Invited Review

Contribution of carbohydrate histochemistry to glycobiology

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Summary. The physiological importance of carbohydrate biology has gradually emerged from a lot of recent information on protein-carbohydrate and carbohydrate-carbohydrate interaction in normal and pathological conditions. After considering the conventional methods which allowed researchers to differentiate glycan-containing macromolecules from other complex compounds (nucleic acids, proteins), selected topics of intracellular and cellular organ architecture are focused upon in which the use of lectins and neoglycoproteins as histochemical reagents has opened new horizons for the localization of glycoconjugates in situ and the elucidation of their often still enigmatic functions. The authors hope to place into perspective that such glycohistochemical studies will strongly contribute to the progress in the dynamically growing field of glycobiology.

Key words: Glycobiology, Glycohistochemistry, Lectin, Neoglycoprotein

Introduction

Up to the late 1960s, the prevailing opinion concerning sugars (or carbohydrates) placed emphasis on their relevance as energy sources in food or as the structural polysaccharides of cellulose in plants and chitin in the exoskeletons of Arthropods (crustaceans, arachnids, insects). The importance of complex carbohydrate chains in the structure and function of glycoconjugates (GCs) in many major biological processes has recently been well documented (Olden et al., 1982; West, 1986; Rademacher et al., 1988; Hughes, 1992; Lee, 1992). Besides nucleic acids and proteins, carbohydrates offer the highest capacity for carrying information because they have the greatest potential for structural variety (Kobata, 1992; Sharon and Lis, 1993).

Recent advances in the field of carbohydrate biology and the ensuing start to unravel their functional significance have underscored the necessity to characterize sugar moieties and oligosaccharide sequences in situ in tissue sections. Thus, the identification of glycans at the microscopic level has developed remarkably in the last ten years and constitutes one of the most fascinating chapters of histochemistry.

The present review is not intended to be encyclopedic. The object of this report is to provide the non-specialist with selected and updated examples of progress within carbohydrate histochemistry that may lead, in combination with biochemical methods, to rational glycobiology approaches.

The first part of this paper reviews the progress in the development and application of chemical and microscopical methods using cationic dyes and other substances (also referred to as conventional or non-lectin methods). In the second part of this article the use of lectins and neoglycoproteins as histochemical reagents coupled to sensitive detection techniques for probing the chemical nature of glycans of GCs in precise loci is considered. Statements and critical viewpoint mainly concern experience from our laboratories. Some novel observations will also be included.

Mucosubstances

Mucosubstance is a collective term that includes macromolecular compounds composed in whole or in part of carbohydrate. The major mucosubstances of animals include: 1) polysaccharides (large aggregates of monosaccharides and monosaccharide derivatives); 2) proteoglycans (long polysaccharide chains, covalently

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attached to a relatively small protein core; and 3) glycoproteins (proteins bearing covalently-bound oligosaccharide chains). Molecules of this class belong to various subgroups, whose structural characterization currently offers a view of steadily increasing complexity. Introduction of modifications like sulphation add to the complexity. Such negatively-charged additions to a sugar chain, together with carboxyl groups, are known from many mucosubstances. Because they contain many negative charges, these molecules have a high affinity for cations. Objects that are stained by cationic (basic) dyes are said to be basophilic in histochemical terminology.

Biochemically, two families of glycoproteins may be considered: N- and O-linked glycoproteins. N-glycosidic bond is established between N-acetyl-D-glucosamine (GlcNAc) and the amine nitrogen of asparagine (Asn). The O-glycosidic bond presents a variety of links; the most important in mucosubstances is represented by oligosaccharide chains linked from N-acetyl-D-galactosamine (GalNAc) to the hydroxyl groups of L-serine and/or L-threonine (Fig. 1) (Montreuil et al., 1986; Montreuil, 1987; Roche, 1991).

![Diagram of glycans](image)

**Fig. 1.** Glycan-protein bonds in N-(upper) and O-(lower) glycosylated oligosaccharides.

**Identification of carbohydrates by means of classical methods**

Only the most popular procedures will be considered: the use of alcian blue (AB); the periodic acid-Schiff (PAS) method; the observation of metachromasia and iron diamines.

These conventional methods have a solid foundation and have been proven over the years to be valuable tools for the localization and characterization of GCs in normal and pathological tissues, providing that three essential demands are met. Firstly, the substance in question must be retained in the site when it occurs *in vivo*. The second requirement is that the chemical group be preserved in a reactive state which will allow characterization by the appropriate histochemical probe. Thirdly, a good preservation of morphological features must be achieved on the basis of the greater acidity of sulphates compared with carboxyl groups. Many papers have reported on AB used at buffer-controlled pH to separate mucosubstances according to their acidity (Spicer and Schulte, 1992). AB is usually applied at pH 2.5 - 3.0 to stain sialic acid and in the pH range 0.5 to 1.0 to selectively stain sulphate polyanions, since at this very low pH the carboxyl groups are undissociated. In case several sections of the same tissue are available, it is possible to determine whether affinity for AB at pH 1.0 and 2.5 is due solely to sulphate esters or to both polysulphate groups and carboxyls coexisting at the same locus. It is quite feasible to identify acid mucosubstance by staining with cationic dyes other than AB. However, the advantage of the latter over most others is that it is not extracted from stained sections by water, ethanol, weak acids, or solutions of other dyes used for counterstaining.

The chemical technique most extensively used in carbohydrate histochemistry is the periodic acid-Schiff
reaction. This oxidative method depends on the cleavage of the glycol linkage by chemical action of periodic acid (HIO₄) that has a high degree of specificity for cis-hydroxyls. Periodic acid oxidizes 1,2 glycol groups in tissue section thereby converting them to di-aldehydes which react with the Schiff reagent to give a magenta colour. This method is positive with structures containing neutral hexoses. The term «neutral hexose» is used by histochemists for monosaccharide residues that do not have sulphate ester, carboxylic acid or nitrogen-containing functional groups (glucose, galactose, mannose and fucose are the principal components of glycoproteins). In tissue sections it has been shown that proteoglycans (containing glycosaminoglycan chains) are not rendered visible by the PAS method usually employed (Spicer and Schulte, 1992). This is rather inexplicable despite content of vicinal hydroxyls in their glucuronic and iduronic acid residues. More rigorous conditions are thus required, but the N-acetyl-hexosamines are still unaffected. On the contrary, mild oxidation by periodic acid produces aldehydes selectively from sialic acid residues. The only potentially periodate-reactive part of a sialic acid glycosically linked at position C2 is the side chain (C7, C8 and C9) attached at C6, which bears three adjacent hydroxyl groups. The side chain reacts with periodate (IO₄) much more rapidly than do the glycols of hexoses. Thus, by using very dilute periodic acid (0.005M) for a short time (5 min), it is possible to oxidize only the sialic acid residues of mucosubstances, and the resultant aldehyde can be visualized with Schiff reagent.

