prevent infection with another strain of virus which, however closely related it might be, is entirely different in its capacity to stimulate the manufacture of protective antibodies. These results confirm previously suspected differences in the kinds of virus causing influenza, and show that such differences go hand in hand with the amount of protection developed by a previous infection.

Information concerning the value of influenza virus vaccine can be gained only from experience with it in the face of an outbreak of the disease. An opportunity to test the laboratory findings in the field presented itself during the winter of 1946-1947 and in the fall of 1947.

Under the guidance of Doctor Thomas Francis, a vac-

cination program was carried out at the University of Michigan in the late fall of 1946, when 10,328 students received the influenza A vaccine and a "control" group of 7,615 did not. With the occurrence of a somewhat belated outbreak of influenza in the spring, it was found that the number of cases developing in each group was the same. According to cold-blooded statistics, the effect of vaccination appeared to be nil. This was due to the lack of protection developed by the vaccine in use against the strain of virus that happened to be prevalent during the epidemic.

On the basis of information derived from experimental studies of recent influenza epidemics, certain facts tend to align themselves against overenthusiastic reports of mass vaccination. The results of tests are based upon accurate methods of determining the amount of protective substances and the length of time these antibodies persist in the vaccinated individual. Such factors, in turn, have been balanced against any demonstrable resistance developed toward a reinfection, with the following conclusions: The duration of resistance does not run parallel to the amount of protective substances persisting in vaccinated persons. Among these are many who lose this protection sooner than others. The effect of vaccine against all the possible variant strains of influenza virus is not known at the present time. Resistance following vaccination is not carried over from one season of respiratory infections to the next.

How effective, then, is vaccination against influenza, and what is its present status? To these questions a final answer cannot be given until there are more opportunities for a complete evaluation of new material. In terms of present knowledge, however, the general use of influenza vaccine by health departments does not seem to be justified. It should be kept in mind that these opinions may be modified by future experimental work, and the verdict sustained or set aside by another court of inquiry.

From all our existing knowledge, it is certain that the

influenza virus is tough and has an extraordinary infective power. It can be kept alive for several months in glass tubes packed in dry ice at a temperature of minus 176 degrees Fahrenheit, which is over two hundred degrees below the freezing point. One part of virus diluted a million times is sufficient to kill experimental animals regularly. Incredible as it may seem, this does not begin to indicate the limit of virulence, for Russian investigators have recently produced influenzal pneumonia with virus diluted one million times a million! An infectious agent capable of such a performance might well be considered an "influence" and the disease resulting from it a strange "visitation."

Parrakeets and Pining Love Birds

Psittacosis is the name for another contagious influenza-like virus disease communicated to man by birds belonging to the psittacus (Latin for parrot) family. In human beings, the illness sets in about one week and in birds after two weeks from the time of exposure. This malady was first made known to the world in 1879 by Professor Ritter, who described an outbreak which occurred in a Swiss household and was traced to some small parrots (parrakeets) brought from Hamburg. A short time after the sudden death of one of these birds, five members of the family and two frequent visitors were stricken with a peculiar ailment resembling a combined pneumonia and typhoid fever. Three died as a result of the infection, which for want of a better name Ritter called "pneumotyphus."

The year 1929 marked the fiftieth anniversary of this event and it was celebrated quite appropriately by an outbreak of psittacosis in various parts of the world. From July of that year until early spring in 1930, approximately four hundred cases of the disease were reported, of which more than one-third ended fatally. As in many smaller epidemics that had occurred in Europe from time to time,

the illness could be traced definitely to contact with sick parrakeets, love birds, or cockatoos imported from South America. In this particular outbreak, the principal seat of the disease was in the Argentine, where, according to local observers, human influenza paralleled a devastating epidemic among cockatoos and other parrot species. During the next few years psittacosis spread to Europe and the United States.

Then no longer a medical curiosity, this disease created wide-spread interest and led to intensive search for the cause and the manner in which the malady was transmitted. There was no doubt that it was extremely contagious. Among birds, psittacosis spread with astonishing rapidity and persons handling them or even entering a room in which they were kept were almost certain to be infected. In 1930, while searching for the causative agent of psittacosis, Doctor Bedson and his two associates at the London Hospital in England inoculated love birds with bacteria-free blood obtained from patients who had been stricken with the disease. The birds developed psittacosis and thus a new virus and a method for its identification were discovered. It was found shortly afterwards by Doctor Krumwiede of the New York City Health Department Laboratories that white mice were susceptible to the virus and could be used for detecting the disease in suspected material taken from birds and man. Unfortunately he did not live to enjoy some of the fruits of these labors, because at that very moment he was suffering quietly from a fatal illness.

Doctor Krumwiede's work was left in capable hands at the Rockefeller Institute, where the investigations begun by him in 1930 were carried on by Doctor Rivers and his co-workers. They promptly recognized the dangers to laboratory workers incurred by using love birds or other parrot species in testing material from patients, and indeed an accidental infection of one of the staff soon afforded an unusual opportunity for studying psittacosis and develop-

ing a more satisfactory method of diagnosing the disease. Taking advantage of the fact that this infection is very similar to a pneumonia, in which material coughed up from the lungs contains the germs, Rivers injected small amounts of sputum from the patient into the abdominal cavity of mice in an attempt to capture the psittacosis virus. When the mice came down with the disease, it was proved that sputum contained the disease-producing agent. In a subsequent test, identical results were obtained with material taken from a group of patients during the third and ninth day of illness. These individuals had been in contact with birds, in which the presence of psittacosis had been definitely proven. For comparison, a number of persons known to be free from the disease were tested in the same way and found entirely negative.

With this excellent diagnostic procedure that was both safe and reliable, it was now possible to discover existing sources of infection, but the manner in which the disease spread from birds to man was still another problem that awaited solution. Rivers and his virus detectives lost no time in getting evidence from the naturally susceptible host, the parrot. Parrots, it was learned, are easily infected through the nasal passages and, once the disease has manifested itself, virus is discharged continuously in secretions from the nose and intestinal contents. This material, when fresh, is scattered abroad among birds in cages or other surroundings, and in a dry state the virus-laden discharges are carried to the nose and throat of persons exposed to the contaminated atmosphere. Bite wounds inflicted by diseased parrots are generally fatal because abundant virus in the mouth of a bird gains entrance directly into the blood stream.

Infected birds, in addition to being an undisguised menace, frequently carry the disease without showing any signs of illness. Outbreaks of psittacosis in aviaries can be traced to such hidden infections by means of laboratory tests, which reveal the presence of virus in the organs of

parrots and related breeds. The love bird or budgerigar, in particular, has been incriminated as a notorious carrier. It is said of these birds that they pine away at the death of their mates. Perhaps psittacosis and not romance alone has had something to do with this interesting behavior.

Experts of the United States Public Health Service have worked out effective administrative methods for control of the disease by enforcing strict regulations against shipment of birds from any foreign port. Only after a suitable period of detention, generally fifteen days, during which they must remain free from illness, are they released for transportation. Local and interstate control measures require a clean bill of health from all owners of aviaries engaged in breeding and marketing activities. Since it is known that parrots are not the only carriers of psittacosis, a watchful eye is now trained on other members of that tribe, which includes cockatoos, parrakeets, budgerigars (love birds), and birds of gorgeous plumage, such as Amazons, lories, and macaws. As a further safeguard, these must be at least eight months old before lawful shipments are allowed in interstate commerce, because young birds are extremely susceptible to the disease. As generally happens, new problems arise constantly to plague the harassed public health officials, for quite frequently it has been discovered that a large number of birds not members of the parrot species are also susceptible. An observation of unusual economic interest in this connection is that chickens can be infected by feeding on material contaminated with psittacosis virus.

While preventive measures in the field have struck at the source of this disease and thereby lessened the chances of infection, those who are obliged to risk exposure have benefitted only indirectly. Some form of protection was needed against the virus when accidental exposure had occurred or when, as in the laboratory, workers could not run away from psittacosis. Experience had shown that seasoned bird handlers as a rule escaped infection, pre-

sumably owing to a resistance that had been developed either by recovery from an attack of the disease or as a result of a gradual self-inoculation of virus. Vaccination therefore seemed to be a logical procedure and, with this in mind, attempts were made at the Rockefeller Institute to produce an artificial immunity by inoculating the virus into human beings. Before attempting any experiments with human subjects, however, a psittacosis pneumonia was first produced in monkeys by injecting virus directly into the windpipe. Those animals that survived were tested for their ability to withstand a second inoculation given in the same manner from six weeks to six months later.

The amount of protection was measured by observing how much pneumonia was produced in the lungs of these animals and of another group which had not been inoculated previously. From a comparison of the results obtained, it was concluded that monkeys that had recovered from psittacosis were more resistant to the virus introduced into the lungs than were animals which had never experienced an infection. This could be shown in another and much better way by comparing the virus-destroying power of blood from monkeys that had overcome a pneumonia due to psittacosis and from those that had not been exposed to the disease. In such experiments, mice were injected with a mixture of blood serum and virus in order to determine the mortality rate and the average time of death in the two sets of test animals. The serum from normal monkeys was without effect, all the mice dying within one week on the average. In contrast to this, about four-fifths of all the animals receiving serum from recovered monkeys survived, the average time of death in this group exceeding that of the first group by a considerable margin. Furthermore, the serum, even when diluted ten million times, protected four out of every five animals, whereas none could be saved by means of serum from normal monkeys.

The study had now reached a stage where vaccination on monkeys was justified prior to its actual trial in man.

Proceeding cautiously, Doctor Rivers determined, first, whether repeated small doses of psittacosis virus could be inoculated with complete safety into the muscle tissue of monkeys, second, whether they became resistant towards virus subsequently introduced into the lungs, and third, if the vaccinated animals developed protective substances in their blood. To all three questions an affirmative answer was given and seven human volunteers cheerfully and bravely submitted to an experiment which was skilful, courageous, and, it should be added, successful.

Inoculations into the muscle of each subject were given at weekly intervals. The first of six injections contained an amount of virus sufficient to kill ten thousand mice and the last dose ten million of them. Aside from relatively slight discomfort, no ill effects were experienced during the vaccination, although two individuals reacted more vigorously following the last inoculation. Considering the nature of the material used—emulsions of liver and spleen from infected mice—it is not in the least surprising that the human tissues rebelled at this foreign substance. Unpleasant reactions might be avoided entirely or reduced to a minimum in the future by using virus freed from irritating material. This should be possible with the newer methods of purification by means of high powered supercentrifugal machines or by growing the virus in tissue cultures, as Doctors Bland and Canti have done at the London Hospital in England.

Two to four weeks after the final inoculation, a small amount of blood was withdrawn and tested for its capacity to destroy psittacosis virus in the body of susceptible mice. Without exception, the blood serum from vaccinated individuals contained protective substances. For the time being, we shall have to be contented with this single yet very important criterion of an artificially produced immunity. Obvious reasons make impracticable the inoculation of virus into the nose or lungs of vaccinated subjects in order to ascertain how effectively they will resist psittacosis. A

final answer to this question cannot be given until opportunities arise for exposure to natural infection.

The Virus of Infantile Paralysis

Fifty years have elapsed since it was first hinted that "infantile paralysis" or poliomyelitis, described originally by Doctor Jacob Heine in 1840, was probably an infectious disease. Medin, reporting an outbreak in Sweden in 1887, mentioned paralysis as only one of the features of what he considered an acute general infection which occurred in epidemics. The modern conception of the disease was developed over thirty years ago by his pupil, Doctor Wickman, who published a monumental work on the great epidemic of 1905 in Sweden. His most noteworthy contribution was a theory as to the manner in which infantile paralysis was spread from person to person through contact. Today this idea is still generally regarded as sound, although it is difficult to reconcile the theory with many newly acquired facts of virus diseases.

Wickman, like many others before him, had tried and failed to reproduce the disease in rabbits, which were commonly used in these experiments, and he recognized the immediate need of a suitable animal, which up to that time had not been discovered. To many troubled families in Vienna, the Christmas of 1908 brought a gift from the Magi. On the eighteenth of December, two Viennese physicians—Landsteiner and Popper—made an important announcement before a medical society to the effect that they had successfully transmitted infantile paralysis to monkeys by inoculating material prepared from the spinal cord of a child who had died after a very short illness.

Landsteiner hoped to follow up this discovery with experiments designed to keep the disease alive in monkeys, but was greatly hampered by lack of animals and necessary funds for his investigations. Only two animals had been inoculated thus far with material taken from the nervous system of the original paralyzed monkey in an attempt to

transfer the infecting agent, but with uncertain success. Meanwhile diligent search for a microbial clue led nowhere and Landsteiner supposed he was dealing with an invisible virus. A happy turn of events soon enabled him to put this assumption to a scientific test.

At the Pasteur Institute in Paris, Professor Levaditi held out a helping hand to his distinguished colleague. There awaited him chimpanzees, monkeys, and all things that bring delight to a researcher's heart. From Vienna, a small container had already arrived with a piece of spinal cord obtained by Landsteiner from a fatal human case of infantile paralysis. The precious material by that time had been preserved four days in a glycerine solution diluted one-third of its full strength. Since nobody knew what effect this might have upon the disease-producing agent, Landsteiner and Levaditi doubtless awaited the outcome of their experiment with bated breath. Into the abdominal cavity of a valuable chimpanzee, they shot an emulsion of this poliomyelitic spinal cord substance—and it worked! From this animal they took bits of the spinal marrow and prepared a mash, which was then injected into the brain and abdominal cavity of two more healthy monkeys. When within six days both were paralyzed, it was concluded that the organism causing infantile paralysis had multiplied and transmitted the disease from one animal to the next. This classic experiment also gave the first indication of an extraordinary resistance of the infecting agent towards chemical substances, since it had survived in glycerine solution capable of destroying ordinary germs.

Under the microscope, the brain and spinal cord of diseased animals gave evidence of some destructive agent which attacked nerve cells and vital tissues, spreading ruin seemingly everywhere, but not a trace of anything resembling a microbe could be found. If this were an ordinary germ, it should be held back, Landsteiner thought, by a fine-grained filter, whereupon he passed an emulsion of infected monkey brain through a stone filter and inocu-

lated some of the resulting water-clear bacteria-free solution into a second healthy monkey. This animal, like all the others which had received unfiltered material, developed infantile paralysis, showing that the infective substance could pass readily through exceedingly fine pores which restrained ordinary microbes. The conclusions from all these experiments were now clear. Infantile paralysis was caused by a filterable virus attacking the nervous system in man, was reproducible in newly found susceptible animals, the monkey and ape, and exhibited hardy qualities rarely, if ever, attributed to disease-producing organisms.

Almost simultaneously with this report late in November, 1909, from Vienna, Flexner and Lewis, who had been working independently at the Rockefeller Institute in New York, announced the results of their investigations. To them belongs the credit for having first propagated infantile paralysis in an uninterrupted series of monkeys by injecting material containing the virus directly into the brain, thereby insuring a rapid and certain method of transferring the disease from animal to animal. This route has been used almost exclusively for experimental work, in which Doctor Flexner and his associates have played a leading part during the past twenty-five years or more. The inoculation is made under ether anesthesia and with strict surgical asepsis through an opening drilled in the skull, just sufficient to admit the fine needle of a hypodermic syringe containing the virus. A very small amount is introduced, either as an emulsion or a filtered preparation of infected spinal marrow or brain. This operation, which lasts only a few minutes in skilled hands, is followed by uneventful recovery from the effects of ether inhalation and the monkeys lose none of their accustomed agility after being returned to their roomy cages. Six to seven days later the effects of a successful inoculation may become noticeable.

For the Benefit of Man

The course of infantile paralysis in the monkey is quite dramatic from beginning to end and while some of the incidents are frequently less pronounced in man, the disease as a whole is accurately reproduced. Relatively few experimental infections can be studied to better advantage than this one, which exhibits an infinite variety of puzzling features that baffle even the most skilful physicians.

As the disease develops in the monkey, subtle changes can be recognized in its disposition and behavior. One of the first signs to attract attention is a peculiar excitability. This is quite different from the intense occupation with meddlesome or trifling activities so characteristic of a normal animal. Its restlessness seems to be part of a general nervous disturbance from which there is no escape, and to say that the monkey bristles with irritability is not a figure of speech. The fur is ruffled and the voice changes to a high pitched staccato cry. In the midst of extraordinarily quick movements, a practised eye will detect unmistakable signs of abnormal fatigue following such exertion. As the animal pauses to rest, one can observe the evidence of an overwrought nervous system in a twitching of various parts of the body or a rapid trembling of the face muscles or head.

After a day or more of symptoms caused by disturbances in the nervous system due to the virus of infantile paralysis, its damaging effects become visible in another series of changes. Muscular movements of arms and legs begin rapidly to lose their coordination and the monkey walks with a staggering drunken gait. Attempts to use the arms in climbing or reaching for objects become ineffectual and the weight of the body cannot be sustained any more with the aid of arm, shoulder, or back muscles. With the development of muscular weakness, paralysis is not far off, although this is not invariably so. Now an eyelid may droop or one side of the mouth refuse to grimace equally

with the opposite half, indicating a beginning facial palsy. If the animal is tempted with a bit of food, held near the cage, one soon discovers whether or not an arm is weakened or partially paralyzed, for the sound limb or the stronger one is generally thrust forward. In various ways it is possible to locate exactly the region and extent of paralysis. Any muscle or a group of muscles may be affected without further damage to other parts of the body.

Frequently, however, the loss of function occurs suddenly and a monkey apparently well one day is found next morning completely paralyzed and flat on the floor of the cage. Every effort is made to hasten recovery and forestall permanent damage by providing a soft pallet, upon which the animal is carefully placed and treated in every respect as one would a human patient. As long as the muscles used in the act of swallowing have not become affected, feeding is not a serious problem because the appetite generally remains unimpaired and strangely enough the jaws at one extreme and the tail at the other seem to retain their functions to the very end. When animals are hopelessly stricken, they are painlessly dispatched with an over-dose of ether.

In contributing to our knowledge of infantile paralysis, Flexner and his co-workers utilized these experiments to study by every conceivable method the effects of the virus when inoculated into the animal body and, by inference, to learn something about the disease in man. The way was now opened for investigating one of the most important problems connected with any communicable disease and that was the manner in which the germs entered and left the affected body. Testing a variety of materials taken from monkeys previously inoculated into the brain, Flexner and Lewis discovered the virus in discharges from the nose and throat. The fact that it escaped exclusively by this route gave them a clue as to its portal of entry. Very quickly they found that healthy monkeys could be infected and paralyzed by placing into the nostrils pledgets of cot-

ton soaked with virus or by instilling it with a dropper. The recovery of virus from the spinal cord of these animals showed conclusively that the infective agent had ascended to the brain and then traveled downwards.

There is no shorter or more direct natural route for disease to enter the brain than through the nasal passages, and for the same reason none could be more efficient as an exit for the microbe. The floor of the skull is connected in front with the roof of the nose by means of a thin horizontal plate of spongy bone, which is perforated by small openings for the nerves of smell. Directly on top of this sieve-like structure (known as the cribriform plate, from Latin *cribrum*, a sieve) rests a portion of the brain that carries the nerve fibers to the upper parts of the nasal cavity and of the thin partition or septum, which divides it in two. The virus of infantile paralysis follows along the course of these nerve threads through miniature caverns leading into the main vault directly above, with its floor supporting the vulnerable brain tissue that communicates by various channels with the spinal marrow. If these nerves are first cut through, an experimental infection cannot take place, as has been demonstrated by Doctors Schultz and Gebhardt of Stanford University, California, and later by Doctor Brodie of McGill University of Montreal.

In human beings this mode of infection has been accepted quite generally as correct on the basis of Flexner's early experiments, in which the virus of infantile paralysis was demonstrated in nasal secretions of persons afflicted with or exposed to the disease. It was left to other investigators to show the exact pathway of the virus as it moved gradually downward from the brain to the spinal cord. But before this could be accomplished with any degree of certainty, a more dependable method of infecting monkeys by the nasal route had to be found. Instillation of virus into the nostrils was a hit or miss procedure, in which the chance of success seemed to be governed by unknown factors.

Doctor Schultz at Stanford had an idea that the nasal secretions or the mucous lining of the nasal passages might account for the irregular results with this mode of inoculation. It occurred to him to wash the nasal passages thoroughly with a slightly acid phosphate solution before instilling the virus and by this method nearly one hundred per cent of his monkeys could be infected successfully. Alkaline solutions were much less efficient for this purpose, as might have been suspected from the fact that the alkalinity normally present in nasal secretions generally had an unfavorable effect upon the virus. In actual practice, Schultz's new method has been a great improvement over the old one, which required instillations over a period of from three to seven successive days. Now the virus can be inoculated at suitable intervals on the same day with the further advantage of fixing quite accurately the time when an experimental infection presumably has occurred.

With this as a point of departure, Doctor Faber at the Stanford University Medical School in San Francisco performed a series of basically important experiments on monkeys, in which the gradual descent of infantile paralysis virus was traced through various portions of the brain into the spinal cord. The progress of the disease was determined by removing pieces of tissue from the nervous system in animals that had been sacrificed on successive days, following inoculation with virus by the nasal route. Testing these materials for their power to infect healthy monkeys, it was found that, even as early as three days and before any evidence of disease had become apparent, the virus was already well on its way to the spinal cord and by the sixth day had become established there prior to first signs of muscle paralysis. Flexner's theory of infection with infantile paralysis was thus clearly sustained, for the virus did pass from the mucous membranes of the nose into the central nervous system, where it multiplied and produced the recognizable disease.

As we shall see later on, these views regarding the portal

of entry and exit of the virus have been revised. A more recent discovery that poliomyelitis virus leaves the body via the intestinal tract has revolutionized methods of recognizing and studying the disease.

Serums, Vaccines, and Chemicals

In dealing with virus diseases, expectations are not always justified by the results. The practical achievements in the prevention of epidemic poliomyelitis (infantile paralysis) have lagged far behind the perfect experiments witnessed in test tubes and monkeys. There is always the possibility, of course, that other channels of infection will be discovered and enable us to place an effective barrier between the virus and its susceptible host. But the chief cause for concern at the present time is an avowed failure of serum treatment in human beings as compared with results observed in experimental animals, a disparity which is in direct conflict with established principles of immunity. Why this should be so nobody knows, although one of the reasons given and already stated ascribes this failure to the inability of any serum or protective substance to reach the infected areas, once the virus has dug into its fortress of tissue cells. To prevent successful invasion of these inaccessible places means a race against time in which, for the present at least, a serum cannot compete with the speedily acting virus. Nevertheless, experimental evidence from first to last has given assurance that some day we shall catch up with this elusive thing.

Early in their work with infantile paralysis, Flexner and Lewis discovered that monkeys that had recovered from an attack of the disease could not be infected again and they tested the blood of such animals for protective (immunity) substances most likely to account for this resistance. Two methods were used successfully in determining whether or not the blood serum from recovered monkeys and from human beings would counteract or "neutralize" the virus. These procedures have become more or less standardized and are regarded today as reliable

tests for measuring the protective power of a serum in question.

By the first method, a virus and serum are combined in a test tube before injecting the mixture into the brain of a healthy monkey. If the serum contains substances harmful to the virus, the animal will show no symptoms of disease, whereas a serum without protective power fails to prevent an infection and paralysis characteristic of the artificially induced poliomyelitis. In the second method, the virus and serum are injected separately into the animal body to imitate more closely the practical conditions of treatment. A monkey receives the virus inoculation into the brain and within twenty-four hours a dose of serum is injected into the spinal canal. Under certain conditions the onset of symptoms can be prevented entirely or limited to a mild attack, which is delayed for a considerable time beyond its normal expectation. A successful outcome, remote at best, is contingent upon the serum being administered before there is any evidence of infection, for once this appears, the disease will run its usual course, regardless of the amount and strength of serum used.

It is obvious that the conditions imposed by these tests are much more stringent than the requirements of a natural infection in man, particularly with regard to inoculating the virus directly into the brain. For this reason, it seemed worth while to investigate the capacity of blood serum to suppress infantile paralysis virus when it was introduced by the nasal route, which, according to Flexner, is the natural manner of infection. Under these circumstances, he found the serum to be more effectual, even though treatment was delayed for a period of time that would be considered unsafe if the virus were inoculated into the brain. Other investigators, using Doctor Schultz's dependable technic of instilling virus into the nose, have been unable to confirm this, more particularly as the nerves of smell are unprotected.

The problem of preventing infection by giving serum before the virus has entered the body is a practical one and

not easily solved. On this question, likewise, experimental evidence seems to favor the possibility of using serum from recovered cases of infantile paralysis, but the facts gathered during several recent epidemics are not very encouraging. Largely at the instigation of Professor Netter at the Pasteur Institute in Paris and on the basis of Doctor Flexner's remarkable studies, the treatment of this disease by means of so-called "convalescent serum" (obtained from recovered and therefore immune patients whose blood contains protective substances) was advocated. It was a foregone conclusion that no benefits could be derived from its use once paralysis had set in, and this expectation was promptly realized in frantic attempts to overcome insuperable odds. For persons in early stages of the disease before paralysis had occurred, the situation, at least from the statistical point of view, appeared brighter. Here, however, interpretation of results depends to a great extent upon the selection of patients and more particularly upon the fact that it is impossible to determine how many will escape paralysis or recover completely without the aid of serum.

This was illustrated in a striking manner during an extensive outbreak of poliomyelitis in New York City in the summer of 1931. A serious effort was then made to assess the value of this treatment on an unprecedented scale. Taking part in this study were the New York City Department of Health, the Public Health Relations Committee of the New York Academy of Medicine, and two hospitals, one in Brooklyn, New York, and one in Hartford, Connecticut. A combined group of more than one thousand patients was studied. Of this number, one-half received convalescent serum early in the disease before paralysis developed, and the other half was given no serum. There was no particular choice in this matter, as it was arranged beforehand that alternate cases would have this treatment, while the others in similar rotation would serve as "controls" or checks.

The resulting statistics showed nothing to indicate that the serum had any value either in reducing the number of

deaths or in preventing paralysis. In fact, better results were obtained with the untreated group. At the end of three weeks, the number of deaths among patients treated with serum was four times as large and paralysis nearly twice as frequent as in the other group. A recapitulation of these results at the end of four to six months showed complete recovery in a higher percentage of the untreated group as compared with those receiving serum and the ratio of deaths in this order was approximately one to three and a half.

At this point the laboratory scientist puts the issue squarely up to the physician, leaving it for him to decide whether or not serum shall be used in combatting infantile paralysis. That no harm will come from its use, there is no doubt and even though paralysis, once it has appeared, cannot be ameliorated, it is thought highly probable on experimental grounds that an immune serum might prevent the advance of virus into healthy nerve cells and thus do some good.

Generally speaking, serum treatment can succeed only in a disease where the virus travels by way of the blood stream and is therefore brought into contact with the protective substances of a serum introduced into the circulation. But infantile paralysis virus is not affected in this manner, as it moves onward to new susceptible tissues along the path of nerve fibers that are well insulated from their surroundings. Certain virus diseases, however, which, like vaccinia (antismallpox vaccine), are carried by the blood stream to distant parts of the body, can be influenced favorably by serums. Rabbits previously inoculated with vaccinia virus instilled into the nasal passages are completely protected against the disease by an injection into the vein of antivaccinia serum given within two days. If administered before inoculation, which can be delayed as long as five weeks, the serum will also protect animals against one thousand times the amount of virus found necessary to produce a fatal infection when serum treatment is withheld. These results, contrasted with the failure of serum

in poliomyelitis, again emphasize the important relation between fixed habits of parasites and the limitations they impose upon our attempts to control their activities.

In a desperate effort to work out some method of preventing infantile paralysis, hope was pinned for a time on vaccination as the sheet anchor, but this too was fouled in a stormy sea of controversy. It has been long known from the work of Flexner and his associates that an antiserum for infantile paralysis can be produced by inoculating the living virus beneath or into the skin of monkeys. Occasionally, however, the animals become paralyzed during this treatment for, in keeping with a well known peculiarity of viruses in general, the infantile paralysis virus fails as an immunizing agent, unless it is in an active, living state. Its application to man, therefore, cannot be advocated with much enthusiasm.

In speaking of immunity to this disease, it should be remembered that while an antiserum can be manufactured quite readily in monkeys, it is not so easy to make them resistant to artificial inoculation. Even after the use of living vaccines, there is little evidence of immunity to infantile paralysis virus when it is given by the nasal route. Hence the appearance of substances in the blood capable of neutralizing the action of this virus by no means proves the development of a parallel immunity to the disease.

