MOLECULAR BIOLOGY

An International Series of Monographs and Textbooks

The Lectins

Properties, Functions, and Applications in Biology and Medicine

Edited by

IRVIN E. LIENER

NATHAN SHARON

IRWIN J.GOLDSTEIN

The Lectins

Properties, Functions, and Applications in Biology and Medicine

Molecular Biology

An International Series of Monographs and Textbooks

Editors

BERNARD HORECKER
Graduate School of Medical Sciences
Cornell University Medical College
New York, New York

NATHAN O. KAPLAN
Department of Chemistry
University of California
at San Diego
La Jolla, California

JULIUS MARMUR
Department of Biochemistry
Albert Einstein College of Medicine
Yeshiva University
Bronx, New York

HAROLD A. SCHERAGA
Department of Chemistry
Cornell University
Ithaca, New York

Recently published titles

TERRANCE LEIGHTON AND WILLIAM F. LOOMIS, JR. (Editors). The Molecular Genetics of Development: An Introduction to Recent Research on Experimental Systems. 1980

ROBERT B. FREEDMAN AND HILARY C. HAWKINS (Editors). The Enzymology of Post-Translational Modification of Proteins, Volume 1, 1980. Volume 2, 1985

WAI YIU CHEUNG (Editor). Calcium and Cell Function, Volume I: Calmodulin, 1980. Volume II, 1983. Volume III, 1982. Volume IV, 1983. Volume V, 1984. Volume VI, 1986

OLEG JARDETZKY AND G. C. K. ROBERTS. NMR in Molecular Biology. 1981

DAVID A. DUBNAU (Editor). The Molecular Biology of the Bacilli, Volume I: *Bacillus subtilis,* 1982. Volume II, 1985

GORDON G. HAMMES. Enzyme Catalysis and Regulation. 1982

GUNTER KAHL AND JOSEF S. SCHELL (Editors). Molecular Biology of Plant Tumors. 1982

P. R. CAREY. Biochemical Applications of Raman and Resonance Raman Spectroscopies. 1982

OSAMU HAYAISHI AND KUNIHIRO UEDA (Editors). ADP-Ribosylation Reactions: Biology and Medicine. 1982

G. O. ASPINALL (Editor). The Polysaccharides, Volume 1, 1982. Volume 2, 1983. Volume 3, 1985

CHARIS GHELIS AND JEANNINE YON. Protein Folding. 1982

ALFRED STRACHER (Editor). Muscle and Nonmuscle Motility, Volume 1, 1983. Volume 2, 1983

IRVIN E. LIENER, NATHAN SHARON, AND IRWIN J. GOLDSTEIN (Editors). The Lectins: Properties, Functions, and Applications in Biology and Medicine. 1986

The Lectins

Properties, Functions, and Applications in Biology and Medicine

Edited by

Irvin E. Liener

Department of Biochemistry
University of Minnesota
St. Paul, Minnesota

Nathan Sharon

Department of Biophysics
The Weizmann Institute of Science
Rehovot, Israel

Irwin J. Goldstein

Department of Biological Chemistry
University of Michigan
Ann Arbor, Michigan

1986

ACADEMIC PRESS, INC.

Harcourt Brace Jovanovich, Publishers

Orlando San Diego New York Austin
London Montreal Sydney Tokyo Toronto

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data
Main entry under title:

The Lectins : properties, functions, and applications in biology and medicine.

Includes bibliographies and index.
1. Lectins. 2. Plant lectins. I. Liener, Irvin E.
II. Sharon, Nathan. III. Goldstein, Irwin Joseph.
[DNLM: 1. Lectins. QW 640 L471]
QP552.L42L425 1986 574.19'245 85-20100
ISBN 0–12–449945–7 (alk. paper)

PRINTED IN THE UNITED STATES OF AMERICA

86 87 88 89 9 8 7 6 5 4 3 2 1

Contents

6 Distribution and Function of Plant Lectins

Marilynn E. Etzler

7 Vertebrate Lectins: Properties and Functions

Samuel H. Barondes

8 Lectins in Cellular Slime Molds

Samuel H. Barondes

9 Bacterial Lectins

Nathan Sharon

Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Samuel H. Barondes (437, 467), Department of Psychiatry, University of California, San Diego, School of Medicine, La Jolla, California 92093, and Veterans Administration Medical Center, San Diego, California 92161

Dominique Buffard (249), Molecular Immunology, Institute J. Monod, CNRS and University of Paris VII, 75251 Paris, France

Marilynn E. Etzler (371), Department of Biochemistry and Biophysics, University of California, Davis, Davis, California 95616

Andre Foriers (249), Medical and Special Biochemistry, Pharmaceutical Institute, Free University of Brussels (V. U. B.), Brussels B 1090, Belgium

Irwin J. Goldstein (33), Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan 48109

Jan Kocourek (1), Department of Biochemistry, Charles University, Prague, Czechoslovakia

Mark Lauwereys (249), Protein Chemistry, Institute of Molecular Biology, Free University of Brussels, (V. U. B.), Sint Genesius-Rode B1640, Belgium

Irvin E. Liener (527), Department of Biochemistry, University of Minnesota, St. Paul, Minnesota 55108

Halina Lis (265, 293), Department of Biophysics, The Weizmann Institute of Science, Rehovot 76100, Israel

Ronald D. Poretz (33), Department of Biochemistry, Rutgers University, New Brunswick, New Jersey 08903

Nathan Sharon (265, 293, 493), Department of Biophysics, The Weizmann Institute of Science, Rehovot 76100, Israel

A. Donny Strosberg (249), Molecular Immunology, Institute J. Monod, CNRS and University of Paris VII, 75251 Paris, France

