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Lectins: Cell-Agglutinating and Sugar-Specific Proteins

Lectins provide new tools for studying polysaccharides, glycoproteins, and cell surfaces, and for cancer research.

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Proteins that possess the remarkable ability to agglutinate erythrocytes and other types of cell are widely distributed in nature. Such agglutinins are found predominantly in the seeds of plants, in particular those of the legumes, but they are also present in other parts of plants such as roots, leaves, and bark (*1–5*). In addition, they occur in invertebrates, for example, in snails and the horseshoe crab, and in lower vertebrates such as fish (*6*). Plant agglutinins are commonly referred to as phytohemagglutinins or phytoagglutinins. However, as cell-agglutinating proteins also occur in organisms other than plants, the term "lectins," proposed by Boyd (*2*), appears more suitable and will be used in this article. Prominent examples of lectins are concanavalin A from jack bean (*7*), soybean agglutinin (*8, 9*), and wheat germ agglutinin (*10–12*).

As well as the ability to agglutinate red blood cells, which makes easy their detection, lectins exhibit a host of other interesting and unusual biological and chemical properties (Table 1). Some of the lectins are specific in their reactions with human blood groups (ABO and MN) and subgroups (A_1) and have therefore been used in blood typing and in investigations of the chemical basis of blood group specificity (*1, 2, 13, 14*).

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Certain lectins are mitogenic (*15*) in that they can stimulate the transformation of lymphocytes from small "resting" cells into large blast-like cells which may ultimately undergo mitotic division. A most useful outcome of the study of lectins has been the expansion of cytogenetics and the increased understanding of relationships between chromosome abnormality and human diseases. The stimulation of lymphocytes by lectins also provides an important tool for the examination of the biochemical events involved in the conversion of a resting cell into an actively growing one.

The interactions of lectins with cells can, in many instances, be inhibited specifically by simple sugars (*4, 16*). This finding has led to the conclusion that lectins bind specifically to saccharides on the surface of cells, and has provided a new tool for the investigation of the architecture of cell surfaces. Lectins also bind mono- and oligosaccharides and specifically precipitate polysaccharides and glycoproteins; the precipitation is inhibited by sugars, as in the case of the agglutination reaction. This property of lectins can be used for the isolation and purification of carbohydrate-containing polymers, and for the study of their chemical structure. It has also led to the development of new "affinity chromatography" techniques for the purification of the lectins themselves.

The interaction of polysaccharides and glycoproteins with lectins may be likened to the antigen-antibody reaction and can serve as a most useful model for the study of this reaction. Because of the high specificities of some lectins, and the homogeneity of their saccharide-binding sites, they provide a unique opportunity for the investigation of homogenous combining sites on antibody-like molecules. An added advantage of lectins is that their combining sites are small, which makes the investigation of these sites simple and attractive. Furthermore, the reaction of lectins with saccharides may serve as a general model for protein-carbohydrate interactions.

The recent observations on lectins specific for tumor cells (*10–12, 17, 18*) have led to a great surge of interest in these compounds, especially among scientists engaged in cancer research. Some lectins preferentially agglutinate mammalian tissue culture cells that have been transformed by oncogenic viruses (Fig. 1) or by chemical carcinogens, as well as spontaneously transformed cells. These and related findings indicate that the surface of a transformed cell differs from that of its untransformed counterpart, and raise the hope that studies with lectins may lead to a better understanding of cancer. Moreover, they have prompted investigations to use lectins as inhibitors of growth of malignant cells in vitro and in vivo (*19*), and lectin-binding synthetic polymers for the immunization of mice against tumors (*20*).

Lectins are frequently toxic to animals, a property that may in part be responsible for the poor nutritive value of some plant proteins (*21*). Because of the great economic value of lectin-rich plant seeds, such as the soybean and castor bean, they have for a long time attracted the attention of food scientists, food technologists, and nutritionists.

Although hemagglutinating activity has been detected in extracts obtained from over 800 plant species (*1–5, 22*), and from numerous species of snails and other animals (*6*), only a small number of lectins have been isolated in purified form and investigated to any extent. However, as lectins have be-

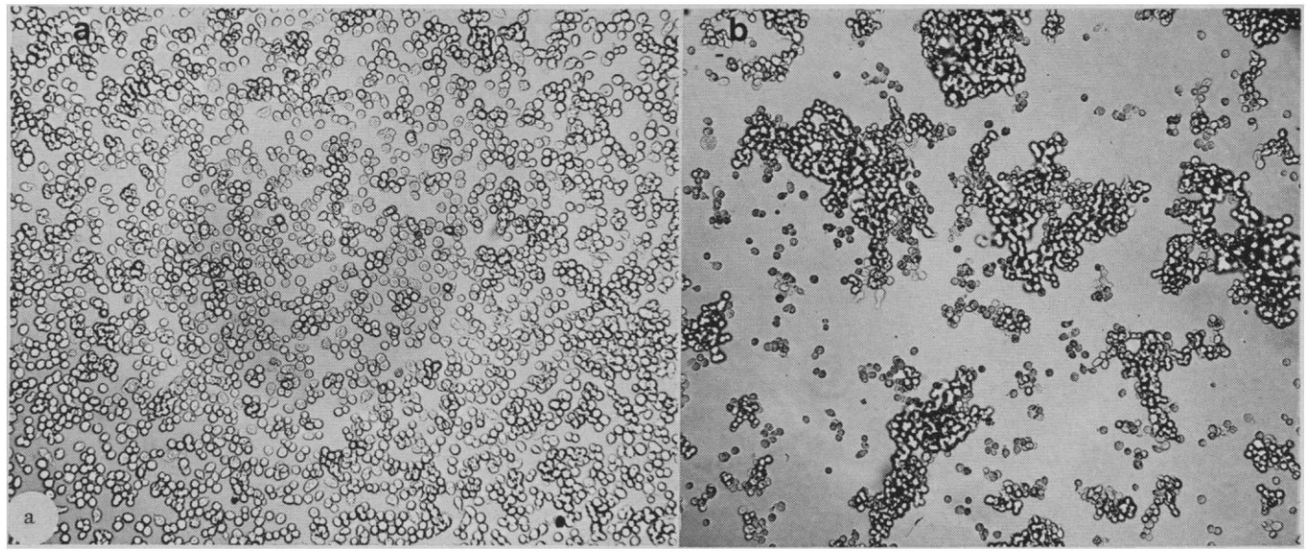

Fig. 1. (a) Normal rat cells and (b) polyma virus–transformed rat cells after incubation for 30 minutes with 100 μg of soybean agglutinin per milliliter ($\times$ 80) (*18*).

come a subject of intense interest in many laboratories, this article seems timely. In addition to reviewing the present status of research on lectins, we demonstrate their great potential in elucidating the structures of carbohydrate-containing polymers, and in particular their use in investigations of the architecture of cell surfaces. We also discuss briefly the possible role of lectins in nature. Information about the mitogenic action of lectins is available elsewhere (*15*), and there are reviews and books (*1–6, 21*) that give more detailed information on various other aspects of lectins.