Chemical or other methods designed to destroy the virus render it useless as a vaccine, and while a lessened activity through contact with injurious substances preserves the power to immunize, it does not deprive the virus of its paralyzing power. Indeed, chemical treatment, short of actual destruction, seems to reduce the quantity of infective material by diluting it, so to speak, rather than weakening it uniformly throughout. In monkeys, for example, which have been paralyzed after inoculation with chemically treated virus, it is possible to recover virus that is as deadly as the original untreated material.

A practical test with a vaccine of this type was made several years ago in a large city on the Atlantic seaboard,

where over ten thousand children were inoculated during an epidemic of infantile paralysis. Unfortunately twelve of these children later contracted the disease and ten died, all after six to fourteen days following the injection. Consequently the vaccine was condemned as unsafe, although scientific opinion is divided on this point because it is believed in some quarters that infection was already present before the vaccination and that disease could not have resulted from it.

A decision in this controversy rests between the statistical evidence and information obtained from laboratory experiments. From the latter, only one conclusion is permissible and that, as we have seen, does not favor the use of chemically treated virus, which, when tested in animals, has either no value at all or may be dangerous. On statistical grounds, however, it might be argued that if the vaccine was responsible for the alleged accidental infections, more than twelve cases of infantile paralysis would have occurred in a group of over ten thousand who were vaccinated. It might be contended further that the vaccine was at fault in this instance because the number of cases developing was greater than normally expected during epidemics among the general population from which the group was selected. Such calculations, of course, are subject to wide variation at different times and raise the question as to how much trust can be placed in such figures and who is to decide their usefulness in a dilemma. Even these matters are debatable.

Doctor J. P. Leake, Medical Director of the United States Public Health Service, emphasizes two things which stand out accusingly in this unfortunate occurrence; an unusually high fatality in the stricken group and the unfailing regularity with which paralysis affected those parts of the body that received the vaccine. If the injection was made into an arm or leg, that limb or its corresponding one became first involved. Here is a confirmation, if any were needed, of the remarkable attraction on the part of the virus for the nerve pathways. Its extraordinary virulence, accounting for the high death rate, is presumably

the result of numerous passages at short intervals in monkeys.

With serums and vaccines for infantile paralysis in disrepute, a new line of attack, chemical prophylaxis, which is now being studied, holds out greater promise than anything hitherto attempted. This is the idea of using protective chemicals to prevent a virus from entering the nose and throat. It occurred to Doctor Peter Olitsky of the Rockefeller Institute, while working with encephalitis (inflammation of the brain) in 1934, that a suitable chemical solution, sprayed into the nose, might act as an effective barrier to the virus of this disease, which, like infantile paralysis, enters through the upper respiratory tract. Tannic acid (obtained from tan bark), he discovered, shrinks and toughens the nasal lining, thus interfering with progress of the virus to the olfactory nerve (of smell) that leads into the brain. A one per cent solution of tannic acid instilled into the nostrils of mice three times a day on three successive days protected against encephalitis virus inoculated by the same route. The results showed conclusively that direct communication could be cut off with the central nervous system through nerve fibers in the nose by coating the mucous membrane with a protective chemical. In effect, this was equivalent to cutting the olfactory nerve, an experiment which Doctors Schultz and Gebhardt of Stanford University had performed somewhat earlier in monkeys and found protective against infantile paralysis virus inoculated into the nasal passages.

It was not long before other chemical substances were found to have the same effect as tannic acid in preventing experimental encephalitis. Doctor Charles Armstrong of the United States Public Health Service, who was himself a victim of this disease and narrowly escaped death as a result of it, reported successful experiments in mice with a nasal spray of alum or picric acid or a mixture of the two. With Doctor Harrison's assistance, he later showed that these solutions, preceding an inoculation of the virus by the nasal route, were effective also in infantile paralysis.

An opportunity was given to try this picric acid-alum method during the 1936 epidemic of the disease in Alabama, but the results were disappointing. Doctor Armstrong attributed the failure largely to improper spraying, done in many instances by anxious parents and others uninstructed in details of the method, which requires a high degree of skill professed by the most competent nose and throat specialists.

In their experiments with monkeys, Doctors Schultz and Gebhardt found, after testing the protective value of forty chemical substances, that a one per cent solution of zinc sulphate diluted with salt water and sprayed with an atomizer protected better than any of the chemicals hitherto tried. This method has been put to a hard test during an epidemic of poliomyelitis in several eastern cities. On a mathematical basis, the outcome thus far of this large-scale experiment with many young adults and children is a frank disappointment. The failure is due obviously to insufficient spraying of the nasal passages, with the result that the nerve endings along which the virus travels into the body are left intact. Doctor Schultz justly emphasizes the importance of determining whether or not an effective chemical blockade of the nerve endings has been produced. If these are destroyed, a simple test will show that the sense of smell is lost and only then, he believes, is the child protected against infantile paralysis. Fortunately the nerve endings grow again and the sense of smell is restored after four or five days, although in adults this may not occur for several months. If the disease is prevalent, Doctor Schultz advocates repeated spraying with zinc sulphate as soon as its effect has worn off, that is to say, when the sense of smell returns. In this way, it is thought, the virus will be stymied completely. Regardless of what statistics will prove or disprove, as an epidemic gradually dies out of itself, the method of nasal spraying does not loom at present as more hopeful than any existing preventive measures.

Until something better is discovered, chemical warfare

may provide the extra amount of protection needed to make the recognized natural defences of man more efficient. It is a remarkable fact that the blood serum in nearly ninety per cent of healthy adults in city communities selected at random contains protective substances against the infantile paralysis virus. So far as is known, these persons have never been in contact with the disease and if so, must have developed it in such a mild form that it could not be detected. Yet such serums, if mixed with the virus and injected into monkeys, will prevent infection and, what is even more surprising, frequently excel in protective power the serums obtained from convalescent patients. These observations have been interpreted as evidence of a mass immunization resulting from repeated exposure to the disease or as a natural resistance developing with age of the individual. Both theories are very important in considering some of the perplexing problems that have to be met during epidemics and will be discussed at greater length in a succeeding chapter.

The question frequently arises as to why there is such a to-do about infantile paralysis, when the attack rate in a given population is so small (perhaps one to two in a thousand persons), and nature seems to be handling the situation by building up a general resistance towards the disease. Answering that question would not help solve the real difficulty. Despite the fact that the number of persons affected even in severe epidemics is not large, the disease can and does in some unexplainable way spread over wide areas. Paralysis, while not characteristic of the disease nor as common as its name would suggest, is nevertheless one of the alarming possibilities, and in itself sufficient to cause mass hysteria. The modern automobile, destroying and maiming at an appalling rate year in and year out, is taken for granted, but man reserves his greatest fear for his invisible and mysterious enemies.

CHAPTER 10

LITTLE MICROBE—WHAT NEXT?

Microbe-eaters

Sometimes there is more truth than poetry in what a poet has to say:

> "So, naturalists observe, a flea
> Has smaller fleas that on him prey;
> And these have smaller still to bite 'em
> And so proceed ad infinitum."

It was about the end of the seventeenth century, long before the discovery of bacterial life, when Jonathan Swift penned this bit of doggerel verse, which if not poetic is at least prophetic. For now the germs that on us prey are smitten with microbes smaller than they.

More than two hundred years elapsed before another Englishman proved the truth of this prophecy and thus made one of the most revolutionary discoveries in bacteriology. This came about as the result of a chance observation by Doctor Twort in 1915, during some experiments with calf vaccine virus, in which there happened to be present a harmless micrococcus (berry-like microbe). In trying to propagate the virus, he spread the material over the surface of a culture medium containing foodstuffs in a hardened agar jelly. The contaminating microbe alone grew as visible masses or colonies, but among them were some with a peculiar glassy appearance and in other areas only clear transparent spots in which no bacteria were found. Attempts to transplant colonies lying close to these spots failed and later on these colonies also became transparent.

This was a puzzle indeed, for ordinarily growth could be renewed from generation to generation by merely

touching a colony of germs with a sterilized loop of wire and then drawing it across the surface of fresh culture medium. Twort soon found that he could produce spreading transparent areas indefinitely by touching normally growing colonies with a wire previously applied to the clear portions. Material from such areas, when filtered through a fine stone filter, dissolved organisms brought into contact with the resulting clear fluid even when it was diluted one million or more times in salt solution or water. Under the microscope, these colonies had a moth-eaten appearance and frequently showed numerous dissolved patches like punched out craters.

Twort believed at first that the transformation might be due to a filterable virus because the effect observed resembled an infectious disease of the micrococci he was studying. There was also a possibility, he thought, that something given off during their growth was responsible, since similar changes frequently occurred in cultures of microbes which had shown nothing unusual for months. According to this point of view, the chemical substance manufactured by the organisms eventually destroyed them and in so doing liberated more of the same substance, which was now free to act upon a new crop of similar germs. He observed the same phenomenon while studying an organism belonging to the colon-typhoid breed of bacteria recovered from the intestinal tract of a dog, an observation of particular historical interest as it was related to present-day theories concerning the origin and properties of the mysterious substance. As to the nature of this solvent agent, Doctor Twort wisely refrained from adopting any particular theory, although he recognized several possible explanations.

Interest in this problem was re-awakened two years later by a similar discovery made at the Pasteur Institute in Paris by Doctor d'Herelle. While working on a disease of locusts, he isolated a microbe which grew in colonies showing peculiar uneven borders resembling the broken

rim of a saucer; at other times, an abundant surface growth was riddled with clear areas that were free from organisms. Shortly afterwards, d'Herelle was interested in finding out what caused dysentery germs to disappear from the intestinal canal of patients during their convalescence. If an antagonistic agent was responsible, as he suspected, then by adding some of the bowel contents to a culture of dysentery organisms in a test tube, it should be possible to hinder their growth or destroy them completely. He found by experiment that stools as well as filtrates prepared from the fecal material during convalescence, but not in the early stages of the disease, contained a substance which would dissolve the organism causing dysentery. Now he took a fresh batch of these germs growing in bouillon, filtered it through a fine clay filter, and tested the clear filtrate in the same way.

The effect produced by adding a trace of this material to another actively growing culture was miraculous. If such a mixture, which was heavily clouded because of the organisms it contained, was set aside in an incubator and examined several hours later, the contents of the test tube had become crystal clear. A portion of this dissolved culture, when added to a new culture, caused it in turn to go into solution and so on indefinitely. No matter how many times the solvent agent was diluted in the act of transferring it from one generation of microbes to the next, its activity remained unimpaired. D'Herelle maintained that this substance is an ultramicroscopic germ smaller than those upon which it feeds and destroys, and gave it the name of bacteriophage or "microbe-eater."

According to d'Herelle, the bacteriophage enters the body of a microbe and multiplies within it precisely as a germ does in the cells of the human body. Thereupon an extraordinary swelling of the bacterial cell occurs, until finally it goes to pieces all at once, in a veritable explosion, and the microbe disappears, leaving behind only a "cloud" of "dust," the granular debris of bodily wreckage. Consider-

ing its size, if one might use that term, the bacteriophage puts on quite a show of power. Ranging, as measurements have shown, between 20 to 25 thousandths of a thousandth of a millimeter in diameter—one millimeter is about one twenty-fifth of an inch—it would be necessary to place nearly five hundred such particles edge to edge in a stippled circle to form a body that is just visible under a microscope. One need but remember that an object 1/50,000th of an inch in diameter can be seen quite readily with the aid of a good instrument.

The nature of the bacteriophage is still a deep mystery. D'Herelle contends that it is alive because it propagates itself without any limit and retains its solvent power after successive transplants, which, in the case of a weakly active strain of bacteriophage, can raise its activity to a higher level. Additional evidence of something living is found in its variable behavior towards different breeds of the same microbe, similar to variations in infective power often found in strains of any bacteria. Adaptation to different environments lends further weight to this argument, for, just as there are some germs that attack sheep and not pigs and others that cause disease in men and not in dogs, so there are highly specialized strains of bacteriophage that are partial to a certain microbe. And just as some viruses, like that of foot-and-mouth disease, will attack sheep, pigs, dogs, and men alike, so there are some breeds of bacteriophage capable of preying upon different species of germs.

Many observers do not accept d'Herelle's explanation of bacteriophage as living agents and attribute its behavior to the action of a chemical ferment or enzyme which is generated by the microbes themselves and set free when the cells break down and die, as all cells must sooner or later. These substances are then supposed to attack other susceptible cells and continue the process indefinitely as long as new material is made available. Every objection, however formidable, that has been raised to the chemical nature of bacteriophage has been countered with equally valid

reasons for denying its independent existence as a living agent. The situation in this respect is quite similar to that of viruses in general and creates a fundamental problem, the solution of which will give a decidedly new twist to the origin and control of infectious diseases.

In the history of bacteriology, probably no subject has created such widespread interest because it touches upon every phase of microbic existence and offers unlimited opportunities of giving free rein to the imagination. The amount of published work on bacteriophage, as can be surmised, is enormous, leaving one with the hopeless feeling that nothing more will ever be said to advance our knowledge of the subject. But there is always the possibility in research problems of this sort that a veritable tyro might find the answer in the laboratory instead of a library.

From the practical standpoint, the most important characteristic of bacteriophage is its complete dependence for "growth" and "multiplication" upon living bacteria, which has led to the use of "microbe-eaters" in treating infectious diseases. According to this theory, one or more races of bacteriophage are naturally present in the human and animal body, which is continually being invaded by germs. When the bacteriophage cannot overcome the microbes, sickness develops and when it acquires a special virulence for its germ victims, the patient becomes convalescent. Inasmuch as each type of microbe varies in its resistance to invasion by bacteriophage, just as the patient does to an infecting germ, successful treatment will depend upon finding the proper breeds of bacteriophage in each case. Such breeds, if selected during convalescence, would, according to d'Herelle, come to the aid of other persons suffering with the same disease. He prophesied that in the future epidemics might be checked by adding the right kind of germ solvent to a city water supply. This prediction has not been fulfilled, although the necessary requirements for such a practical test are found under natural conditions, exemplified by the presence in soil and surface

waters of numerous races of bacteriophage which can be picked out and held captive in a test tube.

"Microbe-eaters" are widely distributed and are most abundant in common sewage, particularly during seasons when certain infections happen to be unusually prevalent. The simplest way to recover these germ killers is to filter human waste matter through fine porcelain filters, which hold back all microbes and allow the bacteriophage to pass. A drop of the crystal clear fluid obtained in this manner will contain billions of bacteriophage particles capable of destroying the organisms that cause dysentery, cholera, typhoid and paratyphoid fever, and other diseases of the intestinal and urinary tracts. Considering their source and manner of leaving the body, it is not surprising to find these protective substances under the circumstances, but the hope of isolating others equally specific for infections of non-intestinal origin has not been realized. However, the filth of cities has occasionally yielded several new varieties of bacteriophage which attack streptococci (cocci in the form of chains) responsible for sore throat.

If, as d'Herelle believes, the bacteriophage is widely disseminated during epidemics and communicated from person to person like the germs upon which it feeds, the problem of controlling infection would solve itself. A contagious immunity of this sort would soon deprive microbes of their susceptible hosts and thereby prevent further spread of disease. This idea is somewhat difficult to follow, as it fails to explain how the bacteriophage can propagate itself while destroying its means of existence. Incidentally, a study of carefully controlled epidemics produced experimentally among laboratory animals at the Rockefeller Institute shows conclusively that bacteriophage activities, although abundantly present, seem to have no part in limiting the spread of infections.

Although this "invisible ally of man" is said to have miraculous curative powers, its mysterious properties are for the most part speculative and the best that can be said of it thus far is that its use does now and then bring about

a striking improvement, but more often does not. Doctor d'Herelle, who was the first to try the new form of therapy in a small group of patients with bacillary dysentery, reported remarkable successes. The results of other investigators have been less spectacular, however, and opinions differ also with regard to similar "cures" observed in typhoid fever and infections of the urinary tract due to colon bacteria. Various reasons are given for this lack of uniformity in results, which is still a problem under careful investigation.

Some of the difficulties and obvious limitations in applying this new therapy can be explained partly on the basis of certain well known peculiarities of bacteriophages. Laboratory tests show that they differ markedly in their native activity towards certain microbes, while these in turn vary individually in their susceptibility to any one race of bacteriophage. Typhoid fever organisms, for example, are not uniformly sensitive to the dissolving power of a particular antityphoid bacteriophage, which may attack one species and not another, whereas dysentery bacteria do not exhibit such irregularities. The problem then is to find the most suitable race of bacteriophage and that means one having the greatest virulence for the microbe in question. This can be determined by preliminary laboratory tests made with the patient's organism, which is first isolated as a pure culture growing in bouillon. The bacteriophage that is selected must surpass all others in clarifying the contents of the test tube quickly and permanently.

Another factor that has to be considered is the tendency of bacteria to develop a marked resistance to the solvent action of the bacteriophage. In adapting themselves to the situation, they become defensively immune just as the bacteriophage originally adapts itself offensively by a gradual increase in the power to dissolve the microbe. As a result of this mutual antagonism, organisms acquire the ability to flourish again after the peak of solvent action has been reached. In the test tube, such a turning point can

be observed by the reappearance of clouding due to bacterial growth in a culture fluid which the addition of bacteriophage had previously made limpid. The same effect is produced in the human body when a bacteriophage lacks vigor necessary to destroy invading microbes completely. It is quite possible under these circumstances for the surviving organisms and bacteriophage ultimately to reach a state of armed neutrality, during which they can be found together in certain chronic bacterial infections. Reasons for this are not apparent, although a similar relationship between parasites and their hosts is not at all unusual.

There is still another peculiarity of "microbe-eaters" which limits their usefulness. They give rise in the body to substances that counteract their power to destroy bacteria, thereby creating a peculiar situation in which the bacteriophages are ruined by their own devices against the enemy. This raises a question as to how the bacteriophage can produce antibodies against itself and yet manage to retain the specific germicidal property ascribed to it. An explanation of this irregularity introduces the familiar bone of contention as to whether the active substance is an independent self-governing body foreign to and multiplying at the expense of germs, or whether it is merely one of the products given off during their growth.

The question of an immunity being developed in the animal body towards a germ that feeds on other germs is one upon which a critical test of the validity of the bacteriophage theory might hinge. This test must be based on experimental evidence that bacterial products accumulating during growth in a test tube or in the body of a patient are in all respects distinct from the dissolving agent itself. Up to the present time, it has not been possible to prove this crucial point by means of every available method.

Whether or not bacteriophages are the cause of microbial dissolution or a result incidental to it still remains an open question. There is no doubt that they induce

antibodies which react with the bacteriophage alone but never with the accompanying substances that are derived from bacteria and are present in the same solution. Nevertheless it would be difficult to accept this as evidence of two separate and unrelated organisms. It is not unusual for a highly specific chemical reaction to be associated with *one* portion of the products liberated during the growth of a *single* microbial species. In this connection, one need but recall some of the facts pertaining to the complicated chemical structure of bacterial cells, in which separate fractions derived from the same germ have remarkably different properties.

The bacteriophage has given us something to think about, even though its immediate general application to the treatment of infectious diseases is not yet apparent. All the claims made for "microbe-eaters" as a new weapon in the fight against germs have not survived the test of cold scientific analysis. For the time being, at least, this "germ that destroys germs" is one of the strangest mysteries in the world of microbes.

Man-eaters

When Elie Metchnikoff, promoter of longevity, died in 1916, he had spent the greater part of his seventy-one years in studying single-celled animals and other lowly progenitors of man, composed of bundles of cells organized to perform a few single functions, such as eating, drinking, and multiplying. In these microscopic living units, Metchnikoff discovered fundamental laws of growth and decay, disease and death, and from their activities set forth certain principles of infection and resistance to bacterial invaders. He came to regard abnormal cell behavior as the inevitable reaction to microbial parasites and with prophetic vision looked forward to the time when improved scientific methods would demonstrate the parasitic nature of tumors or cancerous growths.

Today, as in Metchnikoff's day, the cause of cancer still

lies hidden within the fragile walls of microscopic cells which carry life and death as they grow and multiply to form the tissues of a human body. When the mysterious forces that regulate this growth become better known, the problem may be solved, for the difference between normal and abnormal cell development is simply that of moderation as compared with unbridled excesses. A healthy response to ordinary wear and tear or to injury stimulates the formation in the proper place of new tissues in the proper amount required for restoration of the damaged part. Further growth then ceases under the influences of an automatic control, which, like the governor of an engine, regulates the explosive energy of healthy growing cells and prevents them from running wild and causing unpredictable damage. Cancer cells, on the other hand, are not restrained in this way and grow like noxious weeds at the expense of neighboring tissues, which they destroy. Such cells are undisciplined, growing of their own accord, and are in this respect virtually immortal. Whatever the cause of this growth, it does not obey the laws of normal tissue replacement and once it has commenced, continues long after this need has been satisfied.

Cancers or malignant tumors literally consume the victim, eating their way into all parts of the body and pushing forward relentlessly like a stream of lava, obliterating the familiar architecture of healthy cells and organs. Death may result from the effects of mechanical obstruction due to the tumor mass or from a general wasting away of the body through long continued depletion of its vital resources. To call these destructive new growths parasites would be somewhat inaccurate because they originate within the host itself, yet they are as parasitic as if introduced into the body from without. They are man-eaters indeed and take their toll of nearly 150,000 deaths in the United States alone every year.

Quite naturally the problem of cancer control has revived interest in theories that have been proposed to explain

the cause of this disease. The parasitic theory, which had a vogue during its early history, was inspired more by the finding of various microbes than by any evidence suggesting it as a possible explanation of abnormal growths. Until the disease was reproduced artificially by Doctor Leo Loeb in 1901, nothing was known about its cause and only then did the study of cancer become a subject for scientific research. He succeeded in transplanting certain kinds of tumors found in rats and mice and kept these growths alive indefinitely by transferring a fragment of diseased tissue from one animal to another. In this way the behavior of cancer cells could be observed through many generations of tumor-bearing animals after the animal from which the original material was taken had long since died of old age.

The recognition of transplantable tumors lent considerable weight to the possible part played by an infective agent in producing these new growths. Especially significant, therefore, were the observations of Doctor Peyton Rous at the Rockefeller Institute in 1910 and 1911. He found that a tumor (known as sarcoma) peculiar to hens could be transplanted to other domestic fowls of the same breed, in particular barred Plymouth Rocks. After a time, this tumor became so well adapted to growth and frequent transplantations that it was possible to inoculate hens of other breeds by inserting into their tissues a needle which had merely been drawn through the tumor mass.

Doctor Rous then made a remarkable discovery, which put a new construction upon the old parasitic theory. From the tumor itself, he was able to separate something which caused a similar growth having all the characteristics of the original when injected into healthy chickens. The causative agent was so finely divided that it passed readily through the pores of stone filters capable of holding back the smallest microbes. By means of this clear solution freed of cancer cells, new growths could be produced in normal fowls and from these transmitted to others in

turn by inoculation of similarly prepared filtrates or of tumor tissue in a dried and powdered state.

It was foreordained that the nature of a transplantable growth thus produced should become a subject of controversy. Although there had never been any question that the Rous sarcoma was a genuine tumor, doubts were expressed as soon as its filterable character was discovered, one of the principal objections raised at this time being the failure to demonstrate similar filter-passing agents in tumors of mammals. This line of reasoning implied that only such tumors are genuine as cannot be transmitted by means of agents which are not recognized as a cause of tumors. If this seems involved, it is less so than the devious processes of argument used by the objectors to the theory of virus tumors.

But the nature of this agent required some sort of explanation for its unprecedented behavior. Whatever it was that stimulated the tissues into growth would have to be intimately associated with the tissue cells, multiply with them, and accompany them on their wanderings from place to place. Since nothing had been discovered that could do all these remarkable things, it was assumed that the tumor filtrates contained minute fragments of cells which had passed through the filter without any loss in power to multiply and produce the same kind of growth.

As the puzzle deepened, new properties of viruses were being revealed on every hand and it was soon recognized that their newly found attributes coincided perfectly with the character of certain tumors. Just as some viruses destroyed tissue, others caused growth and in the developing cells found pabulum, shelter, and a means of transportation. With the discovery of filterable agents in a variety of chicken tumors, infectious growths of dogs and cattle, and in common warts, the rôle of a virus came to be regarded as an important phase of cellular activity that went hand in hand with tumor formation.

The question now arises: Is cancer caused by a virus

or is the virus produced by the cells of the tumor? The only satisfactory answer to this is the fact that viruses change the character of tissue in the manner peculiar to tumors and that these growths are caused by some viruses. While this is going on, the amount of virus increases just as it would in a tissue culture of living cells. Does the virus have any share in producing changes associated with malignant growths or cancer? Over a period of years the Rous tumor has shown itself to be highly invasive with a marked tendency to spread to distant parts of the body. In this instance, disregarding for the moment its virus origin, the growth is unquestionably malignant, but to what extent malignancy depended upon the virus activity was learned from a chance observation.

Cottontail Rabbits and Virus Tumors

In 1909, Ernest Seton Thompson, writing in "Life Histories of Northern Animals," described "horns" on jackrabbits and cottontails found in Colorado, Nebraska, and Oklahoma. Similar finger-like, warty, or horny growths, known as *papillomas,* have been seen frequently on the skin of wild cottontail rabbits inhabiting Iowa, Kansas, and Texas. In 1933, Doctor Richard Shope of the Rockefeller Institute branch at Princeton, New Jersey, isolated from these "horns" a virus with which he was able to transmit the disease first in its natural host and later to domestic rabbits. The virus is exceedingly small, passes through the finest filters, and, when rubbed into the skin of the healthy animal, produces a growth which is now known to be a typical mammalian tumor.

This might well be considered a "missing link" in the evolution of cancers in so far as it provides an example of a recognized virus disease which only later proved to be a genuine tumor. The discovery, like that of virus sarcoma in chickens, enables one to follow the progress of malignant changes developing from an unmistakable virus infection. Doctor Rous has tattooed rabbits with Shope's

tumor-producing agent and seen cancer appear after four to seven months. Significantly, these changes, occurring only in cells that are affected by the virus, advance from a benign to a malignant character precisely as in human tumors.

The implications of this work are enough to stagger the most cautious and conservative scientists, but Doctor Rous will not go beyond the assumption that certain tumors of unknown cause may be due to viruses. In the end, the question as to what incites the cancerous condition still remains unanswered, for there are numerous stumbling blocks in the way of a direct line of reasoning. Rabbit cancers, for instance, do not yield a virus with which one can produce similar malignant growths. As a matter of fact, in domestic rabbits the virus cannot be recovered, even from the original inoculable tumors (papillomas) which eventually become cancerous, whereas it is always found in the papillomas of wild cottontails, the natural hosts.

There is another promising approach to this problem in the cleverly contrived experiments of Doctors Rous and Kidd with tumors artificially produced by applying tar to the skin. Painting a rabbit's ear with tar will cause the growth of warty tumors that disappear after tarring is stopped. Under no circumstances do such masses ever become cancerous. Now if the papilloma virus is injected into the blood stream of such animals, cancerous growths quickly develop on the skin and spread to other parts of the body. In rabbits receiving none of the virus, the ordinary "tar warts" never change into a cancer.

That the transformation into malignancy depends upon the introduction of the virus can be shown in a different manner. When some hashed warty material is steeped in a solution containing the papilloma virus and bits of this infected tissue are then planted under the skin of healthy rabbits, a genuine cancer will result, whereas the original tissue immersed in a solution that contains no

virus never produces any growth. It is well known, of course, that certain viruses can live in tumors. But they do so without giving rise to any remarkable changes in the tissue other than those found regularly in the growing cells of a suitable culture medium that allows any virus to multiply. The papilloma organism, however, is undoubtedly a tumor-producing agent capable of progressing into cancer and here quite definitely stimulating a simple tar wart to active growth, changing its architecture and transforming it into a malignant tumor.

Until there is stronger evidence to warrant a different point of view, one is impelled to the belief that no agent, chemical or living, other than a virus, does more than prepare cells for the type of unrestrained growth by which a true cancer is recognized. The prolonged action of tar, chemicals, heat, mechanical irritation, bacterial infection—all these influences and perhaps others—give to the tissues a certain amount of impetus in that direction but nothing further. If one wishes to be more conservative, one can think of tumors as arising from the interaction of viruses and one or more of the helping factors which have been enumerated. Or, one can be non-commital and think nothing at all.

