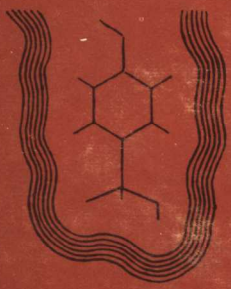
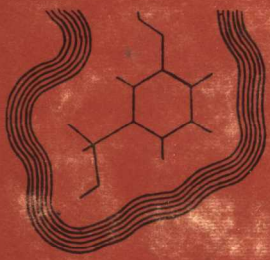
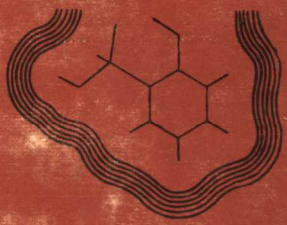
Radiation and Immune Mechanisms







W. H. TALIAFERRO
L. G. TALIAFERRO
B. N. JAROSLOW

AN AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES AND UNITED STATES ATOMIC ENERGY COMMISSION MONOGRAPH

ACADEMIC PRESS
New York and London

Radiation and Immune Mechanisms

WILLIAM H. TALIAFERRO
LUCY GRAVES TALIAFERRO
BERNARD N. JAROSLOW
Argonne National Laboratory, Argonne, Illinois

Prepared under the direction of the American Institute of Biological Sciences for the Division of Technical Information, United States Atomic Energy Commission

ACADEMIC PRESS • New York and London

ACADEMIC PRESS INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
Berkeley Square House, London W.1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 64-20325

PRINTED IN THE UNITED STATES OF AMERICA

FOREWORD

This monograph is one in a series developed through the cooperative efforts of the American Institute of Biological Sciences and the U. S. Atomic Energy Commission's Division of Technical Information. The goal in this undertaking has been to direct attention to biologists' increasing utilization of radiation and radioisotopes. Their importance as tools for studying living systems cannot be overestimated. Indeed, their application by biologists has an added significance, representing as it does the new, closer association between the physical and biological sciences.

The association places stringent demands on both disciplines: Each must seek to understand the methods, systems, and philosophies of the other science if radiation biology is to fulfill its promise of great contributions to our knowledge of both the normal and the abnormal organism. Hopefully, the information contained in each publication will guide students and scientists to areas where further research is indicated.

The American Institute of Biological Sciences is most pleased to have had a part in developing this Monograph Series.

JOHN R. OLIVE
Executive Director
American Institute of Biological Sciences

October 1963

PREFACE

The present volume briefly outlines the humoral and cellular phases of innate and acquired immune mechanisms and describes, in greater detail, the effects produced on these mechanisms by subjecting the body to hard X- or γ-radiation. The coverage has had to be selective because of the wealth of literature available.

The desirable actions of immune mechanisms, such as those which overcome or prevent disease, are largely associated with the fact that they are exquisitely sensitive in recognizing and removing from the body's economy proteins from invaders or other objects that have gotten past the outer boundaries of the body. At the same time, these mechanisms can be the basis for disturbing afflictions, such as hay fever. This same intolerance of foreign proteins interferes with successful organ transplantation. It is thus characteristic of the scientist that, having accepted and exploited the desirable actions of immune reactions, he is now interested in understanding their biochemical aspects in order possibly to eliminate them.

It is hoped that this book will be of interest to advanced students and to scientists working in other fields and that the descriptions of radiation damage may serve as an introduction to immunologists who have not worked on the radiobiological aspects of their subject.

We are indebted to various authors for permission to use their data to illustrate certain aspects of radiation damage. We wish to thank Dr. C. Philip Miller and Dr. Frank J. Dixon for helpful criticisms, and we are grateful to Mrs. E. Bohlman Patterson for her willing cooperation in drawing most of the figures.