History

The study of lectins was initiated by Stillmark who, in 1888, was the first to describe the phenomenon of hemagglutination by plant extracts. Stillmark studied the toxicity of castor beans (*Ricinus communis*) and of the press cake formed during the production of castor oil (*1, 2*). He found that castor beans contained a highly toxic protein which he named ricin, and which was capable of agglutinating the red cells of human and animal blood. Soon after the discovery of ricin, another lectin was found, abrin from the jequirity bean (*Abrus precatorius*). Ricin and abrin immediately drew the attention of Ehrlich, who chose to work with them on problems of immunology rather than with bacterial toxins, such as the diphtheria toxin, which were so popular among bacteriologists at that time. Using ricin and abrin, Ehrlich discovered during the 1890's some of the most fundamental principles of immunology. For instance, in 1891 he reported that mice were rendered immune to a lethal dose of ricin by repeated small subcutaneous injections of the toxin. He also showed that the serum of an immune mouse could neutralize the toxicity of ricin and, furthermore, that this action was specific, since the "antiabrin," which developed in the serum of an animal immunized with abrin, would not neutralize the toxic effects of ricin nor would "antiricin" neutralize abrin. Other classic studies in immunology, such as one of the first experiments on the reversibility of the antigen-antibody reaction, were also conducted with these two lectins (*23*).

In 1908, Landsteiner and Raubitschek (*24*) established that the relative hemagglutinating activities of various seed extracts were quite different when tested with red blood cells from different animals, and compared this specificity with that of antibodies of animal blood serum (*25*). Nevertheless, for several decades it was presumed that plant hemagglutinins were nonspecific. It was not until the end of the 1940's that the independent studies of Renkonen (*26*) and of Boyd and Reguera (*27*) led to the discovery that certain seeds contain agglutinins specific for some human blood group antigens. Thus, it was found that crude extracts of the lima bean (*Phaseolus limensis*) are virtually specific for the A antigen, although when concentrated they react weakly with type B cells as well. Similarly, seeds of the tufted vetch (*Vicia cracca*) contain powerful agglutinins that act much more strongly on A than on B or O cells. Other lectins specific for type A cells, as well as lectins specific for blood types H(O), M, and N are now known.

Most of the studies described above were conducted with crude extracts of plant origin. The first lectin that was obtained in crystalline form was concanavalin A from the jack bean (*Canavalia ensiformis*). Although crystallized by Sumner as early as 1919, it was only in 1936 that Sumner and Howell (*7*) identified the hemagglutinin of jack bean with concanavalin A, and showed that it agglutinated horse erythrocytes at concentrations as low as 0.1 microgram per milliliter. They observed that the protein reacted with glycogen and suggested that hemagglutination may be a consequence of the reaction of concanavalin A with carbohydrate in stroma proteins. Ricin was purified by Osborne and his co-workers in 1905. Further purification and crystallization of ricin was achieved in the late 1940's (*28*). It was observed, however, that the material, even after several crystallizations, consisted of more than one compound.

Specificity

Lectins differ widely in their specificity in the cell-agglutination reactions, and in the susceptibility of this reaction to the inhibition by saccharides. Most lectins agglutinate the erythrocytes of all human blood groups, acting at approximately the same dilution with

different blood types, and are usually referred to as nonspecific lectins, or panagglutinins, or lectins with broad specificity. The abundance of nonspecific lectins is evident from a recent study (*22*) in which extracts of 2663 varieties of plants were tested for human blood group activity. Of these, 711 were nonspecific and 90 were blood group specific. An additional 227 extracts hemolyzed red cells of all groups, whereas the remaining 1635 extracts were inactive.

The specific lectins preferentially agglutinate human erythrocytes of a given blood type and form precipitates with corresponding soluble blood group substances. Some of the crude plant extracts which exhibit blood group specificity are shown in Table 2. Certain lectins act equally well on erythrocytes of blood types A and B (A + B lectins), but no lectin is available that is specific for type B or for Rh. The unavailability of a potent type B lectin imposes a serious limitation on the routine use of lectins for blood typing. Some lectins are nevertheless used in hematology. In particular, a crude preparation from seeds of the gorse (*Ulex europeus*) is used in the identification of "secretors," human beings who secrete blood group substances A, B, or H in saliva or other body fluids (*1, 2*).

Although it would be desirable to use highly purified lectins for blood typing, crude preparations are equally suited for this purpose. Only rarely does an extract from a single source contain two or more lectins with different blood group specificities. Adsorption with one type of human erythrocyte of a lectin preparation that agglutinates two different types of erythrocyte (for example, A and O) nearly always removes both types of activity. Different species of the same genus may, however, contain lectins of different specificities: *Vicia cracca* is specific for blood group A, *Vicia graminea* is specific for blood group N, while *Vicia faba* is nonspecific (*5*).

Type specific lectins are inhibited best by saccharides which serve as part of the immunodeterminant of the corresponding blood group substance: lectins specific for group A antigen by *N*-acetyl-D-galactosamine (or its α-glycosides) and lectins specific for group H(O) antigen by L-fucose. Indeed, the first information concerning the role of sugars as determinants of blood group specificity was obtained in 1952 by Watkins and Morgan (*13*; see also *14*) who studied type A–specific agglutinins of plant origin (*Vicia cracca* and *Phaseolus limensis*) and type H(O)–specific agglutinins from the serum of the eel (*Anguilla anguilla*) and the seeds of *Lotus tetragonolobus*. Since *N*-acetyl-D-galactosamine specifically inhibited type A–specific lectins, Watkins and Morgan (*13*) concluded that this sugar serves as a determinant of human blood group A specificity. Similarly, the agglutination of group O cells by the type O(H)–specific lectins was best inhibited by methyl α-L-fucopyranoside, indicating that the α-L-fucosyl residue is a determinant of H(O) specificity. Both conclusions have been fully substantiated in subsequent studies.

Table 1. Properties and uses of lectins.

Property	Application
Specificity for human blood groups	Blood typing; structural studies of blood group substances; identification of new blood types; diagnosis of secretors
Toxicity in animals	Studies of nutritional value of animal foodstuffs
Induction of mitosis in lymphocytes	Studies of chromosomal constitution of cells and detection of chromosome abnormalities
Agglutination of malignant cells	Investigation of architecture of cell surfaces, and its change upon transformation
Precipitation of polysaccharides and glycoproteins	Isolation, purification, and structural studies of carbohydrate-containing polymers; model for antigen-antibody reactions
Binding of sugars	Studies of specific combining sites on proteins

Among the large group of lectins not specific for human blood groups, a few are known to be highly specific with respect to the binding of saccharides. The sugar specificity of these nonspecific lectins is not necessarily related to that of blood group determinants, as shown by the following examples. Concanavalin A, soybean agglutinin, and ricin are powerful, nonspecific lectins, but they exhibit distinct sugar specificities: concanavalin A toward α-D-mannopyranosides, α-D-glucopyranosides, and α-*N*-acetyl-D-glucosaminides (*29*); soybean agglutinin toward *N*-acetyl-D-galactosaminides (α and β) (*30*); and ricin toward D-galactose (*6, 31*). We may therefore conclude that these lectins interact with erythrocytes by binding to saccharide receptors different from the blood type determinants, and that such receptors are present on all red blood cell surfaces. This would explain the case of concanavalin A, since as far as is known, none of the soluble blood group substances contain D-mannose or D-glucose. Moreover, although blood group substances are now known to contain terminal nonreducing *N*-acetyl-α-D-glucosaminyl residues as an immunodeterminant, this determinant is not blood type specific (*32*). In the case of soybean agglutinin and ricin, the lack of blood type specificity may be the result of their inability to distinguish between α- and β-linked *N*-acetyl-D-galactosamine or D-galactose. In blood group substances, specificity determinants are α-linked; for example, *N*-acetyl-D-galactosamine in type A and D-galactose in type B, whereas on the erythrocyte surface we may assume that these saccharides occur both in α and β linkages, with the latter type of linkage perhaps predominating.