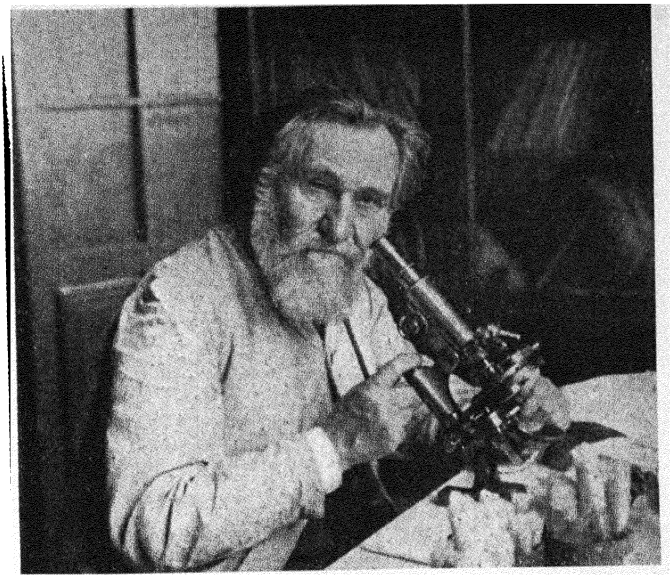
It is possible that the changes from one kind of tumor to another in different hosts is responsible for the failure to reveal the causative agent, which thus far has never been demonstrated in mammalian tumors generally. This might be explained on the reasonable assumption of changes taking place in the virus itself, as it is exposed to unusual conditions arising within the tumor tissue. There is no reason to doubt that viruses, like ordinary microbes, can change into other strains when environmental influences favor such variation. These variants of the original virus, Doctor Rous believes, may be responsible for cancers. In his explanation one finds nothing inconsistent with the natural behavior of viruses as understood at the present time. Stated simply, the tumor is a tissue culture medium

for the virus, which as it proliferates brings about alterations in the growing cells. These now act upon the virus, changing its properties and transforming it into different breeds that incite the tissue cells in turn to cancerous rebellion.

A striking illustration of this is found in the already familiar behavior of the wild cottontail rabbit virus when it is transplanted from its home surroundings to a foreign soil in domestic rabbits. Under the influence of this transition, the virus takes on new characters, so that instead of producing typical growths it may give rise quite suddenly to a different type of disease, having all the distinctive features of cancer. The slightest possible alteration in a microbial agent seems to be significant for stimulating the growth of new varieties.

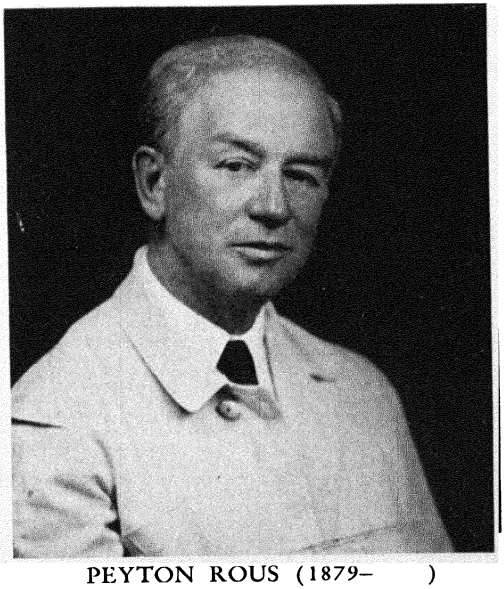
In a tumor, the mechanism is somewhat involved, owing to the inter-action of the causative agent and the cell constituents, but it is possible by experiment to show how readily some viruses can be made to change their properties. A number of years ago at the University of Rochester in New York one kind of virus was successfully transformed into another by adding to the first variety a preparation of the second that had been made inactive by exposure to heat. If such a combination is inoculated into rabbits, the first virus acquires the ability to produce a disease which is identical with that caused by the unchanged second virus alone. The disease thus produced runs true to form even when transferred from animal to animal many times in succession.

The remarkable thing about this transformation is that two distinct viruses are implicated, each capable of developing its characteristic infectious disease. One of these is the infectious tumor of cottontail rabbits (virus papilloma); the other is a disease of certain rabbit breeds, towards which wild cottontails are naturally resistant, and is characterized by the formation of a gelatinous swelling under the skin (*myxoma,* or mucus tumor). Notwith-



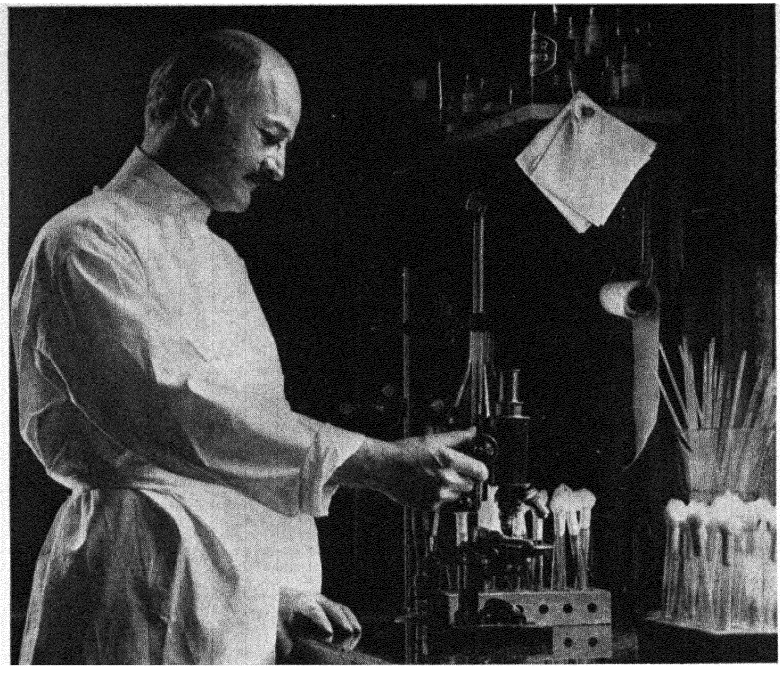
ELIE METCHNIKOFF (1845–1916)

This great scientist referred to himself as "a zoologist who had wandered into the domain of biological chemistry and medical science." He developed the theory of the struggle between the white corpuscles of the blood (phagocytes) and disease germs, which today is proving of vital importance in the war against disease. This contribution has been of greater value than another favorite theory of Metchnikoff's to the effect that the consistent use of sour milk will prolong life.



PEYTON ROUS (1879–)

A pioneer in the study of transplantable tumors by means of inoculation. His work with viruses in cancer has opened up a new field of cancer research



EDWARD FRANCIS

Doctor Francis, U. S. Public Health Service, Washington, D. C., was awarded the gold medal of the American Medical Association in 1928 for "his thorough and important scientific contributions to the knowledge of tularemia."

CARLOS FINLAY (1833–1915)

A brilliant, hard-headed Scotchman, whose stubbornness vindicated his theory of mosquito transmission in yellow fever

standing a mutual exclusiveness of the two diseases created by racial and environmental barriers, the cottontail virus acquires distinctive infectious properties of the myxoma virus, by undergoing a change that necessitates an alteration in the kind of disease produced!

While the ability to mutate or change might make up for their shortage, it is strongly suspected that in all the world there are not enough viruses to account for the extraordinary variety of tumors and cancers. This suspicion grows less tenable as one considers the nature of any disease, whether it be due to common microbes or viruses. Although the causative agent may be and often is widely distributed among human and animal populations, it does not become effective except under unusual circumstances. Various diseases that are due to viruses frequently arise as a result of some trivial incident which fans a smouldering fuse.

Through a study of dormant infections, we are only now beginning to appreciate how extensive and versatile our virus population really is. Just so long as there are cells in the body and these continue to divide, there the virus will thrive and become as specialized as may be necessary for its purpose. Whereas microbes are obliged to find hideouts away from devouring phagocytes and lethal substances that circulate in the blood stream, viruses need only infect a cell to obtain assured protection, unless perchance they lay themselves open to the attack of antibodies by eating their way out of house and home. Actually it is possible to demonstrate these antibodies in the circulating blood of rabbits during the stage of outspoken destruction of the tissues resulting from an experimental infection with a cancer virus. Such blood serum effectually counteracts the virus if a mixture of the two is inoculated into healthy animals, while this property is universally lacking in the non-cancerous bloods.

The natural defences of the body, however, cannot subdue these viruses completely, much less prevent some

external stimulus from rousing them into activity. Chemical or mechanical irritants or bacterial infection may be contributing factors that are necessary to set in train a latent cancer virus. In other diseases, the influence may be less obvious, as when a faulty diet will call forth a crop of fever blisters on the skin of persons in whom the herpes virus has been lying dormant.

Taking into consideration all the available facts of host-parasite relationship, it is almost a certainty that viruses are as commonplace in man as fleas on a woolly dog. Yet these companionships are not inseparable in a strict sense and the question is raised whether the laws of disease transmission can be applied to cancer as to other virus infections. Some of these, we already know, are disseminated by means of biting insects, but there is not the slightest evidence thus far that virus tumors can be communicated to man in this way. In animals, however, experimental transmission of the Shope papilloma by an insect was accomplished by Doctor Larson and associates of the University of Minnesota.

The probability of insect transmission occurred to these workers as they observed many examples of natural infection among cottontail rabbits in certain localities of the state which were never free from the disease and noted the peculiar distribution of tumors on the head and neck, as well as other parts of the body. In their experiments, rabbit ticks were allowed to feed twenty-four hours on the virus-infected ear of a cottontail and then put to pasture on one ear of a healthy animal. Three weeks later typical tumors developed in the circumscribed areas of tick bites, while the other ear remained normal. These tests showed that the virus could be inoculated successfully by an insect bite, even though the method of transmission were purely mechanical. The possibility of natural tick infection and the spontaneous development of tumors was carefully ruled out by collecting both insects and experimental animals from a region where the disease had never

been found. Thousands of rabbits caught and banded over a period of ten years in this large area and later recaptured and examined, along with many more thousands of wild cottontails, failed to show any evidence of virus papillomas. Furthermore, in view of the enormous tick population in rabbits during the summer months, widespread dissemination would have occurred had the insects been naturally infected.

These observations constitute additional proof of the infectious nature of a certain kind of tumor, which is obviously caused by and transmitted in the manner of a virus. Nevertheless, when everything is said and done, the readily demonstrable cause of this type of new growth makes it all the more difficult to accept a similar explanation for ordinary tumors. Inevitably we come to a fork in the road, asking ourselves which way—only to discover that we had been there before. Is the cancer virus—any virus—a living substance or dead? Is it a protein carrying a live agent? Can it be that virus activity is a special quality of protein substance and its infective power a property of a pure chemical compound?

Chemical Whirligigs

In an attempt to answer these questions, science is now riding dizzily on a new kind of merry-go-round. Ultravisible life is being studied along entirely different lines by means of the ultracentrifuge, a "super" machine which is to a common cream separator what the modern telescope is to a spyglass. About twenty years ago, Professor Svedberg at the University of Upsala, Sweden, developed a method of analyzing the various properties of substances which cannot be extracted or separated in a pure state by ordinary chemical procedures. With a motor-driven pressure oil-turbine capable of whirling solutions in a container at incredibly high speeds, he was able to concentrate by sedimentation dissolved substances having a definite structure. This machine, spinning at a maximum speed

of 75,000 revolutions per minute, creates a gravity pull more than 400,000 times that of the earth. As the centrifugal force varies with the speed of rotation, particles of different types can be made to settle out, according to their size and shape. In a perfectly clear solution, for example, which might contain egg white and sugar in chemically undetectable amounts, the two ingredients are readily separated without undergoing the slightest possible alteration in their natural properties. Thus one can purify even protein molecules, which are enormously large bunches of atoms that have jelled or coagulated. Living matter is chiefly made up of just such atoms. Once the substances are isolated in a pure state, the actual size of the molecule can then be determined by precise measurements which help identify different types of protein and their specific activities.

Owing to the prohibitive cost of Svedberg's machine, his methods were not adopted by other investigators until years later, when a less expensive ultracentrifuge developed in this country has made possible the study of a number of disease-producing viruses of plants and animals. This new air-driven apparatus is an improvement upon the oil-driven turbine in that large columns of liquid can be spun in a centrifuge so as to yield virus proteins in sufficient quantities for a thorough analysis of all their mysterious properties. Some of these proteins contain molecules that differ from the kind found in ordinary substances in their remarkable size and weight and tightly packed arrangement. These are known as giant molecules, yet they remain invisible under the highest powers of magnification. If such a molecule were placed alongside of one taken from a grain of table salt and magnified equally, the proportionate bulk would be like that of an elephant to a mouse. In chemical language, the molecular weight of salt is given as fifty-eight, whereas that of a giant molecule can be expressed in the millions. To take another illustration, the number of ordinary molecules required to fill a small

glass tumbler would, if replaced by an equal number of giant protein molecules, occupy the space of a large room.

A great forward step was taken in 1935 when Doctor Stanley of the Rockefeller Institute station at Princeton, New Jersey, using such a centrifugal machine, crystallized the virus of mosaic disease that ruins tobacco plants and discovered that it is composed of giant molecules. Similar heavy proteins were found to be responsible for mosaic disease of potatoes, from which was concentrated the virus found in the juice of infected plants. Following these successes, Doctors Beard and Wyckoff began studying the virus causing infectious tumors in cottontail rabbits and from the warty growths isolated a heavyweight protein, with which infectiousness was intimately associated. The virus activity in rabbits of this purified substance is thousands of times greater per unit than that of the original tumor tissue from which it was extracted, indicating a progressive concentration of the infecting agent in the giant molecules of protein. Not a trace of heavy protein is found, however, in the luxuriant tumor growths of domestic rabbits that have been inoculated with the virus taken from cottontails. This negative result is very significant, as it will be recalled that the papilloma virus cannot be recovered in the cancerous tissues. The obvious conclusion, therefore, is that the infective property must reside in the protein.

If the theory is correct, some future day it may be possible to change these infectious proteins into harmless compounds by injecting a chemical substance into a tumor or the body of a patient suffering from any of the diseases caused by viruses. The ultracentrifuge has begun to make plain some of the strange things that happen to these infectious agents when they are brought into contact with chemicals or are otherwise subjected to disturbing influences. The changes taking place can be photographed by means of delicate optical devices controlled electrically while the machine is in operation.

Certain animal viruses which have been studied in this way show that they become inactive as a result of obvious alterations in the structure of their protein substance. Sometimes, as with the virus of horse encephalitis (inflammation of brain and spinal marrow), it breaks up into exceedingly small bits of material that no longer settle out as a solid mass; or, in the case of rabbit tumors, the virus protein breaks into a few large fragments which progressively diminish in size as the disturbance continues. Under like conditions, plant viruses, such as tobacco mosaic and potato blight, do not disintegrate in this manner, but instead appear to undergo a change in the shape of their molecules.

However the damage is inflicted, these altered proteins are harmless when inoculated into animals or plants that are susceptible to the original substance. Although stripped of infective power, these denatured or inactivated plant viruses retain their property of manufacturing protective antibodies against themselves. Especially significant is the recent discovery that the protein extracted from rabbit tumor virus can be made harmless in a solution which is carefully adjusted to the proper degree of alkalinity, without affecting thereby the nature of its protein molecule.

Taking these facts together as a basis for controlling virus diseases by chemical means, it is hoped that the body will be aided in organizing its defences to better advantage. Two of the most important requirements of successful vaccine or serum treatment have been fulfilled, at least theoretically, in that the material for injection is harmless and at the same time resembles closely the unchanged living agent, even to its internal structure.

What all this will lead to is still problematical, but a way has been opened for a novel method of attacking virus tumors and perhaps some virus diseases. One might be tempted to regard the newer chemistry as the nearest approach to a visible transformation of virulent into non-

virulent microbial breeds. It is fortunate indeed that we can set bounds to their activity, as new kinds of infection constantly appear to warn us of the possible limit which we may be reaching in controlling our germs and viruses. Are these new forms of disease or old ones in disguise, or are certain microbes still in the process of evolution eventually to end in a surprising array of hitherto unknown plagues? Unwittingly, man may be the innocent victim of his hospitality to a straying parasite and perpetuate it as a new disease of higher animals, including himself. If he should need assistance to accomplish this end—and generally he does not—a suitable transmitting agent is introduced somehow from without and a new disease makes its appearance.

In more recent times the air-driven centrifuge has become a useful instrument for purifying individual viruses, precisely as the various protein substances have been identified heretofore, but with a difference. Whereas viruses could be recognized and easily identified by means of their particle size and the diseases they caused, it was difficult and often impossible to purify the virus. This was due to the fact that other substances of an entirely different nature were similar as to size of their particles. Now, by means of various centrifugal speeds ranging from more than 20,000 and up to 40,000 revolutions per minute, the particles of virus can be thrown down in the form of a sediment and graded according to size and kind. This technic, applied to the study of poliomyelitis, has made possible the recovery of virus from intestinal contents and the blood of experimentally infected monkeys.

Quite recently, investigators at Stanford University in California reported an improved and much simplified method, using an ordinary air-driven centrifuge. At a running speed of only 18,000 revolutions per minute for four hours, they recovered poliomyelitis virus in the sediment from stools of paralyzed patients during the first week of the disease. It was hoped that this would be a

better method for detecting the presence of virus in sources of material where it might otherwise be missed. While the results achieved in this regard were not conclusive, the technic furnished a means for preparing virus suspensions free from contaminating impurities and other organisms. By itself this was a step forward in isolating viruses from suspected materials and identifying them with greater accuracy.

Vision of the Future—The Electron Microscope

These were only the beginnings of studies which led eventually to direct observation of virus bodies. Although known to exist, they were too small to be revealed by any form of illumination. An instrument was needed capable of seeing more and more of less and less, until the "invisible" micro-world should become visible. With the ordinary optical microscope anything approaching this ideal is out of the question because its resolving power and range of useful magnification are limited by the nature of light itself. By the term "resolving power" is meant the power of the eye or a lens to make separately visible the small objects or particles that are close together. In this manner the structure of an object is revealed, depending upon the source of light used and the wavelengths composing it. Microscopes using a beam of ordinary light with its long wavelengths, or of invisible ultraviolet light with much shorter wavelengths, cannot detect the size of objects or their details when these are much smaller than the range of wavelengths found in the kind of illumination used.

It took a generation in time to develop the ultraviolet microscope, which at its very best can reveal particles only one half the size of those seen with visible light. In 1940 the introduction of an electron microscope designed and built by Doctor Zworykin and associates, gave us a new precision tool with boundless possibilities. In this instrument of extraordinary resolving power, a beam of electrons is used in place of the beam of light as in the ordinary micro-

scope. These electrons have wavelengths one one-hundred thousandth that of visible light. Moving at extremely high velocities, a stream of electrons generated by a heated wire filament in an "electron gun" is concentrated in a magnetic field and the beam of rays shot into the specimen, which is mounted in a vacuum-locked chamber. "Illumination" of the object through a system of lenses and electromagnetic controls forms an image on a viewing screen or photographic plate. The amount of darkness and brightness seen in the image depends on the thickness and mass contained in a unit of exposed substance. In this respect, electron pictures may be said to resemble those made by X ray.

With this new type of microscope it is possible to see and photograph particles and details of structure too small to be visualized by any other optical device. Unlike the ordinary microscope, resolving power and magnification of the electron microscope are not limited by the wavelength of its "illuminating" electron beam. Thus it is now possible to obtain useful magnifications up to 100,000 diameters, or 50 times greater than is possible with the best optical microscopes.

Electron microscopy, already a highly specialized science and art, is extending a newly discovered sight beyond the range of visual speculation into the world of matter, the beginnings of life itself. Incalculable benefits should result from knowledge gained by observing the changes produced within bacteria as a result of treatment with serums, antibiotics, chemicals, and drugs. What is now clearly discernible in the cell-structure and composition of viruses and microbes will someday contribute to a better understanding of disease-producing agents and their control. But it would be premature to estimate the effects of these first glimpses into unknown worlds where the dividing line between all living and dead matter remains on the last frontier.

Chemical analysis has given us an inkling of the strange

powers residing in a bacterial cell with its highly specialized activities. These depend now upon a coating of sugar-like substance, again upon a layer of fat or wax and more often, as we have just seen, upon the internal arrangement of protein molecules. But notwithstanding all the epoch-making discoveries concerning microbes, they still manage to outstrip man's ingenuity in combat, for he takes his germs with him wherever he goes.

CHAPTER 11

MICROBES RUN AMUCK

When microbes run amuck the result is pestilence, which, together with war, death, and famine, is one of the four great scourges of mankind. It is difficult, in these days of comparative enlightenment as to the causes of disease, to realize the terror that these sudden visitations of epidemic infections brought to the inhabitants of the Middle Ages. Striking suddenly, like a dark cloud, with devastating effect, it is not surprising that the most feared of these diseases was given the name of "plague," meaning in ancient languages a "stroke."

The history of the great plague epidemics has come down to us through the centuries, their horrors dramatized in narrative and story. In the literature of the Middle Ages, this fatal disease of pestilence is commonly referred to as the Black Death, which swept through Europe and over the known world in the fourteenth century, taking a toll in Europe alone of at least 25,000,000 inhabitants and about 60,000,000 in the entire world, or one-quarter of the world's population. The reason for the term "black death" is found in the following quotation from Giovanni Boccaccio, a great Italian novelist, who lessened the tedium of a self-enforced isolation in Florence at that time by writing the hundred tales of the Decameron, all told during ten days. "Great pits were dug, wherein the bodies were laid by the hundred . . . packed in layers, even as men pack bales in a ship's hold . . . and it was a peculiarity of the disease to show itself by black or blue spots, which would appear on the arms of many, others on their thighs, and every part else of the body. Almost all died within three days of the appearance of the fatal signs, some sooner

and some later. . . . This pestilence was all the more violent because, by communication from the sick folk to the sound, it spread no less rapidly than a fire . . . and there arose divers fears and phantasies among the survivors" leading to panicky flight because "by this means each hoped to win his own safety."

Guy de Chauliac, physician and chaplain to Pope Clement Sixth, gave a remarkable account of the plague in Avignon, France, where he saw it twice, the first time in 1348 and again in 1360, when the scourge was introduced from Germany. The first outbreak is said to have carried off in the space of three months more than one hundred thousand inhabitants, according to the eminent physician and chronicler, who himself caught the disease but survived. In this epidemic Petrarch's wife, Laura, of whose beauty and character the celebrated poet had written so much, sickened with fever, spat blood, and died after three days. The history of the plague in 1630 in Milan, where it took its most deadly form in the invasion of the blood stream, known as septicemic plague, forms the closing chapters of Allessandro Manzoni's remarkable novel entitled "I Promessi Sposi," rendered in English as "The Betrothed."

These great epidemics, which were none other than oriental plague that had been carried from its ancestral home in Asia by way of the Near East into Africa, resulted in the first constructive efforts to introduce sanitation and public health measures. In the early days of the disease, as in every destructive pestilence of unknown cause, the great mortality was quite naturally attributed to poison. In most countries the victims of this delusion had to be found in some convenient scapegoat, usually the Jews, who were suspected of having poisoned the wells and fountains and so were put to death. But the epidemic did not cease. When the more enlightened came to realize that plague travelled in the galleys sailing from port to port, *quarantine* laws came into existence. The first of these originated

with the Genoese in 1348, the year following the arrival from the Levant countries of several vessels full of plague patients. Suspected ships were thereafter forbidden entrance into the port of Genoa. Marseilles, France, followed this practice in 1383, with the detention of vessels and passengers in the harbor for a period of forty days ("quarantaine"), during which the ships were exposed to sun and wind and fumigation. A century later Venice, the "queen of the Adriatic," stationed sanitary officers at various ports to enforce similar regulations. Medical historians believe that this time limit was dictated by ancient doctrines, according to which inflammatory diseases literally burned themselves out on the fortieth day and were no longer acute. We still use the old word which means forty but suit the period of quarantine to each disease.

Celebrated physicians of the fourteenth century, even though devoted to astrology, were convinced of contagion in epidemic diseases, an idea that had been handed down from the second century after Christ when segregation of sick from healthy persons was practiced in order to prevent infection with leprosy through contact. In Italy, early in 1374, plague patients were ordered out of the cities to die or recover in the fields, and all who came into contact with the sick were forbidden on penalty of death to associate with other persons or to return from infected localities before the expiration of at least ten days. Notification of disease, searching for the sick, isolating and marking infected houses (the beginning of our modern quarantine placards) by such methods as "putting out wisps" and the "showing of white rods," were already in force as early as the year 1500. Before the close of the fourteenth century sprinkling homes with vinegar and rose leaves came to be regarded as a futile gesture, whereas we moderns even as late as the twentieth still believed in the virtues of stinking sulphur fumes.

From the vivid descriptions by reliable witnesses of the plague, it is evident that two forms of the disease were

prevalent during the Middle Ages. Spitting of blood, a common sign, with death occurring after one to three days in all who were thus afflicted, is characteristic of pneumonic plague. The frequent mention elsewhere of tumors, the so-called "pest-boils" or "buboes," appearing in the groin and arm-pits, clearly indicates that a second form of the disease was bubonic, especially since it was not invariably fatal and then only after six to eight days. "Pestilential buboes" are accurately described in an account of the plague at Naples in 1656, "of which there died in one day 20,000 persons."

At first glance it might appear that there were two different diseases, but both are caused by the same germ, Pasteurella pestis, and its manner of entering the body alone accounts for the striking differences. When inoculation takes place through the skin from the bite of an infected flea or handling of diseased rodents, bubonic plague results, causing glandular swellings (buboes) in certain regions of the body that are connected by natural drainage channels with the site of infection. If, instead, the portal of entry happens to be the respiratory tract and pestis microbes enter the lungs, plague pneumonia develops.

Accordingly there is a vast difference in the manner in which each type of disease will spread. While the bubonic form requires a continued supply of infected rodents and suitable insects to feed upon them, the pneumonic variety can be communicated readily from person to person by inhalation of droplets expelled in coughing or talking. Infection through contact, however, will occur frequently in bubonic plague when pneumonia is produced in a roundabout way by microbes breaking loose from the glands, entering the blood stream, multiplying there, and finally lodging in the lungs. Blood stream invasion is quite common to both types of plague and may cause death in a few days, even before the appearance of any recognizable signs. This form of the disease has been

met with under circumstances suggesting that it often hastens the decline of an epidemic because an extraordinarily high mortality before droplet infection can take place prevents its spread.

Fleas and Rats Are One Jump Ahead

Plague is mainly a disease of rodents transmitted to man by certain kinds of fleas or other insects that choose their meals indiscriminately and jump from one host to another. When animals die of plague, the fleas, already gorged with germ-laden blood, immediately leave the corpse and wait for a fresh host. In the meantime, the germs have been multiplying in the insect so as to block the entrance of food into the stomach. The pump, which is in the pharynx or opening to the digestive tract, continues to function, however, and during frantic efforts to appease a fierce hunger, the sucking of blood merely distends the canal leading from mouth to stomach. As the pumping ceases, its contents are forced into the punctured skin. Sometimes the obstruction is not complete and a temporary opening in the mass of bacteria causes the flea to discharge the entire stomach contents repeatedly. Under these circumstances a bite is exceedingly dangerous because of the enormous infective dose injected into the victim.

Measures designed to prevent plague must take into account the close connection of this disease with rodents and their vermin and the part, accidental though it be, that is played by man. Cleanliness and strict personal hygiene effectively dispose of the flea problem, as far as man is concerned, but a campaign to exterminate rats is doomed to failure. Rats are nothing if not prolific and a modest pair, living on the average three years, will produce several million descendants. Calculations like these soon lead to fantastic figures and show the folly of any attempt to eradicate this pest. Someone has suggested in all seriousness that by killing only the female rats caught

and sparing the males, the disturbed balance between the sexes would wipe out the species.