A number of chemical procedures of known reaction mechanisms have been developed and applied in the histochemical study of carbohydrates. The reactive groups of glycan are carboxyls, hydroxyls and sulphate esters. These can be chemically blocked to prevent their subsequent histochemical visualization. Treatment of tissue sections with acetic anhydride dissolved in pyridine for sufficient time converts hydroxyl groups to acetyl esters (esterification); N-acetylation of amino groups can also occur. When the PAS technique is applied subsequently, carbohydrates that are normally PAS positive are no longer stainable by this procedure. This is attributed to the acetylation of adjacent hydroxyl and amino groups required for a positive PAS reaction. Hydrolysis of acetyl esters is achieved by saponification with KOH or with a mixture of concentrated ammonia and absolute ethanol.

Adequate methylation with a solution of HCl in methanol forms methyl esters with carboxyl group. The same reagent, if applied for a sufficient period, also affects O- and N-sulphate residues and methylates amino groups. Staining of acidic mucosubstances by AB, or toluidine blue metachromasia (see below) no longer occurs after sufficient methylation.

Under the same circumstances certain dyes in solution will react with a substrate to produce a colour that is different from that of the original dye solution. This phenomenon is known as metachromasia and dyes possessing the property of reacting in this fashion are called metachromatic dyes. Thionine and toluidine blue are such dyes. In most cases the metachromatic colour of the dye is of a longer wavelength than the orthoromantic colours. This metachromasia is of interest in carbohydrate histochemistry because only certain compounds as acid mucosubstances have this property of reacting with the metachromatic dyes. The metachromatic effect is produced when the coloured ions of toluidine blue, the most commonly used metachromatic dye, are brought into close proximity with one another. This occurs when anionic centres (SO₄²⁻) of the mucosubstance are linked in close spatial proximity, as in some proteoglycans. Toluidine blue forms a red or purple colour in most cell granules, in the intercellular matrices of cartilage, in various mucosubstances and in a number of microbial walls.

Using N,N-dimethyl-meta-phenylenediamine together with a fairly low concentration of ferric ions (LID: low iron diamine) or even three times as high a concentration of ferric ions (HID: high iron diamine) it is possible to visualize acidic carbohydrates and to some degree differentiate between polysulphates and polycarboxylates. Sequential combinations of a couple of dyes for dual stainings of tissue sections have proved to be very informative for the study of the precise natures of the stainable substances in the histochemistry mucosubstances. Thus, application of the HID-AB method distinguished sulphated from carboxylated, non sulphated GCs in a wide range of normal tissues (Zaccone and LoCascio, 1981; Reid et al., 1989). This combined staining method has also provided information of diagnostic significance concerning altered properties of sulphated vs sialylated GCs in neoplasms and premalignant lesions (Filipe et al., 1985; Malchiiodi Albedi et al., 1989). Alcian blue - alcian yellow introduced by Ravetto (1964) is another combination using two basic dyes for detection of different acidic groupings. The AB-PAS sequence performed on a single section is one of our most useful routine stains. Acidic groups of GCs stain turquoise; neutral groups of GCs are magenta. Cartilage, ground substance, and the majority of epithelial mucosubstances are coloured in varying shades of purple to very deep blue.

**Enzymatic extraction in carbohydrate histochemistry**

Digestion of tissue sections with enzymes of known specificity can give important information to allow the discrimination between different histochemically demonstrable carbohydrates. Amylase, hyaluronidase and neuraminidase are the most commonly used. Amylase catalyses the hydrolysis of the glucosidic linkages of glycogen. This can therefore be identified as a substance whose stainability by the PAS method is abolished by treatment of the section with amylase. Testicular hyaluronidase can remove hyaluronic acid, chondroitin-4- or 6-sulphate. Elimination of basophilia by digestion with bacterial hyaluronidase provides
evidence for presence of hyaluronic acid. Removal of basophilia by neuraminidase localizes glycoproteins possessing terminal sialic acid residues.

Other enzymes that split glycosidic linkages are histochemically valuable and are especially used in conjunction with lectins (see below).

These conventional methods alone (Bremer, 1987; Gilks et al., 1988; Boer and Kits, 1990; Chieffi-Baccari, 1990; González de Canales et al., 1992; Saint-Girons and Zylberberg, 1992), or in combination with lectins (Mallinger et al., 1986; Danguy and Genten, 1990; Genten and Danguy, 1990a,b; Hill and de Bruyn, 1991; Getchell and Mellert, 1991; Castells et al., 1991; Ferri and Liquori, 1992; Sharma and Schumacher, 1992) have successfully been employed as reported in the given selection of recent papers of carbohydrate histochemistry.

The readers are referred to the following books as sources for detailed complete technical procedures and original publications. We also recommend these books for a further understanding of the theoretical background for histochemical methods, developed for carbohydrate localization: Lison (1960); Gabe (1968); Ganter and Jollès (1969-1970); Pearse (1980-1985); Clark (1981); Kiernan (1990); Lyon (1991). Full details of these volumes are given in the bibliography section.

### Histochemical use of lectins

Despite their potential to differentiate main categories of mucosubstances, the above mentioned procedures are still rather limited in their specificity for particular sugar residues and yield incomplete information on structural details of the glycans. The introduction of lectins in histochemistry has revealed a number of GCs that have not been previously identified, since they are not demonstrable by conventional methods and, often occurring as a minute fraction of the compounds in the organ, are only amenable to biochemical detection or isolation with affinity techniques like lectin affinity chromatography.

Table 1 summarizes selected highlights of the history of research on lectins (adapted from Gabius and Gabius, 1993b).

