ISSN 0006-2979, Biochemistry (Moscow), 2013, Vol. 78, No. 7, pp. 679-696. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © G. Ya. Wiederschain, 2013, published in Biokhimiya, 2013, Vol. 78, No. 7, pp. 877-900.

REVIEW

Glycobiology: Progress, Problems, and Perspectives

G. Ya. Wiederschain

Program in Glycobiology, Department of Biology, 140 Commonwealth Avenue, Boston College, Chestnut Hill, MA 02467, USA; fax: +1 (617) 552-2011; E-mail: Gherman.Wiederschain@bc.edu

Received April 21, 2013

Abstract—This review highlights different aspects of glycobiology with analysis of recent progress in the study of biosynthesis, degradation, and biological role of glycoconjugates and of hereditary diseases related to the metabolism of these compounds. In addition, the review presents some analysis of the papers of other authors who have contributed to this special issue.

DOI: 10.1134/S0006297913070018

Key words: glycobiology, glycans, glycoconjugates, glycosyltransferases, glycosidases, lectins, receptors, lysosomes, glycosidoses, lysosomal storage diseases, hereditary disorders, biosynthesis and degradation of glycoconjugates

In memory of Professor Evgenia Lazarevna Rosenfeld

This special issue of *Biochemistry (Moscow)* concerns different aspects of glycobiology, the science that studies the structure, function, and metabolism of carbohydrate-containing compounds and that has been rapidly developing during the last three decades. Studies in glycobiology are now extending to virtually all biochemical and physicochemical processes inherent in living organisms. The trends in glycobiology include studies on simple and complex carbohydrates, primarily on carbohydrate-containing biopolymers, or glycoconjugates, which include glycoproteins, proteoglycans, and glycolipids. The most important directions in the investigations of glycoconjugates are their biosynthesis and degradation along with molecular and cell biological processes where carbohydrate-containing compounds frequently act as key molecules responsible for the high efficiency and specificity of these processes. The widest range of these can be followed through fundamental lines of investigations of intercellular interactions and adhesion, energy system of the organism, biological signaling, blood coagulation, immune response, provision of receptors and hormones, fertilization, and embryogenesis. Studies related to molecular mechanisms underlying the development of pathological processes in the human body occupy a special place in glycobiology. Disorders (often hereditary) in the metabolism of carbohydrates and carbohydrate-containing compounds include diverse lysosomal storage diseases, diseases associated with disorders in glycosylation and blood coagulation, diabetes, and muscle dystrophies. In some malignant tumors, significant changes in the glycoconjugate spectrum and in activities of the enzymes responsible for their biosynthesis or degradation are used as molecular markers for early diagnosis.

This review cannot describe and analyze all aspects of glycobiology. Many experimental papers, reviews, and monographs highlighting various trends in glycobiology have been published within the last decade. Along with recently published encyclopedic handbooks in glycobiology with more than 3000 pages [1, 2], there are briefer but rather informative textbooks on glycobiology for students and junior researchers [3, 4]. One of these books, *Essentials of Glycobiology* [3], published in 2009 as the 2nd enlarged edition, contains contemporary data on many aspects of glycobiology and is a useful handbook. Because of the limited volume of this review, in some cases we give full titles of recently published monographs and collective comprehensive editions summarizing data of many scientific groups in glycobiology obtained with a great variety of approaches. Some of these publications have been used as a basis for writing the present paper and also other papers of this issue, and the reader can find these sources in the corresponding references. The purpose of the present review is to discuss several trends in glycobiology, progress in these directions, and problems and prospects of the science of glycobiology.

UNIQUE INFORMATIONAL FEATURES OF SIMPLE AND COMPLEX CARBOHYDRATES AND THEIR CELLULAR AND SUBCELLULAR LOCALIZATION

Studies on the structure of simple carbohydrates were begun by E. H. Fischer near the end of the 19th century. As discriminated from nucleotides and proteins, which are linear polymers with the same bond between the monomers, monosaccharides can be D- or L-sugars. They can be furanosides or pyranosides and bind to each other with α- or β-glycosidic linkage between the most reactive hemiacetal hydroxyl at the C1 atom of one monosaccharide and one of hydroxyl groups at the C2, C3, C4, and C6 atoms of another monosaccharide, and at the C1 atom in some cases. Thus, three identical amino acid units can produce only one tripeptide with the same peptide bond, whereas three identical monosaccharides of pyranoside D-glucose can theoretically produce 176 glucose trisaccharides different in structure, and three different monosaccharides (XУZ) can produce 1056 trisaccharides, whereas three different amino acids can produce only six different peptides. The calculation includes not only the possibility of producing different glycosidic linkages between monosaccharides (1–2, 1–3, 1–4, and 1–6), but the glycoside oxygen position (αα, αβ, or ββ) on the formation of a glycosidic linkage. The carbohydrate chains, including those of simple glycans, polysaccharides, and glycoconjugates, can also be branched. Such modifications significantly increase the number of possible carbohydrate structures having the same monosaccharide sequence. Thus, a hexasaccharide consisting of six hexoses has more than a trillion possible structural combinations [5, 6]. Fortunately for glycobiologists, the structure of natural glycans is not so tremendously diverse; nevertheless, it seems that the spectrum of carbohydrate structures can expand and give new combinations under the influence of various factors, including disease-associated changes in the body.

Polysaccharides such as starch, glycogen, and cellulose are polymers of the same monomer, glucose, illustrating how different anomeric configurations of glycosidic linkages between monosaccharide, α- or β-, and even slight changes in the type of bonds, 1–4 or 1–6, change the conformation and properties of each of these polymers.

Starch consists of a linear amylose polymer with D-glucose residues bound with the α(1→4)-glycosidic linkages. Branches of amylopectin consist of the same glucose units, but bound to amylose with α(1→6)-linkages, and a hollow helical structure is formed with about 20 to 25 glucose residues of amylose, and this conformation is the most suitable energy store for green plants.

Glycogen, a high molecular weight polymer with a starch-like helical structure, has an approximately similar set of bonds, α(1→4)-bonds in the linear moiety of the molecule and α(1→6)-bonds in branches that occur every 4 to 8 glucose residues. Glycogen is the main energy store not only in animal cells, but also in insects, plants, fungi, and yeast. The strongest fibers of plant cell walls are composed of cellulose, which is a linear glucose polymer of β(1→4)-bonds unrelated to any of the energy storage polymers [7].

Still more structurally diverse compounds can be produced by combining carbohydrate moieties into other molecules. As mentioned, glycoproteins, proteoglycans, and glycolipids are the most important biological glycoconjugates. The structure of these complex compounds, their non-template synthesis under the influence of highly specific glycosyltransferases and degradation under the influence of glycosidases, are being studied in many research laboratories with remarkable progress [8-15]. However, we are still very far from complete understanding of the construction of glycoconjugate molecules during their advance along the "assembly lines" within cells. Unique biosyntheses occur on the way from the rough endoplasmic reticulum (RER), where glycosylation of proteins and lipids only begins, and further on the assembly line of the smooth endoplasmic reticulum (SER) and different cisterns of the Golgi complex (GC). The GC is the place of final construction of carbohydrate chains including their different modifications: sulfation, phosphorylation, acetylation, and many other changes under the influence of highly specific glycosyltransferases, processing glycosidases, sulfotransferases, and other modifying enzymes. There is no doubt that many enzymes remain to be discovered and studied in detail.

It should be emphasized that various cells, tissues, and organs are characterized by sets of glycoconjugates specific in structure within the cellular, tissue, and organ structure. In turn, this specificity determines the functional specialization of the cells, tissues, and organs, and the highly efficient functioning and cooperation of metabolic processes of the whole organism. The diversity and heterogeneity of glycoconjugate structure are known, as well as existence of their so-called glycoforms, and this shows again that these molecules have really unlimited structural–functional abilities that are not inherent in any other class of compounds of living organisms [16, 17]. The question arises of whether these unique abilities of glycans are actually used in living organisms. The answer is affirmative, because Nature could not miss such a possibility during the long periods of evolution.

Glycosylation of different molecules of living systems is generally thought to be their most frequent modification [18]. To some degree, this modification is found in various molecules of plant and bacterial cells, viruses, and fungi, and it is most fully documented in molecules of eukaryote cells. Polysaccharides of plant cells form their envelope and are their major nutritional stores. In bacteria and fungi, glycans are the most important structural elements of the cell walls responsible for intracellular

homeostasis and protecting against bacteriophages and antibiotics generated by other microorganisms [19].

Due to combined features of their complex molecules – amphipathic (glycoproteins, glycolipids), conformational, neutral or negatively charged (sialo-biopolymers and proteoglycans) – glycoconjugates can be located in membranes of all cells and subcellular organelles, including the nuclear membrane. Carbohydrate chains can be oriented both outside the membranes and into the organelles to perform certain functions including signal transduction and triggering different processes under the influence of events on the cell surface or on the outer surface of the organelles. Proteoglycans, some glycoproteins, and glycolipids occupy intercellular spaces in animals and plants and cover the plasma membrane with a layer called the glycocalyx. The glycocalyx determines cell adhesion, protects cells against deleterious chemical influences, is involved in mineral and water metabolism of cells, mediates the immune response to a large spectrum of microorganisms, recognizes tumor cells, protects leukocyte movement in normal endothelium of blood vessels, and participates in fertilization and embryogenesis.

Thus, the simple and high molecular weight glycans by themselves or as components of glycoconjugates occupy communication "strategic posts", performing the most important functions of intercellular interaction, signaling, stabilization, and protection of biologically active compounds against untimely proteolysis, as well as of binding and neutralizing viruses and bacteria. Moreover, glycan-containing molecules of biological fluids, e.g. many glycoproteins of the bloodstream, various secreted molecules, and mobile cells in blood vessels can be recognized due to their carbohydrate determinants by many animal lectins [20]. Highly efficient binding with lectins often determines the lifetime and further fate of molecules and cells. It is reasonable to at least generally consider some functional features of glycoconjugates – their biosynthesis, degradation, and mechanisms of delivery to particular places in cells and tissues where these molecules "are in service".