This page intentionally left blank

Preface

Since the introduction of the term "lectin" (from the latin *legere,* to pick out, choose) by Boyd and Shapleigh (1954) to describe a class of proteins of plant origin which agglutinate cells and exhibit antibody-like sugar binding specificity, the subsequent discovery of many similar substances in both animal as well as plant tissue has prompted several attempts to reach common agreement as to how to define a lectin (Goldstein *et al.,* 1980; Kocourek and Horejsi, 1981; Franz *et al.,* 1982). We have chosen to define a lectin simply as a carbohydrate-binding protein of nonimmune origin that agglutinates cells or precipitates polysaccharides or glycoconjugates (Goldstein *et al.,* 1980), a definition adopted by the Nomenclature Committee of the International Union of Biochemistry (Dixon, 1981). This definition implies that lectins are multivalent, that is, they possess at least two sugar binding sites which enable them to agglutinate animal and plant cells and/or to precipitate polysaccharides, glycoproteins, peptidoglycans, teichoic acids, glycolipids, etc. The sugar specificity of lectins is usually defined in terms of the monosaccharide(s) that inhibits lectin-induced agglutination or precipitation reactions. The emphasis on "nonimmune origin" is included in the definition in order to distinguish lectins from anti-carbohydrate antibodies which may act as cell agglutinins. Furthermore, many lectins are found in plants and bacteria that do not synthesize immunoglobulins. Also, in contrast to antibodies which are structurally similar to each other, lectins are structurally diverse and are known to vary in molecular size, amino acid composition, metal requirement, and three-dimensional structure. In this regard, as well as with respect to specificity, lectins are more akin to enzymes.

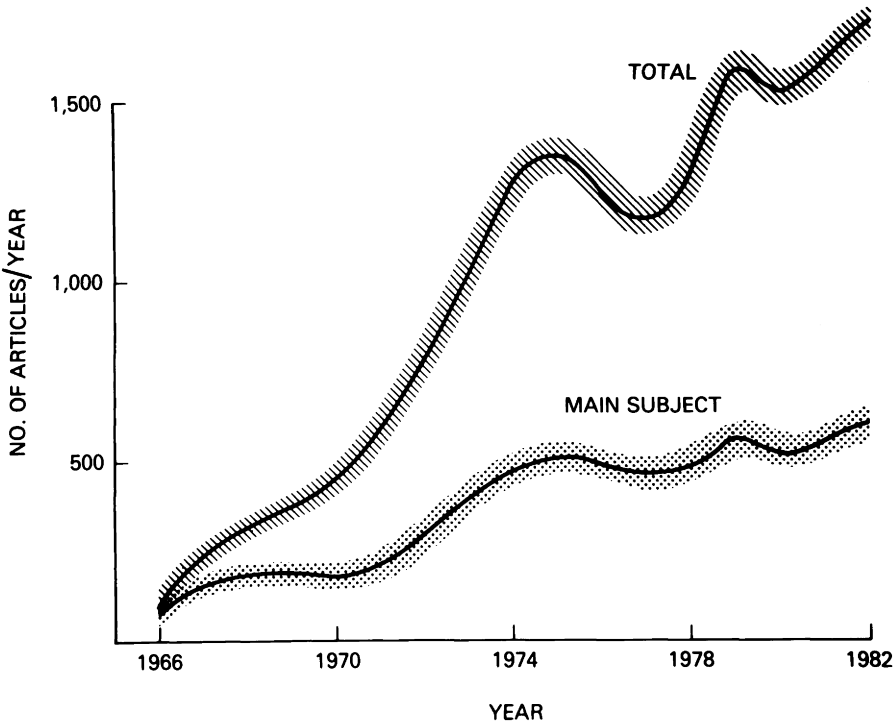
The definition of lectins as used here is an operational one (i.e., evidence of agglutination or precipitation) which requires the presence of more than one sugar binding site. Accordingly, this definition would exclude a wide variety of other sugar-binding proteins such as sugar-specific enzymes [i.e., glycosidases, glycosyltransferases, glycosylkinases, glyco-

sylpermeases, glycosylepimerases, sugar transport proteins, hormones (e.g., thyroid-stimulating hormone), chemotaxis receptors, bacterial toxins, and interferons]. Perhaps the most controversial aspect of this definition is the fact that, if rigorously applied, it would exclude such toxic plant proteins as ricin, abrin, and modeccin. These are proteins which may be found in the same plant in which "true" lectins are found and closely resemble the latter in terms of amino acid composition and possibly primary structure. However, they are poor agglutinins, if at all, and for this reason were once thought to lack multiple sugar binding sites. More recently, however, ricin has been shown to possess two carbohydrate binding sites (Houston and Dooley, 1982).

Although known for nearly a century, it is only during the last decade or two that lectins have become the focus of intense interest. This is evidenced by the rapid growth of the literature on the subject which, since the 1960s, has increased nearly twentyfold. The total number of publications that deal with various aspects of lectins is fast approaching 2000 per year (see figure). There are many reasons for the current interest in lectins. Prominent among these is their usefulness in detecting and studying carbohydrates in solution and on cell surfaces. As a result, lectins have become indispensable tools in many areas of biological research. Another reason is that lectins are believed to serve as recognition determinants in a variety of biological systems in microorganisms, plants, and animals. Bacterial surface lectins mediate the sugar-specific adherence of bacteria to epithelial cells, which is an essential prerequisite for infection. Plant lectins, on the one hand, may act as protective agents against fungal phytopathogens and, on the other hand, may mediate the attachment of nitrogen-fixing bacteria to the roots of leguminous plants. In animals, membrane-associated lectins appear to function in the clearance of glycoproteins from the circulatory system and in the intracellular translocation and targeting of glycoproteins.

