CLINICAL IMMUNOLOGY AND ALLERGY

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Preface

The field of hypersensitivity or allergy has made tremendous strides since the appearance of the author's last book, *Essentials of Allergy,* 15 years ago. This progress is particularly evident in the experimental phases of the immunology of hypersensitivity, an understanding of which has become essential for the enlightened allergist. A glance at modern medical literature and a review of the scientific programs of the various allergy societies indicate the extent to which clinical immunology has become a basic constituent of allergy. It is for this reason that the first part of this book is devoted to a consideration of fundamental immunology. This is written, not for the immunologist, but for the student, the resident, the internist, the pediatrician, the dermatologist and the allergist. It is intended to impart an understanding of the basic principles of immunology and an acquaintance with the immunologic tools employed in the study of hypersensitivity.

The second part of the book deals with the subject of hypersensitivity conveniently divided into four classes. These are the "immediate" response associated with circulating antibody; the "delayed" response, *not* associated with circulating antibody—cellular sensitivity; the response resembling immunologic reactions associated with circulating antibody and/or cellular sensitivity; and finally, diseases characterized by morphologic and clinical similarity to immunologic reactions.

In many conditions, basic immunologic considerations are not subject to direct positive and dogmatic interpretation since our knowledge at the present is not conclusive. Hence, various shades of gray exist between what is considered black or white. This will account for the lack of general agreement among immunologists in many of the areas treated in this volume.

Ancillary subjects such as pulmonary function tests, corticosteroids, agammaglobulinemia, chronic vascular headache, diffuse connective tissue diseases, hematoimmunology, autoimmune disorders and rejection of homotransplants are discussed briefly and only to the extent to which they are of interest to the student of allergy. An effort is made to place the consideration of such topics within the framework of the classification given above. Insofar as it is possible, lengthy controversial discussion of various views and theories is avoided. Obviously, in a book of this scope, there are bound to be a number of omissions. Some subjects are presented briefly. For additional information, the interested reader is referred to recent reviews and to the list of reference books appended at the end of this volume. In the available space, we can present only a working version of these topics. In order to avoid much repetition, the reader is often referred to other sections of the book.

This volume covers modern and generally accepted concepts of diagnosis and treatment of allergic diseases. These conditions are considered important

not only because of their relatively high incidence, but also because of their persistence and high morbidity.

The contents of this book truly reflect the ever-widening scope of allergy. The allergist is no longer content to concern himself only with the conventional concepts of clinical allergy. He has become aware that clinical immunology is an essential part of his domain. Through an understanding of the newer phases of hypersensitivity, he has grasped the significance of the ever-increasing list of subjects related to this field.

In writing this text, the author has drawn freely from the available literature as well as from his own experience. He therefore acknowledges his gratitude to the many named as well as the many unnamed collaborators and investigators without whose brilliant work the present volume could not have materialized.

The author owes gratitude to the Schering Corporation of Bloomfield, New Jersey, a grant from whom made printing of the color illustration possible.

The author is grateful as well to to the editors and publishers of the medical journals with whose permission certain material is reprinted; and to Thaddeus Danowski, M.D., Hubert Bloch, M.D., Rudolph L. Baer, M.D. and Wallace N. Jensen, M.D., who have been very kind in critically reviewing parts of the book; to Miss Catherine M. Leslie, his secretary; his laboratory personnel, Miss Romayne A. Teufel, B.A., M.T., Miss Nada Cindrich, B.A. and to Mr. J. Mineo and Staff of the Medical Illustration Department of the Veterans Administration Hospital. Special acknowledgments are due to Frank J. Dixon, M.D., for his valuable assistance in carefully reviewing the part of the book dealing with fundamental immunology and the pathogenetic-classification which is followed in the book; to E. R. Fisher, M.D. for writing on the anatomy and pathology of connective tissue diseases, and to P. A. Bromberg, M.D. for writing on the pathophysiology of bronchial asthma. The author expresses his indebtedness to his wife for her patience and help in reading the manuscript and proofs; and finally to his publishers for their kindness and cooperation.