A more complex pattern of specificity emerges when the agglutination of erythrocytes, as well as other cells from different animals, by a variety of lectins is examined and compared with the sugar specificity of these lectins (*33*) (Table 3). Lectins that do not act on erythrocytes but will agglutinate sarcoma-180 cells (*34*) or leukocytes (*35*) are present in the red kidney bean and appear to be distinct from the hemagglutinin found in the same bean.

The susceptibility of erythrocytes and of other types of cell to agglutination by lectins is markedly enhanced by mild treatment with proteolytic enzymes, such as trypsin or Pronase (*4, 6*). For example, the concentration of soybean agglutinin (0.1 to 0.2 μg/ml) sufficient for the agglutination of trypsinized rabbit red blood cells is about 200 times lower than that which will cause agglutination of the untreated cells (*30, 36*). Therefore, a search for lectins should include investigations of cells from a variety of sources, both before and after they have been treated with proteolytic enzymes. Moreover, the agglutination tests should be carried out in the presence of different additives. This is important, since some lectins behave as if they were "incomplete" in that they

do not agglutinate erythrocytes in saline, but do so in the presence of bovine serum albumin or synthetic polymers such as polyvinylpyrrolidone (*4*). Metal ions (for example, calcium and magnesium ions) should also be included in the assay mixtures, because certain lectins have been shown to require such ions for their activity (see below). An interesting incomplete lectin is the agglutinin from the seeds of *Lotus tetragonolobus*. This lectin, when purified, agglutinates human type O cells at a concentration of 38 μg/ml. Upon the addition of a crude extract of the same seeds, diluted to a point at which the extract by itself would not cause agglutination, 2 μg of the purified lectin per milliliter is sufficient for the agglutination of type O cells (*37*).

Purified Lectins

Lectins can be purified from plant extracts or other sources by conventional techniques of protein chemistry such as salt fractionation and chromatography on ion exchangers or other types of adsorbent (for example, hydroxylapatite). More recently, use has been made of the specific binding properties of lectins to develop new and convenient "affinity chromatography" methods for their purification. Thus, concanavalin A can be purified by adsorption on cross-linked dextran (Sephadex) gels (*38, 39*) and elution with either D-glucose or a buffer of low *p*H. Specific adsorption on Sephadex gels has also been used for the purification of lectins from the lentil (*Lens esculenta*) (*40, 41*) and the seeds of the garden pea (*Pisum sativum*) (*42*). In the case of the type A–specific lectins of the seeds of the horse gram (*Dolichos biflorus*) (*43*) and the vineyard snail (*Helix pomatia*) (*44*) purification was achieved, essentially in a single step, by adsorption of the crude extracts on a column of insoluble polyleucyl blood group A substance and subsequent elution with *N*-acetyl-D-galactosamine. A similar step was used in the purification of the lectins from lima bean (*45*), whereas the type A–specific lectin from *Vicia cracca* was isolated by specific adsorption to blood group A substance coupled to Sepharose B (*46*). The L-fucose-binding protein of the seeds of *Lotus tetragonolobus* was specifically precipitated by a trifunctional fucosyl dye and the dye removed from the lectin by treatment with an ion exchange resin (*37*). By means of these techniques, it is often easy to obtain large quantities of purified lectins—several hundred milligrams from a kilogram of starting material.

Table 2. Human blood group specificity of some crude plant lectins (*1, 2*).

Blood group agglutinated	Origin of lectin
A	*Phaseolus limensis* *Vicia cracca* *Dolichos biflorus** *Crotalaria aegyptiaca*
A + B	*Sophora japonica* *Calpurina aurea*
H	*Cytisus sessilifolius* *Laburnum alpinum* *Lotus tetragonolobus* *Ulex europeus*†
M	*Iris amara*
N	*Vicia graminea** *Bauhinia purpurea*

* In use in blood banks. † Used for the identification of secretors.

The limited number of lectins that have been obtained in highly purified form, and whose chemical and physical characteristics have been investigated in greater or lesser detail, form a somewhat variable group of substances (Table 4) and it is difficult to single out properties that may be characteristic of the group as a whole. Most of the purified lectins contain covalently bound sugars, and can thus be classified as glycoproteins. A notable exception is concanavalin A [and possibly also the lectin of the garden pea (*42*)], which is devoid of sugar residues (*39, 47*) and is, therefore, not a glycoprotein. The carbohydrates found in lectins include mannose, glucosamine, and galactose, which are typical constituents of animal glycoproteins; other sugars such as xylose and arabinose are sometimes found, but in smaller amounts.

Most lectins are relatively rich in aspartic acid, serine, and threonine, which may comprise as much as 30 percent of their amino acid content and are very low in, or completely devoid of, sulfur-containing amino acids. Such a pattern of amino acids is characteristic of many plant proteins. In the snail lectin (*44*), aspartic acid and serine are the most abundant amino acids, but a significant concentration of cysteine and methionine (23 and 12 residues, respectively, per mole) is also present. Cysteine is also found in the lectins of the meadow mushroom (*Agaricus campestris*) (*48*), of the lima bean (*45, 49*) and of the horseshoe crab (*Limulus polyphemus*) (*50*). Whenever tested, lectins have been found to contain the metal ions Mn^{2+} and Ca^{2+}, and it has been shown that there is a requirement of metal for the carbohydrate-binding and agglutinating activities of these lectins (*41, 45, 51, 52*).

Values reported in the literature for the molecular weight of different lectins vary markedly from 26,000 for wheat germ agglutinin (*11, 12*) to 269,000 for the lima bean lectin (*49*) and to 400,000 for the horseshoe crab lectin (*50*). Moreover, markedly different values have sometimes been obtained for the same lectin, for example, 55,000 (*53*) to 100,000 (*54*) for concanavalin A. This may be partly because many (see Table 4), and perhaps all, lectins are made up of subunits and undergo association-dissociation reactions. Moreover, the subunits themselves may be comprised of polypeptide fragments which are complementary and which assemble to form the intact subunit, as has been shown in concanavalin A (*55*).