Rats are exceedingly clever in eluding baits and traps, especially the black variety with an amusing scientific name, Rattus rattus, which lives near, yet invisible, to man and is therefore a dangerous plague menace. It is generally known as the "house rat" and because of unusual climbing ability finds satisfactory shelter also in ceilings and walls of buildings. Experience has taught that nothing is accomplished by trying to match wits with these undesirable tenants. They cannot be evicted once they have moved in, but it is possible to keep them out by making houses and surroundings unsuitable as breeding or nesting places, that is to say, "rat-proof." This is the most effective antiplague measure yet devised and the method, which has already proved its worth, can best be summed up by saying that a human habitation must have no dead spaces or outside openings other than necessary doors, windows, and guarded roof outlets. But attention to the details of house construction will not suffice unless food is kept under cover and garbage, rubbish, and refuse are disposed of properly. In the control of bubonic plague, rat-proofing is said to have been used for the first time by the city of San Francisco, California, the year following an extensive outbreak in 1902. When the epidemic of 1907 came along on the heels of the earthquake and fire, Chinatown had rat-proof buildings and consequently suffered little, whereas the Italian quarter with its shacks rooted to the ground bore the brunt of disease.

There are some who condemn systematic rat destruction in plague centers because of sound evidence that the rodents from such areas tend to develop a resistance to infection and pass this immunity on to their offspring. It has been argued, therefore, that to destroy them is to encourage rather than prevent future epidemics. The advocates of this idea probably fail to appreciate the danger entailed in letting natural disease kill off animals which may

AN ACCOUNT OF THE PLAGUE at *NAPLES* in the Year 1656.

Of which there died in one Day 20000 Perſons. With the Symptoms that appeared upon Diſſection: And the approved Method of Cure.

Facsimile page from "An Account of the Plague at Naples in the Year 1656," describing the "peſtilential buboes" of bubonic plague.

IN the Opening and Diſſecting at that time of two dead Bodies, one of a Man, the other of a Woman, on the Firſt of *June* in the Year *1656*, by Order from his Excellence and the moſt illuſtrious Magiſtrates deputed by this moſt faithful City, about the Sickneſs then riſe, it was obſerved by the moſt expert Anatomiſts *Marco Aurelio Severino*, and *Felice Martorella*, (with the Aſſiſtance of the chief Phyſician and other Doctors) that

As for the diſtinction of the Spotted Fever and Purples, and that which is called the Tokens, thus much is obſerved, That upon the Inciſion of the Spots of the firſt two, there will ariſe a little Blood or Gleering; but if the Tokens be cut there will nothing of either ariſe the whole Blood or Juice being ſuppoſed to have ſome Touch of a Gangrene or *Sphacelation*.

And as to the Buboes Venereal and Peſtilential, they are ſuppoſed to be thus diſtinguiſhed, that the Peſtilential Tumours lie lower in the Groin

Neceſſary DIRECTIONS FOR THE *Prevention* and *Cure* OF THE PLAGUE in 1665.

With divers Remedies of ſmall Charge, by the College of Phyſicians.

I *Doctors, Chirurgeons and Apothecaries.*

THE Church-Orders for Prayers being firſt obſerved, as in former Times, it might be deſired, that by the Government of the City there be appointed ſix or four Doctors at leaſt, who may apply themſelves to the Cure of the Infected; and that theſe Doctors be Stipendiaries to the City for their Lives; and that to each Doctor there be aſſigned *two Apothecaries, and three Chirurgeons*, who are alſo to be ſtipended by the City, that ſo due and true Cure may be taken in all things, that the People periſh not without Help, and that the Infection ſpread not, while none take particular Care to reſiſt it, as in *Paris, Venice* and *Padua*, and many other Cities.

Facsimile page from an old history of the Great Plague in London. Especially noteworthy are the quarantine and isolation measures (Sections II and III) and the emphasis placed upon "correcting" the atmosphere by means of perfumes (Section VIII). A novel use for toasted bread is described in Section X.

The Tarbagan

Some of the Inns at Manchouli. The structure seen on the right is the entrance to one of the underground type.

and often do carry the same disease to man. It is generally agreed that a definite relation exists between plague epizootics (epidemics among animals) and epidemics in man. As a safeguard, it is necessary to treat an outbreak among the rodent population exactly as one would elsewhere, by eradicating the source of infection.

As soon as rat plague is discovered in a community, control measures are immediately put into effect to confine the outbreak, lest it spread far and wide. Since the mortality is so great among them, an increase in the number of dead rats means a corresponding increase in the quantity of plague-infected fleas turned loose on man. Three days generally elapse between the time an insect leaves its cold host and attacks a human being and then another three days before plague asserts itself in the victim, so that within one week a fair-sized epidemic may get under way in the human population. After biting a plague-infected rat, a flea, though starved for two to three weeks, is still infective. Survival of plague organisms in the flea is favored by moderately low temperatures (about 50 degrees Fahrenheit) and moist atmospheric conditions, although the germ itself can withstand extreme cold and remain fully virulent, even when recovered from bodies that have lain frozen underground for months, as during a typical long and severe Manchurian winter.

It takes a great deal of hard labor to discover a simple truth and generally as much or more to make others believe it. The obvious connection between rats and bubonic plague was already recognized during the Hong Kong epidemic in 1894, the year when the microbic cause first became known, but there was no proof of the correctness of this theory, although its workings were clearly perceptible to everybody. A doctor by the name of Simond one year later published an article in a journal of the Pasteur Institute, in which was stated the view that fleas carried plague from rat to man. Of course this idea seemed preposterous, and when the First Indian Plague Commission

later failed to sustain it by means of inadequate experiments, the matter rested there. Meanwhile observers in plague hospitals in Bombay were puzzled as to why a microbic disease should be so peculiarly non-infectious, considering the close contacts of the patients with their relatives and friends who never caught the germ.

Doctor Glen Liston, for many years director of the Bombay Bacteriological Laboratory, and other investigators now began a systematic hunt for the hiding place of the plague microbe. They carefully examined the soil, as it was supposed that buboes which commonly developed in the groin might be caused by an infection travelling upwards through the feet. But no plague bacilli were found in the soil or for that matter any place outside the animal body, where under natural conditions the microbe could not survive for any appreciable length of time.

Liston took his disappointment quite casually and addressed himself wholeheartedly to the study of fleas as possible carriers of the pestis germ. One of the first things to catch his eye was that certain kinds of fleas jumped at the chance to attach themselves to another host in the absence of their natural host, the rat. To test this further, he used guinea-pigs as flea traps by exposing them in plague-infected houses. When the animals were chloroformed, the fleas that had been caught in the fur were examined and in the stomach plague bacilli were found and seen to multiply. Some of the guinea-pigs, incidentally, died of plague, showing that fleas carried the germs in blood sucked from infected rats. Another thing he discovered was that rat-fleas could often be found on the human body, suggesting the possibility of plague transference from rodent to man. It was now clear why buboes occurred so frequently in the groin, from the observed fact that fleas got a foothold, as it were, by jumping from the ground and attacking that part of their victim which was within easy reach. Six inches, the upper limit of a flea's jump, is quite sufficient to start the insect well on its way up the body.

These and subsequent experiments forged the chain of evidence that bubonic plague was carried from infected rats to man by rat-fleas. Preventive measures, accordingly, must attack the two strongest links, insects and rodent host, for there is no longer any doubt that plague epidemics depend upon the prevalence of this disease among rats. Day after day and year after year, in cities where plague may be a matter of concern, laboratory experts who are vitally interested in the health of local rat populations keep careful tab on the number of plague-infected animals found in daily catches. The work goes along unobtrusively and the general public rarely, if ever, hears of this important phase of preventive medicine.

It is interesting to see how instructive such patiently accumulated statistics can be. During the six years of Liston's pioneer work in Bombay from 1907 to 1912, more than 700,000 rats (!) were examined in the laboratory, among which slightly less than one-tenth had plague. When the total percentage of human plague deaths was compared with the number of infected rats in corresponding months, the rise and fall of epidemics year by year ran closely parallel to the outbreaks among rodents. Thus the general relation between rat and human plague was clearly shown.

In certain towns of India, however, it was noticed that epidemics gradually became less and less severe, until finally these localities enjoyed immunity from the disease for many years. Although the decline of rat plague at any given time went hand in hand with a reduction in human plague, it did not explain why the disease tended to die out until the possibility that immunity or resistance of rats to the disease might account for this was put to an experimental test. Rats were collected from several different places, such as Bombay, Madras city, and Poona, and each group of animals, numbering over one thousand, was inoculated with a fixed dose of plague germs. This is what happened. The Bombay and Poona rats made the best showing, with more than two-thirds surviving, while all

but three out of every hundred Madras rats died. The animals from Madras, which had been comparatively free from plague for years, had no resistance at all, whereas those from the other two cities, which had passed through a number of severe epidemics, were remarkably tough.

The immunity of rats from these various places evidently had a definite relation to the number and kind of epidemics that had occurred there. One should like to know how this immunity or resistance has come about. Either it has resulted from recovery, following an attack of the disease, or from the elimination of most susceptible animals and a selection of those having an inbred immunity. In order to answer this question, it would be necessary to test the resistance of young rats that had been bred in captivity and had never been exposed to plague. Accordingly such animals were inoculated with pestis germs, and it was found that the offspring of Bombay and Poona stock did not take the disease, while the young of Madras stock were as highly susceptible as their parents. It was concluded, therefore, that frequent outbreaks of plague in the former cities had produced a hardy race of immune animals.

The history of modern epidemics of bubonic plague suggests that such a selective process might account both for the long intervals between successive outbreaks and their declining severity. Nevertheless, it would be unwise to explain the disappearance of the disease in this way because natural immunity is so variable and tricky at best. So long as there are susceptible animals to keep infection alive, epidemics must occur, no matter what measures are taken to check them. With these facts in mind, it has always been considered good practice to try to maintain the normal balance between the rat population and their quota of insect followers, as well as the more or less stabilized ratio of immune to susceptible animals. Lest the disease get entirely out of hand, a wholesale slaughter of rats therefore is never attempted during epidemics of bubonic plague.

Heiser's method, which is widely used today, takes this into account. At the first sign of an outbreak, the place where a plague-infected rat is found is taken as a central point of attack, from which radiating lines are drawn in all directions like the spokes of a wheel, enclosing an area divided up into districts. The outermost points where diseased rats are discovered mark the boundaries of these plague centers, which ordinarily have a radius of several blocks. A "round up" of rats follows along the rim of these circular areas, always working towards the center, and as the rat catchers close in, the territory behind them is searched systematically for plague cases and all dwellings are made rat-proof or burned. The disease is thus confined to a limited space without endangering any outlying districts.

Rodents of the Field Are up to Mischief

In some parts of the world, plague is endemic. These regions are the permanent depots from which epidemics originate and depend for their maintenance upon the native rodent population. The disease in such places slumbers, but always, it seems, with one eye open, so that it is difficult to decide whether the activity of the pestis germ is being interrupted by sleep or its sleep broken by activity.

Although plague has been more or less quiescent during the period of about two centuries following the major epidemics in Europe, the disease has shown a disquieting tendency of late to spread among rodents in various countries. India, Manchuria, and North China, Asiatic Russia in the Lake Baikal region, South Africa, Argentina, California, and perhaps a few other places have their animal reservoirs of infection. In Manchuria and Transbaikalia the tarbagan or marmot is infected, in the Steppes of southwestern Russia the spermophiles, in South Africa the gerbilles, and in California the ground squirrels. From time to time epidemics break out among these rodents, die out as most of them are exterminated, and the disease then

becomes dormant until the next breeding season, when the arrival of susceptible animals starts another outbreak.

It has always been a mystery how plague is carried over from one season to the next. In the case of domestic rats, the survival of infected fleas might alone account for keeping the disease alive in different localities. But another problem is introduced by wild rodents, which undergo a prolonged seasonal hibernation. The tarbagan or Siberian marmot and the Russian suslik awaken at the end of their winter sleep and frequently become ill and die, showing enormous numbers of plague microbes in their blood and organs if examined at this time. Although as a rule plague-stricken animals remain outside their shelter to die, the tarbagans, even when infected, will retire to an underground burrow and hibernate like any healthy animal. Doctor Wu Lien Teh, for many years Director of the Manchurian Plague Prevention Service, has recorded some instructive experiments with sleeping tarbagans, which were inoculated with plague germs and continued to hibernate until spring, when after a few days of normal activity the animals died of typical plague. The fact that infection under these conditions takes the form of a latent or resting stage does not prevent its spread among wild rodents wherever they happen to be found. It is also easy to understand how the tarbagan fleas help maintain the disease during winter and from year to year by feeding in the usual manner upon their hosts.

Because plague is a disease of rodents and plague is always plague, little can be gained from present day academic discussions of the nature of the disease in wild rodents as though this were something unique and needed a fancy name to distinguish it from ordinary rat plague. Whether we call it *silvatic, selvatic,* or *sylvatic,* depending upon how little we know about the origin of a word that refers to *wooded places* or *rural surroundings,* the fact remains that the disease of wild rodents can and does spread to rat populations in nearby cities. Presumably by this

method plague has become firmly entrenched among domestic rats in some parts of China through a close association with the tarbagan.

The tarbagan or true marmot of Manchuria, which has become a central figure in the history of plague because of the dramatic part it played in an epidemic unparalleled in modern times, numbers among its well-known relatives the squirrel, prairie dog, woodchuck, gopher, porcupine, rat, and mouse. It ranges over the plains of Mongolia and Central Asia and is highly prized for its fur, which has a fine texture and can be converted into imitation sable and seal. Weighing from nine to twelve pounds and averaging nearly eighteen inches in body length, the animal is ferocious in captivity and does extraordinary things with its powerful jaws and sharp teeth. On one occasion a recently trapped tarbagan housed for an experiment in a cage constructed of boards one inch thick and fitted with parallel iron bars one-half inch in diameter, escaped by simply twisting the bars between its teeth and gnawing through the boards of its prison.

The demand for its fur and the deceptive habits of this animal readily explain why it has been such an important factor in Manchurian plague epidemics. The healthy animal is not easily caught and runs about uttering a deceptive cry which means, in the Chinese language, "no harm" or "be not afraid," but when sick with plague and truly harmful, the marmot cannot cry out or run away from pursuers. Experienced native trappers, noticing this strange behavior and with a knowledge of the mysterious sickness handed down from ancient Mongolian legends, learned to shun these quiet sluggish animals and leave for other hunting grounds. Although plague broke out from time to time in certain outlying areas, until about thirty years ago it made little headway because the settlements were thinly populated and the sick were promptly isolated when left alone to die by those who hurriedly fled the camps.

But in 1910 an increased demand for skins and the high prices they brought attracted a great influx of Chinese to the town of Manchouli, one of the principal fur centers in the Manchurian provinces. October of that year, towards the close of the hunting season which lasts about three months, found over ten thousand hunters gathered in and about the market-places, waiting to sell their pelts before returning south. Most of these men were newcomers, who were seeing a tarbagan for the first time and knew nothing about plague. They hunted with improvised snares, frequently making excellent catches of slow-footed animals. Frequently these innocent trappers would dig the marmots out of the burrows and expose themselves to plague infection.

In Manchouli the conditions under which these people lived were ideal for the spread of disease, and the lodging houses occupied by the hunters were perfect plague traps. These "inns" had mud-covered roofs and were built underground or only partly above ground, in which case the so-called windows allowed feeble light to enter through paper-covered openings that conserved the heat of the room. The underground dwellings were naturally more desirable in winter when bitter cold temperatures prevailed and the ground was frozen generally to a depth of eight feet. Warmth was provided by means of several horizontal brick flues known as k'angs about five feet wide and two feet high, open at one end to receive millet stalks (kaoliang) or firewood and leading outside at the other end through a hole in the wall. These made choice sleeping bunks for a number of lucky patrons.

The rooms, usually from fifteen to twenty-five feet square and twelve to fourteen feet high, contained two or three tiers of "berths," accommodating forty or more persons. There were no partitions between and spray expelled in coughing was liberally shared by adjoining occupants. Winter garments, worn next to the body in three layers with frequently a sheepskin garment over all, topped with

a fur cap, were seldom if ever removed, thus providing a permanent lodging for fleas, lice, and vermin. The men cooked and ate their food in these rooms and slept with decomposing marmot pelts beside them. During the hunting season, when business prospered, not an inch of space was wasted, and additional bunks were erected if possible to handle newcomers. Mongol caravans outside these hostelries added to the growing mobile population amidst scenes of disorder and confusion typical of a mining town that springs up during a gold "strike." Into one of the Manchouli inns came a tarbagan hunter who was incubating plague germs.

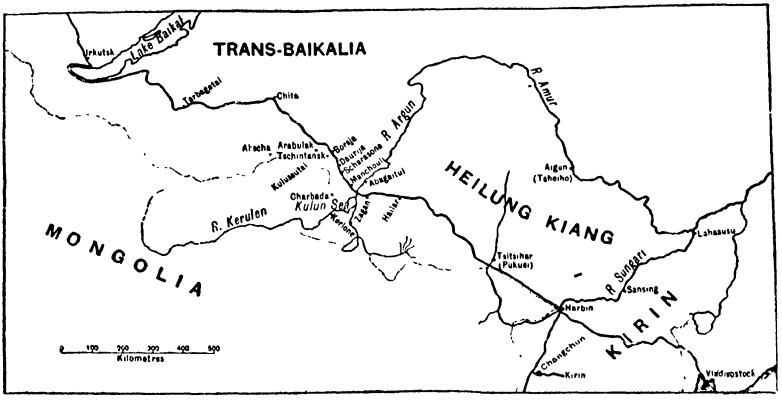
A Great Plague Fighter

The exact date when pneumonic plague broke out in Manchuria cannot be fixed with certainty, but the first case that was recorded occurred in the town on October 12, 1910. There were several earlier reports of hunters who had been stricken suddenly with fever, headache, and blood-spitting and had died after a short illness. Creditable work on the part of Russian officials checked this outbreak effectively and during the ten weeks it lasted there were less than five hundred deaths.

More than five hundred miles southeast of Manchouli lay the city of Harbin, administrative center of the Chinese Eastern Railway and the focal point of travel in all directions. Late in October a tarbagan hunter from Manchouli arrived here and plague broke out with explosive violence in the crowded quarters of the Chinese village, consisting of a jumble of vermin-infested, mud-built huts, without the semblance of floors, with windows of pasted paper, housing men, women, and children packed like sardines in a tin. Plague was soon on the march and during the winter months followed the lines of travel taken by thousands of migrating Chinese homeward bound for the New Year holiday. From its starting point in the north, the epidemic spread southward for a distance of nearly 1,700

miles to Shantung. More than sixty thousand died in the six months before the plague burned itself out literally and figuratively.

Principally through the efforts of Doctor Wu Lien Teh, the first and last great medical leader in China and one of the foremost in the field of public health under any flag,



MAP OF TARBAGAN REGION

cremation of the dead was resorted to in a desperate attempt to stem the tide of pestilence. It was difficult indeed to gain this point without offending the religious feelings of the Chinese people, who worship their dead with the deepest respect. Nevertheless, as corpses were constantly being thrown out into the street, stark naked in most cases, the clothing having been kept for further personal use, the only hope that remained was to dispose of these bodies according to modern sanitary practices.

Those who took part in fighting this unusual epidemic had to contend with superhuman obstacles. Ideal conditions for the spread of pneumonic plague were already present in the unbelievable surroundings under which these teeming populations existed. The foul-smelling huts filled with people huddled in masses trying to keep warm provided ideal conditions for the spread of infection by contact. To this was added a winter climate with tempera-

tures outdoors ranging from twenty-five to thirty-five degrees below zero (Fahrenheit).

The importance of discovering as soon as possible the first cases of sickness and of erecting barriers between the sick and the well has never been more clearly shown than in this type of epidemic disease. When all else fails, epidemiological control remains the sheet anchor. There was no cure then and none now. All efforts to check the advance of pneumonic plague by curative means were even as now useless. Some of the serums and vaccines upon which great hopes were centered during this epidemic proved ineffective. Years later it was said that the left-over stock of a certain one of these highly exploited products was liberally scented with musk and marketed as a hair oil. It "sold like hot cakes."

When the ashes of thousands of Chinese dead had cooled in April, 1911, an International Plague Conference with representatives from all parts of Europe, the United States, and the Orient met at Mukden, South Manchuria, within the grounds of an old temple. Here the ancient old-fashioned medicine was sacrificed on the altar and in its place a newer science of public health was dedicated. Doctor Wu Lien Teh, then Assistant Director of the Imperial Army Medical College at Tientsin, was chairman of this conference. He had been living and working tirelessly for months in the worst plague-stricken areas and struggled through it all to realize his dream of an institute for modern preventive medicine in China. More far-reaching than the many valuable scientific papers and discussions which came out of this notable gathering was the recommendation that there be established a central organization for the control and prevention of plague. Thus one year later was born the North Manchurian Plague Prevention Service, with headquarters at Harbin and a chain of auxiliary hospitals placed at strategic points.

For ten years Manchuria remained free from plague and when in 1920–21 a second epidemic struck, again from the

north, this splendid organization, which Doctor Wu Lien Teh had been directing since its beginning, met the situation fully prepared. The tarbagans, which were heavily infected with plague as in 1910, cried "pu p'a, pu p'a"—"no harm, no harm," and again carried the pest on their journeys through Transbaikalia. This time the first plague cases, which were bubonic, originated in the town of Hailar, a fur center second in importance only to Manchouli and approximately one hundred and thirty miles distant. Early in October a number of Chinese coolies employed in a skinning plant died suddenly of a mysterious illness and were promptly cremated. By the time Doctor Wu Lien Teh arrived in the town, bubonic plague had spread among some soldiers and shortly afterwards took the more deadly form known as septicemic plague, in which the pestis germs invaded the blood stream. In this stage of the disease pneumonia developed as the lungs became infected through the blood circulation and gave rise to the first cases of pneumonic plague.

The epidemic spread quickly to outlying districts when a gang of undisciplined soldiers set free a number of quarantined contacts. Some travelled to a coal mining town about one hundred miles westward and here the epidemic started in earnest. The mine workers lived in partly underground barracks with space barely adequate for one-fourth the number of men who were crowded into them. Neither sun nor air ever penetrated these dungeons and from the day those Hailar plague contacts entered one of the barrack-rooms, the fate of all the occupants was sealed. Within a few days only three among sixty-four coolies who were exposed to the infection survived, and one-quarter of the total Chinese population of four thousand was wiped out before the epidemic spent itself.

In the meantime, plague had been carried into other towns along the railway line by escaping contacts and sick persons. According to Doctor Wu Lien Teh's reports, these were responsible for more than six thousand deaths

in three areas several hundred miles apart. By mid-winter the plague moved eastwards from Harbin, eventually reaching Vladivostok in the spring, and when the last fatality occurred there in October, the epidemic had lasted one year and taken a toll of over nine thousand lives.

Compared with the first great Manchurian outbreak, this one was less severe, but only because of the remarkable work of the Plague Prevention Service. At the very outset, the civil war in Transbaikalia interfered with all anti-plague measures, which soldier ruffians turned into a farce. Border traffic was not controlled and quarantine was not observed anywhere. The usual custom of throwing unclaimed bodies into the streets prevailed in every community, so great was the fear of being isolated for safety! Despite these difficulties and overt acts of violence to which the plague fighters were subjected, the epidemic was kept within bounds. Harbin, the key city of Manchuria, virtually broke the southward march of plague, so that less than two hundred cases occurred in all South Manchuria as compared with over five thousand in just one of its cities during the 1910 epidemic. In Harbin, with a population more than four times as large in 1920, the mortality during this second outbreak was considerably less than half that of the first.

For twenty years Doctor Wu Lien Teh carried on with his staff of workers at the plague outposts of Manchuria. Political events have changed the map of his country as the result of what history has recorded as the Mukden incident of 1931. He and his loyal associates were obliged to flee Harbin, leaving behind the greatest monument to Chinese medical progress erected on what was formerly barren Siberian waste. In Shanghai, Doctor Wu, as he was generally known to his friends, became Director of the National Quarantine Service. Following the military occupation of the city and the dissolution of the Chinese Plague Prevention Service, Doctor Wu returned several years later to Ipoh, his homeland in the Federated Malay

States. The war caught up with him finally in his own courtyard. Here the story comes to an end.

In California's Golden Hills

Like its Manchurian cousin, the marmot, the California ground squirrel generally remains in a torpid state throughout the winter. Sometimes, however, it chooses the summer months for sleep or combines both seasons, so that the animal might be called *aestivating* as well as *hibernating*. It breeds in spring, the pregnant female after one month giving birth to a litter of from four to eleven young.

In the peaceful rolling country and foothills of California the sleeping plague germs have been stirring uneasily during the past years. Since 1908, when ground squirrels were found to be infected in a coastal county neighboring on San Francisco, plague in these wild rodents has increased and spread to an alarming extent over a wide territory. As late as 1934 new endemic areas were discovered in three northern counties and after a few years the disease among ground squirrels reached epidemic proportions. At the present time, wild rodent plague seems to be more or less permanently entrenched in the Coast Range, the interior valleys, and in the Sierra Nevada mountains. The region involved extends from the northernmost point of the state southwards to the city of Los Angeles and covers an area of approximately 1,500 square miles. Whether or not these nests of disease can be eradicated, time alone will tell, but the ancient and modern history of plague speaks against it.

There was a foretaste of what might happen, if rodent plague should break loose, in two small outbreaks of pneumonic plague which were promptly and effectively localized. The first one occurred in 1919 in Oakland, a city directly across the bay from San Francisco, with thirteen cases and twelve deaths. A similar outbreak five years later in Los Angeles was confined to the Mexican district, where out of thirty-two cases only two recovered. The Oakland

outbreak was undoubtedly due to infected ground squirrels, a hunter having first contracted the disease, which started out as bubonic plague and later took the form of a pneumonia that spread by contact to other persons. While some doubt existed as to the connection between wild rodents and the Los Angeles episode, a systematic round-up of rats and squirrels in the area at the time showed that both were infected with plague.

Disturbing as it is to find wild and domestic rodents interchanging pestis microbes, a matter of even greater concern is the nature of the disease found in affected ground squirrels. There is an unmistakable tendency on the part of the germ to cause a plague pneumonia, which cannot be distinguished from the disease in human beings. From evidence collected during recent outbreaks, it appears likely that the organism during its passage from the original host to squirrels is becoming more virulent for this animal. Unlike the rats, the wild rodents frequently die with an overwhelming infection of the blood stream before there has been time for any other signs to develop. In this respect, plague in ground squirrels and marmots shows similar characteristics which, taken together with a marked preference for the lungs in these animals, emphasize the connection between the type of rodent disease and the character of epidemics that might result from it. Perhaps we are seeing nothing more nor less than the natural interplay of environmental influences that bring about variation in microbial breeds. There is no reason to suspect that the plague germ alone would be exempt from these effects and require a novel theory to explain a change in its behavior resulting from passage through a different breed of host.

While the mortality of rodents has been great in the newly discovered plague areas of California, the only human case thus far occurred in 1934 in Tulare County, one of the nineteen counties where squirrel plague is now endemic. The present situation is fraught with danger,

which only the foolish will ignore entirely or, going to the opposite extreme, regard with unnecessary fears that plague may at any moment literally rise out of the ground and sweep the West Coast. Social conditions have as much to do with epidemic disease as the type of microbe involved in its spread. Just so long as the foci of infection are limited to sparsely inhabited regions, the problem is one of prevention. Should persons infected through contact with plague-bearing rodents carry the disease into the more populated areas, an epidemic situation might arise calling for drastic suppressive measures. In the meantime, wild rodent control and education of the public to the existing dangers in handling or skinning these animals should go a long way towards holding the pestis microbe at a safe distance.

A Tale of "Brer Rabbit" and the Squirrel

There are in various parts of the world more than fifty species of wild rodents known to suffer from spontaneous plague and as many different varieties of fleas capable of transmitting it. The gloomy side of this picture is perhaps not the hopelessness of efforts to keep the disease within bounds, should it ever break through our barriers. Rather it is a growing appreciation of the difficult task ahead of man to subdue any one of his germ enemies completely. No sooner are they checked in one direction than they find another outlet—a new and unsuspected animal host or some other go-between that brings microbe closer to man.

An illustration of this fact is found in California, where of all the "unusual" things native to this state the ground squirrel is the most pestiferous, for it carries, in addition to the notorious plague, another disease with which it is often confused, so close is the resemblance between the two. The plague-like malady, however, has its own special microbe, which was discovered in 1910 by two surgeons of the United States Public Health Service in Southern California during an intensive warfare against the Pacific Coast ground squirrels.