With the expansion of our knowledge about lectins, it became apparent that they deserve attention in their own right. Some of them exhibit unusual structural properties. They constitute a convenient source of well-defined plant glycoproteins, and are excellent models for studying protein–carbohydrate interactions. Furthermore, the availability of lectins from different species and tribes of the same family (e.g., of plants or bacteria) makes them suitable objects for taxonomic and phylogenetic studies as well as for evolutionary correlations (Goldstein and Etzler, 1983).

Contributing to the increasing popularity of lectins is not only the ease of their purification (mainly by affinity chromatography on immobilized carbohydrates) but also their increased availability from commercial



Growth of the lectin literature from 1966 to 1982, based on citations in the Medlars System. The decrease in the annual number of publications in the middle of the 1970s was due to a change in the data base. Data obtained from Dr. Elizabeth J. Van Lenten at the National Library of Medicine, National Institutes of Health, Bethesda, Maryland.

sources. Well over a hundred lectins have been purified to date, and more than forty of these are available from a large number of companies.

The voluminous literature generated by this interest in lectins has made it extremely difficult for the nonspecialist to keep abreast of the latest developments as they might impinge on the researcher's particular area of specialization. Numerous reviews and monographs dealing with selected aspects of lectins have appeared. Among these, mention should be made of reviews covering such specific topics as lectins from plants (Liener, 1976; Kauss, 1981; Lis and Sharon, 1981; Goldstein and Etzler, 1983), slime molds and animals (Barondes, 1981, 1984; Olden and Parent, 1986), invertebrates (Yeaton, 1982; Cohen, 1984), and membranes (Ashwell and Harford, 1982; Monsigny *et al.*, 1983). Other reviews deal with the possible role of lectins in nature (Sharon, 1979, 1984b; Schmidt, 1979; Schmidt and Bohlool, 1981; Sequeira, 1978; Dazzo and Sherwood, 1983) and the application of lectins to the study of glycoconjugates in solution and on

cells surfaces (Lis and Sharon, 1984), immunology (Lis and Sharon, 1977; Sharon, 1983), blood typing (Bird, 1978; Judd, 1980), microbiology (Pistole, 1981; Doyle and Keller, 1984), isolation of glycoproteins (Lotan and Nicolson, 1979; Lis and Sharon, 1984; Hedo, 1984), cell identification and separation (Sharon, 1983), and histochemistry (Schrevel *et al.*, 1981; Leathem and Atkins, 1983; Alroy *et al.*, 1984). A monograph on concanavalin A describes the many applications of this lectin (Bittiger and Schnebli, 1976), but the procedures described are also applicable to other lectins. Techniques for the purification of lectins and some of their applications can be found in several volumes of *Methods in Enzymology* (Agrawal and Goldstein, 1972; Ashwell and Kawasaki, 1978; Jackson *et al.*, 1982; Barker *et al.*, 1974).

Valuable as these reviews undoubtedly are, there is clearly a need for a comprehensive, up-to-date treatment of lectins which would serve not only as an introduction for the nonspecialist but also as a sourcebook for the specialist whose research involves the use of lectins. In an attempt to accomplish this goal, this book includes chapters devoted to diverse but selected topics of lectins written by active researchers in their respective fields. Chapter 1 provides a fascinating insight into the historical development of lectins and sets the stage for the more specialized topics that follow. Chapter 2 should be of principal interest to the more chemically oriented investigator desiring detailed information on the physicochemical properties of lectins, their isolation, and remarkable specificity toward sugars.* Chapter 3 should be of interest to those whose research deals with the molecular aspects of protein evolution, since the lectins provide an excellent example of a family of homologous proteins. One of the most interesting and important features of lectins is the diversity of their biological activities (Chapter 4) and how these properties have been utilized for the isolation and characterization of carbohydrate-containing compounds in solution and on cells (Chapter 5). Chapter 6 attempts to answer the ever-recurring question regarding the functions of the lectins in their natural milieu. Chapters 7, 8, and 9 serve to emphasize the importance of lectins in nonplant systems as exemplified by lectins that occur in vertebrates, slime molds, and bacteria respectively. Chapter 10 deals with an area which, until recently, has received scant attention, namely, the nutritional significance of the occurrence of lectins in plant foods such as legumes.

The literature pertaining to lectins continues to proliferate at a seemingly unabated rate. For this reason, a book such as this can at best portray only the current status of the field, but even so, many facets of the

* All sugars referred to in this book are of the D-configuration unless otherwise noted.

subject may not have received the attention that some may feel they deserve. Nevertheless, it is our hope that this book will serve as a reference source for those who choose, for whatever reason, to learn more about this unique class of proteins and their applications to biology and medicine.

Irvin E. Liener
Nathan Sharon
Irwin J. Goldstein

REFERENCES

Agrawal, B. B. L., and Goldstein, I. J. (1972). *In* "Methods in Enzymology" (V. Ginsburg, ed.), Vol. 28, pp. 313–383. Academic Press, New York.

Alroy, J., Ucci, A. A., and Pereira, M. E. A. (1984). *In* "Advances in Immunohistochemistry" (R. A. DeLellis, ed.), pp. 67–88. Masson, Inc., New York.

Ashwell, G., and Harford, J. (1982). *Ann. Rev. Biochem.* **51,** 531–554.

Ashwell, G., and Kawasaki, T. (1978). *In* "Methods in Enzymology" (V. Ginsburg, ed.), Vol. 50, pp. 287–372. Academic Press, New York.

Barker, R., Chiang, C.-K., Trayer, I. P., and Hill, R. L. (1974). *In* "Methods in Enzymology" (W. B. Jakoby and M. Wilchek, eds.), Vol. 34, pp. 317–367. Academic Press, New York.

Barondes, S. H. (1981). *Ann. Rev. Biochem.* **50,** 207–231.

