

# Chapter 2

## The History of Lectinology

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A salient precondition for progress and uncomplicated communication in research is the general agreement on definitions and terms. As the headline attests, the term 'lectin' is commonly accepted. This chapter will explain how this term is rooted in the experimental work of the field's pioneers. The impressive demonstration of capacity of agglutinins for selecting distinct blood-group epitopes like an antibody was a cornerstone for lectins to broadly enter laboratories as tools. Further discoveries, e.g. lectin-dependent cell stimulation, paved the way to realizing the enormous scope of lectin-carbohydrate interactions documented in this book. Our historical survey, summarized in Table 2.1, focuses on early developments up to coining the term. Selected more recent advances, which spurred the publication rate as a measure of the field's momentum, are also included in Table 2.1. It presents a graphic overview on the chronology how lectinology developed.

### 2.1 How Lectinology Started

A seminal report of lasting impact was published in 1860. Therein, the physician S. W. Mitchell gave a detailed account on what happened when performing the following experiment: "One drop of venom was put on a slide and a drop of blood from a pigeon's wounded wing allowed to fall upon it. They were instantly mixed. Within 3 mins the mass had coagulated firmly, and within ten it was of arterial redness" [1]. In the catalogue of his works printed in 1894 he described the situation encountered during the preparation of the cited treatise as follows: "this quarto with its many drawings was the result of 4 years of such small leisure as I could spare amidst

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**Table 2.1** Brief historical account of lectinology

1860	Observation of blood “coagulation” by rattlesnake ( <i>Crotalus durissus</i> ) venom (S. W. Mitchell)
1888	Detection of erythrocyte agglutination by a toxic protein fraction from castor beans (termed ricin) and seeds of related plants (H. Stillmark)
1890	Detection of a toxic lectin in the bark of black locust ( <i>Robinia pseudoacacia</i> , O. Power, O. Cambier)
1891	Toxic plant agglutinins applied as model antigens (P. Ehrlich)
1898	Introduction of the term “haemagglutinin” or “phytohaemagglutinin” for plant proteins that agglutinate red blood cells (M. Elfstrand)
1902	Detection of bacterial agglutinins (R. Kraus, S. Ludwig) and demonstration that blood “coagulation” by snake venom (seven to nine decades later shown to depend on presence of a C-type lectin) observed in 1860 was due to cell agglutination but not to blood clotting (S. Flexner, H. Noguchi)
1906	Detection of an agglutinin in bovine serum (later characterized as the C-type lectin conglutinin) by use of activated complement-coated erythrocytes (J. Bordet, F. P. Gay); detection of a haemagglutinin in mushrooms ( <i>Amanita</i> sp., W. W. Ford)
1907/1909	Detection of non-toxic agglutinins in plants, of their nature as proteins and of “deagglutination” of erythrocytes by hog gastric mucin (K. Landsteiner, H. Raubitschek)
1913	Use of intact cells for the purification of the agglutinin ricin (R. Kobert)
1919	Crystallization of a globulin from jack bean, concanavalin A, which was later defined as lectin and used in pioneering studies (please see below) (J. B. Sumner)
1935/1936	Concanavalin A identified as jack bean haemagglutinin; precipitation of starch, glycogen and mucins by concanavalin A defines carbohydrate as ligand and points to “a carbohydrate group in a protein” as binding partner on erythrocytes (J. B. Sumner, S. F. Howell)
1941	Detection of viral agglutinins (G. K. Hirst; L. McClelland, R. Hare)
1944	Description of anti-O(H) haemagglutinating activity in serum of <i>Anguilla anguilla</i> (B. Jonsson), following earlier work on a similar activity in <i>Anguilla japonica</i> (S. Sugishita; 1935)
1947/1948	Detection of plant agglutinins specific for the human histo-blood group A (W. C. Boyd; K. O. Renkonen), “good keeping qualities” and low “cost of producing them” are emphasized as advantageous properties
1952	Carbohydrate nature of histo-blood group H(O) determinant proven by eel-serum-mediated agglutination of respective erythrocytes and its $\alpha$ -L-fucose-dependent inhibition (W. M. Watkins, W. T. J. Morgan)
1954	Introduction of the term “lectin” for plant (antibody-like) agglutinins, primarily for those which are specific for a distinct histo-blood group (W. C. Boyd)
1956	Detection of an agglutinin specific for the human blood group B in the seeds of the African shrub <i>Griffonia (Bandeiraea) simplicifolia</i> (O. and P. Mäkelä)
1960	Detection of the mitogenic potency of lectins toward lymphocytes (P. C. Nowell)
1963	Introduction of affinity chromatography for the isolation of lectins, published in 1965 (I. J. Goldstein, B. B. L. Agrawal)
1968–1974	Detection of rapid serum clearance of asialoceruloplasmin in rabbits and isolation of a Gal/GalNAc-specific lectin (asialoglycoprotein receptor) from liver, the first mammalian lectin (G. Ashwell, A. G. Morell and colleagues)
1972	Determination of the amino acid sequence and the three-dimensional structure of a lectin, concanavalin A (G. M. Edelman, K. O. Hardman, C. F. Ainsworth et al.)