In Tulare County, so-called by early Spanish settlers because of the immense tracts of bulrushes or "tules" found in the vicinity of a great lake bed, the squirrels flourished as luxuriantly as the reeds that grew there. In addition to being a plague menace, they were destroying annually millions of dollars worth of crops. When a strange fatal epidemic suddenly broke out among them at this time, destroying in plague-like fashion enormous numbers of these rodents, it seemed like a boon to the exterminator squad. But the Public Health Service wanted to get at the bottom of this mystery and sent two experts, Doctors McCoy and Chapin, to study the disease. They found what looked like bubonic plague but instead of the pestis microbe, the cause was a much smaller and different organism to which they gave the name Bacterium Tularense in recognition of the locality where infected rodents were first discovered. This germ is now known as Pasteurella tularensis.

How this disease was transmitted did not come to light until some years later when farmers in the state of Utah reported that they were suffering from a peculiar ailment which they called "deer fly fever." The infection followed the bite of deer flies during the summer months, when these insects were prevalent, and because of the long drawn out illness that resulted, crops could not be harvested. In 1919 Doctor Edward Francis, who was sent to Utah by the United States Public Health Service to investigate this disease, found many persons who were suffering with high fever, chills, intense headache, and swollen glands; others had ulcers on the hands or in the eyes. Most of these patients, he learned, had taken part in a very popular pastime of rounding up the common western jack rabbit in "rabbit drives" organized frequently to check the inroads of this pest. It was customary to run the animals into a corral and bludgeon them to death. Some of them were skinned later and brought home for food.

Guinea pigs inoculated with some of the blood taken from a sick man died after a few days, showing all the

signs of tularensis infection. Doctor Francis himself then contracted the disease, which he had named *tularemia*, and returned to Washington for a long stay at the Naval Hospital, where he recovered. In the meantime, his assistants pursued the problem further and found that tularemia could be contracted easily from wild rabbits, the germs apparently gaining entrance into the scratched or even unbroken skin of persons handling diseased animals. Before long, the five investigators who had been assigned to this problem also came down with tularemia in the course of their work. Experience with this microbe has shown it to be extraordinarily invasive and nearly all who handle it in the laboratory become infected sooner or later, despite the extra precautions that are taken.

Having settled the rabbit question, attention was next directed to the blood-sucking deer-fly. Some of these insects were caught and allowed to bite healthy guinea pigs and rabbits. In less than a week the animals developed tularemia and died, their blood containing the organism which Doctor McCoy had identified ten years before as the tularense of ground squirrels. To infect any kind of rodent, it sufficed merely to place a drop of this blood on the skin. That other blood-sucking insects were guilty of transmitting this disease soon became quite evident. "Tick fever," as it was also known in Utah, turned out to be none other than tularemia, which was spread by biting ticks habitually infesting not only the jack rabbit and squirrel but the coyote, woodchuck, opossum, and larger animals, such as sheep, cows, and horses.

Under natural conditions, insects play the most important part in keeping alive epidemics of tularemia among animals. In the field, man is infected by wood ticks and deer flies indirectly or more directly through contact in handling their animal hosts. The human disease shows itself in several different forms, one resembling plague and another, without any enlargement of glands or other visible changes, closely imitating typhoid fever. Infection car-

ried to the eyes by fingers contaminated from diseased animals or crushed ticks causes still another variety of tularemia, in which there is an enlargement of the glands in the neck and in front of the ears.

Epidemics in the usual sense among human beings are not likely to occur, since the disease does not spread from person to person, but there have been sizable outbreaks in different parts of the world. Known at one time as the "all-American" disease, because it was discovered in this country and until 1926 was presumably confined to the United States, tularemia is now no longer a rarity. Norway, Sweden, Japan, and Russia have since had extensive epidemics, one Russian province alone reporting over eight hundred cases. Here the hamster, a large rat with cheek pouches for carrying grain and much sought after for its marketable skin, was found to be a carrier of the tularensis microbe. In Japan, Sweden, and Norway, human infection was due to the handling of dead rabbits. The main sources of tularemia in the United States are shipments of jack rabbits from western markets for food (about one per cent are naturally infected) and the stocking of hunting preserves with cottontail rabbits. The disease is now so widely distributed that hardly a state is free from it.

Thus far no serum or other specific cure or preventive for tularemia has been discovered. Well cooked rabbit flesh, a highly delectable dish, is absolutely safe for human consumption. Care in handling and skinning animals and avoiding insect distributors of infection are the only known protective measures. To eradicate deer flies, ticks, and rabbits is out of the question. The tick remains infected for life, transmitting the disease through its eggs from generation to generation, and the rabbit with chronic tularemia is probably here to stay, littering the world. New Zealand had a rabbit problem over fifty years ago. With the idea of annihilating this rodent pest, mongooses and weasels were imported into the country, but instead of killing rabbits, they took a fancy to the poultry, which

happened to be the only article of food the rabbits did not seem to like. New Zealand and Australia still have their rabbits.

It is a curious fact that Tulare County in California, the place after which tularemia was named, has never had a case of human infection with this disease until recently. Those who are historically inclined might be interested also in the belated evidence that the very first record of the disease in the United States has been traced to San Diego, California, where in 1904 a physician's son contracted tularemia while dressing a catch of wild rabbits. Twenty-five years later this patient's blood serum was tested for its power to react against the causative germ, which by then was well recognized, and the original diagnosis was thus verified. This method of identifying Pasteurella tularensis by clumping of the microbes in a suspected sample of blood is the only reliable laboratory procedure known and used in practice at the present time.

But for this authentic report, it might have been necessary to go back to another historic document dated 1907 from Phoenix in Arizona Territory, as the state was known before admission to the Union five years later. Here lived a certain Ancil Martin, a physician of repute, with an inquiring turn of mind. Among his patients, he observed a peculiar ulcerated condition of the eyes, not unlike that in trachoma which, incidentally, was very common, especially among the Indians of that settlement, and a disease which he couldn't mistake. The new illness, however, was more like typhoid fever than anything else, except for the ulcers on the eyes and hands of these patients, who recovered gradually after from one to three or more months. What impressed the doctor was the story every patient told of having handled jack rabbits. Frequent "drives" on these pests in and about Phoenix were being encouraged at that time by a bounty, and these patients had skinned and dressed wild rabbits before coming to him with this strange malady. He wrote to Doctor Frederick Novy, professor

of bacteriology at the University of Michigan in Ann Arbor, telling him about these cases and giving a description of the disease, to which Doctor Martin gave the name "rabbit septicemia." Nearly twenty years later, in 1926, it was suggested that the name "Martin's disease" be given to tularemia in honor of the Arizona physician who, as far as then known, was the first to describe the disease, but the name coined by Doctor Francis in 1921 has stuck and tularemia it is.

Because tularemia and plague are so similar, there is a growing tendency to regard them as modified forms of the same disease. Both are carried by rodents, transmitted to man through insects, and produce in the natural host abnormal changes that cannot be told apart easily. Having so much in common, it might even be supposed that they are caused by different breeds of the same germ. This idea is no longer a novelty in the natural history of microbial diseases that are common to man and animal and exposed to environments affecting the host-parasite relationship. Just so long as the microbe can continue to exist in similar animal species, virulence will be kept up without any drastic changes in its mode of adaptation. When this spark-gap between susceptible hosts becomes too wide, man himself helps to narrow it through a long continued association or casual contact. Thus the parasite, deprived of an "indispensable" host, always manages to find others that are equally suitable. One does not have to go far afield in search of examples, such as cow-pox, sheep-pox, and horse-pox, encephalitis in the horse, rabbit, and fox, trichinosis in hogs and rats, paratyphoid infections of rodents and swine, or rabies (hydrophobia) in dogs and cattle.

Strange as these instances of microbial wanderlust may appear, the end is not yet in sight as new and bizarre germ carriers are being discovered. Only in recent times, an unheard of thing happened on the island of Trinidad in the British West Indies. Great numbers of blood-lapping or vampire bats were seen flying about in the daytime in

bright sunshine, attempting to bite man and animals. The islanders, who were well acquainted with the nocturnal habits of bats, were mystified, the more so as live stock owners had noticed an unusual prevalence of bats in certain districts where, over a period of years, thousands of animals had died of a malady resembling paralytic rabies. Human beings were similarly affected and more than fifty died of this disease. Although rabies in animals had been known in Trinidad for some time and the first cases in man were recognized about ten years ago, the incident of blood-lapping bats flying over infected areas drew attention to a possible new source of the disease. It was soon discovered that the vampire bat actually carried the rabies virus and was the cause of this unprecedented outbreak.

The mystery as to how the virus gained a foothold in Trinidad was solved by an epidemiologist and a map. Trinidad lies within a zone about nine to twelve miles from the mainland of South America, where rabies is prevalent. The northern part of the island is connected to a tip of Venezuela by a series of small islets which establish a short line of communication between Trinidad and the continent, while to the south lies only ocean. The vampire bat—a queer sort of carrier pigeon—plied a repulsive trade between the virus reservoir on the mainland and susceptible hosts on the island.

We can see how, in one form or another, the plagues of mankind tell the life history of parasites running amuck in frenzied thirst for blood. Whether they jump, run, or fly, they carry along microbes that have an unlimited capacity for adjustment to new conditions and experiences. Were it not for this extraordinary ability to meet such exacting requirements, they would soon be eliminated from competition. But microbes, we no longer doubt, derive their power of survival from new characters acquired in the host or while passing from one host to another. During these migrations, the organisms tend to revert to the virulence of their ancestors, a natural result of judicious ad-

mixture with new stock. Less resourceful "pure" breeds of microbes, the "poor mixers," have always been at a great disadvantage in this struggle and sooner or later have fallen by the wayside, while the tough mongrel races have survived. Whether in microbe or man, purity of race is an out-and-out myth—just unadulterated propaganda.

CHAPTER 12

EPIDEMICS TO ORDER

Community disease begins where human disease ends—with the story of microbial wanderlust. The history of the rise and fall of epidemics is a record of the spread of disease from one host to another. To the extent that microbes depend upon such human and animal hosts for distribution, the latter are responsible for keeping the disease alive. Thus the factors that determine the progress and outcome of a bacterial infection, whether in an individual or a crowd, are more or less the same.

In dealing with mass infection, bacteriology moves from the laboratory into the open, where disease as it occurs in nature becomes the problem of the epidemiologist, whose field is preventive medicine. One of his tasks is to apply the knowledge of germs and their habits to tracing communicable disease to its source, for every infection must have originated from a preceding one. Some of the problems he is expected to solve in the prevention and control of sizable outbreaks often require seemingly far-fetched measures, depending for the most part upon the sum of what is known about the supposed cause. But the basis of all these practices is to break the connecting links between a source of infection, its disseminating agent—insect, animal, or man—and the regularly susceptible hosts, which may in turn become new sources of disease or even far more troublesome carriers of infection hiding beneath a healthy exterior.

It is not surprising, in view of the bizarre things parasites are known to do, that measures taken to suppress them nowadays should appear to the uninformed as no less fantastic than the devices of medieval times. Actually there

is little difference except in the better results of modern methods, which have replaced an irrational fear of the mysterious or unknown with a more rational fear of known causes. In the days of superstitious mysticism the natural thing to do in times of pestilence was to run away from it, thereby aiding its spread, whereas today disease-producing microbes are held in check by lessening the opportunities of contact between those who have been infected or exposed to infection and those who have not. Restriction of their movements accomplishes precisely what blind flight, although failing dismally in its purpose, was supposed to do.

From the simple fact that germ diseases are communicable, it is possible to control or prevent epidemics by paying attention to the various ways in which microbes are passed on from person to person and from place to place. Of the important part played by animal and insect life, nothing further need be added here other than to emphasize the value from an epidemiological standpoint of careful observation and thorough knowledge of parasitic tricks. The flight of an insect may be more important than the fact that germs do not fly and atmospheric heat and moisture may have greater significance than knowledge that there is no basis for the ancient Hippocratic theory of climate as a factor in causing disease. Modern epidemiology distinguishes clearly between fanciful or even remote causes in the environment and the recognizable effects which surroundings can produce upon known agents.

The epidemiologist who would control a communicable disease successfully must carefully scrutinize and interpret accurately every available bit of evidence or clue that may have some bearing upon the way in which it is spread. He is expected to play the part of both detective and keen bloodhound and not to lose his way along the circuitous routes taken by germs in their travels. Since we are already familiar with facts in the microbial world that are stranger than fiction, we need not be surprised at some of the con-

clusions resulting from epidemiological studies. For example, if someone in an unguarded moment were asked what cats had to do with the abundance of red clover, the sanity of the questioner might be regarded with suspicion. Yet the facts which prompted this serious inquiry were obtained by Charles Darwin, the naturalist, in much the same way as an epidemiologist goes about the task of tracing the source of a communicable disease in which an insect takes part in the complicated process of spreading infection from host to host.

Over ninety years ago, Darwin, studying the rôle of insects in the dissemination of plants, observed that effectual pollination of red clover could not take place without the aid of bumblebees, all other insects being unable to reach the honey. He noted, further, that in certain country districts where clover was scarce, a reduction in the number of bees corresponded to an increase in the number of field mice, while in other localities near populous towns, the bees could always be found in large numbers. Hence he correctly concluded that field mice habitually destroyed the nests of bumblebees, which were protected against their destructive enemies close to human habitations by the numerous cats, which preyed upon the mice.

Attempts to discover the mode of transfer in microbial disease by similar reasoning are not always successful, even when the situations are less involved. As a rule the insect vector is not known at all or, if suspected, may be discovered only after exhaustive studies of the haunts and habits of many species. A few notable achievements of this order have been touched upon already.

Why Epidemics?

What are some of the things that affect the outbreak and subsidence of microbial diseases? Why do these outbreaks have ups and downs like cycles of business prosperity and depression? Theoretically an individual with a highly

contagious disease is the starting point of an epidemic in any community, assuming for the moment that nothing is done to prevent the transfer of his germs to other persons. Yet epidemics are not common, despite the frequently careless and sometimes accidental exchange of microbes in the home and in public places. Perhaps this is due to the fact that there are said to be about one hundred kinds of germs that are pathogenic or disease-producing, as compared with half a million harmless varieties scattered over the face of the earth. If the underlying causes of this remarkable inequality were better known, natural infection might be controlled more successfully by methods which accomplish more than merely limiting the activities of harmful microbes.

In any locality absence of disease results from the same sort of balance between germs and their environment as that which occurs on a smaller scale in the human body, but with one marked difference; the external factors which determine whether mass infection will or will not take place are much more complicated. Disease in an individual is the outcome of a successful invasion by microbes of a fully susceptible host. For the uninterrupted spread of such disease there would be required an unlimited number of persons who are equally susceptible, but in any given population already in the process of being infected, conditions are not quite so uniform.

Among uninfected individuals as a class are found many who are resistant, while those that have been infected fall into a number of different groups, each capable of modifying in varying degree the severity of an epidemic and the way it spreads. The typical cases, the unmistakably sick, are easily recognized and do not become a serious menace because they can be segregated promptly. Frequently this is automatic, as when illness confines such persons to bed and takes their germs out of public circulation. In many instances, however, the illness may be so slight and its characteristics so unusual as to escape detec-

tion by a physician. More often the patient, because he does not feel sick enough or for other reasons, fails to obtain medical advice. These atypical or "missed" cases are important sources of new infection. Again, the germs do not always make a person sick when they have gained access to the body, and are then responsible for many healthy "carriers" who, while going about their business, infect other susceptible persons with whom they may come into contact directly or indirectly by various means, as through the handling of food products. This group might be justly regarded as an example of latent or dormant infection, illustrating the perfect balance between parasite and host.

The laboratory has provided accurate and reliable methods of detecting all these elusive distributors of pathogenic microbes. If it were only possible to find such sources early enough and to break the chain of susceptible contacts as soon as they can be located, the control of communicable disease would be less difficult. Practical considerations, however, make this well nigh impossible, because an irreducible minimum of undiscovered germ infections will always be present and only a sudden or progressive increase in their number attracts attention to the beginnings of a possible outbreak. If the various groups of individuals among an infected population, like distributing bases for the advancing microbial army, are strategically placed and environmental conditions are otherwise favorable, disease may explode suddenly along every line of communication, like the preliminary barrage on an extensive battlefront.

To single out any one factor as more important than another in the building up of epidemics is not easy. It would certainly be far simpler to regard an epidemic as the expression of all the factors already enumerated, acting in unison but varying with time and place and under the influence of environmental conditions to which they are sensitive. Modern preventive measures, in emphasizing the need of keeping a watchful eye on healthy carriers rather than sick persons, follow a sane course. The carrier, because

he is not incapacitated and is rarely recognized, can do greater harm. He is found not only among those who have never been ill, but is also recruited to a large extent from among those that have recovered from a disease, yet continue to discharge the causative germs for variable lengths of time.

A great difficulty in analyzing the progress of community disease is to distinguish between cause and effect, for the majority of bacterial infections give no clear indication that an increase in the number of germ carriers first precedes and then starts an epidemic or that this increase is the consequence of it. In certain diseases, however, such as epidemic meningitis, the prevalence of carriers at a given time seems to determine the frequency and severity of an outbreak. During epidemic periods, the prevalence of healthy carriers among those who have not come into contact with a known case of the disease may be as high as among the group that has, and there is no doubt in this instance that an epidemic will surely follow in the wake of a rising tide of carriers. Yet the facts do not explain how the cases of illness arise under these circumstances. The disease may result from microbes getting into the nose and throat of those who happen to be unusually susceptible, or it may conceivably develop in persons carrying the meningococcus for a long time. From the evidence gathered during such outbreaks, we do know, however, that the carriers themselves are generally resistant to infection.

As the puzzle deepens, it becomes quite obvious that an explanation of the ebb and flow of epidemic diseases must be sought in when, where, and how the germs are distributed among the interacting groups of susceptible and immune persons in the entire population that is exposed to risk. All these factors, of course, are subject to change, depending upon whether the outbreak has just begun, reached a peak, or has almost subsided. At any stage of its progress, moreover, the relationship between parasite and host will be influenced largely by whether or not this popu-

lation has experienced a similar epidemic in the recent past. If it has, then the natural resistance to disease will be unusually high, resulting in a lesser outbreak with few cases and many carriers; if not, the situation will be reversed. During the quiescent periods between epidemics, diseases will be absent, carriers scarce, and the number of susceptible persons on the increase. These interrelationships are complicated further by the activities of insect vectors or rodent reservoirs of infection, which may individually and jointly play a prominent part in transmitting certain diseases from man to man, animal to animal, and from animal to man.

Having all these facts in mind, one should like to know why it is that some bacterial diseases occur regularly in certain localities only, where they smoulder or slumber year in and year out and are therefore *endemic,* while in other regions they become unusually prevalent or *epidemic* at a given time, gaining momentum suddenly and then gradually dying out. An outbreak of disease like influenza may either sweep with terrifying speed across the entire world as a *pandemic* or by contrast occur *sporadically,* that is to say, with scattered cases arising here and there without any tendency to spread very far. These four "behavior patterns" of microbial distribution are not in any sense fixed and under natural conditions can be expected to overlap. Thus the epidemiologist in practice is usually confronted with endemic disease which flares up in a community from time to time, interrupting periods of quiescence with highly fatal outbreaks of epidemic intensity.

These perplexing questions have made it quite evident that mere exposure of a susceptible individual to virulent or extremely invasive microbes cannot alone account for epidemic disease. It is also plain that a study of widespread bacterial infections occurring in crowds under natural conditions must fail unless the variable factors are investigated one at a time as in any well regulated experiment.

When these are subjected to such a method of analysis, epidemiology becomes dignified as a laboratory science and only then is it possible to distinguish more precisely between cause and effect. Presently we shall see what experimental epidemiology has accomplished in this direction, but it might be profitable to examine first some of the acceptable evidence accumulated in preceding years which led to the development of this new field of inquiry.

Epidemics Made to Order

All the phenomena of epidemics are explainable in terms of a weeding out process or natural selection that is at work both on the parasites and on the population group among which they are distributed. Three principal factors are seen to contribute to the spread and termination of a mass infection; the resistance of the host to a specific microbe, the dosage of the microbe in the host, and the virulence or infective power of the microbe. Exceptionally virulent germs are eliminated not only as a result of the high mortality and early death of their hosts, but also because active measures are taken to isolate infected persons. While this is happening, two things come into play to raise the tolerance (immunity) of the general population to a particular disease. One is the natural elimination of the most susceptible, who are killed off by the infection, and the other is a gradual building up among the survivors of an individual immunity towards the disease, chiefly by doses of germs in quantities too small to do any damage. As a balance is established, the immunity of the group increases and the epidemic comes inevitably to a close. According to this prevailing view, the course of an outbreak of infectious disease is determined largely by the rise and fall in the acquired resistance of the host and similar variations in the virulence or invasive power of the particular microbe.

From the days of Pasteur and Koch, these epidemiological theories have come down to us in the traditional man-

ner—from one test-tube generation to another. Until recently, as modern bacteriological science is reckoned, we have had no experimental proof of what actually happens under conditions resembling those found in nature. Less than thirty years ago, a group of investigators in London began a study of contagious disease among mice and shortly afterwards, in this country, at the Rockefeller Institute, New York, a similar project was inaugurated on a larger scale. Both groups of investigators approached the problem with somewhat different methods, but from this work, which as it progressed, became more or less cooperative, emerged the science of experimental epidemiology.

Their studies, while not comparable in certain details, have a similar aim in attempting to observe how natural or experimentally induced infections among laboratory animals tend to spread. Nine diseases of the intestinal and respiratory tract of rodents and fowls—mouse typhoid, mouse pneumonia, fowl cholera, rabbit pneumonia, and certain others—have been studied according to a fixed plan. The experimenters, wishing to reproduce natural conditions faithfully, inoculate the micro-organisms by way of the normal channels of entry, either through the nose or into the stomach. Under regulated conditions the essential factors involved in a relationship between the microbe and host are analyzed in terms of virulence, effective dosage, and host resistance, all of which can be measured. The amount of protection resulting from exposure to such test epidemics can also be ascertained in a population at any time during the actual spread of infection.

Before these experiments were undertaken, we had been taught for years that microbes, when first brought into a community, are weak and only later, as they are passed successively from person to person, become harmful; that after an epidemic has reached its height, this virulence, presumably diminishing because germs cannot fight successfully against a resistant (immunized) population, is finally lost almost entirely, and thus the epidemic comes

to an end. Laboratory observations, too, had given us every reason for believing in such a "virulence cycle." It was known, for instance, how easily a culture of bacteria that had been bred in test-tubes a long time could be stepped up in activity by frequently repeated passage through susceptible animals, and also how weakly infective certain organisms were when first inoculated into a foreign animal host. Similarly, the spread of diseases such as diphtheria, smallpox, measles, or scarlet fever, varying in severity as they do from time to time, had come to be regarded as an expression of altered microbic virulence.

This theory was promptly exploded at the Rockefeller Institute, where the problem was attacked experimentally from several different angles. In one set of tests it was found that there was no difference in the invasive power of organisms recovered from animals early and late in the course of a fatal mouse typhoid infection. The mortality in two separate groups of healthy mice inoculated with germs from both sources was the same, showing that the virulence was uniform. Another series of experiments proved that breeds of microbes from healthy carriers were just as deadly as similar strains obtained from fatal cases of mouse typhoid, pneumonia, and fowl cholera. Most surprising was the discovery that throughout all epidemics in animal populations the microbic virulence had nothing to do with the sequence of events, since no differences could be detected in germs recovered before, during, and after an epidemic.

When the British investigators did not agree with these results, maintaining that unequal virulence is responsible for wide variations in mortality during an epidemic, Doctor Webster at the Rockefeller Institute demonstrated that microbes from *different* communities may differ in virulence, but will not exhibit such variation in any one type of organism from epidemics confined to a single community. At the same time he found that whereas epidemic strains had unusual killing power and did not tend

to spread among survivors, the opposite was true of microbes recovered from populations among which the disease was endemic or slumbering.

It is difficult to consider the second variable factor in the course of epidemic events, that of microbe dosage, apart from the question of virulence of the organisms and resistance of the individual or total population. Only by keeping the relationship between these other two factors constant is it possible to determine the effect produced by a single large dose of germs in a given time, as compared with the same number of organisms acquired in many small doses. Under these conditions, the experiments at the Rockefeller Institute showed that mortality can be increased simply by increasing the dosage of bacteria at a given time. It is possible to set in train either an explosive type of epidemic or one that closely resembles the smouldering endemic variety by changing the number of bacteria discharged into the surroundings by infected individuals. As infection spreads through the community, there is an increase in the sources of contagion before an epidemic starts and a decrease preceding its decline. Both increase and decrease take place only after a lapse of time corresponding to the incubation period or time necessary for the disease to develop. How important this circumstance is in causing the ups and downs of epidemic waves can best be seen in its relation to the general resistance of a population, otherwise known as host resistance, a third factor that has been studied experimentally.

Immigration Problems

It is almost self-evident that the spread of infection through a community will depend upon the general resistance of the group, no matter how deadly the organisms may be. The crux of the problem, then, is to find out what determines this resistance, a vague term all too commonly used without particular reference to underlying causes or to any effect which these might have upon the trend of epidemic events. Although population resistance

is thought of in terms of the immunity found in the individuals making up such a group, it is not fair to assume that this is an accurate gauge of immunity for the group as a whole. Some of its members may be completely resistant, others highly susceptible, and still others may strike an average resistance in between. Obviously, as the numerical proportion of these individuals varies from time to time, the effective immunity of the whole population at risk must do likewise, and so determine the prevalence of infections to which it may be exposed.

From the early observations made by Doctor Topley and his associates in England, one is led to believe that such variations in host resistance do indeed play an important part. The probable train of events is illustrated in experimentally produced epidemics among animals, which for practical purposes are the same as segregated human populations. When animal communities are infected with mouse typhoid or certain other diseases, an epidemic, after some delay in starting, gathers momentum, reaches a peak, and then gradually dies out. If it is allowed to run a natural course, the ensuing mortality will greatly reduce but rarely exterminate the population.

At this point a number of surprising things will happen if healthy susceptible animals are added to the small colony. In the beginning, the recent immigrants alone succumb to the disease but during the later stages of this new epidemic wave the survivors of the previous epidemic also swell the death total. It is an interesting fact that the rate at which susceptible individuals enter the community plays a more important part than the total number of such immigrants in keeping an epidemic alive. If large groups of healthy animals are added at irregular intervals, the resulting deaths will be less than those which follow the frequent and regular additions of small colonies. As immigration is made to proceed rapidly, the quiescent periods between epidemic waves are shortened and the fatalities are kept at a high level.

Apparently a dilution of immune with highly suscepti-

ble individuals will reduce the general resistance of the population and lead to explosive outbreaks of disease. To incite a succession of epidemics, all that is needed is a continuous supply of inflammable material in the form of new immigrants. A smouldering infection is always being maintained as a result of previous outbreaks, which seem to confer a certain amount of immunity upon the survivors. Although resistance so acquired will enable these members of an infected population to live longer than others within the same community, it fails to protect against a steady influx of healthy susceptibles. It is the latter who are responsible for recurring epidemic waves.

There is nothing in these experiments consistent with a theory long held by mathematically minded students of disease that epidemics occur periodically. Statistics have been marshalled to prove this contention, but such figures have as little bearing upon the behavior of microbial incitants as almanacs have upon the frequency of equinoctial storms. Although certain diseases make their appearance in a locality from time to time and give an impression of regular intervals, the rhythmic ebb and flow of outbreaks cannot be explained by logarithmic figures and no amount of calculation will predict future cycles of epidemics.

In the last analysis, the main reasons for an epidemic are frequent opportunities for close contacts between susceptible individuals and easy transit of parasites from host to host. As we have seen, these mutual relationships are readily modified not only through natural happenings during exposure to disease but can also be changed artificially by specific preventive measures. If, for instance, vaccination has been practiced in a community, it is reasonable to suppose that the resistance of the population has been increased. Immunity, moreover, may be natural as well as acquired and depend upon inborn or inherited characteristics of a group. Thus in the long run, as collections of individuals differ in their vulnerability to microbes at different times, the resulting epidemics are more a matter of

favorable conditions for their expression than of particular cycles.

Methods of controlling communicable diseases in the future will probably undergo many changes as the knowledge gained from experimental epidemiology is applied in practice. The science is an infant prodigy, new and strange, and nobody can foresee its possibilities until an opportunity is given for its development. Yet even in its present stage, the evidence has brought into question the effectiveness of some methods used in warring against certain microbial diseases, for example, the custom of isolation and quarantine.