Barondes, S. H. (1984). *Science* **223,** 1259–1264.

Bird, G. W. G. (1978). *Proc. XII Congr. Int. Soc. Haematol. Paris*, 87–95.

Bittiger, H., and Schnebli, H. P., eds. (1976). "Concanavalin A as a Tool." Wiley, London.

Boyd, W. C., and Shapleigh, E. (1954). *Science* **119,** 419.

Cohen, E., ed. (1984). "Recognition Proteins, Receptors and Probes: Invertebrates." Alan Liss, Inc., New York.

Dazzo, F. B., and Sherwood, J. E. (1983). *In* "Chemical Taxonomy, Molecular Biology and Function of Plant Lectins" (I. J. Goldstein and M. E. Etzler, eds.), pp. 203–223. Alan Liss, Inc., New York.

Dixon, H. B. F. (1981). *Nature* **292,** 192.

Doyle, R., and Keller, K. (1984). *Eur. J. Clin. Microbiol.* **3,** 4–9.

Franz, H., Ziska, P., and Mohr, J. (1982). *Acta Histochem.* **71,** 19–21.

Goldstein, I. J., and Etzler, M. E., eds. (1983). "Chemical Taxonomy, Molecular Biology and Function of Plant Lectins." Alan Liss, Inc., New York.

Goldstein, I. J., Hughes, R. C., Monsigny M., Osawa, T., and Sharon, N. (1980). *Nature* **285,** 66.

Hedo, J. (1984). *In* "Receptor Biochemistry and Methodology. Vol. 2: Receptor Purification Procedures" (J. C. Venter and L. Harrison, eds), pp. 45–66. Alan Liss, Inc., New York.

Houston, L. L., and Dooley, T. P. (1982). *J. Biol. Chem.* **257,** 4147–4151.

Jackson, K. W., Esmon, N., and Tang, J. (1982). *In* "Methods in Enzymology" (L. Lorand, ed.), Vol. 80, pp. 387–391. Academic Press, New York.

Judd, W. J. (1980). *CRC Rev. Clin. Lab. Sci.* **1,** 171–214.

Kauss, H. (1981). *Encyclopedia Plant Physiol.* **13B,** 627–657.
Kocourek, J. and Horejsi, V. (1981). *Nature* **290,** 188.
Leathem, A. J. C., and Atkins, N. J. (1983). *In* "Techniques in Immunocytochemistry," Vol. 2 (G. R. Bullock and P. Petrusz, eds.), pp. 39–70. Academic Press, New York.
Liener, I. E. (1976). *Ann. Rev. Plant Physiol.* **27,** 291–319.
Lis, H., and Sharon, N. (1977). *In* "The Antigens," Vol. 4 (M. Sela, ed.), pp. 429–529. Academic Press, New York.
Lis, H., and Sharon, N. (1981). *In* "Biochemistry of Plants," Vol. 6. (A. Marcus, ed.), pp. 371–447. Academic Press, New York.
Lis, H., and Sharon, N. (1984) *In* "Biology of Carbohydrates," Vol. 2. (V. Ginsburg and P. W. Robbins, eds.), pp. 1–85. John Wiley, New York.
Lotan, R., and Nicolson, G. L. (1979). *Biochim. Biophys. Acta* **559,** 329–376.
Monsigny, M., Kieda, C., and Roche, A. C. (1983). *Biol. Cell.* **47,** 95–110.
Olden, K., and Parent, J. M., eds. (1986). "Vertebrate Lectins, Recent Research." Van Nostrand and Reinhold Co., New York (in press).
Pistole, T. G. (1981). *Ann. Rev. Microbiol.* **35,** 85–112.
Schrevel, J., Gros, D., and Monsigny, M. (1981). *Progr. Histochem. Cytochem.* **14,** 1–269.
Schmidt, E. L. (1979). *Ann. Rev. Microbiol.* **33,** 355–376.
Schmidt, E. L., and Bohlool, B. B. (1981). *Encyclopedia Plant Physiol.* **13B,** 658–677.
Sequeira, L. (1978). *Ann. Rev. Phytopathol.* **16,** 453–481.
Sharon, N. (1979). *In* "Glycoconjugate Research," Vol. 1 (J. D. Gregory and R. W. Jeanloz, eds.), pp. 459–491. Academic Press, New York.
Sharon, N. (1983). *In* "Advances in Immunology," Vol. 34 (F. J. Dixon and H. G. Kunkel, eds.), pp. 213–298. Academic Press, New York.
Sharon, N. (1984). *Immunology Today* **5,** 144–147.
Yeaton, R. W. (1982). *In* "Developmental Immunology: Clinical Problems and Aging" (E. L. Cooper and M. A. B. Brazier, eds.), pp. 73–84. Academic Press, New York.

The Lectins

Properties, Functions, and Applications in Biology and Medicine

This page intentionally left blank

1

Historical Background

JAN KOCOUREK

THE LECTINS: PROPERTIES,
FUNCTIONS, AND APPLICATIONS
IN BIOLOGY AND MEDICINE

The concept of lectins as a class of proteins or glycoproteins different from immunoglobulins, having binding sites for carbohydrates without inducing any chemical changes on them, is relatively new, actually newer than the term "lectin" itself (Boyd, 1954). However, the existence of lectins in biological materials, especially in plant seeds, where they manifest their presence by their physiological effects, can be traced far back into history. This is true especially of the toxic effects of some lectins like ricin (Tschirch, 1912; Olsnes and Pihl, 1982) and abrin (Tschirch, 1925; Olsnes and Pihl, 1982). In fact, the first investigations of lectin-containing seeds were aimed at elucidating toxic principles of the plant materials used for medicinal purposes. This research, undertaken in the penultimate decade of the last century in different parts of the world, can be considered a prelude to lectin history. Bruylants and Vennemann (1884) in Belgium and, simultaneously, Warden and Waddel (1884) working under Robert Koch in India reported on their investigation of the toxic principle of the jequirity seeds (*Abrus precatorius*). The last named authors were the first to anticipate the protein nature of the toxin, which they characterized as a "phytoalbumose." Dixson (1887) demonstrated that the toxin of castor beans (*Ricinus communis*) is contained in aqueous extracts of the beans; he also assumed that the toxin was a protein.