BIOSYNTHESIS AND DEGRADATION OF GLYCANS AND GLYCOCONJUGATES

Biosynthesis. Glycosylation in animal cells is initiated in the RER through the interaction of three main components: acceptors of carbohydrate residues, their donors, and highly specific glycosyltransferases located on the inner surface of the RER membranes and in different compartments of the Golgi complex.

Acceptors of carbohydrate residues can be proteins, lipids, or other simple and complex carbohydrates. Two classes of compounds can act as donors of carbohydrate residues. The first class includes nucleoside diphosphate sugars (NDPS), which are α- or β-glycosyl esters of nucleotides (nucleotide sugars). They can contain adenine, guanine, cytosine, thymine, or uracil as nitrogenous bases. There are more than 50 different NDPSs. The NDPSs are mainly produced during interaction of nucleoside triphosphate and a 1-phosphate sugar in the cell cytoplasm. In animal tissues every monosaccharide corresponds to a certain nucleoside diphosphate, more strictly to its heterocyclic base. Thus, for L-fucose only GDP-fucose, for L-rhamnose – TDP-rhamnose, and for glucose – UDP-glucose are donors.

By contrast, plants and bacteria contain multiple nucleoside diphosphates that can be donors, e.g. glucose can be bound with UDP, GDP, ADP, and CDP. Plant cells contain a set of enzymes responsible for production of different glucose-bound nucleotides that serve as donors of glucose residues during biosynthesis of more complex carbohydrates. An activated form of sialic acids is CMP-sialic acid, which is the only nucleoside monophosphate sugar acting as a donor of sialic acids in animal cells.

NDPSs can be donors of carbohydrate residues because they have greater free energy than the product, as well as individual conformations recognizable by specific transferases on glycosylation [21, 22].

The other class of donors of carbohydrate residues includes lipid carriers, often polyprenol phosphate sugars, which are much more hydrophobic than the hydrophilic NDPSs. The lipophilic moiety of these compounds is a polyunsaturated alcohol consisting of isoprene residues with some carbohydrate residue bound to one or two phosphoric acid residues.

In mammals, the most widely distributed polyprenol is dolichol. The term *dolichol* (from *dolichos* – *long* in Greek) has been proposed for all prenols having a saturated α-isoprene residue carrying a hydroxyl group to discriminate from the bacterial polyprenol undecaprenol having an unsaturated α-terminal unit. The hydrophobic chain of dolichol consists of 80 to 110 carbon atoms and is one of the longest aliphatic molecules known. The undecaprenol chain consists of 50 to 60 carbon atoms. The alcoholic hydroxyl can be replaced by one or two residues of phosphoric acid with production, respectively, of dolicholmonophosphate or dolicholpyrophosphate, and sugar residues bound via a phosphate or pyrophosphate bridge.

The resulting dolicholmono- and dolicholdiphosphate sugars are known to be the main lipid carriers during biosynthesis of carbohydrate chains of N-glycoproteins [23, 24]. The functional difference of these two types of activated donors of carbohydrate residues is that NDPSs donate only one monosaccharide residue to peptides, proteins, lipids, and carbohydrates. Dolicholmonophosphate sugars also donate carbohydrate residues (monosaccharides) on their transfer either directly to the peptide core of the synthesized glycoprotein molecule, to the nonreducing end of the constructed oligosaccharide

chain, or to dolicholpyrophosphate sugar (DPPS), which in this case acts as an acceptor of carbohydrate residues increasing its oligosaccharide chain by 20 monosaccharides. Upon completion, the entire oligosaccharide block can be transferred by the corresponding glycosyltransferase to a protein or another acceptor of the carbohydrate component. Thus, DPPSs act as acceptors of carbohydrate residues during the first stage of glycosylation and as donors during the second stage.

In animals, nucleotide disaccharides and nucleotide trisaccharides have been detected, but their biological role is unknown: no enzyme systems capable of carrying oligosaccharide blocks from such donors to the corresponding acceptors are known. In addition, a possible role of some lipid-soluble vitamins, such as vitamin A (retinol) and vitamin K, is also discussed. From animal cells retinylphosphate, retinylpyrophosphate-galactose, and retinyphosphate-mannose have been isolated, as well as some sugars bound through phosphate or pyrophosphate with vitamin K [25]. It is still unclear whether sugars as components of phosphorylated lipid-soluble vitamins act as donors of carbohydrate residues during the biosynthesis of glycans, or if they play another role in carbohydrate metabolism. Monosaccharides and their activated NDPSs penetrate across the plasma and intracellular membranes through a series of specific transport proteins [26-29].

Glycosyltransferases and specific glycosidases modify the structure of carbohydrate chains during their maturation, which is the third important enzymatic system of glycan biosynthesis. Glycosyltransferases are a highly specific and very numerous group of enzymes responsible for non-template biosynthesis of glycan chains. These enzymes are specific to the nature of transported sugar and to the donor and acceptor structures, and they form a unique glycosidic linkage with one of two anomeric configurations [9, 30-32]. The known glycosyltransferases subdivided by their specificity into families and subfamilies is so great that it allows us to consider the non-template biosynthesis postulate: "one enzyme – one type of glycosidic linkage".

Although many of these enzymes are membrane-bound and had been difficult to separate, modern methods of isolation and purification allow researchers to isolate these enzymes in homogenous states, to crystallize them, and to study their structural features. Studies on the glycosyltransferases at the level of their genes have revealed comparatively elongated regions (motifs) similar in structure and sequence in genes encoding the same family of enzymes, e.g. galactosyl-, fucosyl-, or sialyltransferases, and also short regions that characterize a common feature of the enzymes, e.g. glycosyltransferases of eukaryotes [33-35].

However, many problems related to certain stages of biosynthesis of carbohydrate chains need further interpretation. These unsolved problems include mechanisms of time- and space-correlated interactions of glycosyltransferases with donors and acceptors, regulation of carbohydrate chain growth and termination, influence of metal ions, optimum pH, effective multienzyme complexes, energy and mechanics of mutual movements, and interactions of the complex biosynthetic assembly with carbohydrate chains [12-14].

This type of biosynthesis is rather accurate as evident by the structure of the resulting molecules, but synthesized molecules of the same type are often not identical even if their biosynthesis occurs in the same cell. This microheterogeneity is inevitable in molecules produced by non-template biosynthesis. These minute changes in positions of one or two monosaccharide residues increase the heterogeneity of carbohydrate structures, and the function of such structural diversity is still unknown, but may become apparent under certain conditions, including pathology, that is still unknown for us in detail.

The macro- and microheterogeneity of glycoconjugate molecules make it difficult to establish their structural–functional interrelations. During their biosynthesis, these molecules undergo multiple modifications due to many enzymatic systems operating at all stages of the intracellular assembly. The activity of some glycosidases involved in creation of N-bound carbohydrate chains is to cleave the terminal glucose residues initially bound on the biosynthesis of N-glycans with a branched chain of mannose on a dolicholphosphate acceptor.

"Cutting" or "trimming" by glucosidases 1 and 2 in the endoplasmic reticulum starts sequentially by detachment initially of one glucose residue by α-glucosidase 1 from the carbohydrate structure $Glc_3Man_9GlcNAc_2$ that has been already transferred onto the proteins and then of the two remaining glucose residues by α-glucosidase 2. Modification of the remaining part of the carbohydrate chain continues upon its movement into the cisterns of the GC by a series of α-mannosidases with distinct specificity to mannose residues in shortening branches of the carbohydrate chain. This specificity is very likely influenced by a new GlcNAc-residue added during the penultimate stage of the trimming.

The biological reason for cooperation of glycosyltransferases and glycosidases during the biosynthesis of glycan chains is still unclear. Putative regulatory mechanisms responsible for the movement of glycoconjugates as they are constructed along the RER–ER–GC pathway are also unknown. Possibly, modification of molecules as they are constructed serves for their recognition by transport receptors responsible for their movement along the biological conveyer, including the delivery of glycoconjugates into the GC, from its *cis*- to *trans*-compartments, and further into other cellular organelles or for export. The GC is a surprisingly organized and multifunctioning biological fabric producing various molecules as they move along a set of conveyer–cistern–domains during their structural completion resulting in their ultimate sub-

cellular orientation and localization. Further studies of this extremely complex cellular compartment seem very promising for advancing in understanding of vital cell activity. At present, some experimental and theoretical works analyzing the GC organization deserve the greatest attention [36, 37].

During the last two decades, glycosylation of proteins has been detected in the cell nucleus and cytoplasm of various living systems including fungi, protozoa, worms, insects, and humans. Yeast seems to be a rare exception from this list [38].

This type of glycosylation includes addition by O-glycosidic linkage of only one N-acetylglucosamine residue (GlcNAc) on its transfer from the activated form, UDP-GlcNAc, to hydroxyl groups of specific serine/threonine within the amino acid sequence of proteins, resulting in production of a β-glycosidic bond. This type of glycosylation is characterized by the absence of the GlcNAc-residue within the protein on other carbohydrate residues; however, this residue has an exceptional ability to repeatedly attach to the protein moiety of the molecules or to detach from them. Therefore, a newly joined GlcNAc-residue can also glycosylate different serine/threonine residues in the amino acid sequence of the protein. Researchers consider this feature of glycosylation to be very different from the classic glycosylation in the endoplasmic reticulum and GC cisterns and to be more like protein phosphorylation.

Note that the above-described dynamic modification occurs by the action of two enzymes: O-GlcNAc-transferase and O-GlcNAc-glycosidase (β-N-acetylglucosaminidase). The two enzymes with oppositely directed activities coexist in the same complex and seem to be controlled by a yet unknown regulatory system responsible for overseeing the O-GlcNAc-glycosylation and deglycosylation.