In order that opportunities of transferring disease from person to person may be lessened, the sick and those who carry germs, even though not sick, must be separated from the well. This is what *isolation* means. Persons who have been exposed to a communicable disease are also potential menaces and they too are restricted in their movements or *quarantined* for the period of time necessary for the disease to develop. Successful application of these measures depends largely upon early and accurate recognition of human and animal diseases and the effectiveness of restraining barriers placed between microbes and their susceptible hosts. Yet with so many undetected carriers and missed cases on the loose at all times, it is unlikely that the extent of infection actually present in a community can ever be determined. As for barriers, these mean little or nothing since parasites can travel in modern style from one part of the world to another.

Notwithstanding the obvious shortcomings of isolation procedures, they have done much good in checking the advance of contagious diseases in densely populated centers, where it is always more difficult to control the spread of infection than in small communities. There have been some interesting experiments which prove that strict quarantine of those who have been exposed to a disease will accomplish more than wholesale confinement of the sick

within hospital walls. A group of healthy mice is allowed to remain in contact with an infected group for a given time and then removed to another cage, where they are quarantined for a similar period. Now another batch of healthy susceptible mice is added to this previously exposed group and if the same procedure is repeated a number of times it is found that an infection will die out after a few successive passages. On the other hand, the free intermingling of a continuous supply of susceptible animals will result in widespread outbreaks of disease lasting as long as fresh contacts are permitted access to the infected groups.

New Problems for Old

Consequently tracking down the sick may one day become as obsolete in preventive medicine as searching for sources of infection in night air or the dirt pile. If modern epidemiology teaches anything at all, it is the importance of finding ways and means to increase the powers of resistance in persons that are susceptible to microbial diseases. Artificial immunization is a fond hope, but to become effective this measure must be applied on a universal scale. There are certain barriers standing in the way of such an accomplishment and at the present time it is difficult to decide which will prove the stoutest hurdle—the undiscovered varieties of microbes or the unreasonable human objectors to vaccination who cannot be induced to protect themselves. Meanwhile, although the goal is in sight, efforts will be made for a long time to come to prevent germ diseases by destroying their causes, when these are known, and by barring their entry into susceptible individuals.

Nobody can tell how or along what path an epoch-making discovery will come to life, but the tendency is to trust to the laboratory for miracles. This attitude forgets or ignores the fact that the history of preventive medicine and epidemiology is replete with achievements that were made in actual practice long before microbes were even thought of, much less seen under the microscope. Control

of smallpox, cholera, and typhoid fever are cases in point. Today the epidemiologist is in a much better position than his predecessors with regard to technical methods of pursuing facts, but in other respects he is at a great disadvantage, because laboratory science has become so highly specialized that observations made from nature must be tested and interpreted under conditions which often bear little resemblance to the original environment.

Accurate and painstaking as these scientific experiments may be, they merely prove that some things are thus and so and others are not, according to certain rules which govern these tests. It happens frequently that logical and valid conclusions follow from inadequate methods of proof, so that while the mental operation is a success the results do not survive. At other times when there is no avenue of approach open to experimentation because none has yet been discovered, observations and practical experience carry little weight with laboratory searchers after truth. Perhaps this accounts for some of the curious epidemiological practices in vogue today, which our better judgment might prompt us to discard but for fear of being held "unscientific." The strange part about it all, however, is that the newer science fails in certain situations where the old-fashioned way of methodical plodding, aided only by a good pair of eyes, managed to pull quite a few rabbits out of a hat.

What will man-made epidemics in animal cages tell us about some of the mysterious virus diseases? How communicable is infantile paralysis and how is the infective agent carried from place to place? Until a more susceptible test animal than the monkey is discovered, we shall have to fall back upon such evidence as can be obtained from studies of the natural disease in man. This material has by no means been fully exploited and there is always the possibility that field epidemiology may yet do with it what has been achieved in other diseases once as baffling as this.

A child that has never had chickenpox or measles,

coming into close contact with another who has it, is almost certain to "catch" the disease in due time. There is an obvious connection between one case and others that follow, and while this is undoubtedly true of all microbial infections that are spread from person to person, infantile paralysis is a striking exception to the rule. When a history of exposure to a case of this disease can be obtained, the evidence of direct transfer is not especially convincing. One would expect to find the disease more prevalent in crowded communities and certainly far more common that it is in families where living conditions favor the occurrence of several cases.

Outbreaks in densely populated areas seem to follow the same sort of haphazard distribution as in sparsely inhabited localities. If spots are marked on a map to indicate new cases of illness reported each day, one sees nothing like the dense accumulations that are so characteristic of other epidemic diseases, in the spread of which contact plays an important part. The question of determining the significance of more than one case in a family is complicated somewhat by a lack of agreement as to whether or not infantile paralysis shall include forms of the disease in which there are no paralytic signs, which introduces an error in estimating the frequency of multiple infections. Another difficulty arises from the fact that we do not know accurately how long a time it takes for the disease to develop in human beings. Consequently it is plain guesswork as to whether successive cases in affected families have arisen from a common source outside the group, from independent sources, or as a result of contagion in the household. If association with paralytic cases alone is taken as a criterion, the time interval between the first and second cases might be useful, but there is no way of telling how many non-paralytic cases are genuine and how many are spurious, because it is extremely difficult to recognize infantile paralysis in its early stages.

When we deal with communicable diseases like measles,

scarlet fever, and diphtheria, on the other hand, the interval between multiple infections is definite enough to enable one to fix the time when exposure probably took place and to predict quite accurately the occurrence of new cases. Here the family group is undoubtedly concerned in the spread of disease from person to person and some evidence for this is based upon experience with a number of epidemics. In scarlet fever, for example, one out of every four among those who are exposed in a family in the most susceptible age groups will contract the disease, and in diphtheria, about one in seven.

Infantile paralysis, however, tells a different story. During the epidemic in 1916 in New York City, only four per cent of nearly nine thousand families had more than one case, and only one in a hundred thus exposed in a family was attacked by the disease. Again, in an outbreak during 1931, among four thousand families affected, less than three per cent of these were found with more than one case. When such figures are compared with statistics gathered for the population at large, there might be a good reason to doubt that infantile paralysis is contagious. For scarlet fever the frequency of disease following exposure in families already infected is approximately thirty-eight times greater, in diphtheria twenty-one times, and for infantile paralysis only six times as great as in the general population.

From an epidemiological standpoint, the conclusions that have been drawn from these facts are, to say the least, disconcerting and make all attempts to control infantile paralysis rather vague and groping. It does not help very much to learn that, although infection of more than one member in a family occurs infrequently, when it does happen there is little delay between cases, generally from five to six days, thereby suggesting a common origin, presumably a carrier of the virus. In order to meet this situation, it is customary to regard the patient as a possible source of infection and to employ the usual means of com-

batting epidemics. On the theory that carriers are most likely to result from contact with the sick, all persons known to have been exposed to the disease are held in quarantine twenty-one days, and the patient is also isolated for a similar period. This time limit has been chosen arbitrarily because it is not known how long the period of alleged communicability may last.

How important a part is played by healthy intermediaries or germ carriers in distributing the virus of infantile paralysis is for the present a complete mystery. If the truth of this carrier theory could be demonstrated, it would give a definite direction to measures of control and some added assurance that they might be applied successfully. Unfortunately the evidence on this point is not convincing. In order to locate carriers of the disease, one must find where the virus is concealed and how easily it can be transferred from person to person. It is a very simple matter, for instance, when looking for diphtheria carriers, to swab the nose and throat of suspects and with the aid of a microscope identify the germs at short notice, but not so with infantile paralysis. Even during epidemics it is impossible, except on rare occasions, to demonstrate the virus in nasal secretions where one should expect to find it if carriers are truly as numerous as they are supposed to be. From experiments with which we are already familiar, we know that the virus can always be found in discharges from the nasal passages of diseased animals, no matter what route of inoculation is used to produce an infection. Therefore the contention that the spread of epidemic infantile paralysis is made easier by contact with healthy carriers of the causative virus will remain an open question until technical improvements become available for using suitable tests. At present the means at our disposal for studying these suspected carriers are so incomplete that we cannot get a clear idea of their number and distribution.

The first successful demonstration of the theory of transmission by virus carriers was made by Doctor Flexner

about thirty-five years ago. In this experiment the nasal passages of two healthy persons, who had been exposed to the disease in a family, were irrigated with salt solution and the combined material, after filtration through a stone filter, was inoculated into monkeys. The animals promptly developed poliomyelitis.

This method had failed in the hands of two other doctors, who reported experiments identical with these from Stockholm one year before. In their next attempt (1914), they accordingly modified their first method of preparing the nasal washings by concentrating them at body temperature in a vacuum apparatus prior to injecting monkeys. An experiment was then tried with combined rinsings obtained from four surviving members of a family, one of whom had died the day before from infantile paralysis. By "boiling down" one to two quarts of this fluid to nearly one-tenth of its original volume, they obtained sufficient virus without destroying its native power to infect. A monkey which received a small amount of this filtered material into the brain died from typical poliomyelitis on the twelfth day following inoculation. Both successful experiments prove without a doubt that the virus can be harbored by persons who show no signs of illness and are not suffering from this disease. It is impossible, however, to conclude from these tests how many of the healthy contacts were carriers, because the nasal washings were pooled before inoculation.

This point received particular attention several years later at the hands of two protégés of Doctor Flexner, who were working in his laboratory at the Rockefeller Institute. In the summer of 1917, an epidemic of infantile paralysis in a small town of Vermont gave these two young medical scientists an opportunity to do a classic bit of field epidemiology. The subject of their study was a family with four children, one of whom, a boy sixteen years old, developed the disease, became totally paralyzed, and died after an illness of five days. On the day of his death, a

younger brother and sister, neither of whom had been away from the village, were given nasal irrigations with sterilized salt solution. These washings were filtered and concentrated separately in a vacuum machine until a very small part of the original material remained, and then were inoculated into the brain of each of two Indian monkeys. Both animals came down with typical poliomyelitis in less than two weeks. A third child, the youngest and least cooperative member of the family, was not included in this experiment because nasal washings could not be taken on the day when they were obtained from the others. Any doubt as to whether or not he carried the infantile paralysis germ was removed two days later when he also became ill and suffered a mild attack of the disease with slight paralysis of a muscle in one leg.

All three children undoubtedly harbored the microbe, but it is especially interesting to follow more closely the story of the first two, whose nasal washings infected monkeys. One child, in perfect health at the time the test was made, fell sick five days later and afterwards had a mild paralysis of certain muscles in an arm and leg. This is an illustration of a healthy carrier who can develop the disease and it is incidentally the first example of its kind to be demonstrated experimentally. The second child, who had been ill three days before the nasal washings were taken, recovered completely without showing any evidence of paralysis. Here we have an example of a virus carrier resulting presumably from a non-paralytic type of infection.

If it were possible to reproduce such results as these often enough, one might be less skeptical regarding the mode of spread by personal contact, but the number of similar reported successes during the past twenty or more years has been extremely small. While it is difficult to accept the idea that every case of infantile paralysis develops from a carrier, some cases do seem to arise during the period of virus carriage. This brings up the question

once more of the part played in the spread of this disease by persons suffering mild attacks of illness that are not accompanied by paralysis or any other tangible evidence of an infected nervous system. During epidemics one sees large numbers of children, particularly under the age of five, affected in this way in families where infantile paralysis has occurred. From reliable estimates, it is believed these cases of vague illness are usually six to ten times more prevalent than outspoken examples of recognized infantile paralysis.

Few attempts have been made to detect the virus in such illnesses, although their frequent association with frank infantile paralysis has often suggested a common cause for both. Fifteen years after Doctors Taylor and Amoss first emphasized this possibility by experiment, their work was confirmed in 1932 by Doctors Paul and Trask of the Yale University Medical School at New Haven, Connecticut. They inoculated monkeys with a portion of the throat rinsings obtained from each of a group of children during an attack of illness following known exposure to a case of infantile paralysis and in two instances out of twelve the virus was present. All these young patients showed signs of what is commonly referred to as "summer grippe," ushered in by fever and drowsiness or sore throat, vomiting, and headache. They became perfectly well within thirty-six hours afterwards. Out of forty-one children in a community, over half had similar illnesses lasting a day or more. Nobody can say with assurance that the presence of virus in the nose or throat under these circumstances makes a person a carrier or a case, but whatever name is given, the association of these peculiar ailments with outbreaks of infantile paralysis is probably not accidental and it is inferred that they are mild forms of the same disease.

The chief objection to this theory is not, as might be supposed, the failure to demonstrate the virus more regularly in these situations. Rather, the problem is to disen-

tangle cause from effect, since the mere presence of virus may be incidental to the patient's condition and in no way responsible for it. Any illness whatsoever can break down the defensive barriers of an individual and thus favor the entrance of loitering parasites. This is particularly so with infections of the upper respiratory tract and therefore would include poliomyelitis. Under normal conditions, it is agreed, the lining of the nose and its mucous secretions protect against invasion by the rank and file of microbes. Experiments have been made to show how effective this defence may be in warding off the poliomyelitis virus. If nasal washings obtained from healthy persons are combined with virus and inoculated into monkeys, the disease will not result, but that protection fails when the same test is performed with washings taken from persons who have colds or other infections of the nose and throat. In similar tests made on virus carriers and other persons suffering from infantile paralysis, the nasal rinsings were likewise devoid of any protective action.

The problem of "carriers" raises a question which concerns the mode of virus elimination: What is the mechanism or the pathway of excretion of poliomyelitis virus? We know for certain that it appears in the nasal secretions and in the stools, but how does it arrive there? Doctor Harold K. Faber and colleagues at the Stanford University School of Medicine have attacked this problem by a direct experimental approach.

In 1946, Doctor Faber suggested the hypothesis of virus passage along the nerve fibers from the body surface inward to the central nervous system, and then back to the places supplied by the same system of nerves. Such a mechanism would be somewhat analogous to the two-way communication over a telephone wire through a central station and its local branches.

Experimental results in support of this theory appear to be highly compatible with the possibility that virus can pass along nerves to branches communicating with the

mucous membranes of the nasopharynx. In brief, this has been demonstrated in monkeys by applying virus to the cut end of a large nerve branch in the cheek. On the third and fourth days following, virus can be detected in the nasal washings from the nasopharynx; intestinal contents and stools contain virus on the fourth day but not earlier, suggesting that virus might have been swallowed. From these findings it is concluded that excretion of poliomyelitis virus takes place in the same way as during invasion, through the nerve channels. This raises the question of a possible mechanism of virus infection through the skin, and brings us back once more to the riddle of virus transmission by insects.

These observations have some bearing on the question of swimming pools in relation to the spread of poliomyelitis. During an epidemic no topic is discussed more frequently than this, and the swimming pool has been condemned on numerous occasions. While it is true that patients often gave a history of having taken part actively in some form of water sport, there was until recently no scientific basis for incriminating water as the source of infection. Quite obviously, swimming offers every opportunity for over-fatigue, chilling of the body, and irritation of the mucous linings of nose and throat, all calculated to bring on a respiratory disease. Irritation of the nasal passages from constant washing and snuffing with water damages the natural barrier by removing protective secretions of mucus. Under such circumstances, especially in diving or under-water swimming, the disease might be introduced through the nose.

Whether or not persons infected this way are carriers to begin with, or become inoculated afterwards, it is impossible to say. If the water supply is in a condition comparable with drinking water, then the pool cannot be regarded as a vehicle of infection. The public is protected by the enforcement of health regulations requiring clean water, frequently changed or provided with a system of

continuous pumping and repurification. With proper methods of chlorination it may be possible to maintain a swimming pool in sanitary condition. This means the constant circulation in the water of pure chlorine, a disinfecting chemical that will destroy germs even in the presence of human discharges, which are known to interfere with any germicidal activity. Yet it appears doubtful whether chlorination that is adequate for ordinary purposes will destroy the virus of poliomyelitis. This question is even now the subject of warm debate, in view of the discovery that the virus is discharged in the intestinal contents of man.

In considering the possible spread of infection from surroundings, the resistance of germs towards environmental influences has to be taken into account. Outside the human or animal body, under natural conditions, microbial life, not excepting the virus of infantile paralysis, is exceedingly short. Yet its tenacity in the face of rigorous exposure to certain chemicals which quickly destroy all other living things, or to drying at low temperatures, is a strange contradiction. Present-day thought, however, is less concerned with inanimate objects than with the living agents that might transmit disease. Air, water, food, dirt, geography, atmospheric phenomena—none of these has given up a satisfactory clue as to how this virus finds its way into man.

Insects and Virus Diseases

Curiously, the possible rôle of go-between hosts belonging to the insect or animal kingdom has not received more serious consideration, although there seems little justification for embalming an idea on the strength, or rather, weakness, of early investigations along such lines. Certain factors in the spread of infantile paralysis, which differ from those ordinarily found in contagious diseases, have suggested the possibility that insects may be implicated. Only a small percentage of the cases, for instance,

presumably can be traced to previous ones, and conversely, the number of infections occurring among persons known to have been exposed is very low. Likewise, there are scattered cases arising constantly, without recognizable exposure to the disease and with no subsequent cases following. Moreover, the greatest prevalence of the disease is witnessed in the warm seasons when insects are most numerous and not when droplet infections, spread by coughing, sneezing, and spitting, are generally epidemic.

For these reasons, some attempts have been made to determine whether transmission of poliomyelitis by insects might be possible. These experiments dealt principally with fleas, bedbugs, lice, the domestic housefly, and mosquitoes. In some instances the insect was allowed to bite a healthy monkey after having bitten an infected animal. In others, insects that had fed on infected monkeys were macerated and the filtrates prepared from such material were injected into healthy monkeys. In still others, fly larvae—just plain maggots—were fed with brain tissue from a monkey that had died of poliomyelitis, but no trace of the virus was found in the adult flies that subsequently hatched from these larvae. All these half-hearted experiments were negative with a single exception, when in one out of many trials the filtrate prepared from a bedbug which had fed upon an infected monkey seven days earlier produced the disease. This result, however, could not be verified.

The theory of insect transmission of poliomyelitis has consequently been abandoned by most epidemiologists. There still remains the possibility of the virus being carried in mechanical ways different from those covered in the above experiments, which claim to disprove the rôle of a biological factor. It is known that the domestic fly can carry the infantile paralysis virus in an active state for several days upon the surface of its body and for several hours in its intestinal tract. The disease, however, is not more common among the poor than among the well-to-do,

as is usually true of germ infections borne by flies, fleas, lice, and bedbugs, the inseparable companions of poverty and filth. On these grounds mechanical transfer of the virus by insects is thought to be of no consequence.

An important question to be answered, nevertheless, is whether flies act as hosts for the virus, in which case transmission of the disease might result from the survival and possible multiplication of virus in the fly. Interest in this problem has been revived again by the recovery of poliomyelitis virus from common houseflies collected in widely scattered epidemic areas of North America during 1941 to 1945. These studies confirmed what was already known about the carriage of virus without adding anything new in regard to the insect carrier. Perhaps it was the kind of bait, perhaps the lure.

Research epidemiologists, working at the Yale University School of Medicine, thought it better to follow a pattern more closely resembling conditions in nature. Instead of using the domestic housefly in these experiments, they selected blowflies, which in previous studies were always found among the tested virus-carrying flies collected from epidemic areas. Another innovation was to allow the insect to feed on human poliomyelitis virus as present under natural conditions in the stools of patients.

Using a human poliomyelitis virus in comparison with strains adapted to the monkey or mouse, feeding experiments revealed a striking difference in results. Human intestinal virus was found in the fly excreta for as long as *three weeks,* and in the bodies of the flies after feeding, for *two weeks.* Of the other strains none survived longer than *five days.*

As to whether the poliomyelitis virus actually multiplies in the fly, the question cannot be answered until more accurate measurements are made of the amounts of virus fed and excreted. On a comparative basis, however, the results are said to be in agreement with demonstrable multiplication. All this aside, it would seem to be just as important

to recognize the fact that the blowfly carries and excretes the virus over a sufficiently long period of time to be a factor in transmission of the disease.

Some day, perhaps, the problem will be attacked with renewed zest by the old-fashioned method of trial and error applied with the dogged persistence of a Theobald Smith, a Patrick Manson, or a Ricketts. Who knows in which breed of myriad crawling, flying, or biting insects a virus might not have to pass through a cycle of development before becoming infective in man? Is the theory that the disease gains access to the nervous system exclusively through the nose and throat passages the only possible explanation? While strong evidence to the contrary is lacking at present, it is due perhaps more to experimental difficulties than to incontrovertible proof.

When all is said and done, this theory is based upon evidence that rests heavily on the limitations of available methods. First comes the greater ease with which monkeys can be inoculated by the nasal route rather than through the skin or by way of the blood stream; second is the failure ever to detect infantile paralysis virus in the blood of diseased persons and only seldom in the blood stream of experimentally infected animals. The obvious inference is that successful transfer of a disease through insect bites cannot take place unless the causative organism multiplies and circulates freely in the blood stream.

During recent years, however, we have learned that the viruses responsible for yellow fever, dengue fever (dandy or breakbone fever) and encephalomyelitis ("sleeping sickness") of horses can multiply within the bodies of certain kinds of mosquitoes. Pertinently, also, it was discovered that human beings are susceptible to encephalomyelitis, a disease which causes inflammation of the brain in horses. When, late in the summer of 1938, an epidemic of this disease broke out in the state of Massachusetts and at about the same time several children in the vicinity died of encephalitis, popularly known as "sleeping sickness,"

rumors were current that these outbreaks were in some way related. Although the idea seemed preposterous at first, the Massachusetts Health Department, with the aid of scientists from the Harvard Medical School, the Rockefeller Institute, and the United States Public Health Service, identified the horse virus in material taken from the brain of human victims. The cause of human encephalitis and of inflammatory brain disease in horses is one and the same thing.

The name originally given to this animal disease, equine (horse) encephalomyelitis, is really wide of the mark, because it now appears that domestic animals have less to do with the spread of epidemics than have wild game and a variety of insects which carry the germ responsible for this malady. It is believed that game birds bitten by mosquitoes carry the disease to far distant points, where other mosquitoes may transmit it to human beings. The migratory habits of these birds serve as a grim warning of the easy way in which the virus can be widely distributed.

The virus has also been recovered from pigeons which died in unusually large numbers in a locality of Massachusetts where horses were affected with brain inflammation. From Connecticut, a nearby state, came another interesting clue when it was discovered that several paralyzed ring-necked pheasants found on a range carried a virus which, if inoculated into mice, would cause the same disease that occurs in horses and human beings. The growing list of suspects also includes vultures, turkeys, chickens, ducks, geese, the European blackbird, and even the fabulous stork, all of which may carry the causative virus without showing any signs of sickness.

At the present time two known varieties of encephalomyelitis exist in the United States, one in the eastern part and another in the West, which in laboratory tests behave like two different diseases. Horses made immune to one breed of virus will die if inoculated with the other, a fact which makes it a simple matter to determine the variety

responsible for an epidemic. In Venezuela there has appeared a similar disease which resembles the Eastern variety of encephalomyelitis more than the Western but is thought to be different from either one. There are also a Russian strain, and the infection known as Borna's disease, the first known encephalomyelitis of horses which was recognized as a virus disease many years ago. It is probably only a question of time before all these varieties will come to be regarded as forms of the same disease, just as pneumonia, caused by thirty-odd breeds of germs, is still pneumonia, no matter where it is found.

In the control of environmental factors related to the spread of disease, no problem has been more vexing than the search for possible carriers among insects. Today there is an ever-growing list of virus diseases that affect man and animals and are known to be transmitted by newly discovered insect vectors. From widely scattered places on all continents evidence is accumulating to show the world-wide distribution of many such transmitters. Some of the advances already made in our knowledge of arthropod (insect)-borne virus diseases emphasize the importance of identifying the disease-bearing culprit with utmost accuracy. For this knowledge we can thank the medical *entomologist,* an insect specialist whose contributions have perhaps not received the recognition they properly deserve. The familiar caricature of a "bug hunter" does not do justice to the important rôle of this present-day skilled investigator. He is not at all like the bespectacled and usually absent-minded professor pictured as chasing butterflies with a net, and stumbling inevitably over or into something. In a similar way, the student of bird lore, a trained naturalist, has also come to play a vital part in discovering new disease-transmitting vectors. This expert on birds, known as an *ornithologist,* will some day help establish the possible rôle of migratory or partially migratory birds as intermediate hosts in the spreading of virus disease.

A besetting problem is the pre-epidemic seeding of causative agents in many as yet undiscovered insect carriers. Mosquitoes, as predicted, have been incriminated in equine (horse) encephalomyelitis of the Western, Eastern, and Venezuelan types; in encephalitis of the St. Louis, Japanese "B," and Russian varieties; and in a disease caused by the "West Nile virus," found in a large area of Central Africa. What was not known until recently is the existence of a true reservoir or "overwintering" mechanism during a non-epidemic period, to explain how virus diseases can be carried over from season to season. A fortunate sequence of events, beginning with the 1937 epidemic of encephalitis in St. Louis, helped bring this about. Doctors Casey and Broun of St. Louis University, in a classic study of the outbreak, showed that every known feature of its epidemiology was characteristic of a mosquito-borne disease. Their concept, while perfectly sound and valid, stopped short of experimental proof. Isolation of the virus from naturally infected mosquitoes was accomplished by Hammon and his co-workers in California several years later. From this pioneer work we learn that during epidemics of Western equine and St. Louis encephalitis, the virus occurs naturally in the mosquito, which transmits the disease to chickens and pigeons. Here might be a suggestive clue to a reservoir—fowl and bird life infected by a blood-sucking insect with feeding habits that suggest a source of supply of virus. However, attempts to prove chickens and birds capable of becoming true carriers of the virus have resulted in failure. Something else must be responsible for the carry-over between epidemic seasons—a missing link somewhere in the chain of infection—from mosquito to fowl to mosquito or, now and then, from mosquito to man to horse.

The scene now shifts to St. Louis, where a group of investigators at the Washington University School of Medicine are trying to solve this mystery. As a starting point in the study it is necessary to find out how much, if any,

immunity or resistance has been developed in the human and animal population exposed to the virus of encephalitis. If it can be shown that protective antibody substances are present in the blood of those tested, there is conclusive evidence that the disease must have appeared in the community at some time or other. A survey of the population in St. Louis County revealed that few residents who came into this area after the last epidemic had any protective substances in their blood serum. However, in flocks of year-old chickens found in this locality, specific antibodies could be demonstrated. This observation, taken together with the negative findings among the human population, suggested the presence of a blood-sucking insect vector, which was maintaining the virus disease in fowl, although not biting man. Suspicion fell, quite naturally, on the common poultry mite with which the fowl were usually infested. The chicken mite is named *Dermanyssus gallinae,* and the bird mite, *Dermanyssus avium* ("derma" = skin; "nyssus" = pierce). When these mites were ground up and inoculated into mice, the animals developed encephalitis. The infective agent, cultivated in the developing chick embryo and maintained in successive generations, also produced the same disease in the mouse. If it could be shown further that the virus is transmitted through the egg of adult females to the larval and nymph stages, the evidence in support of a winter carry-over mechanism would be more convincing. Not only was this essential fact of congenital transmission of virus demonstrated; decisive proof that the virus persists in mites in nature was also given. Thus, when naturally infected poultry mites are kept alive in laboratory colonies by feeding on healthy chickens, the virus is retained in the insect for as long as five months. But for the lack of one important detail, the ability of this vector to transmit the infective agent by bite, it could be stated now that the chicken mite is a true reservoir of St. Louis encephalitis virus during a non-epidemic period.

For the most part, the Western equine and St. Louis encephalitis are practically identical in their epidemiology. It is not surprising, therefore, to find the same insect vector implicated in both diseases. During an outbreak of Western equine encephalomyelitis in Texas in 1944, the virus of this disease also was recovered from chicken mites (*Dermanyssus gallinae*). Similar epidemiological studies, based on the work previously reported from St. Louis, confirmed the presence of equine virus in naturally infected mites.

