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PRINCIPLES OF IMMUNOLOGY

Principles of Immunology

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FOREWORD

With the tremendous expansion of all scientific knowledge in the fundamental sciences, the educational system of the country continually faces the problem of introducing more and more material to the advanced undergraduate to prepare him adequately for graduate work or for living intelligently in our society. To do this, there must be available textbooks suitable for use in undergraduate courses which can bring together experimental data and present unifying concepts which are initially confined to original scientific papers, review articles, and monographs. One such field which has grown almost exponentially in the past two decades is that of immunology and immunochemistry. With its powerful techniques for recognizing differences among proteins and among polysaccharides even from very closely related species, it provides a tool of enormous versatility for the study of living systems.

Drs. Cushing and Campbell have bravely undertaken to assemble the vast amount of data by which immunological and immunochemical methodology and reasoning have advanced the study of biology and to present this information at the level of the advanced college student. In addition, they outline to the student the fundamental principles of immunochemistry and clearly trace, to the extent that current information permits, the origins of biological variation to differences in chemical structure. The interrelations of immunology and such diverse fields as population genetics, embryological development, fertilization, and antigenic individuality are brought together in one volume.

Among the important features of the book are the attempts to deal with mechanisms rather than with masses of data or systematic classification, and the inclusion of substantial numbers of references to original and review articles which will make it possible for an eager student to delve deeper into the field on his own. Naturally, with so broad a field to cover, there may be minor areas of disagreement with interpretations or indications of attempts to oversimplify certain concepts, but such criticisms could be leveled at any textbook designed to reach the college student. The book should prove invaluable in the college or university which wishes to have its courses keep up with this aspect of the advance of modern science.

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PREFACE

Although control of infectious diseases is an important part of immunology, it is becoming increasingly evident that the basic principles and concepts of the "immunity mechanisms" involved are of great general importance to fundamental biology. This book, therefore, has been written particularly for the biology student to acquaint him with these basic principles and concepts and to provide a general background which will enable him to continue to more advanced studies of the relation of immunological phenomena to biological problems. It is designed primarily for college seniors and graduate students, majoring in biology, who are being exposed to the principles and concepts of immunology for the first time. It is assumed that such students will have a reasonable knowledge of at least the elements of the areas covered by their major including those of genetics, embryology, physiology, and evolution, as well as a general knowledge of the physical sciences. Advanced courses in mathematics and physical sciences would be helpful in understanding some of the more complicated aspects of immunochemistry. However, this is not necessary in order to grasp the important basic concepts of antigens and antibodies, and a workable knowledge of the physical factors involved can be obtained from the simple descriptions given in Chap. 1.

Very little medical immunology is discussed, and laboratory procedures are given only where they may aid in clarifying a certain basic problem. An attempt has been made to keep the number of references as small as possible in order to facilitate reading. Statements that are undocumented are those which are common knowledge, and references to many original studies can be obtained from the review articles and more advanced treatises which are given at the end of each chapter. References to original work are, in general, made when such work has not received much attention in immunological literature, has appeared only recently, or illustrates a particular point that is being made. Many controversial problems have been purposely omitted since the authors believe that such discussions merely lead to confusion until the student has acquired a detailed knowledge of the entire field. Consequently, the material presented here will be of little help to the advanced student or research worker in immunology except, perhaps at times, to offer a new perspective, at an elementary level, on some old problems. Some discussions may seem oversimplified, but this has been intentional in order to give the student a simple tangible concept to start with.

The material in this book is divided into three parts. The first reviews aspects of the physical sciences which are important for an understanding of subsequent discussion of antigens and antibodies and also presents a broad general survey of immunology. This survey is intended to give the student an acquaintance with a subject that contains many terms and concepts which are at first difficult to define or grasp. The second part deals with biological problems in which immunological reactions and concepts play an important role both as an intimate part of the biological processes described and in the understanding of these processes. The third part takes up in more detail many of the immunochemical problems which are only briefly mentioned in Chap. 1. In some instances, certain materials or reactions have been mentioned repetitiously in different places in connection with different problems or concepts.