(continued)

**Table 2.1** (continued)

1972–1977	Detection of impaired synthesis of a marker for glycoprotein (lysosomal enzymes) routing as cause for a human disease (mucopolidosis II) and its identification as Man-6-phosphate, the ligand for P-type lectins (E. F. Neufeld and colleagues; W. S. Sly and colleagues)
1978	First conference focusing on lectins and glycoconjugates, termed Interlec (T. C. Bøg-Hansen)
1979	Detection of endogenous ligands for plant lectins (H. Rüdiger)
1981–1988	Further refinements of the definition of the term “lectin” as carbohydrate-binding protein, separated from antibodies and carbohydrate-processing enzymes/sensor or transport proteins for free sugars (S. H. Barondes, I. J. Goldstein, J. Kocourek et al.)
1982	Introduction of serial lectin affinity chromatography as analytical tool for structural analysis of glycans from glycoproteins (R. D. Cummings, S. Kornfeld)
1983	Detection of the insecticidal action of a plant lectin (L. L. Murdock)
1984	Isolation of lectins from tumors (H.-J. Gabius; R. Lotan, A. Raz)
1985	Immobilized glycoproteins as pan-affinity adsorbents for lectins (H. Rüdiger)
1989	Detection of the fungicidal action of a plant lectin (W. J. Peumans)
1992/1993	Detection of impaired synthesis of lectin (selectin) ligands by defective fucosylation as cause for leukocyte adhesion deficiency type II, a congenital disorder of glycosylation (CDG IIc) (A. Etzioni and colleagues)
1995	Structural analysis of a lectin-ligand complex in solution by NMR spectroscopy (J. Jiménez-Barbero and colleagues)
1996–2003	Detection of differential conformer selection by plant, bacterial and animal lectins (H.-J. Gabius and colleagues; L. Poppe and colleagues)
2001–2005	Development of glycan/lectin microarrays for specificity analysis of lectins/structural analysis of glycans and glycoproteomics (various laboratories worldwide)
2001–2007	Advances in lectinology and glycosciences honored by devoting special issues in <i>Advanced Drug Delivery Reviews</i> , <i>Biochimica et Biophysica Acta</i> , <i>Biochimie</i> , <i>Biological Chemistry</i> , <i>Cells Tissues Organs</i> , <i>Chemical Reviews</i> , <i>Current Opinion in Structural Biology</i> , <i>Glycoconjugate Journal</i> , <i>Journal of Agricultural and Food Chemistry</i> (“Liener symposium”), <i>Nature</i> and <i>Science</i> to these topics

From [11], extended, updated and modified

the cares of constantly increasing practice. The story of the perils and anxieties of this research, embarrassed by want of help and by its great cost, is untold in its pages.”

His later experiments using washed erythrocytes and then studies by S. Flexner and H. Noguchi, which S. W. Mitchell himself inspired, revealed that the noted ‘coagulation’ did not result from procoagulants (clotting factors) in blood. As stated by these authors, “the value of the use of washed corpuscles comes especially from the fact that the suspension of lytic phenomena is eliminated. Agglutination, therefore, may be studied purely” [2]. The “venom-agglutination” was especially strong with rabbit erythrocytes—swine and ox cells being less susceptible—and akin to the reaction with “intermediary bodies” (today known as immunoglobulins) [2]. Its biochemical nature was defined in 1984 by purifying the agglutinin of *Crotalus durissus* venom, a C-type lectin [3]. This feat

underscored the pioneering character of S. W. Mitchell's research for lectinology. Groundbreaking as it was, the spirit and attitude, with which he carried out his work, as captured on p. 1 of his report [1], also continue to set commendable example: "for the researches which form the novel part of the following essay, I claim only exactness of detail and honesty of statement. Where the results have appeared to me inconclusive, and where further experimental questioning has not resolved the doubt, I have fairly confessed my inability to settle the matter. This course I have adhered to in every such instance, thinking it better to state the known uncertainty thus created than to run the risk of strewing my path with errors in the garb of seeming truths." Its range was extended to plants as sources for agglutinins by a medical thesis in 1888 (Table 2.1).