The search for reservoir hosts and insect vectors continues. Early in 1948 Hammon and his associates in California reported the isolation of Western equine and St. Louis encephalitis virus from wild bird mites found in the nest of a yellow-headed blackbird. This insect, identified as the northern fowl mite, travels under the scientific name of *Liponyssus sylviarum* ("lipo" = fat; "nyssus" = pierce). Now that migrating or partially migratory birds have been coming into the picture of virus transmission, might not a similar epidemiologic pattern be studied in poliomyelitis? With this thought in mind, it is quite possible that a research project undertaken at the proper time, *early in the course of an epidemic*, would have positive value. Certain localities offer ideal conditions for the study of epidemiologic factors suggesting the probable rôle of birds as intermediate hosts, and of *insect vectors other than flies*, as possible reservoirs.

A wealth of epidemiologic material is contained in the Audubon bird guide dealing with our eastern land birds. Many of these become a pest during the late spring and summer seasons when starlings, red-winged and rusty blackbirds, brown-headed cowbirds and the common grackle darken the skies morning and evening. All are in the blackbird family, they flock together and have nesting, feeding, and ranging flight habits, which seem sufficient to make a good case in evidence against these birds of a feather. They abound in most rural areas, where plowed

croplands, hayfields, and pastures provide ideal surroundings; nesting and roosting places are within flying distance of marsh vegetation, wet woods, swamps, open sewers, and sewage. In cities and towns they roost about buildings or in trees, feeding mostly on the ground in parks, or lawns and sidewalks or, in rustic areas, on ground-dwelling insects such as beetles, grasshoppers, weevils, and leaf hoppers. The grackle includes mice in its diet, while the cowbird species, which originally attended great herds of prairie bison and was once known as the "buffalo bird," now accompanies cattle. Thus we see a multitude of possibilities for picking up the virus in and about sewage-polluted areas, for its dissemination via the intestinal tract, and for its carriage by a host of parasitic insects in and upon these migratory birds.

Now and then an epidemiological situation arises in a town or city with its own or surrounding rural characteristics. Here we can find a natural field experiment for the asking. In one such instance, recently, it was shown that sewage pollution appeared to be directly related to an unusually great number of multiple cases of poliomyelitis occurring in many different households. The popular question of fly transmission was again investigated, although by this time the epidemic had burned itself out and the opportunity for an adequate study of possible insect and bird carriers had slipped away.

Seemingly the subject of virus transmission by insects has by no means been exhausted and some of the evidence obtained in poliomyelitis might be sifted more carefully before throwing it all to the winds. As new facts come to light here and there, one begins to see the possibility of order evolving from a chaotic jumble of scattered observations. Even now the matter of different breeds of virus, for example, may change deeply rooted ideas regarding the mode of spread in this disease and in others closely resembling it. General statements are made as to the portal of entry and inoculation via certain routes in animals without

taking into account the natural tendency of individual virus strains to make the selection that is best suited to their habits.

The fact that stands out as bright as a shiny new nail is that some breeds of virus will infect monkeys very easily when inoculated into the skin. This was first noticed in 1932 by Doctor Maurice Brodie of McGill University at Montreal, while working with potent strains, and several years later by two other investigators at the Yale University Medical School. The contributions to this important subject prove that certain strains of infantile paralysis virus newly isolated from human cases are peculiarly infective when introduced into the skin of monkeys. This property is not shown by old viruses that have been transplanted from animal to animal in a series of brain inoculations. Considering how much difficulty is experienced in "acclimatizing" fresh breeds of virus to the nervous system of monkeys, it is remarkable indeed that infections can follow the inoculation of small doses into the skin. All this means simply that infantile paralysis virus probably does not enter the human body exclusively by way of the nose and throat.

One is tempted to use this discovery to illustrate the manner in which insects might transmit poliomyelitis, because experimental infection by this particular route has not been satisfactorily demonstrated hitherto. However, as mentioned already, the recent studies by Doctor Faber again suggest the possibility that a mechanism or pathway of virus infection through the skin does exist. It seems obvious by now that the question of insect transmission is again wide open. For all that we know, outbreaks of poliomyelitis and other virus diseases may be caused by unusual strains of organisms capable of invading the human body through unsuspected channels.

It is quite probable that this happened in southern California during the 1934 epidemic of infantile paralysis. In this outbreak three separate breeds of virus that were

isolated from patients were highly infectious when injected into the skin and in one instance this peculiar trait was retained even though frequent transfers of the virus were made from animal to animal. But even more remarkable is the fact that all attempts to inoculate such virus through the nasal passages failed consistently from the beginning. This is a radical departure from its customary behavior and suggests that freshly isolated strains may differ in their invasiveness from those having more or less fixed habits. While the old established stock has settled down to an even tenor of life, nobody can predict what path the flighty new generation will take.

Where the transmission of a disease has been controlled effectively, there can be little doubt as to the manner in which it spreads. Is it not odd that those diseases which have until now balked all efforts at control are presumably caused by a microbe or virus that enters and leaves through the respiratory tract? Epidemics past and present tell the same story of a search for causes in the most unexpected places. Virus diseases are still eluding the marksmanship of modern microbe hunters, who in aiming at a moving target might find shotguns more effective than rifles.

Man-made epidemics have taught us much and yet so little. History repeats itself with the same germs, perhaps in different garments, for after all, "the colonel's lady and Judy O'Grady are sisters under their skins."

CHAPTER 13

THE CHALLENGE

How successfully have we met the microbe's challenge? If, in taking stock today, we assess the extent of our advances at their true value and see the long road that still lies ahead, we need but remember that the progress of science in theory and practice depends more upon direction than speed, less upon individual gains than their consolidation. Somewhere along the line of attack existing gaps in our knowledge must be plugged by concentrating forces in places where certain acceptable theories have lacked support. At other points a change in strategy indicates that it would be much wiser to abandon such theories completely.

Although microbes in general are amenable to control, the ones that most stubbornly resist all our efforts to conquer them are those which are carried from one victim to another by the mosquito. This annoying insect, an opportunist if ever there was one, has done more than any other germ-carrier to prick the vanity of man's conquests of disease and turn his optimism into pessimism.

Not so very long ago, medical science had a rude awakening from a pleasant dream in which yellow fever, popularly known as "yellow jack," was pictured as a disease fast becoming extinct on the American continent and destined soon to disappear from the face of the earth. The yellow fever mosquito (known as *Aëdes aegypti*) was being driven back to its lair and seemingly held at bay under the fierce attack of bacteriologists, epidemiologists, and sanitarians. Success had lulled them into security, but a familiar persistent buzzing about their ears soon disturbed their sound sleep. Stirring uneasily at first, they

woke up finally to discover that they had been making a few misdirected slaps here and there and that the tormentor was still at large.

The apparent triumphs over yellow fever that have been achieved in recent years were actually won on earlier battlefields at no little cost to the pioneer investigators who discovered certain simple facts. When, as early as 1881, a hard-headed Scotchman, Doctor Carlos Finlay of Havana, told the medical world that yellow fever was spread by the bites of mosquitoes after they had stung a yellow fever patient, his theory was dismissed as fanciful. It did not occur to anybody nor seem to matter in the least that Sir Patrick Manson, only a short two years before, had shown how the filarial disease was carried from man to man by a certain kind of mosquito. For nearly twenty years Finlay's idea was completely ignored, despite the work of Theobald Smith, who had long since found ticks responsible for the spread of Texas cattle fever and the discovery by Ronald Ross and others that mosquitoes transmitted malaria.

Faith and Fate

Meanwhile Havana and other important Cuban centers were in a deplorable state as the devastating march of yellow fever continued its ravages. This could not have been any worse when, in June, 1900, there came to the infested island a small group of men who had been appointed by the Surgeon General of the United States Army as a medical board to investigate the deadly disease. In this party were Major Walter Reed and three doctors, Aristide Agramonte, James Carroll, and Jesse Lazear. Shortly before Doctor Agramonte's death some years ago, he published an interesting account of the work that was done in Cuba by these four men who made history. In this simple report, he described the experiments which confirmed the theory of Carlos Finlay that yellow fever was transmitted to man by a certain kind of mosquito.

The glorious adventure was a strange hodge-podge of bungled science, comedy, and tragedy. Having been assigned to study an epidemic in a town of the interior many miles distant from Havana, Agramonte was impressed with the singular manner in which this and some other outbreaks seemed to arise at a distance from known infected areas. Out of a clear sky the disease would strike a locality and spread without visible means of personal contact or any direct contamination from bedding or clothing used by the sick. In talking this over with Major Reed, the possibility of an insect carrying the disease was suggested and everyone agreed that the mosquito question, so dear to Doctor Finlay's heart, should be looked into carefully. Such a spirit of openmindedness, praiseworthy in itself, was more or less overshadowed by what these men accomplished, although their heroic tasks were performed in the routine manner expected of heroic personalities.

The very first thing they had to do was to find the guilty mosquito and this was supplied by Doctor Finlay, who turned over to them a partly filled bowl of water, containing some unhatched eggs deposited about one month before by a female mosquito which he had suspected as the trouble maker for a long time. (The eggs of a yellow fever mosquito can withstand prolonged drying and do not hatch below a temperature of approximately sixty degrees Fahrenheit.) When these eggs hatched, the insects were identified by an expert mosquito fancier as *Culex fasciatus*, later known under the name *Stegomyia calopus* and now *Aëdes aegypti* (Aëdes: from the Greek word meaning "unpleasant"). Doctor Lazear, who was in charge of the mosquito work, guarded his pets carefully in their individual glass tubes after permitting them to feed on yellow fever patients, whose blood-shot eyes stared fixedly at some spot high above the ceiling of "Las Animas" Hospital.

The next step in this investigation was to have the infected mosquitoes bite a number of willing human sub-

jects to see if the disease could be contracted that way. Since almost everyone except the experimenters looked upon the mosquito business with amusement, a request for volunteers was taken more or less as a joke and quite a few persons allowed themselves to be bitten by mosquitoes that had first sucked blood at frequent intervals from hospitalized patients. To make the test, a tube containing the insect was inverted over the forearm of the volunteer and held there until the mosquito had drunk its fill and in the act of biting, had presumably introduced the infective agent into the blood of the victim. As it was supposed from previous knowledge concerning insect-borne diseases that this unknown parasite would also require a certain period of time for development before the mosquito could become infective, insects that had been fed yellow fever blood were made to bite their healthy subjects at intervals of two or three days.

Nothing happened to cause the slightest ripple of excitement as both Spaniards and Americans lined up for the sport and allowed themselves to be bitten without the least discomfort or ill effect. Doctor Lazear, by that time quite disgusted with the turn of events, applied to his own person a mosquito which had fed ten days before upon a yellow fever patient in the fifth day of his illness. Like all the other tests, this too was negative and now even the experimenters themselves began to lose faith in their theory.

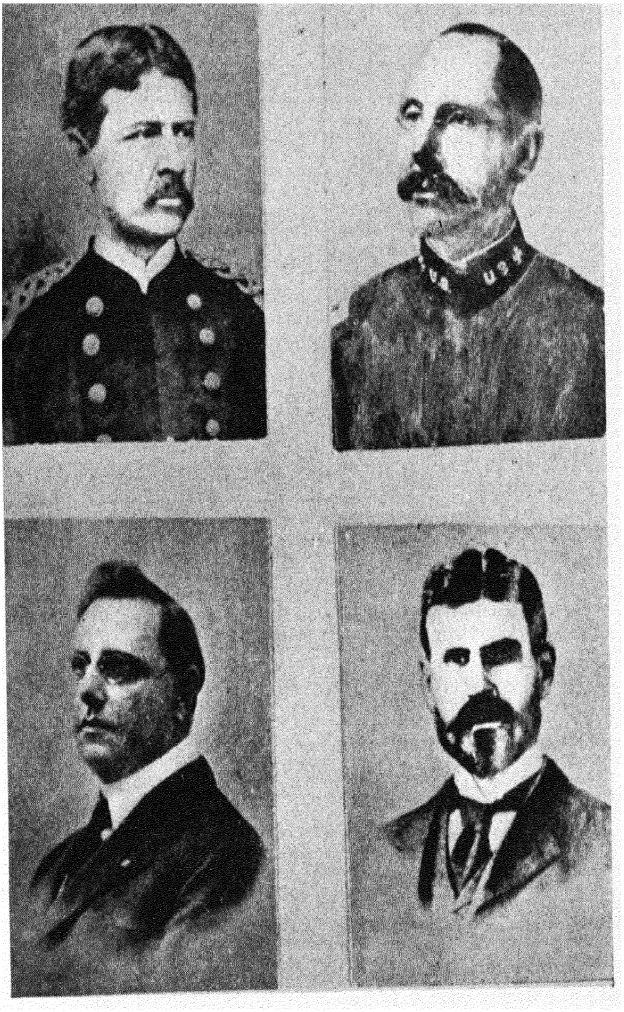
It was this frame of mind and a stubborn mosquito that produced the fortunate and unfortunate accidents which saved the yellow fever research from becoming a fiasco. One day late in August, as Lazear and Carroll were working in the laboratory and discussing the seemingly harmless mosquitoes, Lazear mentioned casually that he had failed all morning long to induce one insect in his collection to bite patients at the hospital. Thereupon Carroll decided to try feeding the insect some of his own blood, lest it die. After much coaxing and a long wait, the mosquito finally took a meal from his forearm, both

men watching the procedure intently. That mosquito bite proved to be the most important event in yellow fever history.

Three days later, Carroll took sick with frightful chills and fever. Thinking that it was malaria, he and Lazear arranged with Agramonte to examine a blood specimen the next day. When Agramonte arrived the following morning, Carroll had already been at a microscope searching for malarial parasites, and failing to find any, decided his illness was only a "cold" caught while bathing. But Agramonte, an experienced physician who had had the disease himself, saw at a glance that this was genuine yellow fever. When Carroll realized that he was actually stricken with the disease, he attributed it to some source other than a mosquito bite. Had not Lazear too been bitten by the mosquitoes and remained unharmed?

Despite Carroll's incredulity, however, events proved that all the factors necessary to produce an infection were provided by the particular mosquito which had bitten him. While his fate hung in the balance, his co-workers hurried to make another test with the same mosquito on the first person who volunteered to run the possible risk. This was a soldier named William Dean stationed at the military hospital, who happened to be passing by the laboratory door and stopped to watch Doctor Lazear at work. As he received the mosquito bite, Dean remarked: "Shucks, they can't hurt me none," but five days later he had yellow fever.

Lazear's notebook recorded that the insect causing these two infections had been hatched in the laboratory and had *fed twelve days before upon a yellow fever patient,* who was then *in the second day of the disease.* This, then, was the proper combination of these two factors necessary for a successful inoculation. All Lazear's previous experiments, including those made upon himself, had failed because the mosquito had bitten the patient too late in the disease and, although gorged with yellow fever blood, was



THE UNITED STATES ARMY YELLOW FEVER BOARD

Major Walter Reed — Dr James Carroll
Dr. Aristides Agramonte — Dr. Jesse W. Lazear

Pioneers of Yellow Fever Research They have not lived in vain Major Walter Reed died in 1902. His widow had been receiving a monthly pension of $125. Major Carroll died in 1907 and his widow received the same amount. Doctor Lazear died in 1900 of yellow fever, his widow and two children receiving an annuity of $125 a month. Doctor Agramonte, until his death in 1931, was professor of bacteriology and experimental pathology in the University of Havana. He never received any material reward for his work.

A SHORT

A C C O U N T

OF THE

MALIGNANT FEVER.

LATELY PREVALENT IN

PHILADELPHIA:

WITH A STATEMENT OF THE

PROCEEDINGS

THAT TOOK PLACE ON THE SUBJECT IN DIFFERENT PARTS OF THE

UNITED STATES.

BY MATHEW CAREY.

SECOND EDITION

PHILADELPHIA:

PRINTED BY THE AUTHOR

November 23, 1793.

Yellow Fever Epidemic in Philadelphia. Mathew Carey was a young Irishman who wouldn't stay muzzled and was banished from his country. He came to the United States and established himself in Philadelphia, where he founded a newspaper, the *Pennsylvania Record*, and set up a prosperous trade as a bookseller. When yellow fever swept over Philadelphia in 1793, Carey was in his early thirties. During the epidemic he busied himself as an active relief worker. His experience furnished the material for a remarkable first-hand account of the pestilence which he graphically described in a classic work entitled "Malignant Fever." In this epidemic more than four thousand persons died in the short space of six weeks from August to September.

not in condition as soon as it bit again to transmit the disease to a healthy person.

Major Walter Reed, who had been away in Washington while these exciting events were taking place, received word there of the great discovery, but before he could rejoin his group of experimenters in Cuba, tragedy struck the happy camp. Both Carroll and Dean had recovered, but in an unguarded moment at the bedside of a yellow fever patient in the hospital, Doctor Lazear had allowed a stray mosquito to settle on his wrist and take its fill. Five days later he came down with a severe attack of yellow fever and on the seventh day of his illness he died. Some say Lazear mistook the kind of mosquito that bit him and consequently ignored it, while others speak of the incident as a planned test. Doctor Agramonte, an eye witness, reports Lazear as having stated emphatically that it was not an experiment, that the mosquito got away before it could be caught, and that Lazear had no fear of the bite because nothing had happened to him one month previously when bitten by an experimentally infected mosquito.

Strange indeed are the paths to discovery! It is hard to decide which incident was the more remarkable—Doctor Carroll's choice at the psychological moment to save the life of a valuable mosquito with his own blood, or Lazear's astounding lack of faith in this theory and his indifference to fate, that made him a martyr to science.

Ordeal by Ordure

What was thought at the time to have been the final chapter in the conquest of yellow fever was written at Camp Lazear, named in memory of the unfortunate victim of chance. Unable to make any headway with experiments on animals, and wishing to exclude all possible sources of error in future tests upon human beings, Reed, Carroll, and Agramonte began the second phase of their researches in a strictly quarantined experimental camp. There was nothing fancy or pretentious about this place,

which boasted some tents for isolating the human guinea pigs and two small, absolutely mosquito-proof wooden houses to be used in demonstrating exactly how yellow fever is communicated to man and what conditions are necessary for its spread.

One of these houses was furnished expressly to test an old theory, then currently accepted, that bedding and wearing apparel soiled with discharges from yellow fever patients were contagious. In this room, which was dark, badly ventilated, and kept at tropical heat by means of a stove, were placed three cots with pillows, mattresses, blankets, and the usual supply of night clothes and linens, all of which had been contaminated beforehand with "black vomit," blood, and unspeakable filth from the sick. These gruesome articles were strewn everywhere about the room and an extra supply was packed in a trunk and boxes.

Three men, Doctor Robert Cook, an officer, and two other American heroes, privates in the Medical Corps, were assigned to these sleeping quarters, where they unpacked the things at night and, in handling them, tried in every possible way to spread any lurking germs over themselves. The various articles were then replaced and the men occupied their foul beds until morning. In the daytime they shared a single tent adjoining this chamber of horrors, to which they returned for twenty successive nights, repeating their unique performance and paying soldierly attention to all its details.

At the end of this period, through some miracle of human endurance, these courageous men staggered out into the delicious fresh air, having survived a horrible ordeal, compared with which the disease itself might have been a blessing. But the experiment was a success in proving that clothing or other articles and excreted material of yellow fever patients do not carry the infection. Medical obsessions sometimes die hard, for history tells us how a century before this ordeal by ordure an ambitious scholar collected similar evidence for a collegiate degree by

swallowing "black vomit" and smearing it, and some "diseased" blood as well, over self-inflicted wounds on the skin, without contracting yellow fever.

Meanwhile, another group of men was settling the question of the importance of infected mosquitoes in the other building, which was well ventilated and lighted and divided into two compartments by a fine-meshed screen partition. The men who occupied one section lay on cots, listening to the weird singing of fifteen yellow fever-infected mosquitoes, interrupted from time to time as the hungry insects settled on their victims to feed, rest, sing, and bite again. There were no mosquitoes in the other section, where the rest of the men were housed. When all the men who were exposed in the mosquito compartment became infected with yellow fever, whereas those in the adjoining section remained well, the rôle of the mosquito in transmitting the disease was established beyond all question.

In the screened laboratory at Camp Lazear, where there was no chance of mistakes getting in with stray mosquitoes, human experimental material was now organized in a business-like way to determine a number of important questions. The saga of Private John Kissinger, who wanted to do something for his country and was the first to offer his services in the cause of science, has been told over and over again. When that young soldier of twenty-three took the salute of Major Walter Reed one morning, it did not appear likely that a private in the ranks would ever live to tell the tale of how an officer had stood before him and said: "Sir, I salute you." Early that day Kissinger calmly watched half a dozen mosquito prisoners in a test tube eat a hearty breakfast on his forearm. Days went by slowly and nothing happened. The mosquitoes were brought back and fed once more, but again without result. The third time was a charm. Six days later he awoke from a sound sleep with a chill that rattled the hospital cot and then he knew the yellow fever at last had "got

him." During the next week, several hundred mosquitoes, to be used in other tests, enjoyed a Roman holiday feasting on his blood. But John Kissinger, a true hero of the Spanish-American War, did live to relate his story and he alone remained to tell it at first hand, while all the others who made history there in Cuba had passed on.

With this noble experiment and others similar to it, three cardinal points of yellow fever transmission by *Aëdes aegypti* were established for all time. First, the mosquito must bite a yellow fever patient during the first three or four days of the disease, if it is to carry the infection. Second, the mosquito then remains harmless until the twelfth day after taking blood from a diseased person. This fact was determined by permitting mosquitoes to bite their willing human victims after varying periods of time had elapsed since feeding upon yellow fever patients. The failure of Doctor Carlos Finlay's attempts to prove his theory of mosquito transmission of yellow fever back in 1881 was due to the fact that he had not waited long enough before allowing his infected insects to bite again. Once infective, however, the mosquito remains so for life, because a number of men developed yellow fever from the bites of insects that had been contaminated as long as two months before the test. Third, once bitten, the patient acquires the disease in not more than six days.

Two things more and the work of the yellow fever investigators was done. The disease should be inoculable if the infective agent is contained in the blood of yellow fever patients. Accordingly a number of volunteers were injected under the skin with blood taken at the height of the disease. Yellow fever resulted. Blood that had been passed through a stone filter was just as effective when inoculated. The cause of the disease was therefore a filterable virus.

From these studies everything concerning the habits and life cycle of *Aëdes aegypti* was learned and from then on the basis of all yellow fever campaigns was merely to

exterminate the insect vector or carrier. Through the years that followed, all control measures applied to this disease were directed first and last against the culpable insect, which was recognized as the most important link in the chain of transmission from man-to-mosquito-to-man.

Beginning in Panama in 1904, with what is probably the most outstanding example of sanitary control ever known in any locality, Doctor Gorgas, then a colonel in the Medical Department of the United States Army, Doctor Henry Carter of the Public Health Service, and their courageous medical staff rid the Isthmus of yellow fever and malaria and made possible the building of the Panama Canal. In the United States, "yellow jack" was driven from its final outpost in 1905, when New Orleans, always most vulnerable to attack, saw its last epidemic and has been free from the disease ever since. Two decades of concentrated effort at exterminating the guilty mosquito now gave assurance that yellow fever, dying in the last ditch, so to speak, had finally been conquered.

Jungle Fever without Benefit of Aëdes Mosquitoes

On all fronts the disease had been routed and appeared to be in full retreat. Until 1925, the world enjoyed a quarter of a century of unprecedented progress that was guided by certain well-established epidemiological laws. If there are no *Aëdes aegypti,* there can be no yellow fever; no epidemics are possible without a supply of new susceptible human material; yellow fever is always a town disease spread by house mosquitoes.

Then came a ten-year period, during which startling discoveries made necessary an about-face in strategy and drove home the lesson that in preventive medicine what is new today can be out of date tomorrow. Yellow fever did not and would not disappear from a small distributing center on the Atlantic seaboard in northeastern Brazil, nor did it obey the rules of epidemiology, laid down at such great cost by ten generations of men. Here the yellow

fever virus outflanked its pursuers and left them completely bottled up, trying to find a way out of the dark jungles. The experts had found a nut they could not crack. The jungle of South America was fighting back.

To the International Health Division of the Rockefeller Foundation fell the task of driving yellow fever from its hiding place in Brazil. The challenge was met with unusual success, chiefly by suppressive measures directed against the mosquito, and within a few years the disease there had virtually disappeared. Then in Rio de Janeiro, which had been free from yellow fever for twenty years, came a surprise outbreak of unknown origin in 1928, followed one year later by similar unexpected flare-ups in Colombia and Venezuela. These two epidemics occurred in regions that were completely isolated and far from any known sources of infection. Although the extreme northern part of Colombia had been visited by yellow fever quite often in the past, a careful search failed to reveal the offending mosquito, presumably the well-known *Aëdes aegypti.* Without a clue as to the nature of these mysterious epidemics, it became evident that if the disease sprang from unrecognized endemic areas, the insects must have some unsuspected methods of tapping these hidden reservoirs of infection.

As a forerunner of later discoveries, which would solve this problem, an interesting observation was made by a group of Spanish investigators, who reported one such epidemic that occurred in Colombia in 1907. They noticed that the yellow fever seen there invariably broke out among laborers engaged in clearing the forests or in persons who had occasion to work near the uncut jungle, but that the disease was not contracted in the neighborhood of the houses. Other investigations made there pointed definitely to a virus carrier other than domestic mosquitoes. The significance of this was not fully realized until years later, when laboratory research caught up and made available newer and better methods of investigation, which proved useful in epidemiological study.

Rockefeller Foundation scientists began to realize that during the golden era of sanitary progress the control of yellow fever had been eminently successful only so far as the disease was then known to exist. Apparently that knowledge had not gone far enough. Recognition of the disease in unsuspected fatal cases was not a simple matter and the extent of previous outbreaks among the living in certain obscure regions could not be measured with any degree of accuracy. Nobody knew how many deaths among natives were due to unrecognized yellow fever, which was generally mistaken for malaria or some other febrile disease; furthermore, it was impossible to arrive at an estimate of the number that had recovered from a previous infection with yellow fever and were therefore immune to it. Without this information, the actual distribution of the disease remained an unknown quantity.

Two important discoveries, coming at an opportune time soon after the major outbreak in Rio de Janeiro, changed the entire complexion of the problem. One was a simple device for collecting and examining liver tissue from the bodies of persons who had died of various febrile diseases. With the new surgical instrument perfected for this purpose, an autopsy was not necessary and the operator, in no danger of infection, could quickly remove a plug of liver as one might withdraw a wedge of watermelon to examine a sample from the inside. Abnormal changes produced in the liver by an attack of yellow fever are characteristic and can be recognized easily under the microscope in thinly sliced chemically dyed preparations made from the specimen.

The second new method was a blood test, by which population groups might be sampled to determine whether or not previous outbreaks of the disease had left behind an immunity. To make this test a mixture of blood and yellow fever virus was injected into white mice. If the blood contained the protective substances which are always developed in persons who have had the disease, the animals survived.

These two laboratory aids gave for the first time a true indication of the prevalence of yellow fever in regions where it had never been suspected. Most startling was the discovery that the disease is never absent from jungle districts where *Aëdes aegypti* mosquitoes, formerly regarded as the only vectors, cannot be found. In an outbreak of jungle yellow fever during 1935 and 1936, there were involved many hundred thousand square miles of Brazilian territory alone, districts which had not been visited by this disease for the preceding twenty-five years. In other South American nations—Colombia, Venezuela, Bolivia, Peru, Paraguay—the story is quite similar, and now we know that the back country of Africa also has its endemic centers. The added threat of these permanent jungle reservoirs of infection has thrown the yellow fever problem right back into the lap of the gods. More pressure than ever will have to be put on the house and town mosquito, Aëdes aegypti, in order to cut off its supply of virus coming from the jungle and to prevent outbreaks in localities where this insect is permitted to flourish.

There is a side to this picture darker than the dense wilderness itself, where jungle yellow fever is being maintained under conditions that are as yet unknown. In this scheme of things man seems to be relegated to an insignificant part, while insect vectors and wild animals are the principal characters. A dozen or more different breeds of wild South American monkeys are already known to be susceptible to inoculated yellow fever virus and there are at least ten known varieties of jungle mosquitoes, including some from Africa, capable of transmitting the disease under natural conditions. It is quite conceivable, therefore, that jungle yellow fever might infect human beings in much the same way as wild rodent plague spreads outwards from endemic areas. Such a possibility is strengthened by the recent discovery of naturally immune monkeys and other wild animals, including opossums, captured in Colombia and Brazil in regions where jungle yellow fever is preva-

lent. How many insect carriers there are, how many undetected animal or insect reservoirs of infection, and how many different hosts other than man himself, remain for the time being dark secrets of the jungle. Any attempt to check the enemy on all fronts would mean declaring a biological war, which even the most optimistic cannot picture beyond a fantastic dream.