Although this book contains little that could be used for laboratory instruction, it has been assumed that laboratory work must accompany any complete course in immunology. Consequently, liberal reference has been made to the laboratory text "Experimental Immunochemistry" by Kabat and Mayer. Liberal use has also been made of such advanced texts as "Specificity of Serological Reactions" by Landsteiner, "Principles of Bacteriology and Immunity" by Topley and Wilson, "Genetics and the Races of Man" by Boyd, "Blood Groups in Man" by Race and Sanger, and other books referred to in connection with specific chapters.

The authors are greatly indebted to the many persons who have generously contributed their time to the review and critical discussion of manuscripts, to their wives who have persevered through all the typing, proofreading, and other preparation of the manuscript, and to Roger Lewis who conceived and made all the original figures. Special acknowledgment is given to Professor Elvin A. Kabat for the time and attention that he has devoted to reviewing this book during various stages in its preparation.

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PART I

INTRODUCTORY SURVEY

CHAPTER 1

A GENERAL SURVEY OF SOME FUNDAMENTAL PRINCIPLES INVOLVED IN IMMUNOLOGY

Immunology, in a broad sense, deals with the mechanisms by which living tissue reacts to foreign living or nonliving biological materials. This is essentially a defense mechanism, although in some instances, such as allergic responses, the reaction may result in damage to the host's tissues. The term *immunology* may be used to include all aspects of defense against external as well as internal environment, but, in general, it considers the *specific* reactions which result from some sort of exposure to foreign biological materials. Reactions such as climatic adaptation, drug tolerance, natural resistance to certain infectious agents or their toxic products, and the protection offered by skin and other mechanical barriers involve mechanisms which are normally considered to be the result of nonspecific physiological factors. These nonspecific factors may be considered as first lines of defense, and if these lines are broken, then the body brings into play the second lines of defense in which immune mechanisms occupy an important position. The basic mechanisms of specific immunity are the same, whether an organism or tissue is reacting against living agents (e.g., infectious organisms, skin grafts, etc.) or nonliving toxic agents (e.g., snake venoms; diphtheria, tetanus, etc., toxins) or relatively bland but foreign biological materials (e.g., egg white, serum proteins, pollens, etc.). Immune mechanisms may be an inherent property of all living material, but as will be seen from discussions in the following chapters, only a few species of animals have been studied extensively and plants have received practically no attention.

Immunity, or the so-called *immune state*, is brought about when foreign materials enter the tissues of the body. Entrance can be accomplished naturally from infections and by absorption through the membranes of the respiratory or gastrointestinal tracts and artificially by injections, "shots." If the tissues develop a specific altered reaction toward subsequent injection of the same material, this is referred to as an *immune state*, and the foreign material is then considered to be *anti-*

genic. The injection of antigen to induce immunity is sometimes referred to as a *vaccination*, and the antigen which is injected is called a *vaccine*. These terms have persisted from the time of Jenner who used the virus of cowpox to immunize humans against the closely related smallpox virus (variola). Immunity brought about by exposure to an antigenic material is referred to as *active immunization* because of the active participation of the host in producing the immunity. Immunity can also be induced in a nonimmune animal by injecting blood or serum from immunized animals. This is called *passive immunization* since the recipient plays no active role in the production of the immune state. The serum from an immunized animal is called an *antiserum*.