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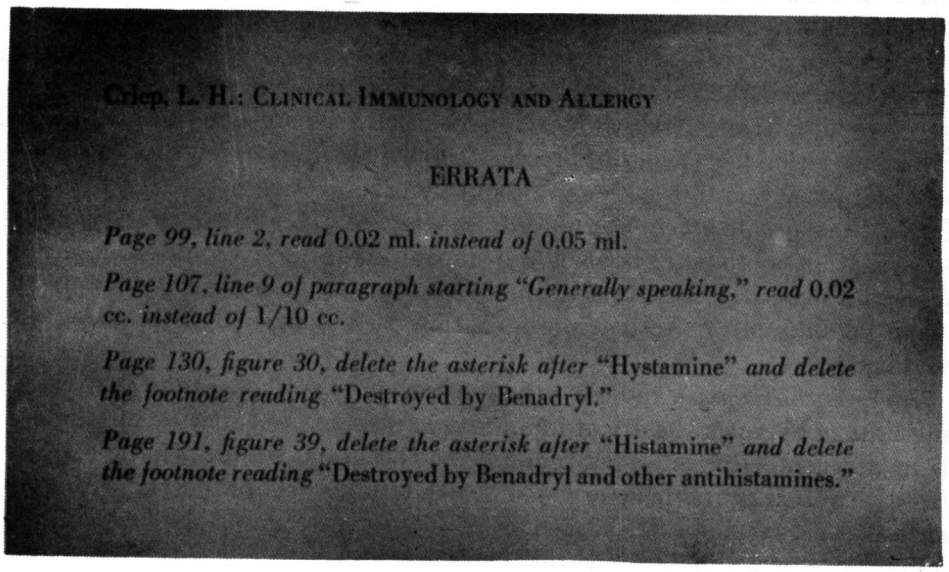
Contents

Part 1

Fundamentals of Immunology



Criep, L. H.: Clinical Immunology and Allergy

ERRATA

Page 99, line 2, read 0.02 ml. *instead of* 0.05 ml.

Page 107, line 9 of paragraph starting "Generally speaking," read 0.02 cc. *instead of* 1/10 cc.

Page 130, figure 30, delete the asterisk after "Hystamine" *and delete the footnote reading* "Destroyed by Benadryl."

Page 191, figure 39, delete the asterisk after "Histamine" *and delete the footnote reading* "Destroyed by Benadryl and other antihistamines."

1

Nomenclature

Hypersensitivity; hyersensitiveness; allergy; sensitivity; sensitiveness; specificity
Immunology; immunity; immunopathology
Immunization: active and passive immunization; immune response; antibacterial immunity; antiviral immunity
Isoimmune reactions

As might be expected in the development of a comparatively new study, there exists a great deal of confusion in the literature on the terminology of allergy. This confusion is due to the fact that a variety of terms have been coined by various workers to denote the phenomenon of *hypersensitivity*. For this reason, it seems advisable to make an attempt to clarify this nomenclature and to indicate the terminology to be adopted in this book.

The terms *hypersensitivity* and *allergy* are used interchangeably, in an all-inclusive sense, to denote a state of specific sensitivity on the part of lower animals or of man to a substance which is harmless to other members of the same species. Thus, an individual is said to be hypersensitive, or allergic, to egg if the ingestion of a small amount of egg gives him unusual symptoms such as asthma. The terms *sensitiveness* and *sensitivity* are used synonymously with hypersensitiveness and hypersensitivity. General usage justifies the employment of the term hypersensitivity, redundant though it may be. The sensitivity in these instances is always *specific*. It results from exposure to a definite substance. An animal sensitive only to egg white will develop manifestations if exposed specifically to egg white alone. The essence of the allergic reaction is altered tissue reactivity. Sensitized tissue reacts in a different manner from that of normal tissue.

Immunology is the study of immunity. *Immunity* is a state of increased resistance to a disease. It is specific. *Immunopathology* is the study of the physiologic and the morphologic changes which occur in a host animal exposed to an antigen to which it is hypersensitive. The process of artificially inducing antibodies (whether protective or sensitizing) is referred to as *immunization*. When this is brought about by the injection of antigen, it is referred to as *actively acquired immunization*. However, when protection or sensitization is brought about by the transfer of antibody-containing serum from the sensitized host to a normal recipient, it is referred to as *passive acquired immunity*. Active immunization lasts longer than passive immunity.