<table>
<thead>
<tr>
<th>Year</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1888</td>
<td>Description of toxic, cell-agglutinating protein in seed extracts of <em>Ricinus communis</em> termed ricin (H. Stillmark)</td>
</tr>
<tr>
<td>1891</td>
<td>Application of toxic plant agglutinins as model antigens (P. Ehrlich)</td>
</tr>
<tr>
<td>1898</td>
<td>Introduction of the term «hemagglutinin» for plant proteins that agglutinate erythrocytes (M. Ellisrund)</td>
</tr>
<tr>
<td>1902</td>
<td>Detection of bacterial agglutinins (R. Kraus)</td>
</tr>
<tr>
<td>1907</td>
<td>Discovery of non-toxic plant agglutinins (K. Landsteiner, H. Raubitschek)</td>
</tr>
<tr>
<td>1913</td>
<td>Application of cells for lectin isolation and desorption by acid (R. Koberl)</td>
</tr>
<tr>
<td>1919</td>
<td>Crystallization of concanavalin A (J.B. Sumner)</td>
</tr>
<tr>
<td>1936</td>
<td>Definition of a carbohydrate group as ligand for concanavalin A (J.B. Sumner, S.F. Howell)</td>
</tr>
<tr>
<td>1941</td>
<td>Detection of viral agglutinins (G.K. Hirst)</td>
</tr>
<tr>
<td>1947/48</td>
<td>Description of blood group specificity of certain agglutinins (W.C. Boyd, K.O. Renkonen)</td>
</tr>
<tr>
<td>1954</td>
<td>Description of the term «lectin» for antibody-like proteins (W.C. Boyd)</td>
</tr>
<tr>
<td>1960</td>
<td>Detection of elicitation of a mitogenic response of lymphocytes by binding of a lectin to the cell surface (P.C. Nowell)</td>
</tr>
<tr>
<td>1963</td>
<td>Introduction of affinity chromatography to lectin isolation (I.J. Goldstein)</td>
</tr>
<tr>
<td>1972</td>
<td>Sequencing and analysis of the three-dimensional structure of the first lectin (G.M. Edelman, K.D. Hardman, C.F. Ainsworth)</td>
</tr>
<tr>
<td>1974</td>
<td>Purification of the first mammalian lectin from liver (G. Ashwell)</td>
</tr>
<tr>
<td>1980</td>
<td>Definition as carbohydrate-binding protein of non-immune origin that agglutinates cells (I.J. Goldstein et al.)</td>
</tr>
<tr>
<td>1988</td>
<td>Definition as carbohydrate-binding protein other than an antibody or an enzyme (S.H. Barondes)</td>
</tr>
</tbody>
</table>

**Table 1.** Selected highlights of the history of research on lectins (adapted from Gabius and Gabius, 1993b).
and Halliday, 1992), the reagents from the Vector-<Elite> kit were the most sensitive and specific. On the broad range of tissues investigated in our research unit, this kit received the highest overall rating for quality with 10 times its recommended dilution. In some cases avidin or biotinylated lectins can bind nonspecifically to tissues. Thus, a blocking procedure must be included in the labelling protocol (Danguy and Gabius, 1993). To overcome such a drawback, the steroid digoxigenin (occurring naturally only in plants of the Digitalis family) has been introduced as a new hapten labelling system (Sata et al., 1990). In our hands this procedure has proven comparable in sensitivity and selectively to the method using the ABC complex.

Colloidal gold has recently acquired popularity as a histochemical marker at both the light and electron microscope levels (Roth, 1984a; Skutelsky et al., 1987; Fujimori et al., 1988; Beesley, 1989; Horisberger, 1992; Roth et al., 1992). Lectins were among the first purified macromolecules to be immobilized onto colloidal gold granules. Their precise locations in various tissues by means of transmission (Vorbrodt et al., 1986; Quatacker, 1989; Herken et al., 1990; Ueno and Lim, 1991) and scanning electron microscopy (Horisberger, 1981; Hodges et al., 1987) are well documented. Furthermore, characterization of GCs at the ultrastructural level has provided knowledge about cellular organization and function. Table 2 provides a summary of the source and binding preference of the lectins most commonly used as histochemical reagents. For detailed protocols and advice on how to circumvent major drawbacks, the readers are referred to a recent paper from our laboratories (Danguy and Gabius, 1993).

Table 2. Sources and specificities of the most common lectins used for histochemical characterization of carbohydrate residues and oligosaccharide sequences.

<table>
<thead>
<tr>
<th>SYSTEMATIC NAME (COMMON NAME)</th>
<th>ACRONYM</th>
<th>SUGAR RESIDUES OR SEQUENCES/OLIGOSACCHARIDE STRUCTURES BINDING PREFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dolichos biflorus (horse gram)</td>
<td>DBA</td>
<td>Terminal FpGalNAc1,3GalNAc&gt;GalNAc1,3Gal</td>
</tr>
<tr>
<td>Glycine maxima (Soybean)</td>
<td>SBA</td>
<td>Terminalα(1,3)GalNAc&gt;Gal</td>
</tr>
<tr>
<td>Vicia villosa (hairy vetch)</td>
<td>VVA</td>
<td>Terminal GalNAc1,3Gal&gt;GalNAc1,6Gal=GalAc-serine</td>
</tr>
<tr>
<td>Griffonia simplicifolia (unknown)</td>
<td>GSI-I</td>
<td>TerminalαGal</td>
</tr>
<tr>
<td>Arachis hypogaea (peanut)</td>
<td>PNA</td>
<td>Terminal Galβ1,3GalNAc</td>
</tr>
<tr>
<td>Ricinus communis (castor bean)</td>
<td>RCA-I</td>
<td>Galβ1,4GlcNAc&gt;βGal&gt;xGal</td>
</tr>
<tr>
<td>Sophora japonica (poda tree)</td>
<td>SJA</td>
<td>Terminal Galβ1,3GalNAc&gt;Galβ1,3GlcNAc&gt;α,βGalNAc&gt;α,βGal</td>
</tr>
<tr>
<td>Artocarpus integrifolia (Jackfruit)</td>
<td>JAC</td>
<td>Unlike PNA, JAC will bind terminal Galβ1,3GlcNAc&gt;Galβ1,3GalNAc&gt;α,βGal even in mono- or disialylated form</td>
</tr>
<tr>
<td>Erythrina cristagalli (coral tree)</td>
<td>ECL</td>
<td>Tetraantennary and triantennary oligosaccharides containing three or four N-acetyllactosamine branches respectively</td>
</tr>
<tr>
<td>Phaseolus vulgaris erythrogglutinin (kidney bean)</td>
<td>PHA-E</td>
<td>Bisected bi- and tri-antennary complex N-linked sequences</td>
</tr>
<tr>
<td>Phaseolus vulgaris leukogglutinin (kidney bean)</td>
<td>PHA-L</td>
<td>Highly branched (in tri-antennary or more) non-bisected complex sequences</td>
</tr>
<tr>
<td>Triticum vulgare (wheat germ)</td>
<td>WGA</td>
<td>GlcNAc[(1,4)GlcNAc]1,2-&gt;β1,4GlcNAc=NeuAc</td>
</tr>
<tr>
<td>Succinyl WGA (wheat germ)</td>
<td>S-WGA</td>
<td>GicNAc[(1,4)GlcNAc]1,2</td>
</tr>
<tr>
<td>Griffonia (=Bandeiraea) similicifolia</td>
<td>GSL-II</td>
<td>TerminalαβGlcNAc, glycogen</td>
</tr>
<tr>
<td>Solanum tuberosum (potato)</td>
<td>STL</td>
<td>poly-N-acetyllactosamine</td>
</tr>
<tr>
<td>Lycopersicon esculentum (tomato)</td>
<td>LEL</td>
<td>Oligosaccharides containing poly-N-acetyllactosamine</td>
</tr>
<tr>
<td>Datura stramonium (thorn apple)</td>
<td>DSL</td>
<td>Galβ1,4GlcNAc(N-acetyllactosamine)&gt;GlcNAc</td>
</tr>
<tr>
<td>Canavalia ensiformis (Jack bean)</td>
<td>Con-A</td>
<td>αMan&gt;αGlc</td>
</tr>
<tr>
<td>Lens culinaris (lentil)</td>
<td>LCA</td>
<td>Fucosylated core region of bi- and triantennary N-glycosidically linked oligosaccharides</td>
</tr>
<tr>
<td>Pisum sativum (garden pea)</td>
<td>PSA</td>
<td>The same as LCA</td>
</tr>
<tr>
<td>Galanthus nivalis (snowdrop bulb)</td>
<td>GNA</td>
<td>Manβ1,3Manβ1,6Manβ1,2Man</td>
</tr>
<tr>
<td>Ulex europaeus (gorse seed)</td>
<td>UEA-I</td>
<td>L-Fucα1,2Galβ1,4GlcNAcβ1,6</td>
</tr>
<tr>
<td>Aleuria aurantia (orange peel fungus)</td>
<td>AAA</td>
<td>L-Fuc</td>
</tr>
<tr>
<td>Maackia amurensis</td>
<td>MAA</td>
<td>NeuAcβ2,3Galβ1,4GlcNAc</td>
</tr>
<tr>
<td>Sambucus nigra (elderberry bark)</td>
<td>SNA</td>
<td>NeuAcβ2,6Gal=NeuAcβ2,6GalβNA</td>
</tr>
</tbody>
</table>