However, the finding by Stillmark (1888) of the hemagglutinating properties of ricin is usually considered to be the beginning of the actual lectin story. The period of almost a century that has elapsed since his discovery can be divided into several stages characterized by some highlights in the advancement of research and knowledge. The division into periods that follows is necessarily an arbitrary one; the importance of some boundary marks may be considered differently by various workers. Nevertheless, the start of the modern era of lectin investigation can be defined relatively easily: it was the first application of a chemically well defined affinity carrier for isolation of concanavalin A in the mid-1960s (Agrawal and Goldstein, 1965). Affinity chromatography of lectins opened the way for both extensive and intensive research of these substances on a molecular level and for their wider application.

The developments in the lectin field within the years 1888–1964 form the main subject of the present chapter. In this period the research of lectins was confined mostly to the "serological" level and, as a rule, only the biological activities of unpurified lectins in extracts of plant and animal tissues or body fluids were investigated. Erythroagglutination was then the most frequently followed activity and "hemagglutinins" the most frequent designation for lectins. It should be borne in mind, however, that in some cases reported here the substances causing hemagglutination have still not been isolated and fully characterized.

I. THE EARLY OR CLASSICAL PERIOD (1888–1918)

A. Plants

During the work on his doctoral thesis in 1887–1888 in Kobert's laboratory at the University of Dorpat (now Tartu), Stillmark (1888, 1889) investigated the constituents of seeds of some plants belonging to the Euphorbiaceae family. When he obtained from castor beans a partially purified proteinaceous preparation that he called ricin, he also tested its effects on blood. He observed that upon addition of ricin the red blood cells stuck together "like in clotting." Erythrocytes of different animals reacted differently. Similar differences were also found in the action of crotin, another toxin isolated by Stillmark from the seeds of *Croton tiglium*. Crotin showed an agglutinating activity different from that of ricin toward erythrocytes of the same animal species. Stillmark also observed agglutination of liver cells, epithelial cells, and leukocytes by ricin. His work started a series of theses and papers on agglutinating toxins by Kobert's school. Hellin (1891) described the agglutinating and serum-precipitating properties of a toxic extract of jequirity (*Abrus precatorius*) seeds. Elfstrand (1897, 1898) showed in a clean-cut experiment differences in behavior of crotin toward different animal erythrocytes. Some of them were hemolyzed (rabbit, crow), some strongly agglutinated (ox, pig, sheep, pike, perch, and frog), some slightly (cat), some very slightly (human), and some were completely unaffected (pigeon, guinea pig, dog, rat, chicken, and goose). About 15 years later analogous results were published by Kobert (1913), who used abrin, crotin, and ricin in a similar comparative study. Also Stillmark's original observation of agglutination of various kinds of animal cells was subsequently confirmed by other workers (Lau, 1901; Michaelis and Steindorff, 1907; Kobert, 1913). An interesting observation was recorded by Woronzow (1910), who found that liver effectively absorbed ricin from a solution introduced by perfusion. The fourth vegetable toxin, designated robin, was isolated by Power and Cambier (1890) from the bark of black locust (*Robinia pseudoacacia*). Later a hemagglutinin from black locust seeds was isolated and found to be different from robin (Mendel, 1909; Kobert, 1913). At that time the agglutinating and/or hemolyzing properties of the preparations were ascribed to the toxic principles and it was to be more than half a century before the lectins responsible for the toxic effects were separated from those causing agglutination; this has been the case at least with abrin and ricin (Olsnes and Pihl, 1982).

The early papers on erythroagglutination by plant toxins had a definite stimulating effect on the research in serology and immunology. Stillmark discovered the erythroagglutinating property of ricin less than a decade

after the first description of erythroagglutination by proteins of animal origin (Landois, 1875). At the suggestion of Kobert the term "hemagglutinin" (Blutkörperchenagglutinin) was introduced for the first time by Elfstrand (1898) for plant proteins that cause clumping of cells, due to the "striking similarity" of their activity to that of human and animal serum agglutinins.

The toxic properties of abrin and ricin very soon attracted the attention of the father of modern immunology, Paul Ehrlich (Fig. 1), who recognized the value of abrin and ricin as antigenic model substances and their advantages over the then frequently used diphtheria toxin (1891a,b). With abrin and ricin Ehrlich carried out a number of experiments that established some of the fundamental concepts of immunology. Rabbits fed with small amounts of jequirity seeds developed a certain degree of immunity against abrin. Immunity could be increased by the additional parenteral administration of the toxic protein. Ehrlich was able to show the specificity of the proteins (i.e., antibodies) found in serum of animals after administration of abrin and ricin. The anti-abrin could neutralize the activity of abrin but not that of ricin and vice versa. The toxins could also be specifically precipitated by proteins found in serum in response to the administration of the toxins, and Ehrlich could demonstrate a quantitative rela-