Immunity which is induced by active immunization takes several weeks to develop. This is due to the fact that the tissues which are stimulated by the presence of antigen must synthesize a relatively large amount of new protein for immune reactions. Consequently, active immunization is usually used as a preventative (prophylactic) measure.[1] Active immunization is commonly used to protect humans against typhoid, plague, tuberculosis, whooping cough, cholera, typhus, Rocky Mountain spotted fever, smallpox, yellow fever, poliomyelitis, rabies, tetanus, and diphtheria by injections of the respective antigen. Passive immunization is used either prophylactically or as a therapeutic measure to combat an infectious agent which is already present. Antiserums used in passive immunization are obtained from animals or humans that have recovered from an infection or have been actively immunized by injections of a specific antigen. Passive immunization is used prophylactically in humans to protect against measles, poliomyelitis, tetanus, and diphtheria. It is used therapeutically against diphtheria, tetanus, snake venoms, anthrax, measles, mumps, and infectious jaundice. Antitoxin antiserums are usually obtained from horses that have been given a long series of injections of the respective toxin antigen. Anti-measles and anti-poliomyelitis serums are obtained from the pooled blood of normal adult humans, since much of the general population has had at some time some form of these virus infections. Although active immunity takes several weeks to develop, it may last for a year or more, while passive immunity rapidly disappears within a few weeks, because of the natural loss of the passively transferred materials which are responsible for immune reactions. Passive immunization is of fundamental importance since it shows that antiserums contain a soluble factor that is involved in immune mechanisms. Much is known about this factor which is a definite protein entity called *antibody*.

[1] There are a few instances (e.g., brucellosis and chronic coccal infections) where attempts are made to boost immunity by injection of *Brucella* vaccine or autogenous vaccines made from cultures of organisms isolated from lesions of the patient.

The immune state can be demonstrated by a variety of tests and will depend to some extent on the nature of the antigenic material being studied. One often has a choice of either biological or chemical tests. Biologically, the immune state can be demonstrated by an increase in resistance to the infectious agents or their toxic products. A paradoxical condition also occurs as a result of the immune state, in which exposure to sufficient amounts of antigen results in a violent physiological response. This is referred to as *specific hypersensitivity*, of which allergy is an example. Antigenic materials disappear more rapidly from the blood stream of immune animals than normal animals, and if one examines the tissues histologically or chemically, it will be found that this increased rate of removal from the blood stream is due in part to the increased activity of cells which ordinarily take up foreign material very sluggishly. Chemically, the immune state is shown by an altered reaction of antiserum with respect to the specific antigen. Before immunization, serum usually shows little or no tendency to react with most antigens, but after immunization a variety of reactions can be performed in vitro, depending again upon the nature of the antigen used for the test. For example, antiserum clumps (agglutinates) particulate antigens such as bacteria or other cells, precipitates soluble antigens such as toxins or egg albumin, and it will break down (lyse) certain kinds of cells. Serums from immune animals do not always give evidence of the immune state by in vitro reactions with antigen. Such failures can be due at times to the inadequacy of known physical analytical methods, and biological reactions, which are more sensitive, must be utilized for testing. However, antibody must be present in any immune reaction, whether it is biological or physical, and the fundamental components involved are antigen and antibody.

One of the characteristics of immune reactions is the high degree of specificity with respect to the antigen used for immunization. Thus, if an individual is immunized against diphtheria toxin, his resistance to diphtheria toxin will be greatly increased, but his resistance to tetanus toxin, snake venoms, or other toxins will remain unchanged. The same degree of specificity holds true for immunity against infectious agents whether they are viruses, unicellular organisms, or metazoan parasites. Specificity also holds true for hypersensitive reactions so that individuals who become immunized (sensitized or allergic) to the pollen of ragweed will react only when exposed to ragweed pollen (or pollens of closely related species). The investigations which led to the solution of the mechanism of immunological specificity constitute one of the fascinating stories in the history of immunology, and the essential parts are presented in Chap. 16.

Immunology incorporates the principles of many physical and biological sciences, and hence it is important to have a broad knowledge of fundamental chemistry and biology. It is also important to have some knowledge of specialized subjects such as biochemistry, genetics, and histology. Therefore, an outline of some of these subjects is presented in the following pages to afford a review to those who have had previous acquaintance with these subjects and to aid those who must become familiar with them for the first time.