The presence of several very similar lectins has been observed in a number of seed extracts (*40–42, 47, 56, 57*), as well as in the snail (*44*). These multiple molecular forms of agglutinins, or isolectins, differ in their electrophoretic mobilities. They may be the product of closely related genes, or they may be formed prior to or during isolation, as a result of side chain modifications, such as hydrolysis of the amide group of glutamine or asparagine in the protein. In the isolectins that are glycoproteins, the differences may reside in the carbohydrate side chains. The two glycoprotein lectins isolated from the seeds of *Ulex europeus* (*58, 59*) cannot be considered as isolectins, however, because they differ markedly in their composition and have distinct sugar specificities (L-fucose and di-*N*-acetylchitobiose, respectively) although, for reasons which are not clear, they both exhibit the same blood type specificity [H(O)]. Another exceptional example of different lectins being isolated from the same source has been reported by Kalb (*60*). He isolated from *Lotus tetragonolobus* three distinct lectins which have in common the capacity for binding L-fucose, but which differ in molecular weight, number of binding sites and values of binding constants for L-fucose, and in chemical composition.

For the agglutination of cells, or for the formation of precipitates with complementary macromolecules, a lectin

Table 3. Agglutination titers* of erythrocytes and murine tumor cells by crude lectins [adapted from Tomita *et al.* (*33*)].

Origin of lectin	Rat		Mouse			Human erythrocyte			Sugar specificity†
	Yoshida sarcoma	Erythrocyte	Ehrlich ascites tumor	Leukemia (L 1210)	Erythrocyte	A_1	B	O	
Phaseolus limensis	**0**	0	0	0	**0**	128	0	**0**	None
Sophora japonica	1	1	0	0	0	32	16	16	None
Ulex europeus	0	0	0	0	0	16	4	16	L-Fuc, $(D\text{-Glc}NAc)_2$‡
Laburnum alpinum	0	0	0	0	0	0	0	4	None
Lens culinaris	128	16	64	32	16	4	4	8	D-Man, D-Glc
Vicia faba	128	32	32	16	4	4	1	4	D-Man, D-Glc*N*
Pisum sativum	256	32	16	16	32	4	4	2	D-Man
Wistaria floribunda	64	1	32	32	1	16	8	4	D-Gal*N*
Sesanum indicum	32	2	8	8	4	32	16	16	D-Glc
Ricinus communis	32	256	256	256	32	1024	1024	1024	D-Gal
Momordia charantia L.	8	64	32	32	4	256	512	512	D-Gal, D-Gal*N*
Triticum vulgaris	128	64	64	64	4	16	8	8	$(D\text{-Glc}NAc)_2$
Solanum tuberosum	0	8	32	16	16	64	128	256	$(D\text{-Glc}NAc)_3$
Phaseolus vulgaris	1024	256	512	256	256	256	256	256	None

* Highest dilution of crude lectin preparation (16 mg/ml) causing detectable agglutination after 1 hour at room temperature. † Saccharides tested for inhibitory action: D-glucose (D-Glc), D-galactose (D-Gal), D-mannose (D-Man), D-glucosamine (D-Glc*N*), D-galactosamine (D-Gal*N*), *N*-acetyl-D-galactosamine (D-Gal*N*Ac), *N*-acetyl-D-glucosamine (D-Glc*N*Ac), di-*N*-acetylchitobiose [$(D\text{-Glc}NAc)_2$], *N*-acetyl-D-mannosamine (D-Man*N*Ac), L-fucose (L-Fuc), sialic acid (up to a concentration of 2 mg/ml). ‡ Data from Matsumoto and Osawa (*58, 59*).

Table 4. Chemical and biological properties of highly purified lectins. Abbreviations: Xyl, xylose; Ara, arabinose; other abbreviations as in Table 3. Roman numerals indicate different lectins isolated from the same plant. For further information see text.

Source		Molecular weights	Subunits	Carbohydrates: Percentage	Carbohydrates: Major constituents	Mitogenic activity	Specificity: Human blood type	Specificity: Sugar	No. of binding sites	Metal requirement	References
Higher plants: Leguminosae											
Canavalia enisformis (jack bean)		55,000	2	0		+	None	α-D-Man	2	+	(*7, 29, 51, 33, 90*)
Dolichos biflorus (horse gram)		140,000		3.8	Glc*N*, Man	—	A	α-D-Gal*N*Ac			(*43, 105*)
Glycine max (soybean)		110,000		5	D-Glc*N*Ac, D-Man	—	None	D-Gal*N*Ac	2	+	(*8, 9, 30, 62*)
*Lens culinaris** (common lentil)		42,000–69,000	2	2	Glc*N*, Glc	+	None	α-D-Man	2	+	(*40, 41, 52, 106*)
Lotus tetragonolobus	I	120,000		9.4	Glc*N*, Hexose		H(O)	α-L-Fuc	4		(*13, 14, 37, 60*)
	II	58,000		4.8	Glc*N*, Hexose		H(O)	α-L-Fuc	2		(*13, 14, 37, 60*)
	III	117,000		9.2	Glc*N*, Hexose		H(O)	α-L-Fuc	4		(*13, 14, 37, 60*)
Phaseolus lunatus†	I	269,000	8	4	Glc*N*, Man, Fuc		A			+	(*27, 45, 49*)
(lima bean)	II	138,000	4	4	Glc*N*, Man, Fuc		A			+	
Phaseolus vulgaris (black kidney bean)		128,000		5.7	Hexosamine Man, Xyl						(*107*)
Phaseolus vulgaris‡	I	138,000	8	8.9	Glc*N*, Man	+					(*108*)
(red kidney bean)	II	98,000–138,000	4	4.1	Glc*N*, Man	+		D-Gal*N*Ac			(*35*)
Phaseolus vulgaris (yellow wax bean)				10.4	Glc*N*, Man, Glc, Ara						(*109*)
Pisum sativum (garden pea)		53,000		(0.3)	(Glc)		None	D-Man		+	(*42, 52*)
Robinia pseudoacacia (black locust)		90,000		10.7	Glc*N*, Man, Fuc, Xyl						(*110*)
Ulex europeus	I	170,000		5.2	Glc*N*, Man		H(O)	L-Fuc			(*58, 59*)
(gorse)	II			21.7	Glc*N*, Man, Gal, Ara		H(O)	$(D\text{-Glc}NAc)_3$			(*58, 59*)
Other high plants											
Ricinus communis (castor bean)		98,000					None	D-Gal			(*6, 101*)
Solanum tuberosum (potato)		(20,000)		5.2	Ara			$(D\text{-Glc}NAc)_2$			(*6, 111*)
Triticum vulgaris (wheat)		26,000		4.5			None	$(D\text{-Glc}NAc)_3$			(*6, 10–12, 93*)
Lower plants											
Agaricus campestris (meadow mushroom)		64,000	4	4		+					(*48*)
Invertebrates											
Helix pomatia (vineyard snail)		100,000		7.3	Gal, Man (hexosamine)		A	α-D-Gal*N*Ac	6		(*44*)
Limulus polyphemus (horseshoe crab)		400,000	18					Sialic acid		+	(*6, 50*)