The O-GlcNAc-glycosylation occurs in many compartments of eukaryotic cells and has been found in chromatin, cytoskeleton proteins, cytoplasmic moieties of intercellular vesicles, membrane proteins, proteins of nuclear pores, RNA-processing proteins, and protein regulatory factors involved in protein translation. Moreover, this type of glycosylation occurs in glycans of proteins of viruses and parasites infecting eukaryotic cells. The number of known proteins that can be targets for O-GlcNAc-glycosylation continues to increase, and this type of glycosylation/deglycosylation seems to compete with phosphorylation/dephosphorylation of proteins by specific kinases/phosphatases. All of these enzymes seem to be parts of multienzyme complexes that are typically finely regulated normally and in some pathological states such as Alzheimer's disease, diabetes, and various stresses, but this fine regulation remains enigmatic and varies for different functionally important proteins [4, 39].

Glycoconjugates with a more complex structure than that of the above-described O-GlcNAc-glycoproteins are found in most cellular organelles, including the nucleus, mitochondria, lysosomes, and in the plasma membrane [40, 41]. These molecules may originate from biosynthesis in the RER and GC and be subsequently transferred into other compartments. Glycosylation can also occur in places that glycoconjugates localize, including the plasma membrane outer surface, and this process is now intensively studied [42].

The detection of glycosyltransferases in many biological fluids and secretions, including the cytoplasm and cell membrane surface, suggests that glycosylation of molecules and their partial deglycosylation are dynamic processes that will be better understood after further study. Modern mass-spectrometric approaches for screening potential carbohydrate acceptors and analyzing glycosylation products may result in discovery of novel glycosyltransferases with unique specificities [15, 43]. New effective approaches for measurement of glycosyltransferase activity based on highly sensitive fluorometry, mass-spectrometry, and electrochemical detection will increase the numbers of known enzymes of this type [14, 44, 45]. Special synthetic glycoconjugates allow researchers to obtain data on the activity and substrate specificity of previously unknown glycosyltransferases, in particular, of xylosyltransferases involved in biosynthesis of glycan chains of O-glycoproteins [46-48].

Degradation of glycan chains. Enzymatic degradation of the carbohydrate moiety of glycoconjugate molecules in living organisms is catalyzed by a large group of hydrolytic enzymes – the glycosidases. Glycosidases catalyze the degradation of O-, N-, or S-glycosidic bonds in different glycosides, oligosaccharides, and polysaccharides, and in glycoconjugates. The N- and S-glycosidases are represented by only a few enzymes, whereas O-glycosidases (carbohydrases) degrade the O-glycosidic bonds, the major covalent bond in the most common glycans. For convenience, we shall use the term "glycosidases" instead of "O-glycosidases".

In addition to hydrolytic activity, many glycosidases can catalyze transglycosylation, i.e. the transfer of a glycosyl residue onto other acceptors to produce a new glycoside or oligosaccharide. According to the ideas of some authors, all reactions catalyzed by glycosylhydrolases and glycosyltransferases utilize the same mechanism of glycosyl proton exchange. Based on this principle, apparently different enzymes are classified as glycosylases producing one glycosidic bond from another [49-51].

The enzymatic activity of glycosidases on natural substrates is studied by identification and quantitative determination, using various physicochemical methods, of a carbohydrate residue or an oligosaccharide fragment of a carbohydrate chain removed as a result of the enzymatic hydrolysis. The use of synthetic substrates significantly simplifies and makes extremely more sensitive the determination of glycosidase activities, because in this case not the carbohydrate moiety of substrates is identi-

fied but their detached aglycon parts, which are free chromogenic or fluorogenic products such as phenol, 2-*o*- or 4-*p*-nitrophenols, 4-methylumbellipherone, α- or β-naphthols, and their derivatives [52-54]. The rate of hydrolysis of some synthetic substrates by certain glycosidases, e.g. hexosaminidase, is 10^3-10^5 times higher than the rate of hydrolysis of natural substrates. The number of synthetic substrates of glycosidases continuously increases, and now they are important products of various commercial firms.

Notwithstanding the above-mentioned advantages, the structure of synthetic substrates does not always satisfy requirements of a particular glycosidase in specificity [55, 56]. There are glycosidases with tenfold lower affinity for synthetic substrates than for natural ones. In such cases, researchers make efforts to synthesize substrates with an aglycon moiety that more closely mimics the structure of the aglycon moiety of natural substrates. Thus, fluorogenic glycolipid substrates of glycolipid hydrolases have been successfully used for determining activities of such enzymes in the normal state and in some glycolipidoses [57-59]. The structure of so-called semisynthetic substrates is more like that of natural substrates for some glycosidases. In the case of glycolipids, these compounds are initially isolated from particular sources, and the fatty acid is detached from ceramide by alkaline hydrolysis followed by substitution by a fluorophore-labeled fatty acid. One such compound (Gal-A-sphinganine) is an effective substrate for galactocerebrosidase [60]. The use of a wide spectrum of synthetically modified analogs of different sugars, in particular, of iminosugars, as inhibitors of glycosidases allows researchers to obtain new data on the specificity and catalytic mechanisms of these enzymes [61, 62].

Since the discovery of lysosomes by De Duve in 1955, in many studies glycosidases have been shown to be mainly located in these cellular organelles, which together with other hydrolytic enzymes – proteinases, nucleases, lipases, and some other enzymes – degrade virtually all components of living cells [63]. However, all of these hydrolases are functioning inside lysosomes enclosed by a single lipid membrane with a unique structure that contains various transport and other functional proteins, many of which are glycoproteins. Lysosomal hydrolases, soluble and bound with the lysosomal membrane, include a large group of glycosidases specific to the structure of monosaccharide residues and to the glycosidic bond type. In addition to their function as an "intracellular stomach", lysosomes together with a wide spectrum of their own enzymes and protein components of membranes are now considered as organelles involved in various processes in living organisms: processing of proteins including antigens, degradation of extra- and intracellular waste structures (autophagy), initiation of apoptosis, import of cytoplasmic proteins, and export of products degraded in the lysosomes [64].

Glycosidases are N-glycoproteins synthesized on ribosomes bound to the endoplasmic reticulum membranes as precursors whose molecular weight is higher than that of the mature enzymes functioning in the lysosomes. The enzyme precursors contain a signaling peptide consisting of 15-30 hydrophobic amino acids responsible for the penetration of the enzyme across the RER membrane followed by detachment of the signal peptide from the enzyme by corresponding proteinases. Subsequent glycosylation and maturation occur in the SER and GC cisterns. A unique phosphorylation process comes into play. Hydroxyl moieties of certain mannose residues are phosphorylated by an N-acetylglucosaminyltransferase as follows: the enzyme catalyzes transfer of GlcNAc-P from UDP-GlcNAc as donor to the hydroxyl at C6 of specific mannose residues in the oligosaccharide chains of lysosomal enzymes. Man-6-P-determinants are exposed upon GlcNAc release by another enzyme, GlcNAc-1-phosphodiester-α-N-acetylglucosaminidase, which differs in specificity, immunological, and catalytic properties from the lysosomal α-N-acetylglucosaminidase [65, 66].

It should be noted that the phosphorylation enzymes are highly specific for carbohydrate and protein moieties of lysosomal hydrolases. Other glycoproteins synthesized in the SER and GC cisterns are not phosphorylated under the influence of this phosphotransferase. Lysosomal hydrolases, including glycosidases, include certain carbohydrate and peptide sequences that determine enzymatic phosphorylation. The uniqueness of phosphorylation of each hydrolase is determined by the high order conformation of enzyme molecules under the essential influence of N-glycosylation.

Phosphorylation of carbohydrate chains of lysosomal hydrolases seems to interdict their further processing and be a prerequisite for binding of these molecules with mannose-6-phosphate receptor (Man-6P-R) recognizing the Man-6P-groups of the enzymes. Upon binding with the receptor, in the *cis*-compartments of GC, where the hydrolase concentration is the highest, the hydrolases are packed into vesicles delivered into lysosomes. The acidic medium in the lysosomes, provided by an ATP-driven proton pump, promotes dissociation of the Man-6P-R–hydrolase complex, whereupon the receptor returns to the GC and onto plasma membranes for recycling. A Man-6P-R with molecular weight of about 300 kDa was isolated in the homogenous state, monoclonal antibodies were prepared, and it was established to be an integral glycoprotein component of intracellular vesicles, with the polypeptide C-end inserted into the membrane and into the cytoplasm. Such organization seems to promote receptor recycling and prevents movement into the interior of the lysosome. The receptor is a protein kinase with the ability to phosphorylate its own serine and tyrosine residues, which reduces its ability to bind lysosomal enzymes. This modification probably promotes receptor–ligand complex dissociation and receptor recycling.

On binding with lysosomal hydrolases, Man-6P-R does not need Mn^{2+}-like cations, and thus it is designated a cation-independent Man-6P-R (CI-M6PR). Some data confirm that CI-M6PR is involved in regulation of cell growth and mobility [67].

Cells have another M6PR with molecular weight of 46 kDa that requires cations for binding of lysosomal hydrolases. This cation-dependent Man-6P-R (CD-M6PR) also participates in intracellular transfer of lysosomal hydrolases into particular lysosomes, and is much less likely to appear on the plasma membrane. Regulatory systems that determine the specialization of both receptors are not yet characterized despite very intensive studies [68-70].

The above-described system of intercellular transport of hydrolases including glycosidases is not universal for all such enzymes. Some of them, e.g. acidic phosphatase and β-glucocerebrosidase of fibroblasts and also lysosomal enzymes in other cells and tissues, are delivered into lysosomes by transport systems independent of Man-6P-receptors, but Man- rather than Man-6-recognizing receptors are involved. One such receptor for membrane-bound β-glucocerebrosidase is more likely to be the lysosomal integral membrane protein 2 (LIMP2). There is no doubt that the delivery of glycosidases into various lysosomes will be further described by new works that will significantly expand our ideas about this complicated system of enzyme delivery from cellular compartments and their targeting into functioning organelles.