Using the same technique of haemagglutination, extracts of plant seeds, initially of toxic castor beans (*Ricinus communis*), were also shown to be active. H. Stillmark described a toxin with agglutinating activity, termed ricin, in his MD thesis in 1888. It was prepared under the guidance of R. Kobert at the University of Dorpat (now Tartu) in Estonia, then belonging to the Russian empire [4]. He defined "ricin" as a protein ("Eiweisskörper, sog. Phytalbumose"), conglomerating (or agglutinating) the red blood corpuscles in defibrinated-serum-containing blood ("Zusammenballung der rothen Blutkörperchen") [4]. Such an activity was also uncovered in *Abrus precatorius* seeds ('abrin'), the respective protein fraction produced and tested in R. Kobert's pharmacological institute, and then preparations of ricin and abrin, made commercially available by the Merck Company in Darmstadt on Kobert's initiative, found immediate usefulness beyond lectinology. They substituted bacterial toxins in P. Ehrlich's fundamental studies on the immune response [5]. Once that activity was measured and degree of purity increased, the need for a name became obvious.

## 2.2 Early Definitions

On the initiative of R. Kobert, who left Dorpat for Germany in January 1897 due to the russification of the Baltics that had been unleashed by an attempted assassination on czar Alexander III, this issue was addressed in 1898. M. Elfstrand introduced the term "Haemagglutinin (Blutkörperchenagglutinin)" into the literature [6]. He also noted the "striking similarity" between agglutinating proteins from plants and from human/animal sera [6]. Indeed, exactly this period was characterized by an equally dynamic development in serology. It led to the recognition of the ABO blood group system based on detecting and monitoring isoagglutination (for historical reviews, please see [7, 8]). The discovery of the agglutination and lysis of erythrocytes by serum compounds is especially linked with three investigators, i.e. A. Creite (a medial student in Göttingen in 1869), L. Landois (director of the physiological institute at the University of Greifswald in 1875) and K. Landsteiner, a Nobel Laureate in 1930, in 1900 working at the institute for pathological anatomy in Vienna [7, 8]. Their studies and the work on plant agglutinins (K. Landsteiner referred to them as "Normalantikörper (normal antibodies)")

revealed that the two protein classes also shared activity as precipitin, selectivity for erythrocytes from different species and inhibition of agglutination by haptens (the info box 2 in Chap. 1 tells the story how an agglutinin from eel serum was instrumental to define  $\alpha$ -L-fucose as a structural determinant of the H epitope). On these grounds it becomes rather obvious to look at plant (and other) agglutinins as antibody-like proteins. That they are capable to select epitopes figures as central factor in coining a handy generic term.

### 2.3 The Current Definition of the Term ‘Lectin’

The work on blood-group-specific proteins laid the foundation for the new definition (Table 2.1). It was given by W. C. Boyd in 1954 as follows: “it would appear to be a matter of semantics as to whether a substance not produced in response to an antigen should be called an antibody even though it is a protein and combines specifically with a certain antigen only. It might be better to have a different word for the substances and the present writer would like to propose the word *lectin* from Latin *lectus*, the past principle of *legere* meaning to pick, choose or select” [9]. The author thus intended—in his own words—“to call attention to their specificity without begging the question as to their nature” [10]. Specificity for lectins today means binding activity for sugars. Because not only lectins exhibit this property, they are at present strictly delimited from carbohydrate-specific immunoglobulins, enzymes using carbohydrates as substrates (glycosyltransferases, glycosidases and any enzymes introducing or removing substitutions such as sulfotransferases/sulfatases) and sensor/carrier proteins for free mono- or oligosaccharides [11].

Historically, selectivity of lectins for glycans was unambiguously delineated first in the case of concanavalin A by J. B. Sumner and S. F. Howell in 1936 (for the protocol how to prepare the crystalline lectin, please see info box in Chap. 17) [12]. These authors surmise that dissolving concanavalin A in solutions with sucrose may have masked this activity in any previous studies. Of note in this context, K. Landsteiner and H. Raubitschek had observed “deagglutination” of erythrocyte aggregates, formed by ricin, abrin or bean extracts, by hog gastric mucin in 1909 [13]. This observation, viewed in retrospect, can now be interpreted as early evidence for carbohydrate-binding activity of the tested lectins. In fact, mucins, actually the first protein types detected to be conjugates with sugars in 1865 (“... das Mucin einen gepaarten Stoff darstelle” (that mucin may represent a conjugated compound)) [14], are potent lectin binders exploitable in one-step purification of lectins (please see Chap. 19). Initially, affinity chromatography for lectins used cross-linked dextran (Sephadex<sup>®</sup>) as a matrix for the isolation of concanavalin A [15]. The enormous potential of this strategy had not immediately been realized. A reviewer judged the report after its initial submission to represent “a modest advance in an obscure area” [16]. In effect, this technical advance was instrumental to markedly increase the publication activity in this field, yielding a surge in the number of papers on new lectins and applications [17]. The weak reactivity

to cross-linked agarose of the Charcot-Leyden crystal protein is of interest in this respect, because it indicates reactivity for sugars of this protein, whose typical hexagonal bipyramidal crystals were first described in post-mortem spleen of a leukemia patient in 1853 [18] and sputum of asthmatics in 1872 [19]. Auto-crystallization in situ of this protein constituting 7–10 % of a mature blood eosinophil in protein content (about 8.5 pg/cell) may thus be considered as physiologic purification step. The technical breakthrough of establishing affinity chromatography in lectin research also opened the door for the first experimental purification of a mammalian lectin (Table 2.1) [20]. How its presence and functional significance was traced is recounted in the next paragraph.