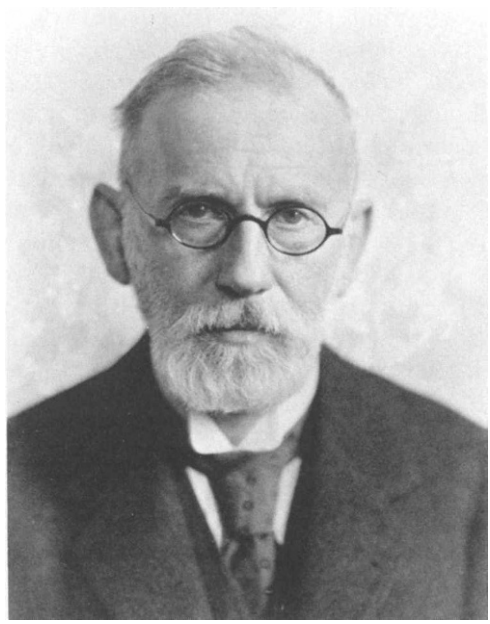


Fig. 1. Paul Ehrlich. [Photograph courtesy of the National Library of Medicine.]

tionship between the amount of antiserum and the amount of toxin that could be neutralized by it. Another important observation revealed that during pregnancy immunity to the toxins was transferred from mother to the offspring in the blood and that after birth it may be passed through the milk. With the ricin–anti-ricin system Danysz (1902) demonstrated the so-called Danysz phenomenon: when toxin is added to antitoxin, the toxicity of the mixture depends partly on the way in which the toxin is added. If an equivalent of toxin is added all at once the mixture is nontoxic, but if it is added at intervals, in fractions, the final mixture is generally toxic.

Another great figure of the classical period of immunology, Karl Landsteiner (1902) (Fig. 2), started to study plant agglutinins in the early years of this century after he had made his discovery of blood groups of the ABO system in 1900 (Landsteiner, 1901). He and Raubischek (1907) described for the first time the presence of some nontoxic lectins in the seeds of plants of the Fabaceae (Leguminosae, Viciaceae) family, such as in beans (*Phaseolus vulgaris*), pea (*Pisum sativum*), lentil (*Lens culinaris*), and vetch (*Vicia sativa*). Landsteiner and Raubitschek (1907) identified these plant agglutinins as proteins and showed that they were water-soluble, nondialyzable, insoluble in alcohol, thermolabile, could be salted out by electrolytes, and gave positive biuret and xanthoprotein reactions. Landsteiner (1902) could also demonstrate the reversibility of the ricin and abrin interaction with erythrocytes. He showed that lectins attached to the red cells can be liberated by raising the temperature to 50°C. Although he also recognized the different reactivity of various

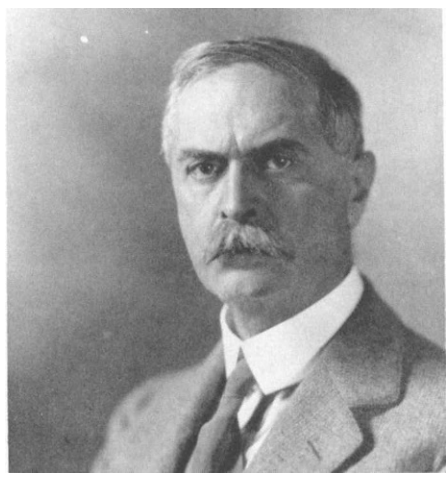


Fig. 2. Karl Landsteiner. [Photograph courtesy of the Rockefeller University Archives.]

lectins with red blood cells, Landsteiner did not seem to search for lectins applicable as blood typing reagents; the first unsuccessful efforts in this direction were recorded some 20 years later by Marcusson-Begun (1926) and Sievers (1927).

Although the inhibitory properties of serum on hemagglutination by ricin were already briefly mentioned by Stillmark (1888), Kraus (1902) was probably the first who made a detailed study of this phenomenon. Raubitschek (1909) demonstrated further that even heat-inactivated serum retains its inhibitory properties in agglutination by ricin. Landsteiner and Raubitschek (1909) also observed that porcine gastric mucin caused "deagglutination" of erythrocytes agglutinated by ricin, abrin, or bean extracts. Although the authors were unaware of it, these early reports showed for the first time inhibition of hemagglutination by carbohydrate substances present in the serum or in the mucin.

The period between the turn of the century and World War I represents one of the most fruitful intervals in the early lectin (hemagglutinin) research. Following the paper of Landsteiner and Raubitschek (1907) a number of contributions appeared reporting on discoveries of new nontoxic plant hemagglutinins (Lenze, 1909; Suga, 1910; Assmann, 1911; Schneider, 1912). Systematic search for hemagglutinins showed that they can be found predominantly in seeds of the Fabaceae (Leguminosae), Euphorbiaceae, and Solanaceae families. There were also first observations on preferential localization of lectins in certain structures of the seeds and various other parts of plants, e.g., in potato tubers (Marcusson-Begun, 1926) or in the milky sap (latex) of plants of the Euphorbiaceae family (von Eisler and von Portheim, 1912). Schneider (1912) followed the fate of hemagglutinin in *Phaseolus multiflorus* seeds and stated that it disappears during seedling development from the cotyledons at the same time as storage substances. No hemagglutinin was present in stems, leaves, or roots.

B. Fungi

Though the presence of a hemolytic agent in *Amanita phalloides* was already reported by Kobert (1893), Ford (1907) seems to be the first who demonstrated the presence of hemagglutinins in mushrooms (higher fungi)—*Amanita muscaria* and *A. solitaria*.

C. Bacteria and Viruses

During the first two decades of this century there were also reports of agglutinins of nonvegetable origin, e.g., bacteria or animals; many of

them would conform to the present-day definition of lectins. Some, however, have not as yet been well characterized.