* Also known as *Lens esculenta*. † Also known as *Phaseolus limensis*. ‡ Two (or more) lectins with distinct activities appear to be present in the red kidney bean, an erythroagglutinin (I) and a leukoagglutinin (II).

molecule must possess at least two binding sites as is found in immunoglobulins. Concanavalin A has one binding site for methyl α-D-mannopyranoside or methyl α-D-glucopyranoside per subunit of molecular weight of about 32,000 (*51*), but this subunit does not exist as such; concanavalin A usually occurs as a dimer (molecular weight 55,000) with two binding sites (*53, 61*). Two binding sites for *N*-acetyl-D-galactosamine have been found in soybean agglutinin (*62*), two for methyl α-D-glucopyranoside in the lentil lectin (*63*), two or four for L-fucose in the lectin from *Lotus tetragonolobus* (*37*), and six sites for *N*-acetyl-D-galactosamine in the lectin from the vineyard snail (*44*). The association constants for the binding of the above saccharides to the corresponding lectins are usually in the range of $K_a = 10^3$ to 10^4 (with the exception of the lentil lectin, which binds α-D-mannose with $K_a = 2.3 \times 10^2$). These values are lower than the values of the association constants found for the interaction of oligosaccharides with antibodies to the corresponding carbohydrates (*64*). In all cases, the combining sites are homogenous and identical with respect to their distribution in different molecules of the same lectin. In this property lectins differ from immune antibodies in that the combining sites of the latter are neither identical with one another nor are they homogenous.

Concanavalin A: Physiochemical Properties

The relative abundance of concanavalin A in jack bean meal (2.5 to 3 percent by weight) (*7*), the ease of its preparation, and the availability of a great variety of low and high molecular weight saccharides with which it can interact, have led to a large number of studies on this lectin. The pace of research on concanavalin A has been markedly accelerated following the demonstration that cells transformed by DNA tumor viruses or carcinogens are agglutinated by this lectin more readily than are normal cells (*17*). As a result, we have considerably more knowledge of the physical, chemical, and biological properties of concanavalin A than of any other lectin.

Although concanavalin A is biologically homogenous, as judged by the fact that 95 to 98 percent of its weight can be precipitated by a dextran (*47*), it is not a homogenous molecular species in the *p*H range where it binds saccharides. Physical heterogeneity of highly purified concanavalin A has, for example, been observed upon examination by free boundary electrophoresis (*47*) or isoelectric focusing (*57*). Very recently, it was found that crystalline concanavalin A can be separated in strongly dissociating solvents into several molecular species (*55*). These have been isolated and identified as an intact subunit or "protomer" of concanavalin A (molecular weight 27,000) and two main fragments which account for most of the intact protomer. It was concluded that the intact chain is the primary gene product and that the two smaller chains (NH_2-terminal, molecular weight 12,500; and COOH-terminal, molecular weight 14,000) were derived from it by chemical or enzymatic hydrolysis. The chains associate strongly and the results suggest that the concanavalin A protomer may consist of a single peptide chain of 27,000 molecular weight or a mixture (ratio 1 : 1) of complementary fragments. In solution below *p*H 6, concanavalin A forms a molecular species made up of two protomers, whereas at *p*H 6.7 and above, it may consist of four protomers. These association-dissociation reactions would account for the different values for the molecular weight of concanavalin A (55,000 and 100,000) which have been reported. Other intermediate values probably reflect mixtures of the two-protomer and four-protomer units (*65*).

These conclusions are in agreement with recent x-ray crystallographic studies of concanavalin A (*66*) as well as earlier studies of the binding of saccharides and metal ions to the lectin (*51*). The crystallographic studies, carried out to 4-Å resolution, clearly show the molecular shape and the packing of the concanavalin A molecules. The asymmetric unit (or protomer) has a molecular weight of 27,000 and forms an elliptical dome with a base of approximately 46 by 26 Å and a height of 42 Å. The protomers are paired to form dimers which are in turn paired to form tetramers of roughly tetrahedral shape. Each protomer has a depression located on the surface which could be the site of saccharide binding. The molecular structure of the protomers is not clear, since the amino acid sequence of concanavalin A is not yet known. A symmetrical structure and even number of binding sites may be a common feature of many lectins.

Concanavalin A has specific binding sites for transition metal ions and calcium ions, in addition to the saccharide binding site. Each set of sites is associated with a protein unit that has a molecular weight of 32,000 and there are interactions between the three sites: calcium ions can be bound only after the transition metal site is occupied and occupancy of both sites is required before the binding of a saccharide such as methyl α-D-glucopyranoside can occur (*51*). The circular dichroic spectrum of concanavalin A shows changes in the near ultraviolet region upon specific binding of methyl α-D-mannopyranoside, which suggests that sugar binding at physiological *p*H is accompanied by a conformational change in the protein (*67*).

The results of studies conducted by Agrawal *et al.* (*68*) and Hassing *et al.* (*69*) have led to the conclusion that free carboxyl groups, but not free amino or phenolic hydroxyl groups are essential for the precipitation of dextrans by concanavalin A. It was suggested that carboxyl groups may be present in or near the saccharide-binding sites of concanavalin A (*68, 69*); however, chemical modifications may influence the saccharide-binding activity by affecting the metal-binding sites. There is evidence of a hydrophobic region on the protein in close proximity to the carbohydrate-binding region (*70*). Slight changes in the ultraviolet absorption spectra of the tryptophyl and tyrosyl residues could be observed upon the addition of saccharides to concanavalin A, suggesting that these residues may be perturbed although not necessarily situated within the saccharide-binding site (*71*).

Concanavalin A: Specificity and Uses

The specificity requirements of the interactions between concanavalin A and saccharides have been thoroughly investigated by Goldstein and his coworkers (*16, 29*). Concanavalin A can form a precipitate with numerous polysaccharides that contain multiple terminal nonreducing α-D-glucopyranosyl (or its 2-acetamido derivative), α-D-mannopyranosyl, β-D-fructofuranosyl, or α-D-arabinofuranosyl residues. These include glucans such as glycogens, amylopectins, and dextrans; yeast mannans and phosphomannans as well as synthetic D-mannans; D-fructans such as bacterial and plant levans; and a mycobacterial arabinogalactan. It will also form a percipitate with many gly-

coproteins, including soybean agglutinin which has been shown to possess terminal α-D-mannopyranosyl residues (*9, 72*), and with certain teichoic acids (*73*).