## 2.4 Recent Developments

The mentioned line of research was intended to elucidate the role of ceruloplasmin in maintaining the copper level and details on the metabolism of this transport protein for copper ions. As reagent to address these issues, a radioactive form of the glycoprotein was produced by tritiation. This reaction required desialylation of N-glycan chains (for structural details on these chains, please see Chap. 6) and oxidation of galactose at its C<sub>6</sub> atom catalyzed by galactose oxidase [21]. Amazingly, the performed engineering of the N-glycan chains, which unmasked galactose residues, was not without consequences. It dramatically altered ceruloplasmin's serum clearance: "evidence is presented to show that, in contradistinction to homologous, native ceruloplasmin, which survives for days in the plasma of rabbits, intravenously injected asialoceruloplasmin disappears from the circulation within minutes and accumulates simultaneously in the parenchymal cells of the liver. The rapidity of this transfer of asialoceruloplasmin from plasma to liver has been shown to be dependent upon the integrity of the exposed, terminal galactosyl residues." [22]. Thus, desialylation turned N-glycans into ligands, and—luckily—the enzymatic oxidation did not impair the bioactivity for the endocytic hepatic C-type lectin (for further information on C-type lectins, please see Chaps. 17, 20, 21 and 28). It became a role model for targeted drug delivery by (neo)glycoproteins [23] (for further application of neoglycoproteins and examples of sugars as pharmaceuticals, please see Chaps. 26 and 29).

What presently is covered by the umbrella term 'lectin' is outlined throughout this book. As exemplarily emphasized in Table 2.1, intriguing clinical correlations between status of glycosylation and functional implications via lectins have turned up (please see for example Chaps. 26 and 28). Moreover, the molecular details of glycan recognition are unravelled inspiring drug design and new technologies for measuring lectin specificity are developed (please see Chaps. 14 and 15). Combined, these examples for dynamic research lines in lectinology afford efficient driving forces, to make sure that this field maintains its currently acquired prominent status, honored by a series of special feature issues (please see last lines of Table 2.1), and its momentum.

## 2.5 Conclusions

Agglutinating cells is common to different types of proteins. Respective assays with erythrocytes led to an early convergence of work on antibodies and on proteins from diverse sources, which are not produced in response to an antigen. In particular, the crucial role of certain haemagglutinins from plants and eel serum in the process to delineate the biochemical basis of the ABO blood-group epitopes attests their selectivity, rivaling that of antibodies. Close inspection of the literature teaches the lesson that this context entailed coining the term 'lectin'. It embodies the aspect of molecular selectivity. At the same time, it separates the agglutinins terminologically from immunoglobulins. As a consequence of the haptenic inhibition of agglutination by sugars, 'lectin' is now the generic name for carbohydrate-specific proteins, different from antibodies, enzymes acting on the ligand and sugar transport/sensor proteins. The current wide scope of structural and functional studies including promising medical perspectives, described throughout this book, ensures lectinology to properly address the challenges of deciphering the sugar code.

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## Author Biography

**Hans-Joachim Gabius** studied biochemistry in Hannover, Germany and received his PhD degree for work on proofreading mechanisms of phenylalanyl-tRNA synthetase in the lab of Prof. F. Cramer (Max-Planck-Institute for Experimental Medicine, Goettingen) in 1982. Work on tRNA splicing in Prof. J. Abelson's lab at UC San Diego was followed by starting research on lectins at the Max-Planck-Institute. Following a postdoc period with Prof. S. H. Barondes (UC San Diego/VA Medical Center) he was appointed as Assistant Professor in 1988 in Goettingen, then in 1991 as Associate Professor at the University of Marburg, followed by the chair position for Physiological Chemistry (Faculty of Veterinary Medicine) at the Ludwig-Maximilians-University Munich. His track record includes over 690 peer-reviewed publications, which have received over 17,000 citations at an h-factor of 71, and edition of five books, among them the textbook "The Sugar Code". Besides research awards he recently received an honorary degree in medicine by the Charles University in Prague (founded 1348) for his achievements in glycosciences.