Glycoconjugates, as natural substrates of glycosidases, enter lysosomes by receptor-mediated endocytosis, which provides the delivery of many biopolymers into cells. In the first stage of this process, the terminal carbohydrate residues of glycoconjugates are recognized by plasma membrane receptors for sialic acids, galactose, fucose, mannose, and other available sugars, and bind to them with rather high specificity. Then the receptor–ligand complex is packed into a vesicle produced at the plasma membrane intrusion, and is separated from it by its covering with the fibrillar protein clathrin. Then the clathrin cover is discarded, the separated vesicle is acidified by a proton pump, and it is converted into an endosome. In the next segregation stage, the receptor–ligand complex dissociates, and the receptor returns to the plasma membrane (recycling). The ligand-containing vesicle fuses with primary lysosomes and produces secondary lysosomes, which can contain the previously delivered enzymes and the corresponding substrates. Only in the secondary lysosomes are glycoconjugates degraded by glycosidases, proteinases, sulfatases, phosphatases, and lipases [69]. The carbohydrate chains are degraded by exoglycosidases and endoglycosidases. Exoglycosidases successively detach monosaccharide units from the nonreducing end of the carbohydrate chain and are specific to the carbohydrate residue, anomeric configuration of the glycoside bond, and to specific features of the structure and conformation of the aglycon moiety of the substrate. Endoglycosidases catalyze detachment of the oligosaccharide block that later is degraded to monosaccharides by successive action of exoglycosidases. The glycosidases have another specific feature – most often they are heterocatalytic enzymes capable of cleaving the same carbohydrate residues from different classes of carbohydrate-containing compounds. Thus, some β-D-galactosidases cleave galactose from glycoproteins, glycolipids, and proteoglycans. The enzyme affinity for various substrates and the rate of their degradation can differ tenfold.

Whereas the chemistry of catalytic processes that determine the activity and specificity of many isolated glycosidases is well studied, their intralysosomal organization still remains poorly understood. Now it is clear that glycosidases, similarly to glycosyltransferases, can exist as multienzyme complexes exemplified by the giant cellulosomal multienzyme complex of many bacteria that hydrolyze some high molecular weight plant polysaccharides [70], or by the galactosialidase complex of animals [71]. These enzymes need stabilizing proteins, activator proteins acting as biological detergents that help glycolipid hydrolases catalyze degradation of the carbohydrate moiety of glycolipids [72].

An important and still unsolved problem is the interaction of glycosidases with the lysosomal membrane and other components of the intralysosomal matrix that seems to inhibit glycosidase action against each other and on proteinases. Certainly, this does not mean an absolute stability of glycosidases within the lysosomes. Every glycosidase is characterized by its half-life, and many enzymes of this group undergo partial proteolysis and deglycosylation within the lysosomes, and the degree of proteolysis and deglycosylation varies for different enzymes. It is also interesting that some glycosidases, e.g. β-glucuronidase, are located on the outer surface of the lysosomal membrane and contribute to modification of the surface of different subcellular organelles and pinocytotic vesicles as they translocate within the cell. They also trigger certain signaling mechanisms. Some glycosidases are located on the outer surface of the plasma membrane where, together with glycosyltransferases, they play an important role in intercellular interactions and in cell defense against bacteria and viruses [73]. Glycosidases are found in the soluble cytoplasmic and microsomal fractions, in GC membranes, and in fractions of the cell nuclei. Some of these enzymes are different from the similarly named lysosomal enzymes in properties and substrate specificity to glycan chains of different molecules, and other glycosidases have similar features but are different in carbohydrate and amino acid composition as shown for β-glucuronidase of lysosomes and microsomes from rat liver.

In the majority of cases, the nature of factors that determine the localization of a glycosidase in a particular

subcellular compartment is still not known. However, considering β-glucuronidase, we suppose that certain anchoring egasin-like proteins should exist to determine the enzyme localization [74]. It seems also that glycosidase molecules can exist without mediators, and their localization is determined during their formation. It is reasonable to suggest that glycosidases of non-lysosomal origin, together with glycosyltransferases, could contribute to modifications of the glycan chains of glycoconjugates in virtually all cellular compartments and membrane formations. Such modifications seem to "switch on", "turn off", or "redirect" certain metabolic links and be an integral system of the organism's general homeostasis. This standpoint is consistent with the existence of multiple forms of glycosidases that detach the same glycon moiety of the substrate molecule and can differ in physicochemical features, dependence on activators and inhibitors, subcellular localization, and substrate specificity [75-79]. Molecular forms of glycosidases have intraspecies, interspecies, and organ-dependent differences. Studies on molecular forms of α-L-fucosidase in human kidney, placenta, liver, and blood serum revealed a polymorphic isoenzyme spectrum [80, 81].

In addition to the intracellular degradation of glycans, glycosidases are involved in numerous other biological processes. First, they are essential to autolysis and autophagy, the processes of cellular elimination of "garbage" and preventing irreversible changes during starvation and under other unfavorable conditions. Glycosidases contribute to cell division, cell transformation during malignant growth, fertilization through acrosomal enzymes of spermatozoa, and to the subsequent division of the fertilized oocyte under the influence of cortical granules located on the oocyte surface. Glycosidases, in particular β-glucuronidase and hyaluronidase, are involved in embryogenesis, growth, and differentiation of organs of insects and birds, and in wound epitheliazation in mammals. There is no doubt that exo- and endoglycosidases of phages and also viral neuraminidase play an important role during infection of bacterial cells, starting from the interaction of influenza viruses with the cell surface and terminating by the virus leaving the host cells and entering the bloodstream. Some glycosidases, in particular, a highly specific N-glycosidase, are involved in the repair of DNA molecules damaged by mutagenic factors [82, 83].

Concluding this short review of biosynthesis and degradation of glycans, it is necessary to accentuate the importance of combined synthetic and enzymological approaches, which allow researchers to purposefully create substrates with desired structures. Studies on enzymatic modifications of such substrates containing in their chains carbohydrate and non-carbohydrate groups (sulfates, phosphates, acyl groups) that often influence the biological activity of the whole molecule are continuously expanding. This is exemplified by studies on fucosidases and fucosyltransferases using synthetic substrates and acceptors and also by sulfotransferase that terminates biosynthesis of the glycan chains of the antigen HNK-1 [84-88].

BIOLOGICAL ROLE OF GLYCOCONJUGATES

Three decades ago information about the biological role of carbohydrates was quite limited, and many research groups enthusiastically studied problems associated with nucleic acids and proteins. Great advances were made in these fields, and it seemed that nucleic acids and proteins could be responsible for all vital processes and realize them extremely accurately and efficiently. However, notwithstanding the great significance of molecular biology and proteomics, during the following years it was shown that control of processes of in living organisms also depend upon an extremely diverse arsenal of simple and complex carbohydrates and glycoconjugates.

Even the simple listing of biological processes contributed to by carbohydrate-containing molecules in the introduction to this paper and the subsequent consideration of informational features of glycans, their biosynthesis, and their degradation show that these processes are relevant not only to glycobiology, but also enter into the domain of molecular and cellular biology, proteomics, and medicine, because disorders in these processes lead to severe diseases.

Glycan labeling in determination of the fate of molecules. Although the glycan content in glycoconjugates is relatively low, they frequently add to these molecules unique properties that elucidate the biological functions of glycosylation [86].

In 1965, the American investigator E. Eylar proposed one of the most interesting hypotheses concerning the role of the carbohydrate component in proteins. He was analyzing data on glycoprotein distribution, and he found that at least 100 of them were located outside the cells and were components of different biological fluids: blood, saliva, milk, and other secretions. According to his hypothesis, the carbohydrate component was a kind of a "chemical passport", the reception of which was a signal for the protein molecule to leave the cell [89]. Later many intracellular glycoproteins were found and, on the contrary, many non-glycosylated proteins, including albumin, α-lactalbumin, and chymotrypsinogen were found in blood and various secretions. Thus, this hypothesis about the role of carbohydrate components failed to be universal, but it contained a far-sighted idea about particular carbohydrate labeling determining the fate of the whole molecule.

Experimental data indicating the key role of the carbohydrate component in determination of the half-life of serum glycoproteins were obtained nearly 10 years later

by the group of G. Aschwell and A. Morell and their coworkers during the study of mechanisms responsible for development of Wilson–Konovalov disease. This hereditary disease is associated with disorders in copper metabolism due to a decreased blood level of ceruloplasmin, a sialoglycoprotein that is the main carrier of copper in the human body.

To label the carbohydrate moiety of ceruloplasmin with radioactive tritium, it was necessary, first of all, to cleave the terminal residues of neuraminic acid and make accessible for labeling with tritium the penultimate galactose residues of the remaining glycans. Upon finishing this modification, the researchers could follow the fate of tritium-labeled asialoceruloplasmin in rabbit blood, and they were surprised that human asialoceruloplasmin disappeared from the blood in a few minutes, although the half-life of native ceruloplasmin under the same conditions was about 56 h.

The same effect of removal of neuraminic acid and availability of galactose as the terminal residue in the glycan chain was also observed with the serum glycoproteins: haptoglobin, fetuin, and orosomucoid. All these glycoproteins left the bloodstream within a few minutes after the injection, and their half-life became normal upon the further removal of galactose residues or their modification under the influence of galactose oxidase. Subsequent studies by many groups established that the surface of the parenchyma cells of mammalian liver containing receptor capable of binding asialoglycoproteins with high affinity determined the terminal galactose. This protein was termed Hepatic Binding Protein (HBP). HBP is the first mammalian lectin characterized in detail, with known amino acid and carbohydrate sequences in its protein and glycan moieties, subunit and domain structure, features of biosynthesis as a phosphorylated glycoprotein, and subcellular localization in the GC and on the surface of plasma membrane of liver parenchyma cells oriented differently from other human receptors. In addition to recognizing the terminal galactose residue in the glycan chains of glycoproteins, HBP is specific also to terminal N-acetylgalactosamine residues in asialoglycoproteins, as exemplified by asialomucin from bovine submandibular glands.