FP: Forssman pentasaccharide GalNAc1,3GalNAc1,3Galβ1,4Galβ1,4GlcNAc; Man: Mannose; Glc: Glucose; Gal: Galactose; GlcNAc: N-acetylgalactosamine; GalNAc: N-acetyllactosamine; Fuc: Fucose; NeuAc: Neuraminic acid. Adapted from Goldstein and Poretz, 1986, Wu et al 1988a,b; Vierbuchen 1991; Spicer and Schulte, 1992 and commercial literature of Vector Laboratories and Sigma Chemical Co.
Some recent advances in the analysis of mucosubstances in situ: insights from lectin methods

In this section we present a purposeful selection of applications to illustrate that the potential value of lectins in histochemistry extends beyond simply identifying sugar residues.

The rough endoplasmic reticulum and the Golgi complex

It is well established that the complex apparatus of stacked cisternae and the vesicular membrane profiles associated around it, first described by the Italian histologist Camilo Golgi in 1898, is a main crossroad for intracellular traffic (Farquhar, 1985).

The initial step in the glycosylation of N-glycosyl-proteins occurs in the rough endoplasmic reticulum (rER), where the inner core of the oligosaccharide, containing glucose moieties at the outer part and mannose and GlcNAc residues in the central and inner parts, is transferred «en bloc» from the lipid carrier (dolichol phosphate) to the asparagine of the nascent peptide chain (Hubbard and Ivatt, 1981; Schachter et al., 1983). Further processing in the glycosylation of N-glycosylproteins occurs in the Golgi complex. O-glycosylation processes, including the initial glycosylation reaction for the attachment of N-acetylgalactosamine to serine/threonine groups of proteins, are thought to take place exclusively within the Golgi apparatus (Roth, 1984b).

Besides classical cytochemistry and immuno-cytochemistry, demonstration of binding sites for lectins of different glycan specificities represents one of the most promising tools to gain further insight into Golgi organization. Reaction patterns of the Golgi complex of diversely specialized cells, covering intestinal goblet, absorptive and Brunner's gland cells (Pavelka and Ellinger, 1986, Ellinger and Pavelka, 1988, 1992; Suzuki and Kataoka, 1992), gastric cells (Madrid et al., 1990; Ihida et al., 1991; Rios-Martin et al., 1993), embryonic pancreatic tissue (Pavelka, 1987), airway cells and glands (Wasano et al., 1988; Castells et al., 1992), epiphyseal chondrocytes (Velasco et al., 1988), cultured human fibroblasts (Virtanen, 1990), and the middle ear cavity (Ueno and Lim, 1991) have been reported. Generally speaking, reactions for Con-A, which particularly binds high-mannose N-linked oligosaccharides, are limited to Cis/medial cisternae. Binding sites for RCA I, GSA I and WGA, recognizing terminal galactose and N-acetylgalactosamine, respectively, predominate in the medial-trans subregions of the stacks. LFA and LPA, which are specific for neuraminic acid particularly label cisternae of the trans/transmost Golgi subsections. These differentiated Con A-Cis- and RCA I, GSA I, WGA, LFA-trans transmost reactions, as observed in some cell types, may mirror the conversion of high-mannose-type N-linked oligosaccharides into complex-type glycans. Furthermore, distinct Golgi stack labelling is obtained with PNA, HPA, PSA, LCA and UEA-I; in various types of the gastrointestinal tract, PNA, which recognizes N-acetylgalactosamine-galactose sequences, has been shown to label constituents of the medial Golgi cisternae. HPA, an N-acetylgalactosamine-specific probe, particularly binds with cis cisternae. The HPA-Cis reaction presumably indicates initial steps in the synthesis of O-linked saccharides. LCA and PSA specifically bind to bi- and triantennary N-glycosically-linked oligosaccharides; a fucose residue attached to the Asn-linked GlcNAc is essential for high affinity binding. Hence, the reactions obtained with UEA-I, LCA and PSA may be considered as chiefly indicating presence of glycopeptides containing Core-fucosylated N-linked saccharides. The labelling patterns show that PSA- and LCA-binding molecules are particularly concentrated in cisternae of the Cis and medial subdomains of the Golgi stacks, the penultimate Cis cisternae being the favoured one. UEA-I and LFA never bound to rER cisterns, whilst UEA-I bound to trans Golgi cisternae, or secretory granules indicating addition of fucose during the final step of ready-made material.