An *immune response* indicates the production of antibodies and the resulting state of hypersensitivity or immunity in response to exposure of the host to an antigen. *Antibacterial immunity* may occur as a result of infection. In the case of toxin-producing organisms, like tetanus or diphtheria, antitoxin-antibodies are formed. These also bring about protection of the host. Under different circumstances, however, exposure to given antigens, living and nonliving, elicits an immunologic reaction which is not protective in nature.

It produces undesirable manifestations, namely allergy or hypersensitivity. It brings about demonstrable, functional and morphologic changes, conditions which may be seen clinically both in man and in lower animals. These changes vary in extent, severity and duration, depending on the location of the reaction, the quantity of antigen and of antibody, methods and route of exposure to antigen and the host response. It follows then that the manifestations of hypersensitivity are not caused by any toxic property of the antigen. These manifestations are entirely different from those elicited by exposure of a normal nonsensitive animal of the same species to the same substance or antigen. Thus, an allergic individual responds with characteristic symptoms when exposed to substances to which he is sensitive, such as aspirin, pollen or certain foods. An anaphylactically sensitive animal responds with characteristic symptoms if exposed to the specific antigen. These symptoms are entirely different from those which aspirin, pollen, foods, or egg white elicit in nonallergic, nonsensitive, or normal individuals and animals. *Antiviral immunity* usually, though not always, follows exposure to and infection with a virus. This leads to lasting immunity or protection. Humoral antibodies against the mumps virus have been demonstrated. Antibodies against the virus of measles and of infectious hepatitis are also present. An *isoimmune reaction* is an immunologic reaction shown by an animal against antigens derived from a member of its own species.

Immunity indicates protection or resistance against disease because of the presence of antibody. Hypersensitivity indicates the production of a disease because of the presence of antibody.

2

Antigen

Definition. Antigen is an agent which stimulates an immune response or the formation of antibody when introduced into a host animal.

Nature of Antigen. Complete antigens are most often proteins in a colloidal state or some high molecular weight polysaccharides. However, nonprotein substances may be antigenic. Thus, lipoids, polysaccharides (Dextran), synthetic polymers (polyvinyl pyrrolidone) and nonprotein drugs (haptenes) may be antigenic. Some proteins are better antigens than others. For example, ovalbumin, serum globulin, diphtheria and tetanus toxoid are better antigens than hemoglobin or pollen. Antigens are, as a rule, soluble in body fluids, and may occasionally pass through the intestinal and respiratory mucous membranes and through the placenta. The serum passive transfer test in man (Chapter 18) proves that allergenic foods may pass unchanged through the intestinal mucosa and reach the sensitized skin area producing a positive reaction. On the other hand, there is no evidence that diphtheria antitoxin (DAT) antibodies present in the milk and serum of immunized cows are absorbed through the gastrointestinal tract following the feeding of such milk.

Antigenic molecules are usually large molecules generally over 10,000. Antigens with molecular weight of less than 40,000 are poor antigens. Antigenicity is frequently observed to increase with an increase in the size of the molecules. Smaller antigenic molecules are weak antigens. They may be rendered more potent if they are absorbed on large inert particles such as collodion.

Composition. The protein molecule is composed of chains of polypeptides (combinations of amino acids). The formula for amino acid is shown in FIGURE 1. The simplest amino acid is glycine (FIG. 2). The NH_2 is basic. The COOH is acid. Another amino acid, alanine, is obtained by replacing one hydrogen with CH_3 (FIG. 3). In still another amino acid, tyrosine, the R group, which is any group attached in this position, is replaced with phenylhydroxyl. These chains of amino acids in the protein molecule fold back on each other to form polypeptide chains (FIG. 4). Free reactive points are left on the surface of the protein molecule such as amino (NH_2), carboxyl (COOH), sulfhydryl (SH) and other groupings.

The free reactive points found alone or in combination on the antigen molecule are the determinant groups which render the molecule specifically antigenic. These free points become electrically charged when the protein

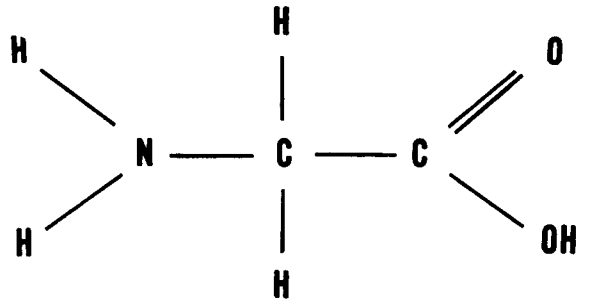


FIG. 1.—Formula for amino acid.