PHYSICAL AND CHEMICAL FACTORS OF GENERAL IMPORTANCE IN IMMUNOLOGICAL REACTIONS

Physical Factors

Physical factors play a very important part in all antigen-antibody reactions, but a specialized knowledge of physical chemistry is hardly necessary to understand most of the basic concepts. The factors involved are those which are normally considered to be characteristic of colloids and hence involve the properties and behavior of large molecules, particles, or in general, surfaces in contact with aqueous solutions. The following is a brief outline of some of the more important factors and phenomena; for more detailed discussion of this subject, reference should be made to textbooks by Bull [1], Gortner [2], or similar sources.

Size and Shape of Molecules. The size of pertinent molecules varies from a few angstroms for amino acids, simple sugars, or the chemical groups which are the determinants of immunological specificity, up to many thousand angstroms for large protein molecules and viruses. Usually size is indicated in terms of molecular weight (i.e., the weight in grams of 6.025×10^{23} particles, which is Avogadro's number) rather than by linear dimensions, and substances which can be given reasonable molecular-weight values may be conveniently considered as molecules. When this value becomes greater than a few million (e.g., for viruses, bacteria, etc.), the designation as molecules becomes ambiguous. Molecules of many sizes are involved in immunological reactions, and Table 1-1 presents the values for the molecular weights of some of the substances which are commonly encountered in immunological studies.

Molecular weights are determined by a variety of methods, and the method of choice will depend to some extent upon knowledge of chemical structure, solubility, and molecular size of the material in question. For example, molecular weights up to several hundred can be determined by the depression of the freezing point or elevation of the boiling point of water. However, when molecular weights become greater than a few thousands, more sensitive methods must be used and calculations made from data obtained from osmotic pressure, ultracentrifugation, and light scattering. Values for the molecular weights of large molecules are sub-

TABLE 1-1. MOLECULAR WEIGHTS OF SOME MATERIALS OF IMMUNOLOGICAL IMPORTANCE

Glucose	180
Arsanilic acid	217
Cytochrome C	18,000
Tuberculin	32,000
Ovalbumin	40,000
Hemoglobin	69,000
Serum albumin	70,000
Diphtheria toxin	74,000
Rabbit precipitin antibody	160,000*
Antipneumococcus antibody (horse)	1,000,000
Hemocyanins	400,000–6,000,000
Tobacco mosaic virus	40,000,000

* This is the approximate value for most precipitating antibodies.

ject to considerable error and hence are usually given in terms of the closest thousand or million gram molecular weights. Many biological materials may be of uniform chemical composition but be present also in a large number of sizes because of aggregation or disaggregation of some fundamental unit. Thus, the polysaccharide dextran and the protein gelatin are of uniform chemical composition, but they are extremely heterogeneous with respect to their molecular weight, which may vary from a few thousand to several hundred thousand in a given preparation.

Although sizes of molecules may be expressed in terms of linear dimensions, such values for macromolecules are subject to considerable error. These dimensions can be calculated from data obtained from molecular-weight measurements, density of the molecules (value usually assumed), and rate of diffusion. Dimensions are then expressed in terms of the quotient of the values for the largest (major axis) and the smallest (minor axis) dimension. This is called the axial ratio. Examples of different shapes and sizes of serum-protein molecules are given in Fig. 1-1. The shape and dimensions of larger particles can be determined easily by use of the electron microscope or the light microscope. Very little is known about the structure of surfaces of macromolecules at an atomic level, but they must present a rough pattern of pockets and protuberances.