WILLIAM H. TALIAFERRO
LUCY GRAVES TALIAFERRO
BERNARD N. JAROSLOW

January, 1964

GLOSSARY

The text is supposed to be self-explanatory, but a few symbols and general terms are briefly defined here. In addition, a few equivocal terms are defined in the sense in which they are used in the text.

Acquired immunity ..... Antibody dependent immunity specifically acquired as the result of the introduction into the body of foreign antigens (see Chapter 1, Section 1).

Adjuvant ..... An agent which when injected with an antigen causes the immunized person or animal to produce above-normal amounts of antibody to the antigen.

Anamnestic response ..... Literally a recall response. It designates an antibody response to any reinjection of an antigen given some time after the primary response at a time when any previous response has passed its peak and has dropped to a low grade. It is usually marked by a shorter latent period, a faster response, and, with many antigens, a higher peak titer than in previous responses (cf. Fig. 1).

Anaphylaxis ..... The syndrome that may follow the reinjection of an antigen. In the guinea pig, it is marked within a few minutes by smooth-muscle contraction, resulting in sneezing, difficulty in breathing, incontinence, and, frequently, death. Other animals show different symptoms—probably in part related to the amount and distribution of smooth muscle (cf. Chapter 3, Section 3).

Antibody ..... See Chapter 1, Section 3.

Antigen ..... Various foreign materials, chiefly proteins, which when injected into an animal engender the production of antibodies. Some particular complex antigens, like foreign red blood cells, and some soluble chemically purified antigens, like bovine serum albumin, are used for special types of investigations because of their special attributes (see Chapter 1, Section 4).

Antiserum ..... Serum containing an antibody which has generally been produced by the introduction of a specific antigen into the body of an animal (see Chapter 1, Section 3). The antiserum also contains complement, which in hemolytic titrations is inactivated at 56°C for 20 minutes (a temperature that does not affect hemolysin) and is then replaced by known amounts.

Arthus reaction ..... A specific hypersensitivity associated with circulating antibody that appears as an inflammatory reaction around the site of an intracutaneous reinjection of the specific antigen. It involves edema, heterophil leucocyte infiltration, hemorrhage, and, eventually, secondary necrosis and reaches a maximum in 8 to 24 hours. It has to be distinguished from delayed hypersensitivity which is not dependent on antibody (cf. Chapter 9).

Avidity ..... The firmness of the antigen-antibody union. It is measured by dissociation of the antigen-antibody complex.

BCG ..... Bacillus Calmette-Guerin, an attenuated strain of tubercle bacilli, used as an antituberculosis vaccine.

BGG ..... Bovine γ-globulin. This and other serum components are frequently used in immunological experiments because they can be obtained in a highly purified form and thus induce the production of relatively specific antibodies.

BSA ..... Bovine serum albumin (see BGG).

Co^{60} ..... See irradiation.

Colchicine ..... An alkaloid extracted from a plant of the genus *Colchicum*. It stops mitosis during the metaphase and is cytotoxic in large amounts. It is effective both *in vivo* and *in vitro* (cf. Chapter 6, Section 4). This alkaloid has been used for many centuries to treat gout.

Complement (C') ..... Five protein fractions in serum which are needed to combine with certain antigen-antibody complexes, such as hemolysin-sensitized red cells, to cause lysis of the cells. It occurs in normal serums and is usually obtained from guinea pigs. It is heat-labile.

Confidence limit ..... The 95% confidence limit contains 95% of the values in a given sample. In this book it refers to the control sample.

Cs^{137} ..... See irradiation.

Delayed hypersensitivity ... An immunologically specific hypersensitivity that is not associated with circulating antibody but results in a widespread sensitization of body cells. It is demonstrated by a delayed skin reaction which is immunologically specific (cf. Chapter 9). It is transferred by cells, preferably lymphocytes, but not by serum.

DNA ..... See nucleic acids.

Electrophoresis ..... The movement of particles, including molecules, in an electric field. The rate of migration is related to the total electrical charge on the molecules at the pH in question. Under standard conditions, each molecule has a characteristic rate of movement. In serum, the rate of migration of albumin is fastest and γ_2-globulin is slowest.