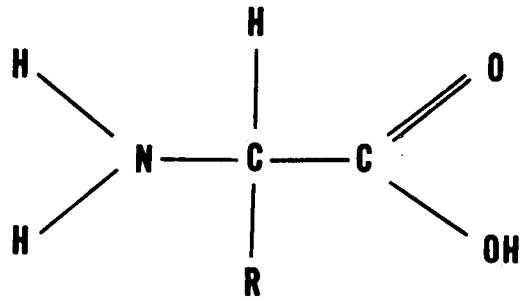


FIG. 2.—Glycine.

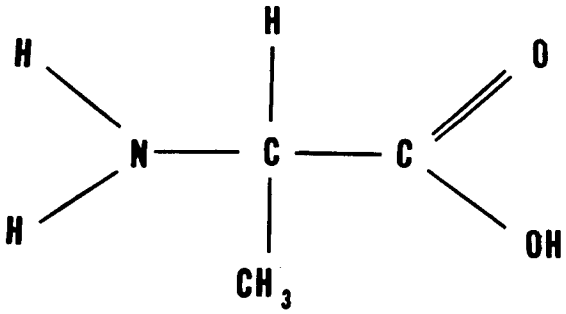


FIG. 3.—Alanine.

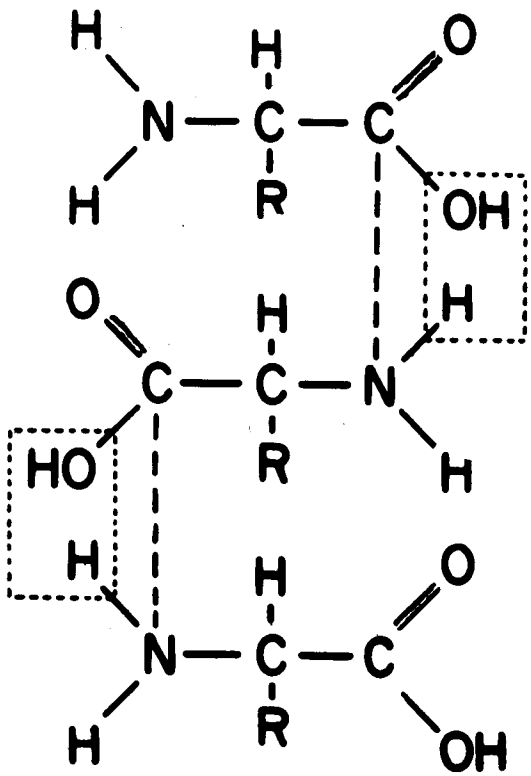


FIG. 4.—Polypeptide.

is placed in solution. Some simple chemicals, dyes and other haptenes may combine with protein molecules and thus become antigenic. As will be seen later, antibodies react and unite specifically with these free reactive groupings of the protein molecules, both because of their complimentary physical configuration and because of their opposite electrical charges.

Factors Influencing Antigenicity. The antigenicity of any agent or antigen in a host is determined by certain molecular characteristics of the antigen and by the genetic makeup of the host. The molecular properties of the antigen include the size, weight, shape and configuration of the molecule, the number of free reactive points on its surface and the degree of solubility. Furthermore, an antigen which is absorbed slowly from the body will usually prove more potent than one which is quickly eliminated. Another factor which determines antigenicity is the alien nature of the substance. The material to which an animal is exposed must in order to be antigenic be "foreign" to the animal and not recognized by the host as "itself" (Chapter 32).

Antigenic Specificity. Antigens are specific. Use is made of this knowledge to identify the origin of certain proteins such as blood stains, etc., by testing with a specifically related antiserum. It appears that the immunologic specificity of an antigen is governed by a small determinant section of the protein molecule, namely the aromatic group. Cross antigenicity is brought about by the close relation or similarity of these determinant groupings in different antigens.