These data suggest a compartmentalized organization in the biosynthesis of glycans in a sequential fashion oriented from the Cis to the trans side of the stacks.

Skeletal muscle, neuromuscular junction and electric organ

It has been known for some time that red (or slow) and white (or fast) muscle fibres exist (Gauthier, 1988). Special methods are required to visualized these fibres and their capillary beds. Determination of capillary density in skeletal muscle is of interest in both normal physiology and pathology. Degeneration and loss of capillaries have been observed in some neuromuscular disorders (Jerusalem, 1982; Carpenter and Karpati, 1984). Moreover, vascularization depends on the fibre type distribution of the muscle type; slow fibres are adapted to regenerate ATP by oxidative processes and contain a dense network of capillaries to supply oxygen and substrates. Fast fibres are predominantly glycolytic and contain a sparse circulation. Some lectins (UEA, GSA), selectively stained capillaries and the remanal surface of large vessels (Holthofer et al., 1982; Capaldi et al., 1985) thus permitting the appreciation of the development of vascularization. Recently, by using rhodamine-conjugated-GSA-I, it has been clearly shown that the lectin method is more sensitive than the enzyme technique for demonstrating capillaries in rat skeletal muscle (Christie and Thomson, 1989). Furthermore, to obviate the need for multiple sectioning, Paljarvi and Nankkarinen (1990) have developed a method compatible for both fibre typing and demonstration of capillaries. They used NADH tetrazolium reductase enzyme histochemistry for fibre typing followed on the same section by lectin histochemistry to visualize the binding of UEA-I, a sensitive marker of vascular...
endothelium (Höflöf et al., 1982).

By comparison of the localization of lectin labelling and activity for myosin ATPase and succinic dehydrogenase in rat skeletal muscles, Kirkeby et al. (1992a) showed that the lectin-binding pattern might be related to development, specialization and function of the individual muscles. No relationship can be demonstrated between the mosaic lectin staining pattern and that obtained after enzyme histochemistry.

Neuromuscular disorders have been associated with changes in glycoprotein composition of the sarcolemma, as demonstrated by lectin methods (Bonilla et al., 1980; Paljärvi et al., 1984; Capaldi et al., 1984). In the dy/dy mouse model of muscular dystrophy Kirkeby et al. (1992b) demonstrated, by use of histochemistry and electrophoresis combined with electron microscopy, changes of the muscle carbohydrate components that might be related to the severe muscular impairment observed in this pathological situation.

The vertebrate neuromuscular junction has received some attention over the last years. Comparative studies on anamniote and amniote species demonstrated substantial heterogeneity on lectin binding properties at the neuromuscular junction (Ribera et al., 1987; Iglesias et al., 1992). The significance of such differences, however, did not reveal a clear relationship with the phylogenesis (Ribera et al., 1987).

Electric organ tissues are believed to be homologous to the neuromuscular junction and it has been postulated that, at least in Torpedo marmorata, the electric organ could exhibit surface GCs similar to those in the neuromuscular junction (Egea and Marsal, 1992). However, these authors demonstrated by means of a lectin cytochemistry and an immunocytochemical electron microscopic approach striking differences in GC distribution between the pure cholinergic synapse of T. marmorata electric organ and the cholinergic endplate of the neuromuscular junction. These observations allow us to conclude that GCs are involved more with the development and maintenance of the synapses than with their functional properties.

The skin

In all vertebrates a stratified epithelium, the epidermis, forms the outer covering of the integument (Bereither-Hahn et al., 1986). In mammals, the epidermis consists of a soft inner portion and a hardened outer cornified layer (Matoltsy, 1986). Proliferation takes place in the basal layer of keratinocytes and cells undergo differentiation as they move through the suprabasal layers to the tissue surface. This tissue thus constitutes an attractive model to study alterations in distribution of GCs as the keratinocytes differentiate. Changes in keratinocyte surface carbohydrate during terminal differentiation were identified by lectin binding some years ago (Danguy et al., 1988, for review). Distinct pattern of lectin acceptors to human skin were identified to epidermal maturation and most of the lectins used exhibited a similar binding pattern on a wide range of individuals. The staining with some lectins, however, shows variation between individuals probably reflecting binding to blood-group antigens (Dabelsteen et al., 1984). Using cultured human keratinocytes, Watt and Jones (1992) were able to characterized PNA-binding glycoproteins. PAGE analysis showed that glycoproteins have respectively a molecular mass of 250 kDa and 110 kDa. These compounds may be related to desmosomal glycoproteins and integrins that play a role in cell-cell adhesion (Watt and Jones, 1992).

Changes in the expression of GCs in hair follicles during the keratinization process have been reported (Ohno et al., 1990). The findings emphasize a high degree of complexity of carbohydrate metabolism in the cells of different layers at various stages of keratinization. Due to their remarkable specificity, the lectins are believed to be useful as markers in the clinical analysis of disorders of hair growth (Ohno et al., 1990). Concerning pathological phenomena, changes in lectin labelling were observed in psoriasis (a chronic disease characterized by increased epidermal cell proliferation and disturbed keratinization) (Bell and Skerrow, 1985). The increased cell proliferation has been shown to be caused by proliferative suprabasal keratinocytes (Karimieni and Virtanen, 1989).

By creating a friction-blistre injury of human palm skin, details of the biosynthesis of oligosaccharides has been investigated. The results obtained by Ohno et al. (1989) offer evidence that at least 2 qualitatively distinct oligosaccharides exist in basal and lower spinous keratinocytes. One belongs to the class of high mannos type, as detected with Con-A; another, evidenced with RCA-I and WGA, is probably a member of the family of complex-type chains.

Lectin histochemical investigations have also been undertaken in the skin and glandular structures of farm mammals. Glycoconjugates of goat nasolabial skin, and secretory substances of the sebaceous glands (Tsukise et al., 1988), the preputial gland of the Japan serow Capricornis crispus (Atoji et al., 1989) and the skin of the bovine muzzle and its associated glandular structures (Meyer and Tsukise, 1989) have been characterized in order to better understand either functional relationships to food intake, or the functions of these particular skin regions.