An idea of the molecular weight and shape of molecules involved in immunological reactions is extremely important for an understanding of many of the antigen-antibody reactions. It enables one to visualize reactions at a molecular level and helps to give logical interpretations of experimental data and reasonable approaches to unsolved problems. For example, if we know the molecular weight and shape of an antigen molecule, we can estimate the limiting number of antibody molecules of a given size that can attach to such an antigen. The fate of injected foreign material can be predicted to some extent on the basis of molecular size and shape, since it is well known that small molecular-weight materials diffuse rapidly into the tissue spaces or through the glomeruli of the kidney and are excreted.

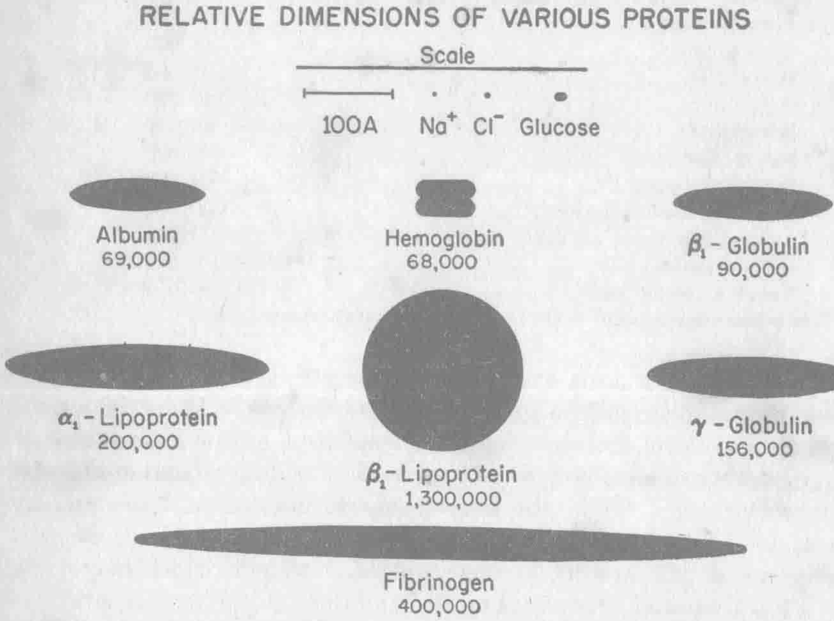

Fig. 1-1. Current concept of the shape and size of some common blood proteins. *(From J. L. Oncley: "Conference on the Preservation of the Cellular and Protein Components of Blood," published by the American Red Cross, Washington, D.C.,* 1949, *with permission.)*

Electrical Charges in Immunological Systems. Electrical charges on the surface of molecules in solutions, or on insoluble surfaces such as glass or tissue membranes in contact with aqueous solutions, play an important role in immunological reactions. The nature and behavior of such charges are referred to as *electrokinetic phenomena,* and they arise mainly from two sources. One source of charge is simply the ionization of groups, such as carboxyl on the surface of protein molecules. When such ionization takes place, the protein molecule is given a negative charge with respect to the surrounding solution. The second source of surface charge is the result of selective adsorption of ions from solution. This selective adsorption may be the result of complicated interactions between atoms or groups of atoms on the surface with specific ions in the aqueous phase. One interaction can be explained on the basis of polar forces. Structures such as amino acids and lipids are, in general, polar substances, since certain portions of these molecules contain atoms with a greater number of negative charges (e.g., oxygen atoms) than another part of the molecule which contains a preponderance of positive charges (e.g., hydrogen atoms). When such groups are an intimate part of the structure of a large molecule, they will tend to attract atoms selectively. Selective adsorption of ions may also result from a tendency of specific atoms on the surface of a large molecule to attract specific ions from solution. This is due to the nature of their electron-shell structure which results in attraction between the respective electron orbits.