Denaturation of Antigen. The active principle in many of the clinically important antigens such as house dust and pollen is not known. Heat, and chemical treatment and denaturation frequently reduce the potency of a protein antigen by changing its physical structure. This may also be effected by enzymatic degradation, by physical agents such as ultrasonic or high voltage x-ray, and by chemical treatment such as acid hydrolysis. In this manner, the complex molecules are broken down into peptides and even into amino acids, losing their specificity and antigenicity. Proteoses and peptides are not antigenic. Boiling or denaturing a food, for example, will occasionally make it possible for a patient allergic to that food to eat it without developing symptoms. Since heat coagulates the albumen fraction in milk or eggs, boiling milk or egg may render these foods nonantigenic. Noncoagulable antigens like ovomucoid are unaffected by boiling, and, therefore, retain their antigenicity. Some derived proteins, like gelatin, are not antigenic.

Valence. The antigen molecule is multivalent, i.e., it has many reactive sites on its surface so that it can combine with several molecules of antibody. Thus, a large protein molecule has more specifically reactive "free" points on its surface available to combine with many antibody molecules.

Complex Antigens. In carrying on experimental work, it must be remembered that many of the natural antigens, i.e., horse serum, pollen, bacteria, etc., are mixtures of proteins or complex antigens. This may well be determined by the process of electrophoresis (Chapter 5).

Haptene. A complete antigen stimulates the formation of antibody and has the capacity of reacting with this antibody. However, antigens such as simple chemical substances, while not by themselves capable of stimulating antibody formation, still have the capacity to react with antibodies. These are referred to as *"incomplete" antigens,* or haptenes. In order to stimulate antibody formation, these substances must be coupled to large carrier molecules which in themselves need not be antigenic.

Conjugated antigen is one whose specific immunologic property is changed or modified by chemical treatment. The coupling of diazotized arsenilic acid haptene with horse serum is an example of this. Thus, diazotizing an aromatic amine with sodium nitrite and hydrochloric acid produces a substance or chemical which has acquired a new or different antigenic specificity.

Botanically and Biologically Related Antigens. Antibodies stimulated by biologically related antigens will cross react. This is seen clinically in the cross reaction between horse serum and horse dander and the cross reaction between botanically related antigens such as cauliflower and cabbage. Likewise, guinea pigs sensitized to horse serum will react, although not as profoundly, to horse meat. Serum albumins and globulins of virtually all mammalian species cross react significantly.

Species and Organ-Specific Antigens. Animals sensitive to horse serum, but not to the serum of any other species, are said to have a species-specific type of sensitivity. This occurs because the chemical composition of horse serum differs from that of dog or guinea pig serum. It is for this reason that a patient allergic to a biologic product like insulin may be able to take insulin obtained from a different source or species. On the other hand, there may be such close similarity in the protein structure of the same organs obtained from different species that they are antigenically similar. Thus, experimental sensitization with lens protein or thyroid extract produces in the recipient rabbit sensitivity to lens or thyroid tissue extract obtained also from *other* species. This is referred to as an organ-specific type of sensitivity. Dixon showed that there is an in vitro cross reaction to the extent of 30 per cent between human and bovine gamma globulin and antisera to human and bovine globulin.

Blood Types. It has been discovered that red blood cells have an inherited antigenic substance in them which can produce antibody in other people. This inherited substance varies with different individuals. There are four main blood groups. Each group is characterized by a different antigenic constitution of the red blood cells of the individual. These are referred to as A, B, AB and O. The latter O symbolizes the absence of both A and B antigens. The counterpart of these antigenic substances is found in the presence of antibodies named agglutinating antibodies in the serum. These are designated also as anti A and anti B. Thus, blood group A has antibody B in its serum. Group B has antibody A in its serum, and group AB has neither; group O has both antibodies A and B. In addition to the A and B antigens, blood cells contain a variety of inheritable antigens for which no antibody is

normally present in the human serum. The more important of these are designated as M and N and Rh. Thus, individuals of the same species may be recognized and classified serologically and immunologically. These groups are of clinical importance because of the role that they play in blood transfusions.

Heterogenetic or Heterophile Antigens. Normally, antigens are specific for the species. Occasionally one may find nonspecies-specific antigens. An example of this type of heterogenetic antigen is the alpha and beta crystalline of lens protein of the eye which is specific for many species. Other organs and tissue specific antigens (Chapter 38), and the Forssman antigen, may be considered as heterogenetic antigens. There are then three heterogenetic antigens; the Forssman, the infectious mononucleosis and the serum disease antigen. The Forssman antigen may be obtained from sheep red blood cells, while the other two are found in ox cells.