In fish, the epidermis is typically not keratinized and has living cells at its surface (Whitear, 1986). Rich supply in various cell types (various mucous cells, alarm substance cells,...) is well documented (Whitear, 1986). A panel of lectins for histochemical mapping of sugar residues and oligosaccharide sequences have been applied in various teleost species. The main conclusions are that interspecies variation is considerable in the lectin binding pattern (Zaccione et al., 1985; Genten and Danguy, 1990a,b) and that taxonomy is no guide to the type of mucus secreted (Genten and Danguy, 1990a,b). The histochemical properties of the alarm substance cells, a cell type exclusively observed in Ostariophysy,
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has also been characterized, and their variability in GC content emphasized (Genten and Danguy, 1990a,b). It is noteworthy that stress factors affect this property. We have demonstrated that pH-induced stress will elicit differences in the pattern in synthesis of carbohydrate moieties as judged by the lectin approach (Genten and Danguy, 1990a).

Sialic acids are one of the most abundant glycan components in fish epidermis. MAA and SNA are two lectins able to visualize these glycans in situ. Moreover, MAA binds terminal sialic acid-linked (alpha 2 -> 3) to penultimate D-galactose whereas SNA detect sialic acid-linked (alpha 2 -> 6) (Shibuya et al., 1987; Wang and Cummings, 1988). By using these two biotinylated lectins and the avidin-biotin peroxidase procedure we have observed that in Heteropneustes fossilis sialic acid is preferentially attached to the subterminal Gal residue via the alpha 2 -> 6 bond (Figs. 2, 3).

These observations emphasize the status of lectins as powerful tools to detect the actual glycosidic linkage, and its anomeric configuration provided appropriate controls are performed concurrently.

In amphibia the epidermis carries out a unique function in the regulation of gas exchange and osmoregulation (Fox, 1986). On the other hand, these anamniotes inhabit a wide spectrum of different habitats. It has been thought that the varied function performed by amphibian skin may be reflected in the composition of its whole stock of mucosubstances (Danguy and Genten, 1989, 1990). Marked differences in epidermis and

Fig. 2. Heteropneustes fossilis. Part of the epidermis stained with SNA. The basal epithelial cells (arrowheads) and the plasma membrane of alarm substance cells (arrows) are strongly labelled. M: melanophores. x 512

Fig. 3. H. Fossilis. Part of the epidermis stained with MAA. The cells are unreactive. M: melanophores. x 512
glandular lectin binding patterns between toads (*Bufo bufo*) and African clawed frogs (*Xenopus laevis*) have been observed (Danguy and Genten, 1989, 1990). Whether these differences may be correlated to the habitat selection or the phylogenetic tree deserves further research on a large number of species. Equally intriguing is the very recent observation that a vertebrate species possesses a chitinous tissue (Wagner et al., 1993). The pivotal tool for proof has been FITC-conjugated WGA. In order to characterize and compare glycan residues and carbohydrate sequences in tissue sections of fish and anuran, extracellular matrices of the skin lectins have similarly proven their suitability (Genten and Danguy, 1990c). A non-uniform binding pattern has been discovered between *B. bufo* and *X. laevis* skin connective tissue. Difference between teleosts and amphibia have also been seen at the cuticle and distal cytoplasmic segments of the superficial epithelial cells of a teleost fish.

**The kidney**

In kidneys, the main nephron functions, filtration, secretion, absorption and maintenance of electrolyte balance, are supposed to be driven by specialized glycoproteins of as yet, largely not well-defined structures; at any rate glycans are thought to play important roles. Application of lectins of tissue sections of animal and human kidney has revealed compartmentalization of GCs along the nephron (Holthöfer et al., 1981; Holthöfer, 1983; Murata et al., 1983; Schulte and Spicer, 1983; Truong et al., 1988; Sharma and Schumacher, 1992). As a rule, mucosubstances in proximal renal tubules differ from those of the distal nephron. The mammalian collecting duct of the nephron is composed of at least three cell types, the principal cells containing Na\(^+\) K\(^+\) ATPase and two distinct populations of intercalated cells (Kim et al., 1992). Both intercalated cell types have a high level of carbonic anhydrase in their cytoplasm. Type A intercalated cells are involved in acid secretion whilst type B cells are believed to be involved in bicarbonate secretion (Kim et al., 1992). Principal cells in rat collecting ducts stain selectively with DBA and WGA for terminal αGalNAc (Holthöfer et al., 1988). Whether the DBA-positive and -negative cells likewise correspond to principal and intercalated cell subpopulations, respectively, deserves further research.

Differences between species in lectin acceptors are also quite obvious. Significant differences were reported between comparable tubular segments of the nephron in mouse and rat, two related species. GCs containing terminal β-Gal and terminal αGalNAc were prevalent on the luminal surface of the proximal convoluted tubule in the rat, but αGalNAc was absent in this site in the mouse (Schulte and Spicer, 1983). Holthöfer (1983) compared the compartmentalization of glycans in kidneys of fourteen animal species including mammals, avians, reptiles, amphibians and freshwater fish. The findings showed that although some GCs of the nephrons are located in a similar way in different animals, each species exhibits expression of some characteristic features in lectin binding.

Kidneys also play a role in the adaptation to desert life (Schmidt-Nielsen, 1991). Therefore, in our laboratory we have initiated a comparative lectin histochemical study on the nephron of two rodent species producing either normal hypotonic urine (the mouse) or highly concentrated urine (the golden spiny mouse which inhabits deserts) (Coppée et al., 1993). Marked cellular heterogeneity has been observed with several biotinylated lectins in both species. Moreover, each of these exhibits a characteristic distribution of some lectin binding sites underscoring the potential association with the physiological process that encourages delineation.

