IMMUNITY

PRINCIPLES AND APPLICATION IN MEDICINE AND PUBLIC HEALTH

An Exposition of the Biological Phenomena of Infection and Recovery of the Animal Body from Infectious Disease, with Consideration of the Application of the Principles of Immunity to Diagnosis, Treatment, and Prophylaxis and Their Usefulness in the Control of Epidemics

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PREFACE TO THE FIFTH EDITION

Through the four earlier editions of this book we have successively modified and expanded the presentation of immunology as this was necessitated by the extraordinary velocity of discovery. There was not, however, in preceding revisions any attempt to alter the original plan or purposes of the book, which was conceived as a critical treatise intended chiefly for medical students and laboratory workers.

The present volume, while retaining much of the character of its predecessors, represents an endeavor to meet the need for increased correlation between the principles revealed in laboratories and their applications to the problems of the clinic and of public health.

When the First Edition of this book was written, immunology was still regarded by the medical profession at large as a highly specialized branch of biology with the fundamentals of which the practitioner had little concern, though some of its methods and results had become a part of his professional equipment. This point of view has completely changed in the intervening years. The scientific training of medical students, and consequently of the profession as a whole, has been extended and intensified to such a degree that the barriers which until very recently separated the experimental laboratories from the clinics are gradually disappearing.

The new type of physician insists on understanding the principles of the procedures which are developed by the specialist and of acquiring critical judgment for the evaluation of such methods in his own work.

Out of this new impulse has grown a spirit of co-operation. As never before, the disciplines infiltrate and fertilize each other. The most notable advances of recent years in this subject have resulted from intimate collaboration of the bacteriologist and the chemist on the one hand, of the bacteriologist and the clinician on the other. Medical progress of the future will depend largely on the intensification of such co-operative effort, made possible by improved fundamental education and the consequently increased mutual understanding of principles and objectives by collaborating specialists.

The present completely revised edition is, to some extent, an acknowledgment of this situation. In the section on principles, we have eliminated much of the older material which has become of purely historical value and have added the newer knowledge accumulated

during an extraordinarily fruitful period of eight years. The fundamental nature of many of the discoveries, especially in the chemical definition of antigens and antibodies and the progress in the field of hypersensitiveness, has necessitated complete reorganization of the text. In Section II, on the application of immunological knowledge to medicine and public health, it has been necessary to expand extensively, to re-write most of the chapters completely, and to add several new ones.

If the new volume fulfills its intended purpose, the laboratory worker, turning to the practical sections, may derive stimulus from the recognition that many of the principles at first conceived without obvious possibility of applied value have ultimately served to enrich diagnosis, prevention or therapy; and the practitioner, referring to Section II for details of specific procedure, can turn back to the chapters on principles and there find discussion which will aid him more fully to understand the observations and reasoning upon which many of the methods used in his practice are based.

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PREFACE TO THE FIRST EDITION

Infectious disease, biologically considered, is the reaction which takes place between invading microorganisms and their products on the one hand, and the cells and fluids of the animal's body on the other. The disease is the product of two variable factors, each of them to a certain extent amenable to analysis, and it is self-evident that no true understanding of this branch of medicine is possible without a knowledge of the biological principles which laboratory study has revealed.

For the purpose of helping to render such knowledge easily accessible this book was written. While it is hoped that it may prove useful to the practitioner and laboratory worker, it is intended primarily for the undergraduate medical student. To many it will seem that the subject in general and our method of treatment especially are too technical and difficult for this purpose. Our own experience contradicts this. During the past three years the writer has had the opportunity to deliver lectures and to give laboratory courses on this subject to medical students of 2d-, 3d-, and 4th-year classes at the Stanford and Columbia universities. It has been a pleasant experience to find the medical student eager for the opportunity to obtain this knowledge, and, under the present increased requirements for preliminary training at our best schools, fully capable of assimilating it. It is not a good plan to attempt too extensively to simplify material that, in its close analysis, presents complex phenomena and intricate reasoning. For this reason no attempt has been made to write an A B C of immunity as a quick road to comprehension. No true insight into any branch of medicine or, for that matter, into any other science, can be attained without a certain amount of labor; however, the concepts of this subject are, indeed, relatively simple after the first principles have been mastered, and the writer has attempted, therefore, at the risk of seeming pedantic in places, to treat the subject critically, separating strictly those data which may be accepted as fact from those in which legitimate differences of opinion prevail.