The Forssman antigen is a heterophile or heterogenetic antigen. It is an antigen which stimulates the formation of heterophile antibody. Heterophile or heterogenetic antigen is found in the red blood cells of sheep but not in their organs. It is also found in bacteria as well as in the tissues and organs of dogs, horses and guinea pigs (kidneys), but not in their blood. Immunization of rabbits with extracts of these organs produces specific antibodies (agglutinins and hemolysins) against sheep red blood cells. The antiserum thus produced (Forssman antibody) is called "heterophile" or "heterogenetic." The lysin which it stimulates in the rabbit will not lyse ox red cells. The Forssman antigen must be injected in combination with some other substance, like serum, in order to stimulate antibody formation. Hence, it is not a "complete" antigen, but rather a haptene; its activity being due to the lipoid-carbohydrate fraction of the organ which combines with the protein component.

Lipids. The exact antigenic role of lipids is not as yet established. The active portion of the Forssman antigen which is a lipid includes polysaccharides.

Adjuvants. An adjuvant is a substance which, when added to an antigen, enhances its antigenic property. It has been observed that antibody production is enhanced by inflammation in the area of injection of antigen. Thus, injection of an aqueous solution of antigen emulsified in oil with dead mycobacteria (complete adjuvant), or without dead mycobacteria (incomplete adjuvant), enhances the phenomenon of sensitization and increases the antibody response. The reason for this is not entirely clear. It is thought that antigen in oil acts as a repository, or depot, from which small amounts of antigen are released slowly over a long period. Another explanation is the occurrence of local inflammatory changes induced by such an oil mixture with the accummulation of mononuclear cells which may enhance antibody synthesis. It may also be that the waxy substances, or lipids, in mycobacteria aid in this process. When this technic of sensitization is used ex-

perimentally, one may obtain the delayed, inflammatory tuberculin-type of hypersensitivity. As a matter of fact, the addition of this waxy substance to egg white as antigen, sensitizes guinea pigs so that they show a delayed type of reaction to egg white, whereas the injection of egg white alone into guinea pigs does not yield this type of reaction but produces only the anaphylactic, or "immediate," type of sensitivity. The injection of the respective tissue extracts in combination with an adjuvant (Chapter 37) has been employed in the production of experimental orchitis, encephalomyelitis and thyroiditis in laboratory animals. This principle is also being used now in hyposensitization therapy for hay fever with pollen in oil emulsions. Influenza and adenovirus vaccination using mineral oil as an adjuvant (repository emulsion) brings about the development of a high antibody titer which lasts for approximately 3 years, so that it is not necessary to revaccinate the patient every year. The preparation of toxoids with aluminum hydroxide as an absorbing agent, and the manufacture of pertussis vaccine, employ the same adjuvant principle. A potent and popular adjuvant (complete or incomplete) is Freund's adjuvant. This consists of dead mycobacteria with or without mineral oil. In addition to the Freund's adjuvant, staphylococcus toxin may also be employed in a similar manner. Also, lipids are being used to concentrate viruses, and the lipid virus mixture itself is used for immunization purposes. This is actually not emulsification but an adjuvant combination. Therefore, particulate insoluble antigen is slowly absorbed and therefore more antigenic. Any adjuvant which precipitates a soluble antigen will enhance its antigenicity.

DNA or desoxyribonucleic acid is found in the chromosomes of all cells. This material is the genetic determinant, dictating the nature of future development. In a similar manner, *RNA* or *ribonucleic acid* molecules are formed in the nucleolus of cytoplasm and cells. It is thought to be instrumental in the synthesis of proteins.

The Fate of Antigen. This subject is discussed at this point because it is so closely related to that of antibody synthesis. The rate of antigen catabolism and the concentration of antigen in the blood and tissue in the rabbit can be determined by measuring the nonprotein bound I^{131} present in the blood, urine and tissues. Dixon found that in the early stages, i.e., the stage of equilibration, the antigen is generaly distributed throughout the tissues. Following this, the antigen begins to disappear slowly and becomes catabolized in a manner more or less similar to homologous serum (*the induction phase*). The third stage consists of rapid *elimination* of antigen and coincides with the appearance of the antibody in the circulation (Fig. 5). This stage is accounted for by the combination of antigen with the antibody and the removal of the soluble antigen-antibody complexes from the circulation. Antibodies remain detectable as such in the circulation and are catabolized at the same rate as the host's own gamma globulins. Metabolism of antigen and antibody gives an insight into the character of the immune response.