Further insight into the stereochemical specificity of concanavalin A has been obtained by examining the extent to which a large number of monosaccharides, oligosaccharides, and modified sugars inhibit the precipitation reaction (the Landsteiner hapten inhibition technique). The carbohydrate-combining site of the concanavalin A molecule appeared to be directed primarily toward unmodified hydroxyl groups at the C-3, C-4, and C-6 positions of the six-membered α-D-mannopyranoside or α-D-glucopyranoside ring (positions 3, 4, 5, 6 of the D-arabino configuration) (Fig. 2). Any modification at these positions completely abolished the inhibitory action of the saccharide. On the other hand, considerable modification of the group at C-2, as long as it was equatorial, affected the inhibitory activity to a small extent only. Thus 2-deoxy-D-glucose, 2-*O*-methyl-D-glucose and *N*-acetyl-D-glucosamine exhibited almost the same inhibitory activity as D-glucose. It was also concluded that the oxygen atom of the C-2 position of D-mannose serves as a polar binding locus for the interaction of the saccharide with the protein (*29*). It appears that D-mannose, D-glucose, and their derivatives bind to concanavalin A in the C-1 chair conformation and that the protein possesses a specific locus capable of interaction with the anomeric oxygen atom of the α-linked glycosides of these sugars (Fig. 2). A β-anomeric oxygen atom interferes with binding, since β-D-mannopyranosides or β-D-glucopyranosides are very poor inhibitors. Indeed, of the large number of glucans and mannans tested, only those containing α-glycosidic linkages interacted with concanavalin A, whereas the β-linked polysaccharides failed to interact. The inhibition studies indicate that polar interactions, such as H bonds, and charge-dipole (but not charge-charge) interactions are the predominant stabilizing forces in the concanavalin A–polysaccharide complexes and that the formation of these complexes involves chiefly the nonreducing chain ends of the reacting polysaccharides.

In addition to glycopyranosides, the binding site of concanavalin A can also accommodate certain five-membered rings, such as β-D-fructofuranosides and D-arabinofuranosides (*74*); this

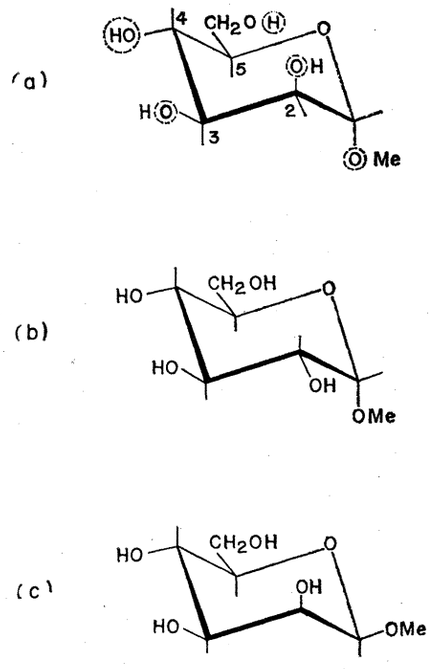

Fig. 2. Methyl α-D-mannopyranoside (a) and methyl α-D-glucopyranoside (b), the best monosaccharide inhibitors of the precipitation of polysaccharides by concanavalin A; and methyl β-D-mannopyranoside (c), which is noninhibitory (*29*). The sugars are drawn in their most stable C-1 chair conformation, and in (a) the groups that interact with the protein are circled.

interaction is considerably weaker than the concanavalin A–glycopyranoside reaction. The binding of furanoid sugars to concanavalin A has been explained on the basis of common configurational features with sugars possessing the pyranoid ring. Binding of furanose ring structures to pyranose-specific sites has also been observed with the lectin of *Lotus tetragonolobus* (*75*), and has been demonstrated by x-ray crystallography for another carbohydrate-binding protein, the enzyme lysozyme of hen egg white (*76*).

Although it was originally postulated that the specificity of concanavalin A is directed exclusively toward a single saccharide residue, there is now evidence that this lectin may possess a more extended binding site. Thus, it has been shown (*77*) that the disaccharide D-Man-α(1 → 2)-D-Man and the trisaccharide D-Man-α(1 → 2)-D-Man-α(1 → 2)-D-Man are much stronger inhibitors of the concanavalin A–dextran interaction than is methyl α-D-mannopyranoside, the disaccharide by a factor of 5 and the trisaccharide by a factor of 20. These findings might also be explained by the possibility of there being two or more concanavalin A molecules associated with extended sequences of α(1 → 2)-D-mannopyranosyl residues.

The results of many other studies of concanavalin A are in perfect agreement with its specificity requirements. The order of reactivity of the various sugars used in inhibition studies is the same, regardless of the polysaccharide used for combining with concanavalin A, and the method of assay of the concanavalin A–polysaccharide interaction (turbidimetry, precipitation, or agar gel diffusion) (*29*). Only sugars that are good inhibitors displace concanavalin A from Sephadex gels to which it has been adsorbed and the relative efficiencies of the sugars parallel their inhibitory activity (*38*). Moreover, direct measurements by equilibrium dialysis of the binding to concanavalin A of methyl α-D-mannopyranoside and methyl α-D-glucopyranoside give constants [$K_a = 1.4 \times 10^4$ liter/mole and $K_a = 0.3 \times 10^4$ liter/mole, respectively (*61*)] which are inversely proportional to the capacity of these sugars to inhibit precipitation [0.34 μmole/ml and 1.3 μmole/ml, respectively, were required for 50 percent inhibition of precipitation in the concanavalin A–levan B-512 system (*29*)].

Knowledge of the specificity requirements of concanavalin A has resulted in its being used as a reagent in analytical and preparative biochemistry. We will mention only a few examples of the increasing uses of this lectin. A dextran from *Streptococcus bovis*, originally believed to be a linear polymer of α(1 → 6) linked D-glucose residues, gave a precipitate with concanavalin A. As a result of this finding, further chemical studies were undertaken and the dextran was shown to be a branched polymer with α(1 → 3) and α(1 → 6) linkages (*78*). An α-D-configuration was assigned to mannosylglycerol of *Micrococcus lysodeikticus*, because this glycoside inhibited the concanavalin A–dextran reaction to the same extent as methyl-α-D-mannopyranoside; subsequent investigations have supported this assignment (*79*). With the aid of concanavalin A, a new antigenic determinant, unrelated to ABO blood group specificity and having a terminal nonreducing *N*-acetyl-α-D-glucosaminyl residue, was detected and identified in hog and some human gastric mucin blood group A + H substances (*32*). Precipitation by concanavalin A of the antihemophilic factor (factor VIII) of blood proved to be a useful step in purification of this factor (*80*). The structure of the car-

bohydrate moiety of rabbit gamma globulin has been investigated with the use of concanavalin A (*81*). It has also been suggested that concanavalin A and appropriate carbohydrates be utilized in the classroom for demonstrating the principles of the antigen-antibody reaction, eliminating the need of antigens and antiserums (*82*).

The recently prepared, insoluble, biologically active derivatives of concanavalin A (*83*) greatly extend the range of application of this lectin. Insoluble concanavalin A can be used for the isolation of immunoglobulins and blood group substances (*83*), and for the purification of glycoprotein enzymes such as glucose oxidase (*84*). In a similar manner, partial purification of concanavalin A receptors from the lymphocyte plasma membrane of the pig has been achieved (*85*).