The osmotic concentration in the blood of freshwater fish (± 300 mOsm/l) is much higher than in the surrounding water. The major problem is thus the osmotic water inflow. Excess water is eliminated as hypotonic urine. Marine teleosts are hypotonic and in constant danger of losing body fluid to the more concentrated environment. To maintain the osmolality of their body fluids they ingest sea water. However large amounts of salts are also ingested and are absorbed from the gut together with the water. The kidney plays a major role in the excretion of divalent ions (Mg\(^{++}\), SO\(_4\)\(^{2-}\)) whilst the gills seem to transport only sodium and chloride (Schmidt-Nielsen, 1991). In our laboratory we have undertaken a comparative study of lectin binding patterns in different segments of the nephron of both a fresh water species (*Salmo gairdneri*) and a marine teleost (*Gadus luscus*). The preliminary results suggest that the glycan distribution is rather specific for each species, although certain common features can also be found between the two teleosts. Some differences in lectin binding pattern are illustrated in Figs. 4, 5. Recently, GC distribution in the renal tubule was assessed histochemically in the dogfish marine elasmobranch by using a variety of lectins as tools (Hentschel and Walther, 1993). Interestingly, despite marked differences in renal organization between elasmobranch fish and other vertebrates, the sequence of nephron segments as visualized by lectin-binding patterns in different segments of the nephron of both a fresh water species (*Salmo gairdneri*) and a marine teleost (*Gadus luscus*). The preliminary results suggest that the glycan distribution is rather specific for each species, although certain common features can also be found between the two teleosts. Some differences in lectin binding pattern are illustrated in Figs. 4, 5. Recently, GC distribution in the renal tubule was assessed histochemically in the dogfish marine elasmobranch by using a variety of lectins as tools (Hentschel and Walther, 1993). Interestingly, despite marked differences in renal organization between elasmobranch fish and other vertebrates, the sequence of nephron segments as visualized by lectin-binding pattern was rather similar to that of tetrapods. Hentschel and Walther (1993) have postulated that the similarity between the complex nephron in the dogfish and the well developed nephron in the frog is that cartilaginous fish and tetrapods have evolved from archaic freshwater fish possessing a well-developed nephron.

More studies are of course needed concerning the physiological relevance of mucosubstances to renal functions.

Lectin histochemistry has also been used to characterized GC modifications in renal lesions (Aguirre et al., 1993). Using Syrian hamsters as the experimental model these authors have reported some different lectin binding patterns from those reported in other species, emphasizing once more histochemical variations...
between normal species. Moreover, in the kidney of streptozotocin-induced diabetic hamsters, Aguirre et al. (1993) have shown modifications in lectin acceptors when compared to control animals, suggesting that some lectins may be valuable as markers of the development of lesions.

Sex steroid hormones and expression of glyco-substances

When we speak about sex steroid hormones we are faced with major differences between the sexes and we have the added complication of species differences which are often so great that generalizations become impossible. In this part our purpose will be restricted to the main events observed in the uterus and vagina of laboratory rodents and the human with some medical implications. The submandibular (salivary) gland of the mouse will also be considered because it is surprising that it actually is a target for steroid hormones. Laboratory rodents (mouse, rat) have been the workhorse of the experimental endocrinologist for decades, and all our ideas about the sexual cycle have been really derived from it. The oviduct, uterus and vagina of adult vertebrates are under the control of ovarian hormones which influence their histophysiology. These organs reach their full maturity at puberty.

It has been postulated that glycoproteins expressed on

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**Fig. 4.** Binding sites of PHA-L in the kidney of *Salmo gairdneri*. Especially marked reaction products are found at the brush border of P2 tubule cells (arrowheads). Distal tubule cells (D) are unreactive. A junction between proximal and distal tubules of the nephron is indicated by a large arrow. x 512

**Fig. 5.** Binding sites of SNA in the kidney of *Gadus luscus*. The brush border of proximal tubule cells (P1) is heavily stained (arrowheads). P2 tubule cells are unlabelled. x 512
the apical surface of luminal cells of the oviduct or the endometrium play an important role in reproduction. These facts have prompted researchers to undertake investigations of lectin binding to uterus and vagina epithelium in normal as well as pathological conditions. The mouse (like the rat and the human female) is a spontaneous ovulator. In this species the sexual (oestrus) cycle has four phases: prooestrus, oestrus, dioestrus I (metoestrus) and dioestrus II.

Very clear-cut cyclic changes occur in the vaginal epithelium and vaginal contents of the mouse and rat (Allen, 1922; Nelson et al., 1982). Using a panel of fluoresceinated lectins, Vrcic et al. (1991, 1992) have been able to demonstrate that the cyclic changes in the histology of the vaginal epithelium and of vaginal smear entail changes in lectin binding to cellular GCs.

In rodents, the formation of decidua in pregnancy or in hormonally-induced pseudo-pregnancy is accompanied by the appearance of a uterine swelling known as the metrial gland (Bulmer et al., 1987). Granulated metrial gland cells of a not well-defined function have been encountered. It has been suggested that these cells contain bioactive substances stored in their cytoplasmic granules. DBA a GalNAc binding lectin has been used as a histochemical reagent for visualizing these cells in histological sections, enabling researchers to learn more about their functions in pregnancy (Damjanov and Damjanov, 1992).

Hormonal changes and local events within mouse uterus during early pregnancy influence the expression of some endometrial glycoproteins, as demonstrated with *Ricinus communis* lectins (Horvat, 1993). Marked modifications in lectin binding patterns have also been demonstrated during porcine uterine development, suggesting alterations in the distribution of some GCs during ontogenesis (Spencer et al., 1992). In these hormone-responsive organs GC expressions are under the influence of trophic hormones and involve a precise interplay between estrogens and progesterone.

In gynecologic pathology it has been shown that lectins can discriminate between endocervical mucosa and endometrium, and between some benign, pre-malignant and malignant conditions of the cervical epithelium (Bychkov and Toto, 1986, 1988; Byrne et al., 1989). They also show a different binding pattern to endometrium with the course of the menstrual cycle, between normal and gestational endometrium, and in transition to malignancy (Bychkov and Toto, 1988).

In the male mouse the adult submandibular gland is larger than that of the female because the granular convoluted tubules are much more numerous and conspicuous (Chrettien, 1977; Pinkstaff, 1980). Biologically-active polypeptides have been localized in this tubular segment (Barka, 1980). However, the impact of hormones on this parameter has not been evaluated. Besides corroborating the presence of lectin-binding sites in the mouse submandibular gland, we have recently demonstrated that the endocrine status has changed the histochemical expression of glycogen residues of glycoproteins as well as endolectins, thus modifying both sides of presumed protein-carbohydrate interactions (Akif et al., 1993).

**Parasitology**

As in a wide variety of biological phenomena, cell surface sugars are implicated in host-parasite relationship. Therefore, commercially available lectins have been employed in attempts to identify trypanosomatid surface ligands and their complementary receptors (Schottelius, 1990). With electron spectroscopic imaging a high concentration of RCA I-ferritin particles has been visualized on the surface membrane of cardiomyocytes of mouse embryos at the site of *Trypanosoma cruzi* attachment (Barbosa and Meirelles, 1993). These findings suggest that migration of host galactosyl residues occurs during the cellular recognition process. Similarly the cuticle of pathogenic nematodes forms interface of the parasite with the host. Controversial results have been reported in the literature using gold-labelled lectins. Peixoto and De Souza (1992) were unable to observe labelling of the cuticle of *Coenorchabditis elegans*, whilst using iodinated lectins Zuckerman et al. (1979) reported the presence of Con-A, RCA and WGA acceptors. Labelling of cuticle thin sections of other nematode species embedded in a hydrophilic resin (lowicryl) has been reported by Rudin (1990).