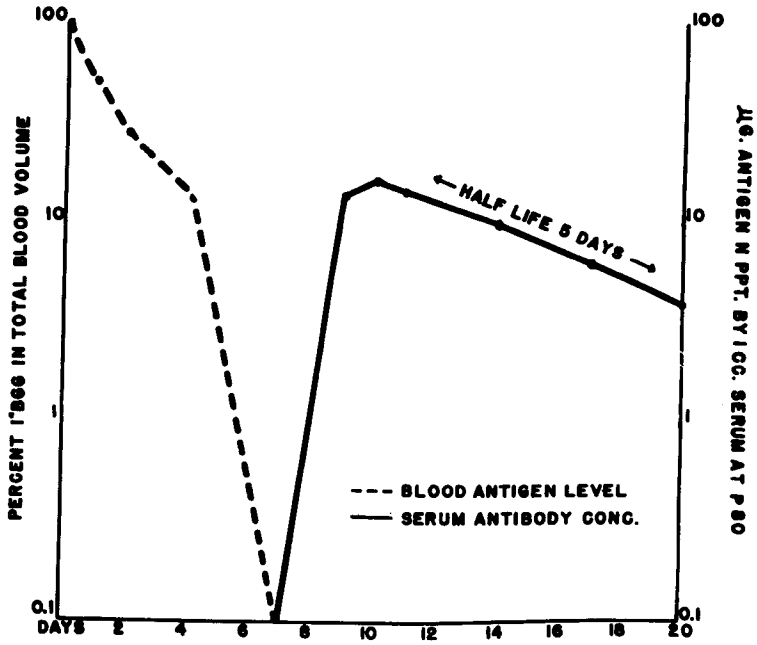


FIG. 5.—Concentrations of serum antigen and antibody in relation to time following the injection of I*BGG (30 mg./Kg.). (From Dixon, F. J., Bukantz, S. C., Dammin, C. J., and Talmadge, D. W.: Fate of I^{131} labeled bovine gamma globulin in rabbits. In Pappenheimer, A. M., Jr. Ed.: The Nature and Significance of the Antibody Response. New York Academy of Medicine, Col. Univ. Press, 1952).

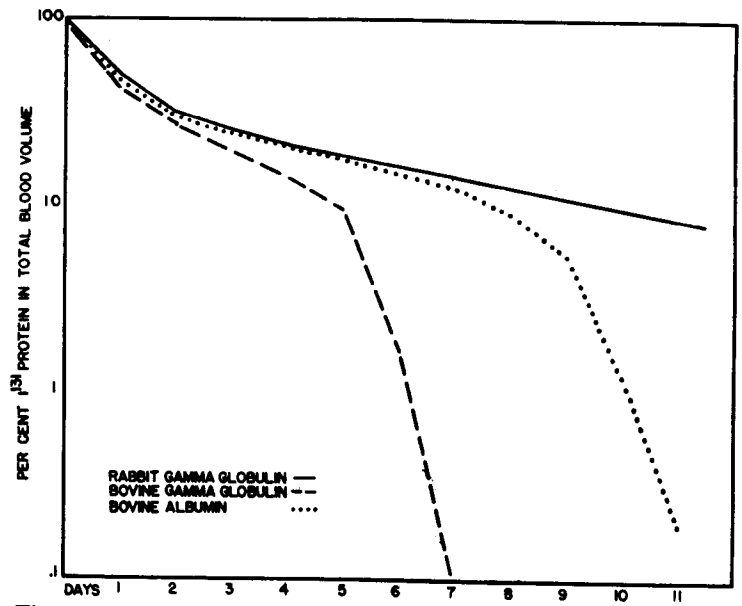


FIG. 6.—The percentage of I^{131} protein found in the total volume of blood plotted against time following initial intravenous injection. (From Dixon, F. J.: Use of I^{131} in immunologic investigation. J. Allergy 24: 547, 1953).