As far as was feasible every chapter has been written as a separate unit. This has necessitated occasional repetition, but, it is hoped, will add considerable to clearness of presentation in each individual subject. Theories have been discussed with as little prejudice as the possession of a personal opinion in many cases has permitted.

The chapter on Colloids was written especially for the book by Prof. Stewart W. Young of Stanford University. Since so many analogies between serum reactions and those taking place between colloidal substances generally have been observed, it has seemed best to devote this chapter entirely to the elucidation of the principles governing colloidal reactions, so that its contents may be utilized as explanatory of the many allusions made to colloids in the rest of the text.

All available sources of information have been freely used. In the large majority of cases we have had access to the original papers and monographs. However, we acknowledge much aid from careful reading of the admirable summaries, written by acknowledged authorities, in the works edited by Kolle and Wassermann, and by Kraus and Levaditi. Similar acknowledgment is made to equally important sources in Weichhardt's Jahresbericht, the Bulletins of the Pasteur Institute, and in such textbooks as those of Paul Theo. Muller, Emery, Adami, Gideon Wells, Marx, Dieudonné, and others. It is needless to acknowledge the use of such classics as that of Metchnikoff or of the many critical writings of Bordet and of Ehrlich — masters who have helped to shape the thoughts of all men working in this field.

The writer takes pleasure in acknowledging many helpful suggestions from his associates, Drs. Hopkins and Ottenberg, and much aid, in the verification of references, from Mr. Walter Bliss, Fellow in the Department of Bacteriology.

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CHAPTER I

INFECTION AND VIRULENCE

THE way to our understanding of infectious disease was first clearly indicated by the studies of Pasteur on fermentation. In fulfillment of the prophecy of Robert Boyle made in the seventeenth century that the problem of infectious disease would be solved by him who elucidated the nature of fermentation, the investigations begun by Cagniard-Latour and by Schwann and carried to a brilliant culmination by Pasteur revealed the living and specific nature of the various microorganisms which cause the several forms of fermentation and especially of putrefaction, and thus led by analogy first to logical speculation, then to experimental proof of the etiological relationship between certain of the minute forms of life and the communicable diseases. The study of putrefaction and of fermentation nevertheless presented a problem far less complex than that of the infection of living tissues with bacteria. For, given any organic material containing suitable nutritive constituents under favorable environmental conditions of moisture and temperature, spontaneously or experimentally inoculated with bacteria or fungi of a suitable species, the phenomena which ensue are essentially those related to the metabolism of the microorganism, in which an active part is played only by the latter, while the organic substrate represents the source whence the chemical compounds and energy essential for growth and reproduction are derived. In the case of infectious disease, however, we have learned that the process is much more involved because here two living entities — the infectious agent and the host — interact with each other. This is made clear from the fact that throughout nature bacteria are abundant, and the environment of man and animals, the outer integuments of skin and hair, the mucous membranes of the conjunctivae, the intestinal respiratory tracts, are constantly inhabited by a thriving bacterial flora. Many of these consist of the ordinarily harmless bacteria (saprophytes), but frequent contacts with many varieties of microorganisms termed "pathogenic" or disease-producing take place without production of manifest disease. Thus perfectly normal individuals may, on occasion, harbor organisms of the latter variety over varying periods of time. The accomplishment of a clinically recognizable infection, therefore, is not determined merely by