Endotoxins ..... See toxins.

Exotoxins ..... See toxins.

50% endpoint ..... Within a controlled standardized carefully performed titration, this point gives the least statistical and technical error.

Forssman antigen ..... A highly antigenic lipopolysaccharide complex with a protein carrier that varies according to source. It is found in different cells of a large variety of animal and plant species. In some animals, the presence of the Forssman antigen in the blood cells is associated with its absence from the general cells of the body, and vice versa.

Forssman hemolysin ..... The antibody that is formed by a Forssman-negative species, such as the rabbit, in response to the Forssman antigen. It is usually titrated by its hemolytic activity against sheep red cells or other cells which contain the Forssman antigen.

Freund's adjuvant ..... A commonly used adjuvant composed of a lanolin-like substance emulsified with (sometimes without) a suspension of tubercle bacilli. It is used mixed or emulsified with the antigen.

HeLa cells ..... Cells cultivated *in vitro* from a human epitheloid carcinoma of the cervix. The strain is continuously cultivated and readily available for experimental work (cf. Chapter 6, Section 4).

Hemolysin ..... An antibody that with the cooperation of complement lyses red cells (cf. Chapter 1, Section 4.1).

Homologous ..... Denotes some type of agreement, i.e., an antigen and its homologous antibody; also denotes of the same species but not of the same genetic strain, i.e., genetically different.

Immune clearance ..... The accelerated rate of disappearance of a radioisotope-labeled antigen from the circulation because of the appearance of antibody (cf. Fig. 1B).

Induction period ..... See *latent period*.

Innate immunity ..... Inborn nonspecific immunity, including innate resistance to infection (see Chapter 1, Section 1).

Irradiation or radiation .... Refers to X-rays and gamma-(γ-)rays in the present volume. In most cases, X-rays are produced by machines, and γ-rays, which are physically identical, are obtained from naturally occurring or man-made radioactive elements. Cobalt-60 is made in atomic energy piles. It and iodine-131 emit γ- and β-rays. Phosphorus-32 emits β-rays. Cesium-137 emits γ-rays. Tritium (H^3) emits β-rays.

Isologous ..... Denotes agreement with the same genetic strain of the species—genetically identical.

Latent period ..... The interval between the injection of antigen and the first detection of newly synthesized antibody. This period has also been termed the induction period (cf. Chapter 1, Section 4.1).

Myeloid cells ..... Usually refer to white cells of the granulocytic series, i.e., promyelocytes, metamyelocytes, heterophils, eosinophils, and basophils; also used to signify cells of bone marrow origin.

Nucleic acids ..... DNA, deoxyribonucleic acid, and RNA, ribonucleic acid, are the repository and carrier of genetic information. Certain forms, such as viruses, may have DNA to the exclusion of RNA and vice versa.

P_{80} test ..... A precipitin reaction in which the antigen is labeled with a radioisotope, usually I^{131}, and the amount of antigen is measured by its radioactivity. The end point of the titration for antibody is the dilution of serum which precipitates 80% of the antigen containing a known amount of protein.

Primary or initial response . The antibody response to the first injection or series of closely spaced injections of an antigen.

Radioisotopic labeling ..... The substitution of a radioactive atom for a stable atom in a compound or the attachment of a radical containing a radioactive atom to a compound. In either case the compound is measured by the radioactivity of the isotope.