After the discovery of HBP, a lectin with different specificity was found in bird liver that binds glycoproteins having a terminal N-acetylglucosamine residue [90, 91]. For some time researchers believed that the main role of the HBP known in the literature as the Aschwell–Morell Receptor (AMR) or Mammalian Asialoglycoprotein Receptor (ASGPR) was to bind glycoproteins desialylated by the action of blood sialidases, and also some cells, including human erythrocytes, thus determining their lifetimes under physiological conditions. But there is no direct proof supporting this viewpoint.

However recently, AMR was shown to be involved in the binding of some glycoproteins and regulatory participants of blood coagulation and thrombogenesis, including von Willebrand factor and platelets. Infection with *Streptococcus pneumonia* is associated with accumulation in blood of a great number of desialylated platelets under the influence of bacterial neuraminidase. Such platelets are dangerous because they promote thrombosis in blood vessels, but they are removed by AMR into lysosomes of the liver parenchyma cells where they are destroyed, thus preventing general sepsis and increasing the survival of infected animals [92, 93].

HBP/AMR is a member of the C-family lectins capable of binding asialoglycoproteins. This ability is used for development of effective approaches for creating new pharmaceuticals directed from the blood into the liver. The discovery of HBP/AMR initiated many studies resulting in discovery of new mammalian lectins specific to galactose, fucose, mannose, and N-acetylglucosamine terminal residues in glycan chains of different glycoproteins. In some cases, different lectins are specific for different organs and cell types. Thus, a lectin specific for mannose/N-acetylglucosamine residues was isolated from plasma membrane of Kupffer cells of rabbit liver. The same type receptor was found on the surface of alveolar macrophages that had an additional specificity to the terminal fucose. Receptor–lectin systems with different specificities determine important but yet undefined functions that may be present in the majority, if not all, organs of mammals. Thus, liver parenchyma cells carry on their membrane HBR/AMR as an integral component, whereas the reticulo–endothelial system of this organ (macrophages, endothelial, and Kupffer cells) contains receptors recognizing mannose/N-acetylglucosamine/fucose.

The number of known human and animal lectins with different cellular and organ specificities has significantly increased, and functions of many of them are well studied. The mannose-binding lectin (MBL) of blood serum binds glycan chains with the corresponding specificity on the surface of some microorganisms and thus prevents infection by helping macrophages attack pathogens. Various selectins participate in regulation of protective reactions of mobile blood cells, lymphocytes and neutrophils, on their movement towards inflammation foci. C-type lectins are involved in presentation of antigens that are to be neutralized. Lectins of the Siglec family are adhesive and signal receptors on immune system cells recognizing sialic acid residues on the target cells. Extracellular galectins recognizing β-galactose residues are involved in cell adhesion and cell signaling; moreover, lectins of this class modulate T-cell activation and controls cell survival by inhibiting apoptosis. Galectin-1 and -3 are found in the cell nucleus. Galectin-3 is distributed between the cytoplasm and nucleus depending on the stage of cell proliferation. The concentration of nuclear lectins is increased in nuclei of virus-transformed fibroblasts, suggesting a possible contribu-

tion of these proteins to cell proliferation and transformation. Some groups of lectins, e.g. calnexin and calreticulin, are not integral components of plasmatic or intracellular membrane formations, but they are present as soluble proteins in the endoplasmic reticulum cisterns and are involved in the control of normal folding of maturing glycoprotein molecules during their biosynthesis. Under disturbances of normal folding, other types of lectins are involved in degradation of such "improper" molecules [4, 94-98].

Role of glycoconjugates in the immune system. Studies in this field were started by Karl Landsteiner in 1900, with the generation of ideas about carbohydrates as specific markers of antigenic properties of molecules. First were concerns about blood group substances (BGS) – carbohydrate-containing biopolymers of glycoprotein or glycolipid nature that determine the blood group and of different secretions of humans and other animals [99-101]. The antigenic specificity of BGS is determined by a sequence of only a few terminal monosaccharides of their carbohydrate molecules. In the ABO(H) system of human blood, A-antigen specificity depends on a determinant oligosaccharide GalNAcα1,3(Fucα1,2)Galβ1,4GlcNAc with the key GalNAc residue for the A-specificity; the B-antigen specificity is determined by Galα1,3(Fucα1,2)Galβ1,4GlcNAc with the key Gal-residue for the B-specificity; the H-antigen specificity is determined by Fucα1,2Galβ1,4GlcNAc with the key Fuc-residue for the H-specificity. In the MNSs system of blood groups, the specificity of N- and M-antigens is determined, respectively, by one or two residues of N-acetylneuraminic acid. For each determinant oligosaccharide, only one rather rigid conformation is responsible, which is due to noncovalent interactions of carbohydrate residues. This results in the maximum accessibility of determinant residues (Fuc, Gal, GalNAc) for intermolecular interactions that are important for the interaction with the corresponding antibodies.

Carbohydrate determinants of various BGSs are characterized in detail; moreover, corresponding carbohydrate determinants can be detached or added using specific glycosidases and glycosyltransferases to change the antigenic specificity of erythrocytes or soluble BGSs of biological fluids. The carbohydrate determinants can be specifically cleaved with bacterial preparations of glycosidases that allows researchers to transform erythrocytes of the A and B groups to the H(O) group cells of the universal blood group, especially important for blood transfusion. Glycosidases of animal origin are inefficient in the BGS transformation. Fucosidase of animal origin could not cleave fucose from native molecules of BGSs, but this monosaccharide was released from glycopeptides fragments of these molecules [102-104].

Histocompatibility antigens responsible for the immune response upon transplantation of organs or tissues are, in most cases, glycoproteins, and carbohydrate antigenic determinants have been identified for some of them, e.g. for the mouse H-2 complex. The T-lymphocyte receptors responsible for immunological control and recognition of foreign antigens most often are glycoproteins [105].

The majority of cell surface antigens are glycoconjugates, the qualitative and quantitative composition of which changes during growth and tissue differentiation and tumor transformation. Membrane glycosphingolipids uncommon for normal cells are accumulated in tumor cells. Excretion of tumor glycolipids, in particular gangliosides, into the extracellular medium changes the glycolipid composition of serum relative to normal serum. Gangliosides are suggested to suppress immunocompetent cells by decreasing antitumor immunity [106, 107].

Glycolipids of tumor cell membranes are associated with production of new fucolipids and simplification of their structure. Fucolipids are synthesized due to activation of fucosyltransferases in the transformed cells. Fucolipids are rarely found in normal cells and seem to be important during the invasion and metastasizing of tumors due to presence of a fucose-binding protein in some tissues. The glycolipid composition of the tumor cell membrane is simplified as a result of blocking of the elongation of glycan chains during their biosynthesis. The simplest gangliosides GM3, GD3, and lactosylceramide become prevalent [101].

A similar situation exists for sialomucins of tumor cells. Sialomucins are O-glycoproteins with sialic terminal residues in the glycan chains. These biopolymers are present in both mucous secretions and on the surface of many cells. Thus, glycophorin is a sialomucin of the erythrocyte surface. Some authors consider sialomucins as anti-recognition factors favorable for the protection of the tumor cells against the host. Sialomucins exfoliated from the tumor cell surface bind with anti-sialomucins and produce a complex named blocking factor. According to another hypothesis, sialomucins of the cell surface mask the cell antigens against the immune system of the body. Sialomucins have a significant negative charge that creates steric inhibition of antibodies and immunocompetent cells capable of attacking tumor cells [108].

Some pathogenic microbes and viruses have on their surface specific proteins capable of recognizing accessible sialoglycan chains and binding to them. These pathogens include human influenza viruses A and B, A-type virus of avian influenza, *Vibrio cholera*, *Plasmodium falciparum*, *Clostridium botulinum*, and *Helicobacter pylori*. Binding such sialylated glycans is the first and very important stage for the subsequent invasion of a pathogen into the body. Many pharmaceutical firms work toward developing preparations with the active moiety represented by a sialo-containing determinant capable of binding the pathogen and preventing its "landing" onto the surface of the host target cells [109].

Carbohydrates play an important role in the transport and secretion of some glycosylated immunoglobu-

lins, maintain the conformation of domains necessary for function of immunoglobulins, and protect proteolysis-sensitive regions of their molecules [110].

The involvement of two typical terminal sugars in glycans, neuraminic acid, and fucose during the interaction of antigen-sensitized T-lymphocytes with macrophages is exemplified by a lymphocyte mediator that is a glycoprotein. It loses its ability to inhibit the migration of macrophages upon treatment with neuraminidase. The receptor of this mediator on the macrophage surface is also a glycolipid with terminal fucose. The cleavage of fucose by α-L-fucosidase completely abolished the effect of the mediator (macrophage inhibition factor (MIF)) because of its inability to bind with the membrane of macrophages [111].

An important role of glycoconjugates for functions of the immune system is demonstrated by activation of the alternative pathway of complement activation and by establishment of the glycoprotein nature of many surface antigens of T- and B-lymphocytes, the soluble immune mediators, lymphokines, monokines, suppressors, and other immune factors and cells. During recirculation of lymphocytes, an important role is played by carbohydrate-specific receptor systems that determine the binding of lymphocytes with the surface of venules and peripheral lymphatic nodes [112].

Glycoconjugates as components of receptor systems. The majority of known receptors are glycoconjugates that faultlessly and very selectively recognize the corresponding ligands. Some of these receptors have already been characterized above in the limits of discussion of problems related with intracellular movement of lysosomal hydrolases and analysis of mechanisms for removal of different glycoproteins and cells from blood. Examples of glycoconjugates as receptors are their binding of target cells by toxins, viruses, hormones, and other biologically active compounds, as briefly considered below.