the fact that a microorganism finds lodgment in or upon the body of a susceptible individual, but it is further necessary that the invading germ shall be capable of maintaining itself, multiplying and functioning within the living and actively resisting body of the host. We have, thus, a battle of two opposed forces, both intricate in nature, the result of which is infectious disease. It is the initial skirmish between the two which determines whether or not a foothold shall be gained upon the body of the subject, or host, and an infection thus established, and it is the balance between them which decides the eventual outcome of recovery or death. The systematic analysis of these forces in their variable conditions, and of the laws which govern them, constitutes the science of immunity. The facts that have been revealed through immunological researches are as fundamental to the pathology of infectious disease and as essential to the clinical understanding of these maladies as is the knowledge of the mechanism of the circulation, the chemistry of metabolism, or the structural changes of the tissues to the comprehension of other pathological states.

In this and the two subsequent chapters, the principal attributes which have been found to be related to the capacity of the organism itself to cause infection, *i.e.*, its virulence, will be discussed.

Two aspects of the problem of virulence as it relates to the parasite have received by far the greatest degree of study, and these in the last analysis depend upon the chemistry of the bacterial body and its products. The most significant results of these studies which represent immunological fundamentals will be described in the chapters on Antigens and Bacterial Poisons. Nevertheless, variations in virulence occur between strains of a given bacterial species or between the generations of a single strain which cannot as yet always be accounted for satisfactorily on the basis of changes in the chemical or antigenic structure of the parasite or the poisons it may elaborate. Our immediate purpose will therefore be to present a general survey of the problem of virulence and infection and to record what is known concerning these other factors which may be involved in rendering a particular bacterium capable of entering a foreign living body, increasing there, and by its presence calling forth on the part of the host reactions which tend to dislodge or destroy it, and which we recognize as the indications of disease.

The Classification of Organisms According to Their Pathogenic Capacities. One of the fundamental facts, immediately apparent on considering the problems of infection, is the phenomenon that among the innumerable varieties of bacteria and protozoa present in nature there is a very limited group which is capable of becoming parasitic upon the bodies of higher animals, and among these a still smaller proportion which is capable of being "pathogenic" or causing disease.

It is reasonable to suppose that all microorganisms were originally in the condition which we designate by the term "saprophytic." As we have stated, by this term we imply that these germs maintain themselves only upon dead organic matter and do not thrive in or upon the living animal tissues. The class of saprophytes is widely distributed and constitutes, of course, the most important group of bacteria in nature, since upon the activities of these germs depends the unlocking of nitrogen and carbon from the organic complexes in the dead bodies and waste products of animals and plants. Such bacteria if strictly saprophytic, that is, entirely unable to maintain themselves upon living tissues, have on the whole little pathogenicity. Nevertheless, there are cases in which strict saprophytes may cause disease by lodging upon and growing in animal tissues which have been killed by other causes, so-called necrotic areas; and from these through the blood and lymph channels, products of putrefaction or bacterial poisons may be absorbed. While, as a rule, the disease following the invasion of necrotic tissue — such as gangrenous areas, old unhealed sinuses, etc. — may be caused by a large variety of saprophytic bacteria, there are a few very important pathogenic bacteria which are, strictly speaking, saprophytes. Thus the form of meat poisoning caused by the *Bacillus botulinus* is due entirely to the poison formed by this bacillus outside of the body within the substance of the dead foodstuff, and disease ensues as the result of subsequent ingestion of this poison with the food. In the same way the tetanus bacillus and, less strictly speaking, the diphtheria bacillus, at least in its ordinary mode of attack, are closer to the class of saprophytes than to that of the parasites, since neither of these bacteria, under usual circumstances, invades the tissues beyond the point of initial lodgment. The tetanus bacillus, moreover, is not usually capable of maintaining itself and multiplying even at the point of initial lodgment unless the tissues have been injured by trauma, the presence of foreign bodies or pyogenic infection. The condition which ensues is not then, properly speaking, an infection in the sense that invasion of the blood stream by the streptococcus or anthrax bacillus is an infection, but rather a "toxemia," differing from the toxemias resulting from the ingestion of drugs or other poisons only in so far as the toxins are manufactured at some point of bacterial lodgment within the body of the victim. Typical tetanus and diphtheria, for instance, can be produced as readily by injection of the bacteria-free culture filtrates as by inoculation with the bacteria themselves. It should be emphasized, nevertheless, that in the case of many toxicogenic bacteria, including the diphtheria bacillus, the toxin possesses a marked necrotizing action on tissue and as such represents in these species an extremely important means by which a foothold is gained and held within the susceptible host.