Resistance to infection	See innate immunity.
RNA	See nucleic acids.
S	Svedberg or sedimentation constant that denotes the relative sedimentation rate of molecules under high-speed centrifugation. Thus, a 7 S molecule is of lower molecular weight than an 18 S molecule.
Secondary response	See anamnestic response.
6-Mercaptopurine (6-MP)	A purine analog that interferes with protein synthesis.
Titer	The amount of antibody per unit of serum—usually based on the highest dilution which gives the endpoint. See 50% endpoint.
Tolerance	See unresponsiveness in Chapter 8.
Toxin	In immunology, toxin is restricted to those poisons which are antigenic, such as various plant, animal, and bacterial toxins. The bacterial toxins are divided into exotoxins, which are potent poisons and excellent antigens, and endotoxins, which are less toxic as a rule but are good antigens.
Toxoid	Toxin treated in such a manner that it is no longer toxic but still retains its antigenicity and is capable of inducing the formation of antitoxins to the original toxin.
Tritium-labeled thymidine	Thymidine labeled with isotopic tritium (H^3) is an indicator of cell division because thymidine is a specific precursor of DNA and is only incorporated in cells during synthesis prior to division. Isotopic tritium can be detected in the cells by autoradiographic procedures in which disintegrating atoms of the isotope emit ionizing particles which sensitize the silver halide crystals in a photographic film emulsion that can be reduced to metallic silver grains in the presence of a developer used to develop photographic emulsions.
X-rays	See irradiation.

CONTENTS

CHAPTER 1

Immune Mechanisms

Interest in the effects of ionizing radiation on immune mechanisms is fourfold. Adequate radiation (1) will make the person or animal unresponsive to the antigen and thus allow the host to accept a foreign graft indefinitely or at least for a much longer period than would normally be expected, (2) will inhibit antibody formation and render individuals more susceptible to infection under certain conditions, (3) will enhance antibody formation under other conditions, and (4) can be useful as a "dissecting" tool to study the remarkable process of antibody formation. The first of these topics will be covered in another monograph in the present series. The last three will be considered in the present one.

1. Innate and Acquired Immune Mechanisms

The study of immune mechanisms had its origin in antiquity from the observation that one attack of certain diseases, which we now classify as infectious, leads to a greatly lessened chance of having the same disease again. In a broad sense, immune mechanisms are the reactions of the body in recognizing foreign material and in detoxifying it or removing it from the body's economy by digestion or by enclosing it in a dense connective tissue capsule.[18,266,267] Foreign materials include large metazoan parasites, bacteria, viruses, tissues, cells, toxins, and many other living and nonliving materials. Burnet[26] has written a unique résumé of immunity from the viewpoint of its importance in maintaining the structural and functional integrity of the body. Immune mechanisms,* as used here, are divided into (1) those that are acquired as a result of prior contact with the material and (2) those that are innate.

Innate or inborn mechanisms are characteristic of the species or individual, are genetically determined, and are characteristically nonspecific in the immunological sense.

Acquired mechanisms largely involve antibodies or antibody-like substances, which arise as a result of the parenteral introduction of antigens, mostly foreign proteins, into the body of higher vertebrates. Antibody formation has

* Some authors limit immune mechanisms to those involving antibody mechanisms and classify the innate factors as resistance or physiological mechanisms.

been studied chiefly in mammals and birds and has been described in a few fish, amphibia, and reptiles. The antigen-antibody reaction is a highly specific mechanism for recognizing and rejecting foreign material. For example, a specific serum globulin from one rabbit can be differentiated from the homologous serum globulin from another rabbit by antigen-antibody reactions. (These intraspecies differences are genetically determined.) From the viewpoint of the individual, the same mechanism, which protects against certain infections, may under other conditions give rise to disagreeable diseases, such as serum sickness, and at times death. The reaction between an antibody and its antigen or closely related antigens can also take place *in vitro*.

2. Antigen-Antibody Reactions

The original medical interest in acquired immune mechanisms continues to be of great importance in diagnosis and treatment, in the protection against disease by antigens in vaccines, in the acquisition of hypersensitivity to certain antigens, e.g., in hay fever, and in the rejection of grafts from one individual to another.