Large molecules of biological origin, or cell surfaces, have a complex pattern of distribution of charges, so that one must consider not only the net charge of the entire molecule but also the distribution of both negative and positive charges. Surfaces may have a net charge, under certain conditions, that gives them the characteristic of being either positively or negatively charged, but this is merely an indication that there

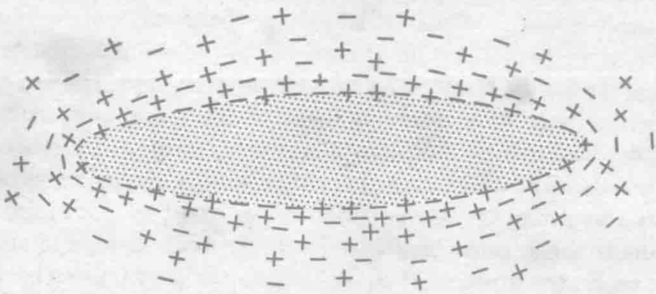

FIG. 1-2. A diagrammatic representation of the distribution of charges surrounding a protein molecule in solution.

is an excess of either positive or negative charges, and if we examine the surface carefully, both types of charges will be found. A diagrammatic representation of charges on the surface of a protein molecule is given in Fig. 1-2. The charges in the solvent (water) are distributed as an ion atmosphere in which the ions are distributed at varying distances from the surface of the molecule. This atmosphere or layer of ions is often called the Gouy diffuse layer, after M. Gouy who first described it.

As might be predicted, the charge density (total net charge) as well as the sign of the net charge is influenced by the concentration and types of ions in the environment, particularly the hydrogen ion concentration which influences ionization for biological systems. In fact, the effect of pH ($pH = \log 1/[H^+]$) on the net charge of proteins is one type of characterization of proteins and is an important factor to consider in all protein chemistry.

Table 1-2 gives a list of some of the important proteins and the pH at which there is an equal number of negative and positive charges. This pH is referred to as the *isoelectric point*. Its significance in the characterization of proteins and its role in chemical and physical reactions are

TABLE 1-2. THE ISOELECTRIC POINTS OF SOME MATERIALS OF IMMUNOLOGICAL INTEREST

	Isoelectric point pH
Cytochrome C	10.6
Hemoglobin	6.8
γ-globulin (average)	6.4
γ_2-globulin of hyperimmune serums	6.5–7.5
γ_1-globulin of hyperimmune serums	5.5–5.9
Ovalbumin	4.7
Serum albumin	4.7
Bushy stunt virus	4.1
Red blood cell (human)	1.7

discussed in the section on proteins. Some materials do not have isoelectric points, since in contrast to the proteins, they have only one type of ionizable group. Thus, most of the bacterial polysaccharides owe their charge to the ionization of carboxyl groups, and although the degree of ionization can be reduced by an acid environment, the net charge cannot be reversed. The same is true of inorganic sols, such as ferric hydroxide, etc., which maintain a positive charge. In general, biological materials have a negative net charge under physiological conditions, and the amount of charge is sufficient to result in a certain amount of repulsion between molecules and, hence, in some degree of stabilization. Furthermore, the amount of charge influences interactions between molecules and cell surfaces and plays an important role in antigen-antibody reactions, phagocytosis (the ingestion of foreign material by cells), and many other reactions.

One of the most important tools for the characterization of large molecules, particularly proteins, is based on an electrokinetic phenomenon: the migration of a charged particle in an electrical field. The rate of migration depends to a large extent upon the net charge on the surface of the molecule, which is more or less characteristic of any particular protein substance. This phenomenon is usually referred to as *electrophoresis*, and one of the most common methods for studying the reaction is with an apparatus developed by Tiselius. The optical systems and the theoretical interpretations are rather involved, so that for more detailed discussion of these aspects of the subject reference should be made to Alberty [3]. The essential parts of the apparatus consist of (1) a glass U-shaped cell to hold the solution, (2) a complicated optical system for detecting changes in the optical density (refractive index) of the solution, and (3) a source of electrical current. Figure 1-3 gives a diagrammatic representation of such a cell containing two different proteins. When an electrical potential is applied to the solution, the two proteins will migrate toward the anode or the cathode at a rate which is depend-