Anopheles Assassins

Malaria, like yellow fever, is another insect-borne disease that has been fighting back and driving home a lesson of man's incomplete mastery of environment. Warring against the malaria-carrying mosquitoes has proved much more difficult and less effective than we, in our first flush of victory, had come to believe. More than thirty kinds of mosquitoes—the Greeks had a name for them, *Anopheles*, meaning "hurtful"—transport the blood-destroying parasite. Many of these insects have different habits, which vary in different localities. In certain regions the insect breeds only in wells or cisterns and not, as ordinarily, in swamps or stagnant pools, while elsewhere running streams furnish ideal breeding places. Thus a method of extermination that works beautifully in Palestine can fail completely in Italy, where the same kind of mosquito, instead of breeding in cisterns and frequenting houses, thrives in the woods, and what is more, does not carry malaria at all!

In mosquito control, an added problem is introduced by the human carrier of this disease. Malaria leaves no genuine immunity, each new infection resulting in a carrier state that lasts from one to three years or longer. During this time the insect is provided with an unlimited supply of parasites from these reservoirs among the general population. If the population is stationary, mosquito control has to be thorough for at least such a period, and when migratory, the suppressive measures must go on indefinitely. The difficulties in malaria prevention are multiplied further by possible reservoirs existing among certain

naturally infected animals, although this possibility has been discredited as a result of some experimental studies.

Notwithstanding all the intensive and costly programs designed to eradicate the Anopheles pest, malaria is still with us, not only in this country but all over the world. Within the past few years, it has shown a decided increase in localities where formerly the disease was not widespread, and has broken out with renewed violence in regions which were thought to be rid of this "Captain of the Men of Death."

By a strange coincidence, in 1934, when malaria started on the upgrade in the United States and became more prevalent than at any time during the two preceding decades, an appalling epidemic swept through the island of Ceylon, India, taking more than sixty-six thousand lives within a period of five months. Nearly half this number died during November, December, and January, and in January alone, out of more than seven thousand deaths, almost eight out of every ten were in children under the age of fourteen years. In a country like India, where malaria on a large scale is taken for granted, this epidemic of extraordinary virulence might pass unnoticed but for the unusual circumstances attending it. The outbreak occurred in what was reputed to be the most healthful part of Ceylon, with a population relatively free from malarial infection, and followed a period of unprecedented drought, broken by a few heavy rains and succeeded by another dry spell. As a result, innumerable breeding places for the Anopheles mosquito were created along the banks of streams and in the dried-up river-beds, where shallow pools of water had become pocketed. Under the normal conditions of abundant annual rainfall peculiar to this locality, the catastrophe could not have happened. It shows clearly how unforeseen natural causes may at any time defeat the aim of preventive measures and emphasizes again the important relation between the environment and the infecting parasite.

As a killing and disabling disease, malaria is probably second to none. More than three and one-half million people in the world die every year because of it. Despite some splendid accomplishments, there are wide gaps in our knowledge of preventing this disease. Its prevalence in man has not been lessened by the use of quinine for nearly a century, nor has the drug made human blood less palatable to the mosquito, which continues to be infected by its hosts and infects new ones in turn. Although quinine serves a useful purpose in cutting short a malarial attack, it is not a preventive in any true sense. The vast sums of money spent every year for this drug by certain European nations alone would go a long way towards the building of even more of their battleships. During World War II, the synthetic drug "atabrine" was widely used to suppress malarial parasites in the blood. Like quinine, however, it fell far short of being effective as a prophylactic. Two new synthetic agents now being introduced are said to be superior to any other—Chlorguanide (recently adopted American name for the British "Paludrine"), for those who are afflicted with periodic attacks of malaria, and an unnamed drug identified as SN 13,274, which, used with quinine, has cured permanently 95 per cent of all relapsing or "repeater" cases of malaria.

It is nearly fifty years since Professor Battista Grassi and Doctor Ronald Ross engaged in literary fisticuffs to settle which of these two gentlemen was the first to discover the life history of malarial parasites. There is glory enough for both in that great discovery, but today we realize our fight has just begun, while theirs is finished.

Environment versus Heredity and Constitution

In a pessimistic mood other weaknesses and failures in our vaunted conquest of germ diseases might be recounted. What of pneumonia, influenza, tuberculosis, infantile paralysis, sleeping sickness, rabies, scarlet fever, measles, whooping cough, chickenpox, and heart disease caused by

rheumatic fever, the deadliest and most crippling ailment of children of school age? As the guns of modern bacteriological science are trained upon microbes, it seems to be only a question of finding the proper range and hammering away at weak points in their defence until sooner or later they yield. Unfortunately, all the various microbes implicated in certain diseases have not yet been discovered. Finding the causative germ, moreover, does not always provide the means for preventing infection or curing a disease like tuberculosis, for example, nor does the inability to discover and see the cause, as in smallpox, invariably result in failure to control it.

Our success in controlling these two diseases is a study in contrasts. Vaccination, wherever practised, has made smallpox a rare disease, while tuberculosis is growing less and less prevalent with the years, following the application of simple epidemiological principles and newly discovered methods of building up natural bodily defences. So many things have been responsible for the astonishing decline in the death rate from tuberculosis that it is impossible even to guess at their relative importance. Knowledge of the ways in which the disease is spread through human contacts and by milk from infected cows has provided the only effective means of attacking the microbe directly and limiting its activity. Yet such preventive measures, based on these facts alone, could not have accomplished the remarkable results witnessed during this period. Besides the artificial control of environment, hereditary and constitutional factors were quietly at work, unknown to those who were interested in mortality statistics to the exclusion of everything else as a barometer of progress in tuberculosis campaigns.

Heredity and constitution as used here are not to be understood in the erroneous sense that tuberculosis can be handed down from parent to offspring, much as the color of eyes or hair is inherited. For a long time after the discovery by Koch of the tubercle germ and its mechanism of

infection, this inaccurate notion was still accepted as correct. The unfortunate result was a belated appreciation of the fact that not tuberculosis itself but *susceptibility* to tuberculosis is tied up in some peculiar way with heredity and constitution. Professor Raymond Pearl of Johns Hopkins University told us that there is often, in the father's kinship, a tendency towards constitutional weakness of the respiratory organs, showing itself in a lack of resistance to pulmonary tuberculosis in early adult life. On the mother's side, also, there sometimes occurs a lack of resistance during infancy and childhood to infections such as bronchitis and broncho-pneumonia. Clearly, then, the offspring of such parents will be extremely susceptible to any kind of respiratory disease. Only in this or a similar sense, perhaps, is it correct to speak of "hereditary tendency" or "predisposition" to tuberculosis. The human constitution, which is the sum total of all that is inherited, is definitely influenced by the environment and determines how successfully an individual will react to the stress of his surroundings, which may break down his resistance to disease. On the strength of these observations, young women contemplating marriage might do well, as they are studying the family tree, to have an X-ray specialist look over the bronchial tree.

A study of the inheritance factor in tuberculosis from a different angle has given us another side of the picture. Two fairly large and representative groups of persons, one without and the other with tuberculosis, have been carefully checked for the presence or absence of the disease in one or both parents, with the following results. Among the non-tuberculous, a history of parental infection was slightly more than twice as common as in the group of tuberculous patients. Reversing the order of this inquiry, the amount of tuberculosis was then determined in a large number of subjects whose parents had no evidence of the disease. In such a group, nearly sixty per cent were found infected as compared with half that number in a second

group, whose parents were tuberculous. Thus the frequency of the disease seemed to be inversely related to the amount of parental infection. Furthermore, there was evidence that patients with tuberculous parents showed a greater tendency to overcome the disease successfully than members of families attacked by it for the first time.

A question might be raised here as to whether or not this evidence excludes any other factors which, apart from heredity and constitution, can influence resistance to infection. On the basis of these observations, one cannot rule out the probability that exposure to disease in the family might have accounted for the difference in susceptibility shown by the various groups of persons. Conceivably, while some acquired immunity from the repeated inoculation of small doses of germs in an infected household, others lacked this opportunity. For the same reasons, patients in families previously tuberculized might be expected to make a better recovery than those who did not have an equal chance of becoming naturally "vaccinated."

It is impossible to guess in which direction further advances will be made in the control of tuberculosis, which up to now has been a triumph based on the application of hygienic and sanitary measures and man's cooperation with natural forces. Perhaps the new conquest will be along strict epidemiological lines in recognition of the fact that this disease is contagious and as such must be controlled by methods found effective in other communicable diseases. For the time being, we are witnessing an almost universal decline of this disease in communities where once it was epidemic. The improvement is now believed to be due, in part at least, to an immunity that is acquired by racial stocks exposed to city life and tubercle infections.

The long-cherished hope of controlling tuberculosis by means of vaccination or inoculation has not yet been realized. Expectations based upon brilliant results in the experimental animal are seldom if ever fulfilled in the human, and then only after many years of carefully controlled

studies. It has taken a full quarter of a century to establish the safety (insofar as this is theoretically possible) of a tuberculosis vaccine, which more than any other has had the benefit of continuous testing throughout the world. This vaccine, first described by the French physicians who introduced it, is widely known as BCG, or the bacillus of Calmette and Guérin. It consists of *living* tubercle bacilli, originally virulent for cattle, but now harmless for calves, rabbits, and guinea pigs, having lost the capacity to produce in these naturally susceptible animals a type of tuberculosis that is uniformly fatal. This change has been brought about by a method of attenuation or weakening, which results from forcing the organism to grow under prolonged adverse conditions. In the case of BCG it was accomplished by cultivating the strain for successive generations in food medium containing potato, glycerine, and ox gall.

Ostensibly, since we are dealing with a phase of microbial transformation artificially induced, there is always the possibility of a reversal to former virulence. Such a hazard, however remote, would immediately condemn and ban the use of the vaccine. Yet it is stated on excellent authority that more than five million children have been inoculated with BCG since 1922 without a bona fide example of the organism having become virulent. So much, then, for the apparent harmlessness of the vaccine.

The important problem now is to evaluate the results of studies reported from different parts of this country and elsewhere over a period of many years. Nearly all the reports have been favorable but only a few of these are controlled adequately, and in the main are not conclusive. Because there are so many variable factors that can change the course of the disease, statistics are often misleading. As a case in point, the effectiveness of BCG vaccination is shown by comparing figures for the death rates from tuberculosis in the unvaccinated and vaccinated groups. The advantage appears to be five to one in favor of vaccination,

unquestionably a significant result, but there is a flaw in the method used here of dividing the subjects for test into two equal groups. Unintentionally, children might be selected for vaccination whose parents were more coöperative and therefore less likely to disregard important details which affect the outcome of the experiment. That such was the case is shown by the results obtained with a different method of selection. In a second comparable group of approximately one thousand children, when routine vaccinations were performed on alternate subjects, the figures for tuberculosis mortality in the vaccinated and unvaccinated groups were found to be identical. Obviously, this conclusion is in complete disagreement with the first.

Many other questions remain to be answered before the efficacy of BCG vaccine can be determined. Shall all children be vaccinated or none at all? Since the immunity produced by vaccination is by no means complete, it is argued that the current public health preventive measures are still the best methods of control. In a controversial matter such as this, an attempt has been made to steer a middle course. On the basis that children with a negative tuberculin reaction are uniformly susceptible to tuberculosis, it would seem wise to limit the use of BCG vaccine to this group. Those with a positive skin test, having presumably been exposed to the disease and already become partially immunized, do not need the vaccine. On the other hand, there is objection to its use for the very reason that vaccination produces a positive tuberculin test, thereby making ineffective one of our most valuable diagnostic aids. At the same time, disturbing irregularities in the development of these positive reactions are not yet understood. They raise additional questions as to the best method of vaccination, the potency of the vaccine, and its method of preparation.

After thirty years of experience with BCG it is concluded that much research is still needed before the vaccine can be accepted for general use. If this seems to be snail-

like progress in view of unremitting labor, it should be remembered that experimental results of a long-range investigation must be observed in the lifetime of the experimental subject.

Recovery from bacterial diseases in man and animal is not the only way in which active resistance is developed. Microbes that brush us lightly in passing by also have an immunizing effect upon the system. Persons who "never seem to catch any disease" probably owe this peculiarity in their make-up to an inherited capacity for responding very quickly to unnoticeable transitory infections. An extraordinary number of city dwellers are thus "seeded" with tubercle bacilli at some time during life without showing the slightest signs of illness. Completely healed "spots" of tuberculosis can be found in the lungs of over ninety in a hundred persons who have died from other causes, a discovery which was made nearly forty years ago. Apparently, then, the ability to withstand this infection, as well as infantile paralysis and many others, depends upon a strongly developed tolerance acquired through exposure to microbes, although it is probable that the burden of defence does not rest on such a protective mechanism alone. This device, working hand in hand with an inherited quality that is characteristic of races or breeds, determines what is commonly spoken of as resistance to disease and protects the individual.

There is reliable experimental evidence bearing upon this question. By selective breeding of mouse populations reared and maintained for twenty years at the Rockefeller Institute in carefully controlled surroundings, two distinct strains of progeny, one resistant and the other highly susceptible, have been segregated. The differences in capacity to react to harmful agents are "bred in the bone," since the tolerance or susceptibility towards germ diseases inoculated into these animals runs parallel with their responses to a deadly poison, such as bichloride of mercury, showing that the nature of this inherited immunity is general rather than

specific. An interesting sidelight on these experiments is the effect which diet may have in raising or lowering native resistance. In the same breed of mice, for example, an adequate bread and milk diet lessens their tolerance towards various microbial infections, while the addition of butter fat and cod-liver oil to this menu makes susceptible animals resistant.

Aside from its theoretical interest, this problem is important from a practical standpoint, because all our efforts to control microbial diseases must take into account certain natural powers, which we seek to stimulate and promote by various methods of artificial immunization. Whether heredity or environment predominates does not concern us so much as the fact that there is a connection between the two. Inheritance and evolution will determine the normal capacity to react to a proper stimulus, by the manufacture of protective antibodies in blood or tissues. The environment, on the other hand, will furnish this stimulus through exposure to natural infection or by means of artificial devices such as vaccination.

Germ Tolerance Grows with Age?

Explanations of natural or acquired immunity raise many more questions than they settle. We do not know how often these stimuli are applied from without, nor can we be sure that this is always necessary in view of the fact that resistance to infection seems to increase with age in circumstances which definitely rule out exposure to the microbes. It is conceivable that gradual development of a protective mechanism for manufacturing antibodies as one grows older may be just as important as the chance of receiving a specific environmental stimulus. In the case of diphtheria, the information on this point is particularly illuminating. The usual insusceptibility to this disease in adults is readily shown by a negative skin reaction (*absence of reddening*) following application of the well-known Schick test, named for its discoverer, the Hungarian bac-

teriologist, Doctor Bela Schick. This test measures the content of antitoxin in the blood sufficient to overcome the irritating action (*a reddening, if test is positive*) of the minute amount of diphtheria toxin which is injected into the skin. The number of Schick-negative individuals shows a definite increase with age up to middle life at least and is proportionately greater in cities than in rural districts, indicating some causal relation between the chances of infection and a progressive development of resistance. On the basis of extensive studies in persons who have never had the disease, the presence of antitoxin in their blood has been interpreted, therefore, as evidence of an immunity following an unrecognized infection with diphtheria or exposure to the microbe.

If this generally accepted explanation is basically correct, it should not apply to those communities where diphtheria has been rare and virtually unknown for generations. Nevertheless, we find that among the Eskimos and Javanese peoples, adults react negatively to the Schick test, a fact which presupposes infection. This assumption, however, cannot be sustained, since the native children show positive tests, a clear indication of their susceptibility to the disease, yet without any evidence of its recognized effect upon those who are most vulnerable. Among the American Indians a similar situation exists with respect to scarlet fever. These facts suggest that an immunity may be acquired without the need of being exposed to the disease in question. Immunity in warm climates has indeed been shown to result from actual infection under conditions that favor a higher resistance.

It is, at all events, difficult to harmonize other conflicting evidence of this nature found in the animal kingdom. For example, in the healthy fowl a high resistance to infection with pneumonia germs is associated with antibodies in the blood capable of reacting with them. Why, one might ask, is it necessary to assume that all fowls become immunized as a result of germ-specific stimuli from their

environment? Similarly, it would be absurd to admit that the dog, cat, and sheep are naturally resistant to pneumonia because they are often exposed to this disease, while man is susceptible because he is only rarely exposed to it.

Many years ago Doctors Roux and Yersin and Behring separated a subtle poison from diphtheria microbes, and learned how to prepare the antidote to this powerful toxin that strangled babies. Antitoxin, a miracle of all miracles, was the result. Today we can count it as the one outstanding triumph of preventive and curative medicine. Yet nobody knows what a toxin is, although it can be identified by its action in the body. Who can say more than that about susceptibility, resistance, immunity—all native endowments of the human and animal organism? For scientists of the future there is a world of unexplored territory to probe in healthy (normal) individuals. These alone hold the secret of natural defences, from which we may hope to learn more about microbial warfare.

The problems that still lie ahead of us are indeed many and at the end of the short journey we have just made is a wonderland that grows "curiouser and curiouser" as new discoveries find us busily occupied with the task of fitting them into strange surroundings. We venture timidly into an orderly kingdom to find quite often a topsyturvydom instead. The language, too, is strange, as we hear words spoken with different meanings for the same thing or words with the same meaning for different things. Paradox, a peculiar animal of inconsistency, pops out from somewhere to remind us that what is seemingly absurd may really be well founded on truth.

And now we are again at the Gate of Resistance opened by Louis Pasteur when he started out on his first journey. After many years the words scratched on the gate posts have almost been obliterated. By trying hard, one can still decipher what he wrote: "It is within the power of man to cause all parasitic diseases to disappear from the world."

GLOSSARY

aerobic—descriptive term applied to microbes that require air for their growth.

agglutination—the collection into clumps of microbial cells in the presence of a serum obtained from animals or human beings that may be immune to attack by these microbes.

allergy—hypersensitiveness to certain substances present in food or produced in the human or animal body as a result of bacterial infection. "Hives" and food rashes, such as might follow a meal of strawberries or shellfish, illustrate one type of allergy; the reaction following a skin test with tuberculin in persons who are infected with tubercle bacilli is the best example of allergy to products of bacterial activity. This appears to be a protective response to foreign substances capable of harming the body tissues. As the body builds up a defence against an invader—microbe or undesirable protein materials—certain cells are "trained" to distinguish between friend and foe. The "guardian" cells become so sensitive to the presence of an enemy that they cause a violent commotion and a general disturbance which is a form of preparedness. "Allergy" seems to be very closely related to immunity, although the hypersensitive condition might exist before an infection as well as after without causing resistance to the offending microbe.

anerobic—growing without the presence of oxygen (see aerobic).

animalcule—a minute or microscopic animal organism.

antibiotic—antibacterial substance of biological origin, such as penicillin, streptomycin, tyrothricin, streptothricin, actinomycin, etc., produced by molds, fungi, or bacteria.

antibody—a substance in the blood and tissue juices of animals made immune by inoculation, and exerting a specific antagonistic effect on the agent under the influence of which the protective substance was produced.

antidote—a remedy for counteracting a poison.

antigen—a bacterial or chemical substance that incites the formation of antibodies following inoculation with or natural exposure to the agent.

antisepsis—prevention of the growth and multiplication of bacteria without necessarily destroying them completely.

antiserum—the serum of persons or animals with protective substances against microbes or their products. In this general sense a serum may be antibacterial or antitoxic. Strictly speaking, antiserum means the serum of an animal which has been injected with that

of another animal and as a result the serum of the first animal, when combined with that of the second in a test tube, will produce a precipitate or cloudiness.

antitoxin—any defensive protein, either present normally or developed in the body as a result of bacterial action, and having the capacity to neutralize toxins or bacterial poisons.

asepsis—freedom from infection by exclusion of germs, as in surgical operations.

attenuation—weakening of the infective power of microbes by repeated inoculation into animal bodies or in artificial food substances that are not suited to the growth of the particular germ.

autoclave—an apparatus for sterilization by steam under pressure which can be regulated automatically.

avirulent—incapable of causing disease, having lost its invasive power.

bacillus—a breed of microscopic rod-like organisms belonging to the plant kingdom.

bactericidal—capable of destroying bacteria.

bacteriophage—a "microbe eater," an ultramicroscopic body that preys upon and kills germs.

bacteriostasis—preventing or arresting the growth and multiplication of bacteria by means of antiseptics, antibiotics, drugs, etc.

biochemistry—the chemistry that is concerned with biological or physical activities of living things.

biotype—individual or organism with hereditary characteristics which breed true to type.

buboes—inflammatory swelling of lymphatic glands, particularly in the armpit or groin, characteristic of the disease known as bubonic plague.

carrier—a person or animal that harbors disease-producing germs somewhere in the body without suffering any ill effects, but is capable of transmitting the infection to others.

centrifuge—a machine for separating the more solid constituents of a fluid by rapid whirling, as in a cream separator.

coccus—a microbe resembling a berry or sphere under the microscope.

corpuscle—any small mass or body, such as blood corpuscles or the red cells that carry oxygen and the white cells or phagocytes, the scavengers that destroy germs; the term "corpuscle" was applied by early investigators to the cause of silkworm disease.

culture medium—a fluid or solid food prepared in the laboratory for growing and propagating microbes.

dissociation—the splitting off from the parent type of unusual microbial forms that can be distinguished by peculiar growth characteristics.

endemic—pertaining to a disease which is neither epidemic nor sporadic, but exists in a certain locality all the time.

enzyme—a chemical substance formed within the body and bringing about a chemical action, during which the substance itself is not permanently changed.

epidemic—pertaining to a disease attacking many people in the same locality at the same time.

epidemiologist—a person who studies and attempts to control and prevent the spread of communicable diseases.

epizootic—occurring as an epidemic disease among animals.

fermentation—a process like that caused by leaven in dough, with production of carbon dioxide gas and alcohol; the breaking down of sugars by bacteria and yeasts.

flagella—organs of locomotion resembling whiplashes attached to microbial cells.

fungus—a class of vegetable organisms of a low order of development, including mushrooms, toadstools, moulds, etc.

genetics—study of the origin of characteristics of an individual; of factors which can account for the resemblances and differences of micro-organisms related by descent.

haptene—a term invented by Doctor Karl Landsteiner to describe his discovery of that part of an antigen which gives it its specific character.

host—man, animal or plant that harbors a parasite.

hypersusceptibility—see allergy.

immunity—the power which a living organism possesses to resist and overcome infection.

immunization—the process of rendering a subject immune.

immunology—the science or study of immunity.

inclusion bodies—microscopic abnormal changes produced within the cells of living bodies by the presence of certain animal and plant viruses.

incubation—(1) the time elapsing between the inoculation of an infectious disease and the signs by which it is recognized; (2) the keeping of a culture of bacteria in an incubator to favor growth.

infusion—the steeping of a substance in a liquid for extracting its soluble portions or the product of such a steeping process.

inoculation—the introduction of a microbe into the body by natural or artificial means.

isolation—the separation of persons having a contagious disease from those who are well.

isomer—one of a number of substances having the same chemical materials in the same proportions but differing in the manner in which these are grouped to form a molecule or small unit of the substance.

lymph—a transparent slightly yellow liquid that fills the lymphatic vessels draining the body tissues and receiving fresh oxygen from the blood in return for accumulated waste materials.

metabolism—the sum of all the physical and chemical processes by which different foodstuffs are transformed and retained in the building up of living tissues.

miasm or miasma—harmful or unwholesome exhalation, formerly believed to be responsible for communicable diseases.

micrococcus—a minute variety of berry-like or coccus microbes.
micro-organism—any minute plant or animal.
molecule—a very small mass of matter; a grouping of the atoms composing it.
morphology—the science of the form and orderly structure of living things.
motility—the ability to move without external aid.
mutation—change resulting in the production of new species.
myxoma—a tumor or growing mass of tissue made up of a mucous or gummy substance.
pandemic—an epidemic of world-wide proportions.
papilloma—a skin tumor, including warts, cutaneous horns, etc., having a nipple-like form of growth.
parasite—a plant or animal which lives upon or within a living organism.
pasteurization—the arrest or checking of fermentation or bacterial growth by heating.
pathogenic—giving origin to disease.
pathology—that branch of medicine which treats of the essential nature of disease, especially of the changes caused by disease in tissues and organs of the body.
phagocyte—a cell that "eats" and destroys micro-organisms or harmful foreign bodies by enveloping and absorbing them.
plasma—the fluid portion of the blood containing the standard foods of the body tissues.
polarization—modification of light rays by means of a special type of prism which causes them to pass through it in one plane instead of being deflected or bent aside in the ordinary manner.
precipitin—a substance formed in the blood serum of animals following treatment with bacterial cultures, blood-serum, or proteins and capable of producing precipitation of the bacteria or proteins of the variety injected.
prism—a transparent crystalline mineral which deflects rays of light passing through the surfaces inclined at a sharp angle to each other.
prophylaxis—the prevention of disease.
protein—any one of a group of compounds widely distributed in the animal and vegetable kingdoms and forming the characteristic materials composing the tissues and fluids of the animal body.
protozoa—a class of single-celled animal organisms, forming the lowest division of the animal kingdom.
putrefaction—the decomposition of animal or vegetable matter brought about largely by micro-organisms.
quarantine—to detain or isolate on account of suspected contagion.
Rickettsia—a group of minute organisms (named in honor of Doctor Ricketts, their discoverer) found in typhus fever, Rocky Mountain spotted fever, and trench fever, tsutsugamushi fever, rickettsialpox, etc.
saprophyte—an organism living on dead or decaying organic matter.

sarcoma—a tumor made up of undeveloped tissue capable of extraordinarily rapid growth.

sensitization—a state of increased susceptibility to chemical or bacterial substances, causing a prompt reaction to such products.

septic—produced by or due to putrefaction.

serum—the clear portion of any animal liquid separated from its more solid blood corpuscles and mass of material produced by coagulation or clotting; specifically the blood-serum from animals or persons inoculated with bacteria or their products in order to develop immunity thereto.

specificity—the property of an immune serum acquired under the influence of bacterial or other agents whereby the serum reacts in a characteristic protective manner if combined in a test tube or in the animal body with these agents; pertaining to the portion of microbial cells or serums in which reside characteristic properties that identify the nature of the particular microbe or serum.

spirochete—a type of flexible organism (causing syphilis or relapsing fever) in the form of a finely coiled spring.

sporadic—occurring here and there; not epidemic.

spore—resting stage of certain bacteria, conferring upon them unusual powers of resistance to destructive agents.

sport—an animal or plant deviating suddenly or strikingly from the normal type.

staphylococci—cocci or berry-like organisms occurring in clusters resembling a bunch of grapes.

streptococci—cocci or berry-like organisms occurring in chains like a necklace.

tartar or tartaric acid—pink crust deposited on cask from completely fermented wine.

tetracocci—cocci or berry-like organisms occurring in groups of four.

toxin—any poisonous substance produced by bacterial action and generally counteracted by means of artificially prepared antitoxic serums.

trypanosome—a spindle-shaped parasite found in the blood of man or animals infected with trypanosomiasis or African sleeping sickness; a parasite that is characterized by a delicate wavy membrane surrounding its body and ending in a freely moving whip-like flagellum.

tsetse—a South African fly transmitting fatal sleeping sickness to horses, cattle, and man.

tuberculin—a material prepared from substances produced by the tubercle bacillus during its growth in artificial culture medium and used in detecting tuberculosis by means of a skin test.

ultramicroscopic—too small to be seen with an ordinary microscope.

ultracentrifuge—a glorified cream separator capable of forming a sediment containing ultramicroscopic particles.

vaccine—any disease-producing virus or microbe modified by artificial methods so as to make it a safe preventative.

vaccination—inoculation with a vaccine to procure immunity from any disease; applied particularly to smallpox prevention.

vector—a carrier, especially the animal or insect host in which the development of disease germs often occurs before transfer from one human host to another.

viable—capable of maintaining life.

vibrio—a microbe in the form of short curved rods or shaped like a comma.

virulence—extraordinary invasive power of certain disease-producing microbes.

virus—name given to a general class of microbes that cannot be seen under ordinary powers of magnification or be cultivated in the laboratory by methods suited to the growth of most known bacteria.

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