In addition, over the past 25 years, immunological reactions have become of increasing use to biologists, chemists, and physicists. This circumstance arises largely from the following two facts. Proteins are the essential chemicals that implement the genetic code of the cells, and the reactions between antigen and antibody are the prototype of the reactions between large molecules. Inasmuch as each chemical reaction within a cell involves at least one protein in the form of an enzyme, the number of different protein enzymes is enormous. In addition, protein antigens may be found in cell structures and in intracellular storage depots of food. The best method of identifying these individual proteins resides in antigen-antibody reactions.[32]

3. Diversity and Measurement of Antibodies

Some days after an antigen is introduced through the external barriers of the body, antibody appears in the serum, increases in concentration to peak or maximum titer, and eventually falls to a very low level. A second injection of the same antigen, as much as a year or more later, is followed by a similar sequence, which is generally faster and in many cases (depending upon the antigen) results in the formation of much more antibody (hence, the common term of "booster" injection of vaccine, i.e., of antigen). A distinction is thus made between primary and secondary (or anamnestic) responses.

Antibody is primarily defined in terms of its ability to combine with the

antigen which induced its formation or with closely related substances. All antibodies belong to several kinds of globulins, and many investigators believe that all globulins are antibodies and that the so-called normal globulins are antibodies to undetermined antigens. A single antigen may also stimulate the formation of antibodies with the same identical immunological specificity but with different physicochemical properties such as electrophoretic mobility, molecular weight, and avidity or firmness of the antigen-antibody union. For example, the heat-stable Forssman lipopolysaccharide component of sheep red cells induces the formation of two antibodies.[269] The large hemolytic one is an 18 S macroglobulin which localizes during electrophoresis with the γ_1-globulins, has a molecular weight of about 1,000,000 and a biological half-life of 2.8 days in the rabbit. The small relatively nonhemolytic one is a 6 S γ_2-globulin, which has a molecular weight of about 165,000 and a half-life of 5.6 days. The macroglobulin hemolysin appears a week or two sooner than the globulin of ordinary size during even multiple injections of fresh sheep red cells. Consequently, it is the one most studied in the radiation experiments on hemolysin in rabbits involving the Forssman antigen. The heat-labile isophile protein component of sheep red cells similarly leads to the production of a large and small antibody, but neither of these appears in appreciable amounts during the first two weeks of immunization.

Antigen-antibody reactions usually involve two stages: the first or primary union of antibody with antigen, and the second or visible stage. The primary stage is fundamentally the same in all reactions and may be demonstrated by absorption and inhibition tests. The secondary stage depends upon the physical state of the antigen, as well as upon environmental conditions, such as pH and salt concentration, and, in some cases, upon cooperative agents, such as phagocytes and complement.[22,199]

Antibodies are designated by the type of specific secondary reaction with which they are associated. An antibody is an hemolysin if it lakes or lyses whole red cells in the presence of complement, a normal component of serum. It is a precipitin if it precipitates a soluble protein in the presence of suitable electrolytes. It is an agglutinin if it causes a suspension of bacteria, red cells, or other particulate matter to aggregate into clumps. It is an anaphylactin or an anaphylactic antibody if it reacts with the antigen *in vivo* to produce anaphylaxis. It is an antitoxin if it neutralizes antigenic poisons, such as diphtheria toxin. Another common practice is to name the antibody after its antigen, i.e., anti-Forssman, anti-BSA (antibovine serum albumin), and antibrucella. Thus, antibodies of the same specificity may vary in their physicochemical structure, but at the same time an antibody preparation containing essentially only one type of molecule may act as a precipitin, an agglutinin, or a lysin if the antigen can be properly manipulated and the

proper accessories are supplied. A simple example is that a precipitin can act as a hemagglutinin if red cells are first coated with the antigen.