Carbohydrate receptor systems are exemplified, in particular, by the well-studied multistage binding of cholera toxin with GM1-ganglioside of the target cell plasma membranes. The same glycolipid attracts the *E. coli* toxin but with lower specificity. Other gangliosides with additional residues of neuraminic acid in the structure, GD1b, GT1, and GT1b, are receptors of the tetanus and botulism toxins. The ricin toxin penetrates into macrophages only upon binding with a mannose-specific receptor on the cell surface. The binding of some pathogenic bacteria with terminal mannose in glycan chains of glycoproteins of target cell plasma membranes has already been mentioned. This list can be supplemented by data on the oral cavity bacterium *Streptococcus sanguinis* contributing to dental caries and binding with saliva glycoproteins via their terminal residues of N-acetylneuraminic acid. The same residue, but as a component of the carbohydrate chains of the erythrocyte membrane, glycophorin, is involved in the binding on the erythrocyte surface of influenza myxoviruses and of paramyxoviruses, e.g. Sendai virus.

The hormonal system for regulating metabolism uses recognition and signal transduction of glycoconjugates of the intercellular space (glycocalyx) of plasma, intercellular membranes, and cytoplasm of target cells. On the surface of liver cells, adipocytes, and lymphocytes there is a specific glycoprotein receptor capable of binding insulin. Treatment of this receptor with neuraminidase and galactosidase abolishes its binding ability. The transduction of the final receptor signal is a very complex multistage process, and it is reasonable to expect new examples of different glycoconjugate involvement in hormone binding and signal transduction, with specificity regarding the localization, structure, and other features of the carbohydrate-containing molecules [21, 22].

HEREDITARY DISORDERS IN DEGRADATION AND BIOSYNTHESIS OF GLYCANS

Glycosidoses are the main group of lysosomal storage diseases. The main group of lysosomal storage diseases (LSD), the glycosidoses, develop as a result of hereditary deficiency of any glycosidase/hydrolase associated with storage in lysosomes of products of carbohydrate nature. The term "glycosidoses" refers to those diseases that lead to a primary storage of complex carbohydrates and carbohydrate-containing compounds. Some types of mucopolysaccharidoses are associated with an insufficiency of sulfatases that are not formally glycosidases, but the defective detachment of sulfate residues on the ends of carbohydrate chains of glycoconjugates finally results in accumulation of glycosaminoglycans due to blocked action of other enzymes responsible for sequential detachments of monosaccharide units from the nonreducing end of the glycan chain. Thus, the term "glycosidoses" is sufficiently capacious and convenient to classify a large group of diseases based on two parameters: the enzyme insufficiency (most frequently of glycosidase) and the nature of the primary storage product – glycan/glycoconjugate [22, 79, 113].

Glycosidoses were described by clinicians before the elucidation of molecular mechanisms of their development. Therefore, many of these diseases are named by the clinicians who were the first to describe the severe clinical picture of these human pathologies. Such are Tay–Sachs, Gaucher's, Fabry's, and Hurler's diseases, Hunter and Sanfilippo syndromes, and others. These diseases are accompanied by disorders in the nervous, muscular, and bone systems, by mental retardation, and other disturbances that finally lead to the death of patients at an early age if the hereditary deficiency was not revealed and the patient was not treated adequately [114-117].

The interpretation of molecular bases of glycosidoses started a half-century ago when H. Hers found in 1963

that in the second type glycogenosis (Pompe's disease) lysosomes of various cells did not contain acidic α-glucosidase and glycogen accumulated [118, 119]. Pompe's disease was really the first glycosidosis that was accurately shown to develop in relation with the insufficiency of a glycosidase. Later Hers formulated a concept of hereditary LSDs as those characterized by four main features: 1) the pathology is a storage disease; 2) the storage products are always located in lysosomes; 3) the accumulated compounds can be homogenous or heterogeneous depending on the deficient enzyme specificity; 4) LSDs are a monogenic group of diseases characterized by deficiency of a single enzyme.

After the discovery by Hers, the number of LSDs approached 50 types and subtypes of these molecular disorders. Their inheritance is mainly of the autosomal-recessive and less frequently of the recessive X-chromosome-linked type. Although each of the diseases is relatively rare, in total LSDs contribute significantly to human pathology and attract the attention of clinicians and researchers as unique models for studies on the aberrant metabolism induced by deficiency of a single enzyme from a multienzyme group. The cell responses to events resulting in storage of uncleavable products in lysosomes are also nontrivial, and as such they contribute to knowledge about previously unknown processes in the body.

Depending on the nature of product accumulation, glycosidoses are subdivided into four main types: mucopolysaccharidoses, glycolipidoses, mucolipidoses, and glycoproteinoses. During the last several decades, these human diseases have attracted the attention of many research groups with very different profiles, and the accumulated experimental data have been summarized in hundreds of papers and tens of monographs [120-123] that certainly cannot be fully covered in the present review. But concluding this section, it is reasonable to emphasize some absolutely new data and ideas obtained as a result of studies on glycosidoses and approaches for their treatment. The more precise definition of specificity of many lysosomal enzymes to natural glycan compounds and detection in lysosomes of numerous protein activators (chaperones) acting as natural detergents necessary for hydrolysis of glycolipids. Some enzymes are secreted by cells, in particular, by fibroblasts, and then these enzymes can be taken up by other cells sufficiently to result in metabolic correction of pathological cells. Receptors responsible for the enzyme uptake and their delivery into lysosomes have been identified. In galactosialidosis, a glycosidase complex is found in the lysosomes. In some diseases (Sanfilippo C, mucolipidoses) the successive degradation of glycan chains is inefficient if the biogenesis of an enzyme involved in this degradation is disturbed because of insufficiency of certain transferases normally responsible for processing of glycoconjugates as substrates or in disorders in the biogenesis of lysosomal enzymes as glycoproteins. Lysosomal membranes contain some transporters of free sugars that detach during degradation, especially the transporter of sialic acids. Its insufficiency can be also associated with an LSD, e.g. Salla disease [124, 125].

Metabolic rearrangements in glycosidoses lead to an appearance in the body of glycans with unusual structure and unusual location in tissues. Thus, a kind of so-called "chemical dedifferentiation" of tissues and organs can occur and be followed by secondary negative influence on biochemical processes [22, 113, 126].

Products stored in lysosomes change the spectrum of secreted enzymes and their molecular forms, the value of the intralysosomal pH, and organization of the cytoskeleton system components [127-130]. Attempts to correct glycosides by substitutive enzymatic and gene therapy gave important information about the organism's immune response, the efficiency of enzyme delivery in different containers including liposomes, and the function of genes incorporated into regions of the genome and controlling biosynthesis of glycosidases. During the treatment of glycosidoses, a new strategy was developed for decreasing glycoconjugate accumulation by suppressing its biosynthesis. In some cases, it has been tested on animal models.

It seems that in the very near future studies on glycosidoses will result in elucidation of mechanisms triggering the cell response to overload of accumulated compounds. There is a pronounced activation of some lysosomal enzymes unrelated with the primary genetic deficiency. Thus, in fucosidosis there is more than a 7-fold increase in α-galactosidase activity, a 6-fold increase in β-xylosidase activity, and a 5-fold increase in α-glucosidase activity. Such hyperactivations were observed in LSDs not only of glycosidases, but also of other lysosomal hydrolytic enzymes, such as cathepsin D, acidic phosphatase, and others. Besides the increase in the total activity of some lysosomal enzymes, in glycosidoses the concentration is significantly increased of protein activators/chaperones that are components of the intralysosomal matrix. Based on these data, we suppose that the hyperactivation of the lysosomal hydrolytic system could be a protective reaction of the cell to products accumulation [22, 79, 113]. The elucidation of systems regulating these processes possibly through certain signaling messengers of lysosomes–cytoplasm–nucleus–cell membranes may lead to discovery of other important pathways of intracellular communication.

LSDs are now in the sphere of interests of some biotechnological companies specialized in creating preparations for correcting glycosidoses [131].

Genetic diseases developing from disorders in glycosylation. Although enzymes encoding biosynthesis of glycans are about 1-2% of the human genome, knowledge of hereditary disorders in these processes was obtained relatively recently, beginning in the 1980s. The knowledge

concerns virtually all stages and forms of glycosylation. Some of them were already mentioned earlier as glycosidoses with deficient glycosylation of glycosidases during their maturation and processing. Initially these pathologies included only biosyntheses of N-glycoproteins, but on expansion of studies the number of such known diseases significantly increased. Now they are a rather large group of hereditary disorders in virtually all aspects of biosyntheses of glycoproteins, glycolipids, and proteoglycans. As in the case of glycosidoses, a disturbance in only one link of glycosylation leads to severe manifestations in various diseases.

Classification and biochemical characterization of genetic diseases associated with disorders in glycosylation were recently reviewed by Freeze [132]. Data on relationships of glycan with more general human diseases, such as cancer and infectious and viral diseases have been summarized [133-136].

To study and treat the whole spectrum of hereditary and non-hereditary diseases related to disorders in the metabolism of glycans and glycoconjugates, a huge arsenal of various highly efficient approaches and medications produced by many pharmacological and biotechnological companies is available [137-142]. These trends have given a powerful stimulus to the development of analytical and synthetic chemistry of carbohydrates. This is one of the most elaborated fields of chemical synthesis and structural analysis now capable of reproducing glycans with more complicated structure, different degrees of polymerization, and modified with other biologically important groups [143-159]. The isolation of biologically active glycans of plant and animal origin from the World Ocean create immeasurably enlarged stores of natural algal and animal sources of novel glycans [160-166]. A new line of science that can be termed "glycopharmacology" already appears and is successfully developing.

CONCLUSION

Periodic scientific journals and virtually all large-scale publishers are increasing publications of new results of intensive studies in glycobiology. The interest for comprehension of molecular processes underlying human diseases is obvious in many of these theoretical and experimental works. Naturally, this interest is not casual. Detection of any deviation from normal leads to understanding many processes in normally functioning living organisms. And in its turn, this helps for elaboration of efficient approaches to correct metabolic disorders at every level. The two trends are inseparable and mutually enriched with new ideas and methods. Glycobiology is becoming a science of the structure, functions, and metabolism of carbohydrate-containing compounds in humans under normal and pathological conditions. Possibly, the term "glycomedicine" will even more reflect advances in this field in the near future.