With this fact in mind we must consider that such pathogenic saprophytes are endowed with a weapon which permits them to establish an infection, although this does not consist of a massive and generalized invasion of the body by the organism.

In the majority of bacterial diseases, however, which are due to organisms in which the capacity to produce toxic substances is limited, it is necessary that they more or less extensively invade the body before clinically recognizable symptoms are produced. At first sight this group of parasites appear to be distinguished by the different degrees to which in any given species of animal host invasion is effected. Some, such as the anthrax bacillus or the bacilli of the hemorrhagic septicemic group, tend to spread rapidly from the site of entrance to cause a generalized infection of the blood and viscera; others, such as the pneumococcus in man or the staphylococcus, after extending through a certain area of tissue frequently remain localized. With these observations in mind, Bail (1) has classified parasites according to their relative powers of invading the living body. Briefly reviewed, his classification is as follows:

I. *Pure Saprophytes.* (Necroparasites, superficial parasites, or external parasites.)

Microorganisms which under no circumstances can be made to develop within the living tissues of a given animal. This does not exclude their pathogenicity for this animal, since, like the diphtheria or tetanus bacillus, they may develop and produce toxins on a localized area of dead tissues.

II. *Pure Parasites.* Organisms like the anthrax bacillus or the bacilli of the hemorrhagic septicemia group which, implanted in small quantity in an animal, rapidly gain a foothold, thrive, and spread.

III. *Half parasites,* organisms which may be infectious if introduced into the animal body, but, not possessing this invasive power to the same degree as the preceding class, require the inoculation of considerable quantities, often a special mode or path of inoculation, or even possibly a preliminary reduction of the local and general resistance of the infected individual. This class includes the majority of the bacteria pathogenic for man.

Such a classification is, however, quite arbitrary and on the whole of little value except perhaps as a mnemonic device, for even though it be made on the basis of the behavior of the various parasites when brought into contact with a single species of host, no rigid schema is obtained. This is clear from the single example of hemolytic streptococcus infection in man, where the organism may at one time induce a rapidly progressive septicemia with little local reaction, while again it may be confined to a small area such as the naso-pharynx, causing severe angina and even ulceration of the mucous membrane, and whence, in the case of scarlet fever, the absorption of a soluble toxin locally elaborated leads to the distinguishing exanthem.

Because of such considerations it is perhaps best to abandon any attempt at systematic classification of the parasitic bacteria or fungi on the basis of pathogenicity, although a loose division can be made between those organisms which practically always remain localized and exert their effect by the production of a potent toxin, and those which must, in order to cause disease to a greater or less degree, invade the tissues, either through their ability to survive in this living environment or to destroy it through the formation of necrotizing substances or through a combination of both these properties.