In addition to the great increase in our knowledge of the chemical properties of concanavalin A, a wealth of information is now available concerning its biological properties. The toxicity to animals of concanavalin A has been recognized for many years (*7*). Recently, concanavalin A has been shown to agglutinate embryonic cells (*86*), spermatozoa (*87*), and certain viruses (*88*), to stimulate the migration of tumor cells (*89*), to exhibit mitogenic activity (*90*), and to confer cytolytic activity on lymphal node cells interacting with fibroblasts (*91*). Whenever tested, these activities were inhibited by the same sugars that inhibit the precipitation of dextrans by concanavalin A.

In spite of its high specificity, concanavalin A may precipitate polyelectrolytes and polysaccharides which are devoid of terminal mannopyranoside (or related) residues (*92*). It is, however, easy to distinguish between the specific and nonspecific reactions of concanavalin A, because the latter are not inhibited by saccharides.

Interaction with Malignant Cells

In 1963, Aub and his co-workers (*10*) reported that wheat germ lipase preparations agglutinated mouse tumor cells more readily than cells from normal tissues. Subsequently, they showed that the lipase preparations contained as an impurity a lectin that was responsible for this agglutinating activity. Wheat germ agglutinin was purified by Burger and Goldberg (*11*) and Burger (*12*). These workers concluded that the agglutinin was a glycoprotein with a molecular weight of 26,000. It agglutinated several lines of virally transformed cells at concentrations that did not cause agglutination of the untransformed parent cells. Burger and Goldberg therefore suggested that the surface of the transformed cells contained a component that was not present on the surface of the normal cells. It was proposed that this component is *N*-acetyl-D-glucosamine or a closely related derivative, because studies of hapten inhibition showed that *N*-acetyl-D-glucosamine and its disaccharide, di-*N*-acetyl-chitobiose, specifically inhibit the agglutination reaction (*11, 12*). The addition of inhibitory sugars to the clumped cells resulted in dissociation of the aggregates, showing that the agglutination was readily reversible. Ovomucoid, a glycoprotein containing a high proportion of *N*-acetyl-D-glucosamine, inhibited the agglutination at very low concentrations. Binding to insoluble ovomucoid and elution with an excess of *N*-acetyl-D-glucosamine was used in a modified procedure for the purification of wheat germ agglutinin (*11, 12*). The specificity of wheat germ agglutinin for *N*-acetyl-D-glucosamine was confirmed in studies of the agglutination of erythrocytes in which it was also shown that this lectin is nonspecific for human blood types (*93*).

Although normal somatic cells are not agglutinated by low concentrations of wheat germ agglutinin, they can be agglutinated after brief treatment with proteolytic enzymes. The agglutination of normal cells treated with proteases is similar in many respects to that of the untreated virally transformed cells, and the chemical nature of the receptors appears to be identical in both types of cell (*11, 12*).

Wheat germ agglutinin is not the only lectin that preferentially agglutinates malignant cells, or normal cells after mild proteolysis. Inbar and Sachs have shown (*17*) that concanavalin A at a concentration of 250 μg/ml agglutinates leukemic cells and tissue-culture cells that have been transformed by the polyoma virus, simian virus 40, chemical carcinogens, and irradiation with x-rays. Concanavalin A does not agglutinate normal cells under the same conditions. The agglutination is reversed by methyl α-D-glucopyranoside, but not by saccharides that do not bind to concanavalin A. Removal of bivalent metal ions from concanavalin A abolishes its cell-agglutinating capacity, in accord with the metal requirements of concanavalin A for the binding of carbohydrates. After treatment of normal cells with trypsin they are agglutinated by concanavalin A. Soybean agglutinin, which is specific for *N*-acetyl-D-galactosamine (both α- and β-linked) (*30*) also agglutinates mouse, rat, and human cell lines transformed by viral or chemical carcinogens (*18*). In contrast, transformed hamster cells are not agglutinated, whereas normal hamster cells can be agglutinated after mild proteolytic treatment, as is the case with the other normal cell lines. The agglutination is, in all instances, specifically inhibited by *N*-acetyl-D-galactosamine, suggesting that this saccharide, or a closely related one, is the terminal nonreducing moiety of the receptor for soybean agglutinin on the surface membrane of the agglutinable cells.

The receptor site of wheat germ agglutinin was isolated from membranes of transformed cells (*94*). Concentrations of the receptor glycoprotein, which fully inhibited the agglutination of cells by wheat germ agglutinin, had no effect on the agglutination with other lectins, such as soybean agglutinin or concanavalin A. These findings suggest that the receptor sites present on the surface of the transformed cells for the different lectins are both chemically and topographically distinct. On the other hand, the glycoprotein receptor for the kidney bean lectin, isolated from human erythrocytes, is a potent inhibitor of the agglutination of human erythrocytes by the lentil lectin, although the two lectins are known to possess different carbohydrate specificities (*95*). It is therefore possible that the lentil lectin and the kidney bean lectin bind to different portions of the same oligosaccharide on the human erythrocyte surface.

Although it was originally suggested that the difference between cells which are agglutinated and those which are not is in the number of saccharide receptor sites, it now appears that both types of cells may contain approximately the same number of receptors. This conclusion is based on studies with radioactively labeled lectins [^{3}H- and ^{125}I-labeled concanavalin A; ^{125}I-labeled wheat germ agglutinin (*96*); and ^{125}I-labeled soybean agglutinin (*97*)], which show that there is no direct correlation between the amount of lectin bound and the agglutinability of the cells. Often normal and transformed cells, as well as cells which have undergone proteolysis, bind more or less the same amount of lectin. This surprising find-

ing has been confirmed in studies of the interaction of soybean agglutinin with untreated and trypsinized erythrocytes (*36*).

Very recently it has been suggested that agglutination by lectins depends on the relative distribution of sugar receptors on the surface of the cell; in normal cells the receptors are dispersed, whereas after proteolysis or malignant transformation a redistribution of sites occurs which results in their clustering, an arrangement favorable to cell agglutination. This conclusion is based on results obtained by means of a new electron-microscopic method developed for investigating the specific localization of antigens and saccharides on cell membrane surfaces; ferritin-conjugated concanavalin A was used in these studies (*98*). Rearrangement of exposed sites upon malignant transformation has also been suggested by Ben-Bassat *et al.* on the basis of binding studies (*99*). However, the possibility cannot be excluded that only a small percentage of the lectin molecules bound to the cells is involved in agglutination, whereas the bulk is bound to sites that contain the appropriate saccharide receptors, but binding to these receptors does not lead to agglutination. If this were true, this "nonproductive" binding could mask any small but real difference in the number of "productive" binding sites. That the medium in which the cells are grown can affect the binding of concanavalin A has also been observed (*99*).

The significance of the changes in the surface membranes of cells to the understanding of malignant transformation are not clear; there is even some doubt whether such changes are a specific characteristic of all malignant cells (*100*). It is clear, however, that lectins, both native and modified, provide a new and useful tool for the study of the chemical architecture of cell surfaces and the changes which they undergo upon malignancy.