Today this viewpoint is confirmed by the papers presented in this issue. Virtually all of them discuss more or less medical aspects of glycobiology. The issue is opened by two methodical reviews elucidating the most effective analytical approaches for studies on the structure of simple and complex glycans and glycoconjugates.

The review by Rohrer et al. is dedicated to carbohydrate analysis of glycoproteins using high-affinity anion-exchange chromatography with pulse amperometric detection. The main principles of the method that allows virtually all carbohydrates and their derivatives to be determined without preliminary modification are described. Technological improvements of the method introduced during the last decade are discussed. The paper concludes by analyzing some modifications of the method for studies on carbohydrate components of various glycoproteins and also by describing possible combination of the method with mass-spectrometry.

The latest advances in mass-spectrometry as of the most effective method for investigation of structure of glycans and glycoconjugates are summarized in the review by Khan and Costello. They not only analyze possibilities of different variants of mass spectrometry and its combinations with other analytical methods, but also consider the main stages of preparing specimens for analysis by mass spectrometry. Their paper focuses on analyses of the structure of carbohydrate components of glycoproteins, which are very difficult objects for structural analysis because of their extreme heterogeneity. The advances of the last decade in the investigation of the structure of simple and complex glycans and glycoconjugates using mass spectrometry essentially contributed to the total database of glycoinformatics and promoted deeper understanding of the interrelation of the structure and function of simple and complex carbohydrate molecules and glycoconjugates in biology, medicine, and the environment.

The review by Pastores et al. considers specific features of studies on lysosomal storage diseases (LSDs) in animals suffering from such diseases or developed in them as a result of the experimental blocking of a gene controlling biosynthesis of a particular lysosomal hydrolase, mainly exoglycosidases. They show that these investigations have resulted a rich body of information for comprehension of mechanisms of development of LSDs, their heterogeneous character, approaches for correction, and degree of correlation with human-like diseases in animal models.

The review by Nugent et al. considers modern concepts about the degree of specificity of heparan sulfates (HSs) binding with various proteins and the functional activity of the resulting conjugates. HSs are considered to be the most informative class of macromolecules in biology. Their functions are mainly associated with their abil-

ity to bind with proteins and supplement them with new features and functional activity. They note that HSs can function as coreceptors for growth factors and cytokines, participate in modulation of lipid absorption by cells, regulate protease activity, play an important role in formation of amyloid patches, be used by opportunistic microorganisms for penetration into cells, and even be involved in epigenetic regulation. A section of their paper is dedicated to development of approaches to treatment of lung emphysema using HS-mediated inhibition of proteases, in particular, of elastase. The authors' concept about the greater dependence of protein binding with HSs on their general domain organization than on their structure is discussed.

The paper by Pshezhetsky and Ashmarina is dedicated to the role of neuraminidases, which recently were considered only as typical hydrolytic enzymes of lysosomes. Their review summarizes the latest achievements in studies on neuraminidases as new structural and functional modifiers of cellular receptors. Many examples are presented about the involvement of this group of enzymes in various vital processes in cells. Hereditary insufficiency of these enzymes leads to severe human diseases.

Glycosidases of hydrobionts are considered in the paper by Sova et al. Their review analyzes catalytic properties, regulation of activity, and structure and functions of glycosidases (O-glycoside hydrolases) from marine organisms exemplified by endo-β-1-3-D-glucanases of marine invertebrates. They note that O-glycoside hydrolases participating in complex vital processes have a relatively simple action mechanism and are convenient models for solution of important problems of enzymology. Marine invertebrates are very promising sources of new enzymes with different specificity and for use in biotechnology.

The review by Chernikov et al. presents literature data and results of their own studies on lectins isolated from some marine hydrobionts. The paper contains data on the properties, specificity, and applicability of the described lectins in a wide range of studies and on potential use of lectin preparations for preventing many infectious diseases, including such a severe viral disease as AIDS. Undoubtedly, data on unique properties and specificity of some lectins of hydrobionts for which there is no analog among lectins of higher animals are very interesting.

The review by Newburg considers many aspects of human milk glycobiology. This source of infant nutrition from birth and later, usually to 1-2 years of age, contains the whole spectrum of simple and complex carbohydrates and glycoconjugates that continuously change qualitatively and quantitatively depending on the stage of breastfeeding. The material of the paper highlights the extreme importance of glycans and glycoconjugates in the child's protection against infectious diseases and for normal development of many systems including the nervous system and brain. Human milk has been under study for several hundred years, and a great store of findings have accumulated in this field by now. However, the author reasonably notes that our current knowledge of the mechanisms of action is only the tip of an iceberg, and many more efforts are required to come nearer to understanding how the complex composition and functions of human milk and the interaction of its components protect the child during breastfeeding. The importance of these problems for glycobiology and medicine and for industry involved in production of artificial mixtures for feeding millions of babies throughout the world is evident.

The review by Bovin presents modern concepts about the so-called natural antibodies to glycans with very different structure. These mainly M-class antibodies are generated by B-1 cells without any external stimulus and are subdivided into three groups. One of these groups, a conserved one, can be found in virtually all healthy donors without significant differences in blood concentration and epitopic specificity. It was quite unexpected that many natural anti-glycan antibodies manifested specificity to the inner (core) part of glycan molecules. The functional significance of the natural antibodies is not quite clear, but intensive studies on them during malignization result in bases for elaboration of tests for tumor diagnosis. The paper critically analyzes methodical approaches used for screening of natural antibodies with different glycochips, determination of the repertoire of these antibodies, and analysis of their specificity.

The structure, serological specificity, genetics, and biosynthesis of O-antigens of the bacteria *Providencia* are considered in the paper by Ovchinnikova et al. These microorganisms include eight species of opportunistic enterobacteria that are agents of intestinal diseases and urogenital infections in humans. The authors have characterized in detail the lipopolysaccharide structure of 36 O-antigens of *Providencia*, their localization and relation to the cell wall outer membrane components, and have noticed that many antigens contain unusual monosaccharides and non-carbohydrate components. The review summarizes data on immunochemistry of O-antigens, organization of their gene clusters, and biosynthesis of nucleotide precursors of sugars from the antigens. Prospects of further studies are discussed.

The experimental paper by Kurbatova et al. describes the elaboration of a test system for assessment of antigenic response to synthetic oligosaccharide ligands similar in structure to fragments of the chain of the capsular polysaccharide *Streptococcus pneumoniae* type 14. This approach can be used for analysis of specimens of natural capsular polysaccharides and immune sera as exemplified by the conjugate of the synthetic hexasaccharide fragment with BSA. The presented data can be used for preparation of various synthetic antigens and effective vaccines based on them without undesired admixtures.

The review by Popov and Ovodov considers the polypotency of the immunomodulating action of pectins.

Based on many literature data and their own results, the authors have shown a surprising ability of various pectins to display immunostimulating or immunosuppressive effect depending on the structure of pectin molecules and their fragments. They analyze in detail the interrelation of the immunomodulating activity and the structure of pectins of the European North of Russia. The possible application of pectins for preventing and treatment of tumors and infectious and allergic diseases is discussed.

The review by Gorshkova et al. presents data on the spatial structure of polysaccharides of plant cell walls. They emphasize that every type of higher plant cell is characterized by a structural specificity of the cell wall that depends on the structural features of polysaccharides and their supramolecular complexes capable of producing spatial structures. The biosynthesis and degradation of polysaccharides of plant cell wall depend on many interacting multienzyme complexes. The paper also discusses approaches for analyzing the spatial structure of complex polysaccharides and their complexes.

In conclusion, I would like to express my gratitude to all the authors who have made all possible efforts for this special issue dedicated to glycobiology, as well to the Editor-in-Chief of the *Biochemistry (Moscow)* Academician Vladimir Petrovich Skulachev, to the Editorial Board, and the whole collective of the journal for their support and inestimable help during the preparation of the issue for publication. I am also grateful to my Moscow colleagues Yuriy Aleksandrovich Knirel, Nikolai Vladimirovich Bovin, and Nikolai Eduardovich Nifantiev for their help during different stages of the preparation of the issue. Together with the authors of the issue, I am especially grateful to the Executive Editor-in-Chief Rada Draganovna Ozrina with whom all stages of the work have been covered. We all hope that the first thematic issue of the *Biochemistry (Moscow)* dedicated to glycobiology will be welcomed with satisfaction by a wide audience of readers and that such issues will be traditional in the future.

This work was supported by the National Institute of Health, USA (projects HD013021, HD059140, AI075563, and HD061930).