Among the methods used for the *in vitro* measurement of antibodies in radiation work are (1) the quantitative precipitin method and modifications of it, such as the P_{80} method using radioisotopes, (2) the Farr test which measures precipitating as well as nonprecipitating antibody, (3) the hemolysin test using a 50% endpoint and photometric methods, (4) agglutinin tests using particulate specific antigens or "passive" hemagglutinin methods in which normal red cells are coated with the soluble test antigen, and (5) diffusion-in-gel methods which aid in separating and identifying various antigenic factors and precipitating antibody components. *In vivo* methods of measuring anaphylactic antibody and antitoxin include endpoints of death or survival and, in the case of delayed hypersensitivity, skin tests, and passive transfer with lymphoid cells. The immune clearance test with I^{131}-labeled antigen is a measure of the length of the latent period. Detailed procedures for these methods are given in current immunological texts[22,28] and in the papers mentioned throughout this text and Table 1. Discussions of the methods may be found in Boyd,[22] Cushing and Campbell,[43] Holub and Jarošková,[103] and Heidelberger and Plescia.[98]

4. The Immune Response in Normal, i.e., Nonirradiated, Rabbits

Since X-rays affect all phases of the immune response, the primary and secondary responses in two types of the immune response will be outlined first in nonirradiated rabbits together with some conditions which influence these responses. As already mentioned, a first injection of antigen leads to a rise and fall of antibody, and a reinjection of the same antigen leads to a second rise and fall of antibody. Such responses constitute the controls by which the effects of irradiation are gauged.

One response to be described involves the Forssman antigen, a complex lipopolysaccharide with a protein carrier that occurs in many plants and animals, including the stroma or colorless framework of sheep red blood cells. When the whole red cell is used for immunization, the anti-Forssman γ_1 hemolysin can be separated from the other antibodies by its early appearance in the serum, or the Forssman antigen can be used for immunization after inactivation of the isophile protein antigen. This inactivation is attained by laking the red cells with water, which largely frees them of hemoglobin, and then boiling the residual stromata.

Another response to be described involves the highly purified soluble crystalline protein antigen, bovine serum albumin (BSA). The antibody is, in part, precipitating and, in part, nonprecipitating. Both types have the capacity

to form complexes with BSA, but one precipitates spontaneously, whereas the other does not.

Serum separated from blood samples taken at frequent intervals from animals injected with either of these two antigens can be measured or titrated quantitatively. The resulting titers or measurements of serum antibody can then be plotted against time, as shown in Fig. 1.

It should be pointed out that work on hemolysins and work on certain soluble antigens complement each other. Thus, the γ_1 hemolysin is so highly hemolytic that small amounts can be detected and a negative test for it is highly significant; whereas precipitins, although they have to be present in relatively large amounts before they can be detected, can be collected in large amounts for purification, analysis, and weighing.

4.1. The Hemolysin Response[28,277,286]

The initial hemolysin response in the rabbit following a first injection of the Forssman antigen is shown in Fig. 1A. It can be conveniently divided into the following three main parts: (1) a latent period* extending from the injection of antigen to the detection of antibody in the serum, (2) a production period during which antibody is rapidly synthesized and liberated into the serum and which extends from the appearance of antibody in the serum to maximal or peak titer, and (3) a period of constant or decreasing antibody synthesis after peak titer that leads eventually to low levels of antibody or, if continued antigenic stimulation is given, to immunological unresponsiveness to that specific antigen.

Major portions of this rise and fall of hemolysin are linear during the period of increase and decrease in levels of serum antibody when log titer is plotted against time.[266,277,279] These linear segments can each be described by the first-order equation

$$\frac{dA}{dt} = kA \tag{1.1}$$

or in the integrated form

$$A_t = A_0 e^{kt} \tag{1.2}$$

In the above equations, the values of the rate constants, k, are determined by the equation

$$k = \frac{\ln A_t - \ln A_0}{t} \tag{1.3}$$

* Through 1962, we used induction period for this interval, but we have now adopted the term "latent period", because this period is itself composed of an early short induction stage and a longer antibody-synthesizing stage, as irradiation and other conditions demonstrate (see Chapters 3–6).