The malarial parasites (*Plasmodium sp.*) are transported by Anopheles. Fluorescein isothiocyanate-labelled lectins have recently revealed interspecific variations of salivary gland carbohydrate moieties together with intraspecific differences between anopheline strains (Mohamed et al., 1991).

Filariosis constitutes a serious public health problem in tropical and subtropical regions. It is evident that the outer structures of the microfilariae play a prominent role in the host-parasite interaction. Previous studies have shown that filarial antigens contain glycans mainly as glycoproteins. A recent investigation confirmed those findings and described the precise distribution of sugar residues in their sections of lowicryl-embedded mature microfilariae of *Wuchereria bancrofti* and *Brugia malayi* using gold-labelled lectins (Araujo et al., 1993).

Based on such glycohistochemically-provided guidelines, further biochemical and cell biological experiments are warranted to contribute to our understanding of the infection process.

**Enzymatic deglycosylation and lectin histochemistry**

Sialic acids are a family comprising about 30 natural derivatives of neuraminic acid, an acidic aminosugar with nine C-atoms (Schauer, 1985; Mandal and Mandal, 1990). Unsubstituted neuraminic acid does not occur in nature but is the most diversely substituted natural glycan component (the amino group is substituted either by an acetyl or glycolyl residue, and the hydroxyl groups
may be methylated or esterified with acetyl, lactyl, sulphate or phosphate groups). Sialic acids have important biological roles (Schauer, 1985). Of the commercially available plant lectins, only a few bind with sialic acids; MAA and SNA are the most popular (Pajak and Danguy, 1993). Sialyl residues primarily occupy the terminal portion in both N- and O-linked oligosaccharide antennae, masking the penultimate disaccharides. Removal of terminal sialic acid residues by neuraminidases (= sialidases), the penultimate disaccharides are stainable with PNA or DBA. Thus, the histochemical sequence involving pretreatment with neuraminidase followed by labelling with PNA and DBA allows in situ localization of terminal tri- and disaccharides such as NeuAc-(1->3,6)-D-Gal-ß(1->3,6)-D-GalNAc.

Enzymatic hydrolysis in combination with lectins is extensively used for the analysis of Gcs in salivary glands and other tissues (Ito et al., 1988, 1989; Acili et al., 1989, 1992; Menghi et al., 1989, 1990, 1991, 1992, 1993; Castells et al., 1990, 1992; Gheri et al., 1992; Iglesias et al., 1992). This is possible because of the wide array of glycosidases of known specificities available for such studies. Thus, structural information gradually emerges by the application of highly specific glycosydases in lectin protocols.

Neoglycohistochemistry

The field of endogenous lectins in all domains of animal biology has remained of minor interest until very recently. The discovery of the essential role of glycan-binding proteins in a wide range of biological phenomena emphasized the ubiquitous role of glycochemistry (Gabius and Bardosi, 1991; Gabius and Gabius, 1991; Zanetta et al., 1992a,b).

Recently, the glycan-binding sites present in sections of fixed tissues have been analyzed using methods which allow the detection of endogenous lectins (Danguy et al., 1991; Gabius and Bardosi, 1991; Danguy and Gabius, 1993; Schottelius, 1992).

They use synthetic probes, biotinylated neoglycoproteins, in conjunction with subsequent visualization with the avidin-biotin peroxidase complex. Neoglycoproteins are built by chemically coupling sugar moieties (monosaccharides or oligosaccharides) to an inert carrier protein (generally bovine serum albumin). These molecules display a strong avidity for endogenous glycan-binding proteins (endolectins) in various tissues (Danguy et al., 1991; Gabius and Bardosi, 1991; Gabius et al., 1993).

Very recently, Gabius and co-workers published in the present journal a review in which we emphasized the versatility and the feasibility of neoglycoconjugates as a tool to detect such carbohydrate acceptors, allowing one to evaluate both sides of lectin histochemistry, namely lectins as probes and lectins as functionally important molecules. Therefore, this topic will not be considered further and the readers are referred to the mentioned publication (Gabius et al., 1993).

Conclusions and perspectives

Besides nucleic acids and proteins, glycans offer a high capacity for carrying information because they have a great potential for structural variety. This extraordinary complexity of their structures discouraged further research. In recent decades however, the importance of carbohydrate biology has gradually emerged. Glycans are common components of animal cell surfaces and are also found in cell organelles and among the products exported by the cell. The cell surface GCS have shown to play prominent roles in intercellular communication, cell adhesion and recognition from the early stages of evolution and ontogenesis. In vertebrates, membrane lectins participate in many protective reactions of the immune system; they are also involved as receptor for hormones and microbes and tumorigenesis. The need for localization and distribution of sugar residues and complex carbohydrate chains in situ has concomitantly appeared. As a general rule the earliest histochemical methods (also called «conventional» or «classical») for localizing glycans-containing macromolecules (glycoproteins, proteoglycans) have mainly served to differentiate these compounds from the other macromolecular constituents in tissue section; i.e. nucleic acids and proteins. The use of carbohydrate-degrading enzymes further adds to the quality of characterization of oligosaccharide sequences in tissue sections. The introduction of lectin methods in histochemistry has added another dimension to the analysis of mucosubstances as evidenced by the vast number of publications.

The well-documented ubiquitous occurrence of lectin acceptors (endogenous glycans) encourages functional consideration. Demonstration of lectins in cells and tissues of vertebrates (endolectins) has raised a pertinent question concerning the physiological relevance of lectin-carbohydrate recognition. The use of synthetic glycan ligand-exposing markers for histochemical purpose has enriched our knowledge in the field of glycochemistry. It is reasonable to propose that the concomitant monitoring of both sides of a protein-carbohydrate interplay, namely the GC structure as well as the glycan-binding protein, is imperative to unequivocally prove attractive concept for functional implications. Currently, the field of glycochemistry is growing due to the joint efforts of scientists from different disciplines; biochemistry, histochemistry, and pathology.

In this article we hope to have placed into perspective the contribution of glycohistochemistry in unravelling the biological roles of glycans.
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