Virulence and Invasiveness. In the foregoing paragraphs we have used the terms *virulent* and *invasive* in such a manner that the impression might easily be gained that they were synonymous, and indeed numerous authors have considered them interchangeable expressions, since in many instances those organisms which invade the tissues most widely lead to the most severe conditions and cause the highest mortality. It is, however, evident that were the phenomena of virulence and invasiveness regarded as identical, it would be impossible to refer to a virulent diphtheria bacillus or a virulent tetanus bacillus, neither of which, as we have seen, ordinarily invades the tissues to any great extent. However, avirulent strains of both are not infrequently encountered. Menkin (2) has recently emphasized the importance of distinguishing clearly between the capacity of an organism to invade and its virulence, and has presented experimental data which show that these two attributes of a given bacterium (Pneumococcus Type III) can be distinguished. We are inclined to regard the term *virulence* as being equivalent to *pathogenic*. Invasiveness, then, would be left to signify that property of certain organisms which permits them to spread through the tissues and body fluids of the host. Accordingly, in certain cases virulence or pathogenicity will be found to be conditioned principally by the characteristic of invasiveness, while in others toxigenicity will be the determining factor; in still others the organism may depend for its pathogenic effect on the possession of both characteristics.

Relationship between Virulence and Dosage. It is self-evident that there must be an inverse relationship between the virulence of microorganisms and the numbers or dosage which can bring about infection. For, even when the bacteria are of a variety known to produce disease, and are brought into contact with the body by a path suitable to their peculiar requirements, the initial quantity introduced must be sufficiently large to preclude immediate annihilation by the defensive powers of the body, which are usually but not inevitably present to a greater or less degree. It is plain, therefore, that in the case of bacteria weak in power to cause disease, given the subject of infection and his defenses as a constant, the quantities to be introduced must be larger than in the case of microorganisms of violent disease-producing properties. Thus we measure the degree of the so-called virulence of bacteria by determining the smallest number which will still cause infection and death in susceptible animals of a standard

weight and strain. In the case of microorganisms of extreme virulence, such as the anthrax bacillus or bacilli of the hemorrhagic septicemia group, the inoculation of a very small number of bacteria may suffice to initiate infection. Indeed, the injection of a single anthrax bacillus of sufficient virulence may produce fatal disease in a susceptible animal such as the mouse. The experiments of Goodner (3, 4) in our laboratory have shown a similar thing with pneumococcus infections in rabbits. Type I Pneumococcus will cause death in rabbits by the intracutaneous route even when only a single organism is introduced.

Contrasting with the maximum virulence of such bacteria is the relatively low pathogenicity of freshly isolated strains of meningococci or typhoid bacilli for mice. The smallest dose of the former which will kill when injected into these animals must be measured in milligrams of bacterial growth (5), and even with the moderately virulent typhoid bacillus, Grinnell (6) found the lethal dose for 80 per cent of mice tested to contain about 30,000 individual bacteria. In these cases where large doses are required, although the organisms have been obtained directly from human cases, and in all probability are equipped with the full complement of pathogenic properties, the factors involved in the natural resistance of the host which resist the attack of the bacteria without much doubt account for the low degree of virulence which they exhibit. Frequently, however, a given species of organism may itself become altered in some way whereby the virulence as measured by the number of bacteria in the minimum lethal dose is greatly decreased. This can occur through the loss of the capacity to synthesize certain protective (to the bacterium) antigenic chemical constituents — a phenomenon which will be subsequently described. In certain instances, however, the virulence may rise or fall independently of any apparent change in antigenic structure. Eaton (7), for example, has described certain strains of Pneumococcus Types I, II, and III which became markedly reduced in virulence for mice, but remained in all other respects identical with the fully pathogenic organism. Practically avirulent pneumococci which retain all their type specificity in respect to agglutination have likewise been studied by Schliemann (8). Wilson (9) has observed a similar phenomenon with *Bacterium Aertrycke*. Obviously the dosage of such "attenuated" bacteria required to bring about the death of a mouse will be large.

That fluctuations in virulence associated with some change in the organism may occur very rapidly which are at once reflected by the increase in the minimum lethal dose is well illustrated by the careful experiments of Webb, Williams, and Barber (10) carried out upon white mice with anthrax bacilli, using the single cell isolation method devised by Barber.