Conclusions

Although much progress has been made in the study of lectins, a large number of questions regarding their properties, uses, and functions remain unanswered. One of the main problems encountered in studies of proteins, especially those proteins that exhibit multiple biological activities, is that of deciding whether they are homogenous. Most, and perhaps all, of the purified lectins (Table 4), have not been subjected to extensive testing for homogeneity, and it is therefore difficult to conclude whether their different biological activities reside in the same molecule. This is true even for the most thoroughly investigated lectin, concanavalin A, which is hemagglutinating, mitogenic, toxic, and sugar-binding. With some lectins, separation of different activities has been reported. Thus, the erythroagglutinating activity of the lectin of the red kidney bean has been separated from its leukoagglutinating activity (*35*). Purified, crystalline ricin has been separated into different fractions, one of which is toxic and devoid of hemagglutinating activity, while another that contains the hemagglutinating protein exhibits low toxicity (*101*). In view of the observations made with "incomplete" lectins (*4*) it would be of interest to know whether the addition of different substances might endow the nonhemagglutinating protein with agglutinating activity.

In addition to the unresolved problems of chemical and biological homogeneity of lectins, there is also the question of the mechanism or mechanisms by which their biological activities are expressed. Are all the activities the result, direct or indirect, of binding to specific saccharide sites on cell surfaces? Are the receptors for the different activities identical, chemically and topologically? Even the relatively simple agglutination reaction is not yet well understood.

The saccharide specificity of many lectins is well established, but for some lectins (Tables 3 and 4) no saccharides have been found that inhibit their agglutinating activity. That the appropriate sugars have not been tested is a remote possibility, since it implies that animal cell surfaces contain unknown sugars, which is unlikely. Another possibility is that these lectins possess extended sites which require complex saccharide structures for efficient interaction. Indeed, in spite of the general agreement that the saccharide binding sites of lectins are small, corresponding in size to a single saccharide unit, there are indications, at least for some lectins, that the sites may be more extended (*43, 77*). This brings us to the question, still far from being answered, of whether there are structural features common to the binding regions of all lectins. In this connection it is also pertinent to ask whether the covalently linked carbohydrate chains, present in most lectins, contribute in any way to their biological activities. Since there are lectins that are not glycoproteins (Table 4) it would appear that the sugar moiety does not play an essential role in these activities.

Although we have pointed out that lectins have a role as analytical tools in the study of carbohydrates, it should be kept in mind that the use of these reagents is not free of pitfalls, because they may interact with apparently unrelated saccharides. Thus, the lectins of eel serum and of the seeds of *Lotus tetragonolobus* are specific in their agglutination of type O erythrocytes but are inhibited not only by L-fucose or its *O*-methyl ethers, but also by some *O*-methyl ethers of D-fucose (*102*).

The role of lectins in nature—whether in plants or in other organisms—remains a mystery. It has been suggested that they are antibodies intended to counteract soil bacteria (*1, 2, 5*); that they serve to protect plants against fungal attack by inhibiting fungal polysaccharases (*103*); that because of their affinity for saccharides they are involved in sugar transport and storage; or that they serve for the attachment of glycoprotein enzymes in organized multienzyme systems. In view of the mitogenic properties of lectins it is possible that their function is to control cell division and germination in plants. There is, however, no evidence for or against any of these hypotheses. It is therefore not surprising that it has been proposed that the biological properties of lectins, as observed in the laboratory, have no relation to their function in nature.

The ready availability, great diversity, and the ease of isolation of these agglutinins and the many interesting and unusual properties they exhibit, make them a most attractive subject for research. They may eventually provide a series of most useful probes, comparable to the carbohydrate-specific antibodies to the pneumococcal bacteria (*104*), for the recognition of individual sugars and specific linkages in polysaccharides, glycoproteins, and glycolipids, and for the mapping of the architecture of cell surfaces. Studies on the primary, secondary, and tertiary structures and of the physiochemical properties of lectins will undoubtedly lead to the understanding of the molecular basis of their sugar specificity and their mechanisms of action on cells, whether normal or malignant. Eventually their role in nature may also be unraveled.

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Coherent Social Groups in Scientific Change

"Invisible colleges" may be consistent throughout science.

Belver C. Griffith and Nicholas C. Mullins

Recent studies have revealed a great deal about the communication and organizational patterns that underlie major advances and changes of direction in science. An important feature of these patterns is their consistency throughout a variety of disciplines, periods of time, and types of research. Biologists on Long Island in the 1940's, ethnomethodologists in southern California in the 1960's, physicists in Copenhagen in the 1920's, and mathematicians in Göttingen in the 1900's acted in very similar ways when, armed with insights radical for their times and disciplines, they faced important scientific problems. Our data on these and other such groups suggest that a single set of social mechanisms evolves in response to the challenge posed by new and major scientific problems. When challenged, some members of a scientific specialty become organized to work toward certain objectives, voluntarily and self-consciously, as a coherent and activist group. This article examines findings from surveys, individual interviews, and biographical essays, and discusses the similarities among contemporary groups that developed into small, coherent, activist groups and that subsequently had major impacts on their "home" disciplines.

Low Levels of Organization and Communication

Communication and some degree of voluntary association are intrinsic in science, and the important question therefore becomes not whether scientists organize, but rather how, why, and to what degree? As a background to understanding high degrees of communication and organization, the first section of this article examines the processes entailed in the "loose" networks, the level of communication and organization that appears normal for science. This level has been repeatedly demonstrated by different methodologies for specialties in various disciplines.

Three groups of psychological researchers studied by Griffith and Miller exhibited this effective, loose communication network (*1*). The workers in these groups had considerable knowledge of the activities of other major researchers, and it is clear that individuals sought out, and interacted very effectively with, one another on the basis of their current research interests. Mullins' data on biologists and Crane's on rural sociologists also show this kind of loose communication network. In both of these studies, respondents named more persons outside their specialty than inside as having had significant effects on their work, thereby suggesting that scientists work in and influence more than one specialty, an apparently normal condition for highly active scientific researchers (*2*). In addition, the Crane study showed that productive scientists were more frequently named as the object of contact, indicating, as expected, that communication is more intense around productive researchers. This finding was later quantified by Griffith *et al.* (*3*).

The Griffith and Miller study (*1*) focused upon persons who were comparatively productive, each of whom headed a team of students and junior colleagues. These respondents employed several special strategies to facilitate information exchange within their specialty. For example, one area, speech perception, was small enough that few communication problems developed, even though the area exhibited low levels of social organization. Of the other specialties studied, those that exhibited loose networks employed, for varying periods of time, mechanisms that are used in highly coherent groups (for example, conference series and exchanges of papers before publication). However, the adoption of a pattern of communication within these specialties was in response to a current scientific problem and to the inadequacy of formal meetings and journals for answering communications needs created by these problems. For example, psycholinguists seemed to develop different patterns of organization depending upon whether they were in the process of applying psychological theories and methodology to studies of language (as they did in the early 1950's) or modifying linguistic theories so they could be used in experimental psychology (as they did after the development of generative grammar). By contrast, research-

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