Kraus and Ludwig (1902) were the first to demonstrate hemagglutination of rabbit erythrocytes by bacterial agglutinins. They used cultures and filtrates of *Staphylococcus aureus* and two strains of *Vibrio*. Flexner (1912) reported the presence of hemagglutinins in filtrates of *Bacillus pyocyaneus, B. typhosus,* and *Staphylococcus aureus*. Kayser (1903) discovered hemagglutinating properties of *Escherichia coli* and Guyot (1908) was the first to report that this property of *E. coli* was associated with the bacterial cell; there was no agglutinin present in filtrates. Fukuhara (1909) found agglutinins in a number of bacteria and showed their selective activity toward erythrocytes of different animals.

At about the same time as hemagglutination by bacteria, chicken plague virus adhesion to erythrocytes was also described for the first time by Landsteiner (1906) and Russ (1906).

D. Invertebrate Animals

Noguchi (1902, 1903) reported the erythroagglutinating properties of the hemolymph of two crustaceans, the horseshoe crab (*Limulus polyphemus*) and the American lobster (*Homarus americanus*). Hemolymph of another crustacean, *Eupagurus prideauxii,* was found by Cantacuzène (1912) to exert erythroagglutination and hemolysis with rabbit and sheep red cells and agglutination of some bacteria. Later the same author (Cantacuzène, 1919) demonstrated the presence of a hemagglutinin and precipitin of mammalian sera in the hemolymph of *Eupagurus bernardus, Homarus vulgaris, Maia squinado,* and some tunicates. He was, however, unsuccessful when he tried to find a hemagglutinin in the hemolymph of the Roman snail, *Helix pomatia* (Cantacuzène, 1915). The lectin that is present in the albumin gland of the animal was found half a century later in its whole body extract (Prokop *et al.,* 1965).

E. Vertebrate Animals

The early period brought only a few papers on lectins or, to use the earlier terminology, hemagglutinins of vertebrates. Probably the first of them were concerned with investigation of the effects of snake venoms and their erythroagglutinating and hemolyzing activities. Mitchell and Reichert (1886), Mitchell and Steward (1897), and later Flexner and Noguchi (1902) observed agglutination by a number of snake venoms. The latter authors did recognize the complexity of the venom composition:

some of the constituents causing agglutination of erythrocytes or leukocytes and others responsible for cytolysis.

F. The Early Methodology and Achievements

In spite of a number of lectins discovered outside the plant kingdom, vegetable lectins from the early period through our time always represented the vast majority. This was doubtless because of the ease of availability of the plant material and relatively simple isolation procedures. In the classical period most of the hemagglutinins were studied in the form of crude saline extracts. Protein fractions were also isolated by precipitating the aqueous extracts with ethanol and ethyl ether to obtain dry preparations soluble in physiological saline. Also fractional precipitation with salts was already in use.

An elegant isolation procedure, the use of which was probably the first case of biospecific adsorption applied to lectin isolation, was developed by von Liebermann (1907) and applied to isolation of ricin by Kobert (1913). Erythrocytes agglutinable by the lectin were used for specific adsorption of ricin from the saline extract of castor beans. After separation of the erythrocytes the lectin was obtained by desorption with an acidic solution. A similar procedure was used also by Münk (1914), who separated lectins (called "phasins" by him) of *Phaseolus vulgaris* and *Canavalia ensiformis* seeds by adsorption onto liver cells and rabbit erythrocytes, respectively.

Just as is currently done, agglutination of erythrocytes was the usual method used for detection of lectins and estimation of their activity; however, in the early studies, the specific procedures used varied considerably. Often whole blood or diluted whole blood was used instead of washed erythrocytes. In some cases extremely high agglutinating titers were reported [up to the order of 10^7, see, e.g., Kobert (1913)] that are difficult to reconcile with our present results. However, in spite of primitive conditions of work, the skilled and persistent scientists of the early period accumulated a wealth of knowledge for years to come. Many of their findings were "rediscovered" several times in the following decades, and it still pays today to look up some of the pioneering papers.

When summarizing the fundamental observations of this early period of lectin research the following seem to be the most important: establishing the protein nature of lectins [although there were also opposing views (Müller, 1899; Wienhaus, 1909; von Eisler and von Portheim, 1926)] and demonstration of some of their properties analogous to those of antibodies—agglutinating as well as precipitating activities, varying selectivity of

interaction with different cells, and inhibition of the activity by certain substances.

II. THE INTERMEDIATE PERIOD (1919–1934)

After an interruption caused by World War I lectin research faced a short period of revival at the beginning of the second decade of the century. Nevertheless, toward the end of the 1920s a perceptible fading of interest set in, especially in the field of plant lectins (phytohemagglutinins). Evidently, no further use for lectins was found, their physiological function remained unknown, and their further exploration was restricted by the state of knowledge and methodology of protein chemistry of the time. The search for blood group-specific lectins undertaken by Sievers (1927) was in vain. There was no driving force of practical applications of lectins in medicine or in any form of commercial utilization. A lag period in the lectin field had started that was to last for 25 years.

A. Plants

In the plant lectin area Sumner (1919) (Fig. 3) and Sumner and Graham (1925) took up investigations of protein extracts of jack beans (*Canavalia ensiformis*) started by Jones and Johns (1916). The last named authors obtained from jack beans a globulin fraction that precipitated upon dialysis. It could be further separated into two fractions from which the minor one, obtained by saturation to 60% with ammonium sulfate, was given the name concanavalin. Though the agglutinating properties of jack beans had already been known (Assmann, 1911), Jones and Johns (1916) did not study the physiological activities of the fraction. Sumner (1919) was able to separate it further into two crystallizable components, one of which, concanavalin B, was sparingly soluble in 10% sodium chloride solution while the other, designated as concanavalin A, was soluble only in concentrated salt solutions. It was subsequently found to represent the agglutinating constituent and in later years was to become one of the most widely used lectins.

The lectins of *Ricinus communis* and *Croton tiglium* still attracted attention. Various aspects of agglutination by "ricin" were discussed by Gunn (1921), di Macco (1923), and Guest (1925). Tsuchihashi (1923) reported that treatment with metallic copper destroys completely the toxic and antigenic structure of ricin. Fujiwara applied the contemporary new techniques of adsorption onto alumina, kaolin, and freshly prepared

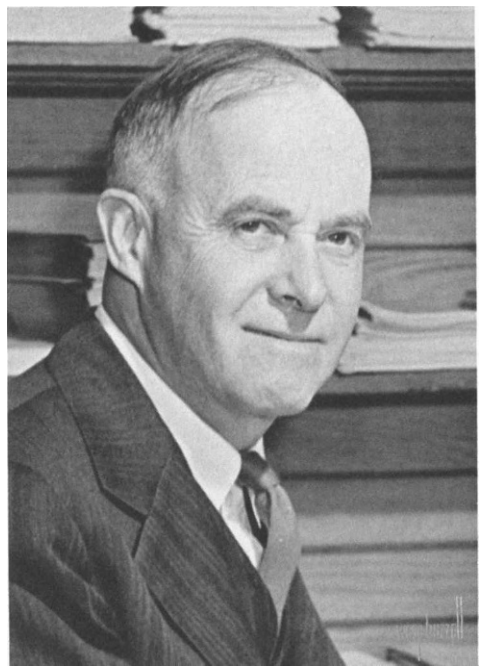


Fig. 3. James B. Sumner. [Photograph courtesy of Cornell University, Department of Public Information.]

calcium phosphate for the purification of soya bean lectin (1923a) and crotin (1923b); in a similar way Karrer *et al.* (1924) purified ricin and crotin and presented the first amino acid analyses of these lectins (Karrer *et al.*, 1925). Marcusson-Begun (1926) investigated the agglutinating principle of potato tubers and von Eisler and von Portheim (1926) found a hemagglutinin in tubers of a member of the Compositae (Asteraceae) family, *Helianthus tuberosus*.

B. Bacteria and Viruses

In addition to new observations of chicken-plague-virus adhesion to erythrocytes (Doerr and Gold, 1932) only a few papers on bacterial agglutinins appeared. Weinberg and Kepinow (1921) demonstrated that bacterial hemagglutinins could agglutinate both red cells and leukocytes. Based on the earlier studies by Guyot (1908) on agglutination by *Escherichia coli*, Rosenthal (1931) demonstrated that *E. coli* can also agglutinate cells

other than erythrocytes, e.g., spermatozoa in a wide range of pH (3–10); he also noted that cells killed by chloroform or heating were agglutinated. However, he did not obtain agglutination with bacterial filtrates as reported by Kraus and Ludwig (1902) and he reported that only a few strains of *E. coli* isolated from feces and urine were hemagglutinating.

III. THE RECOGNITION OF SPECIFIC LECTIN INTERACTIONS (1935–1964)

Although different binding properties to cells of individual lectins were obvious from the very first experiments of the early workers, only the third decade of this century brought the first discovery of a lectin highly specific toward human erythrocytes. The studies by Sugishita (1935) and Jonsson (1944) on eel serum hemagglutinins and later by Boyd (1947) and Renkonen (1948) on phytohemagglutinins can be considered as the first approach to the practical application of blood group-specific lectins and, in general, to the investigation of lectins as recognition molecules.

A. Vertebrate Animals

Sugishita (1935) found two types of agglutinins in the Japanese eel *Anguilla japonica,* one nonspecific toward erythrocytes of the ABO system and the other showing a high titer against group O cells. A similar hemagglutinin with anti-O(H) specificity was demonstrated by Jonsson (1944) in sera of the species *Anguilla anguilla*. Jonsson showed that, after appropriate dilution, eel serum could be used as anti-O(H) blood typing reagent; the lectin was then considered a special type of antibody and it took almost 30 years before its nonimmunoglobulin nature was proven (Bezkorovainy *et al.*, 1971). Investigations of eel sera by Grubb (1949) confirmed Jonsson's results showing the preferential anti-O(H) activity in most sera. In later years agglutinins with marked intraspecies differences in agglutinating specificity were found in several fish sera (Cushing, 1952a; Sindermann, 1958, 1961; Sindermann and Honey, 1964); some were also blood group specific (Cushing and Sprague, 1953).

Other reports of agglutinins in lower vertebrates were rare. Eichbaum (1946) found species-specific erythroagglutinins in the venom of nine South American snakes and the presence of a number of agglutinins of different specificity was described in the serum of the viper *Vipera aspis* by Dujarric de la Rivière *et al.* (1954).

B. Invertebrate Animals

Probably the most important finding in this area was the discovery by Johnson (1964) of an anti-A_1-specific lectin in the butter clam (*Saxidomus giganteus*). The lectin was specifically inhibited by *N*-acetylgalactosamine. This important paper marked the beginning of extensive research of gastropod lectins that commenced in the following years.

A number of interesting results on agglutinins from body fluids and seminal fluids of various invertebrates, especially of the lobster *Panulirus interruptus,* were reported by Tyler and Metz (1944, 1945) and Tyler and Scheer (1945). Tyler (1946) also studied agglutinins of some echinoderms and annelid worms and found erythroagglutinins of selective specificity toward various animal cells.

C. Plants

Boyd (Fig. 4) in 1945 observed that a saline extract prepared from dried lima beans (*Phaseolus lunatus* syn. *limensis*) "agglutinated erythrocytes of some human individuals, but those of others only weakly, if at all" (Boyd, 1970). He realized that the differences were correlated with blood groups. This important observation was mentioned in Boyd's textbook

Fig. 4. William C. Boyd. [Photograph courtesy of his daughter, Sylvia L. Boyd.]