REFERENCES

1. Kamerling, J. P., Boons, G.-J., Lee, Y. C., Suzuki, A., Taniguchi, N., and Voragen, A. G. J. (eds.) (2007) *Comprehensive Glycoscience. From Chemistry to System Biology*, Vols. 1-4, Elsevier, Amsterdam-Boston-New York-London.
2. Fraser-Reid, B. O., Tatsuta, K., and Thiem, J. (eds.) (2008) *Glycoscience. Chemistry and Chemical Biology*, 2nd Edn., Springer-Verlag, Tokyo-Berlin-Heidelberg-New York.
3. Varki, A., Cummings, R. D., Esko, J. D., Freeze, H., Stanley, P., Bertozzi, C. R., Hart, G. W., and Etzler, M. E. (eds.) (2009) *Essentials of Glycobiology*, 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
4. Taylor, M. E., and Drickamer, K. (2011) *Introduction to Glycobiology*, 3rd Edn., Oxford University Press, Oxford-New York.
5. Sharon, N. (1975) *Complex Carbohydrates. Their Chemistry, Biosynthesis, and Functions*, Addison-Wesley Publishing Company, London.
6. Varki, A., and Sharon, N. (2009) in *Essentials of Glycobiology* (Varki, A., Cummings, R. D., Esko, J. D., Freeze, H., Stanley, P., Bertozzi, C. R., Hart, G. W., and Etzler, M. E., eds.) 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 1-22.
7. Dumitriu, N. (ed.) (2005) *Polysaccharides. Structural Diversity and Functional Versatility*, 2nd Edn., Marcel Dekker, New York.
8. Lennarz, W., and Hart, G. (eds.) (1994) *Methods in Enzymology*, Vol. 230, *Guide to Techniques in Glycobiology*, Academic Press, San Diego-New York-Boston-London.
9. Taniguchi, N., Honke, K., and Fukuda, M. (eds.) (2002) *Handbook of Glycosyltransferases and Related Genes*, Springer-Verlag, Tokyo.
10. Hu, Y., and Walker, S. (2002) *Chemistry and Biology*, **9**, 1287-1296.
11. Sansom, S., and Markman, O. (eds.) (2007) *Glycobiology*, Scion Publishing Ltd.
12. Lairson, L. L., Henrissat, B., Davies, G. J., and Withers, S. G. (2008) *Annu. Rev. Biochem.*, **77**, 521-555.
13. Gabius, H-J. (ed.) (2009) *The Sugar Code. Fundamentals of Glycoscience*, Wiley-VCH Verlag GmbHandCo, KGaA, Weinheim.
14. Wagner, G. K., and Pesnot, T. (2010) *Chem. Bio. Chem.*, **11**, 1939-1949.
15. Ban, L., Pettit, N., Li, L., Stuparu, A. D., Cai, L., Chen, W., Guan, W., Han, W., Wang, P. G., and Mrksich, M. (2012) *Nat. Chem. Biol.*, **8**, 769-773.
16. Rademacher, T. W., Parekh, R. B., and Dwek, R. A. (1988) *Annu. Rev. Biochem.*, **57**, 785-838.
17. Bertozzi, C. R., and Rabuka, D. (2009) in *Essentials of Glycobiology* (Varki, A., Cummings, R. D., Esko, J. D., Freeze, H., Stanley, P., Bertozzi, C. R., Hart, G. W., and Etzler, M. E., eds.) 2nd Edn., Cold Spring Harbor Laboratory Press, New York, pp. 23-36.
18. Kannicht, C. (ed.) (2002) *Posttranslational Modifications of Proteins: Tools for Functional Proteomics*, Humana Press, Totowa, NJ, USA.
19. Hattrup, C. L., and Gendler, S. J. (2008) *Annu. Rev. Physiol.*, **70**, 431-457.
20. Vasta, G. R., and Ahmed, H. (eds.) (2009) *Animal Lectins. A Functional View*, CRC Press, Taylor and Frances Group, Boca Raton, FL, USA.
21. Wiederschain, G. Ya. (1976) *Mol. Biol. (Moscow)*, **10**, 957-980.
22. Wiederschain, G. Ya. (1980) *Biochemical Basis of Glycosidoses* [in Russian], Meditsina, Moscow.
23. Shibaev, V. N. (1976) *Usp. Biol. Khim.*, **17**, 187-216.
24. Snider, M. D. (1984) in *Biology of Carbohydrates* (Ginsburg, V., and Robbins, P. W., eds.) Vol. 2, pp. 163-198.
25. Wolf, G. (1984) *Physiol. Rev.*, **64**, 873-931.
26. Caffaro, C. E., and Hirschberg, C. B. (2006) *Acc. Chem. Res.*, **39**, 805-812.

27. Liu, L., and Hirschberg, C. B. (2010) *Semin. Cell Dev. Biol.*, **21**, 600-608.
28. Liu, L., and Hirschberg, C. B. (2010) *Glycoconj. J.*, **30**, 5-10.
29. Xu, Y.-X., Liu, L., Caffaro, C. E., and Hirschberg, C. B. (2010) *J. Biol. Chem.*, **285**, 24600-24608.
30. Taniguchi, N., Suzuki, A., Ito, Y., Narimatsu, H., Kawasaki, T., and Hase, S. (eds.) (2008) *Experimental Glycoscience. Glycobiology*, Springer, Tokyo-Berlin-Heidelberg-New York.
31. Cummings, R. D., and Piers, J. M. (eds.) (2009) *Handbook of Glycomics*, Elsevier, Academic Press, Amsterdam-Boston-Heidelberg-London-New York-Oxford-Tokyo.
32. Sajdel-Sulkowska, E. M., Smith, F. I., Wiederschain, G. Y., and McCluer, R. H. (1997) *Glycoconj. J.*, **14**, 249-258.
33. Kapitonov, D., and Yu, R. K. (1999) *Glycobiology*, **9**, 961-978.
34. Qasba, P. K., and Ramakrishnan, B. (2007) in *Comprehensive Glycoscience. From Chemistry to System Biology* (Kamerling, J. P., Boons, G.-J., Lee, Y. C., Suzuki, A., Taniguchi, N., and Voragen, A. G. J., eds.) Vols. 1-4, Elsevier Ltd., Amsterdam-Boston-New York-London, pp. 251-281.
35. Pasek, M., Ramakrishnan, B., Boeggeman, E., Mercer, N., Dulcey, A. E., Griffiths, G. L., and Qasba, P. K. (2012) *Glycobiology*, **22**, 379-388.
36. Pfeffer, S. (2010) *Proc. Natl. Acad. Sci. USA*, **107**, 19614-19618.
37. Pfeffer, S. (2011) *Cold Spring Harb. Perspect. Biol.*, **3**, a005272, 1-11.
38. Zachara, N. E., and Hart, G. W. (2004) *Biochim. Biophys. Acta*, **1673**, 12-28.
39. Hart, G. W., and Akimoto, Y. (2009) in *Essentials of Glycobiology* (Varki, A., Cummings, R. D., Esko, J. D., Freeze, H., Stanley, P., Bertozzi, C. R., Hart, G. W., and Etzler, M. E., eds.) 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 263-279.
40. Rondanino, C., Bousser, M.-T., Monsigni, M., and Roche, A.-C. (2003) *Glycobiology*, **13**, 509-519.
41. Burnham-Marusich, A. R., and Berninson, P. M. (2012) *Mitochondrion*, **12**, 423-427.
42. Varki, A., Esko, J. D., and Colley, K. (2009) in *Essentials of Glycobiology* (Varki, A., Cummings, R. D., Esko, J. D., Freeze, H., Stanley, P., Bertozzi, C. R., Hart, G. W., and Etzler, M. E., eds.) 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 37-46.
43. Kohler, J. J., and Patrie, S. M. (eds.) (2013) *Mass Spectrometry of Glycoproteins. Methods and Protocols*, Humana Press, Springer Science, New York-Heidelberg-Dordrecht-London.
44. Luzhetskyy, A., Vente, A., and Bechtold, A. (2005) *Mol. BioSyst.*, **1**, 117-126.
45. Wongkongkatep, J., Miyahara, Y., Ojida, A., and Hamachi, I. (2006) *Angew. Chem.*, **45**, 665-668.
46. Krylov, V., Ustyuzhanina, N., Bakker, H., and Nifantiev, N. (2007) *Synthesis*, 3147-3154.
47. Sethi, M. K., Buettner, F. F. R., Krylov, V., Takeuchi, H., Nifantiev, N., Haltiwanger, R. S., Gerardy-Schahn, R., and Bakker, H. (2010) *J. Biol. Chem.*, **285**, 1582-1586.
48. Sethi, M. K., Buettner, F. F. R., Ashikov, A., Krylov, V. B., Takeuchi, H., Nifantiev, N. E., Haltiwanger, R. S., Gerardy-Schahn, R., and Bakker, H. (2012) *J. Biol. Chem.*, **287**, 2739-2748.
49. Vocadlo, D. J., and Davies, G. (2008) *Curr. Opin. Chem. Biol.*, **12**, 539-555.
50. Henrissat, B., Sulzenbacher, G., and Bourne, Y. (2008) *Curr. Opin. Struct. Biol.*, **18**, 527-533.
51. Bojarova, P., and Kren, V. (2008) *Trends Biotechnol.*, **27**, 199-209.
52. Tsvetkova, I. V., Karpova, E. A., Voznyi, Y. V., Zolotukhina, T. V., Biryukov, V. V., and Semyachkina, A. N. (1991) *J. Inher. Metab. Dis.*, **14**, 134-139.
53. Voznyi, Y. V., Karpova, E. A., Dudukina, T. V., Tsvetkova, I. V., Boer, A. M., Janse, H. C., and van Diggelen, O. P. (1993) *J. Inher. Metab. Dis.*, **16**, 465-472.
54. Tsvetkova, I. V., Karpova, E. A., Dudukina, T. V., and Voznyi, Y. V. (1996) *Clin. Chim. Acta*, **248**, 125-133.
55. Bakh, N. L., Beyer, E. M., Bovin, N. V., Zurabyan, S. E., and Wiederschain, G. Ya. (1980) *Dokl. Akad. Nauk SSSR*, **255**, 996-999.
56. Bakh, N. L., Beyer, E. M., Wiederschain, G. Ya., Bovin, N. V., and Zurabyan, S. E. (1981) *Bioorg. Khim.*, **7**, 1024-1033.
57. Shapiro, E., Lockman, L., Kennedy, W., Zimmerman, D., Kolodny, E., Raghavan, S., Wiederschain, G. Ya., Wenger, D., Sung, J. H., Summers, C., and Krivit, W. (1991) in *Treatment of Genetic Diseases* (Desnick, R. J., ed.) Churchill Livingstone, New York, pp. 223-238.
58. Wiederschain, G. Ya., Raghavan, S., and Kolodny, E. (1992) *Clin. Chim. Acta*, **205**, 87-96.
59. Wiederschain, G. Ya., Kozlova, I. K., Ilyina, G. S., Mikhaylova, M. A., and Beyer, E. M. (1992) *Carbohydr. Res.*, **224**, 255-272.
60. Semenyuk, E. P., and Wiederschain, G. Ya. (1985) *Biokhimiya*, **49**, 1556-1560.
61. Rye, C. S., and Withers, S. G. (2000) *Curr. Opin. Chem. Biol.*, **4**, 573-580.
62. Stutz, A. E. (ed.) (1999) *Iminosugars as Glycosidase Inhibitors. Nojirimycin and Beyond*, Wiley-VCH, Weinheim-New York-Toronto.
63. De Duve, C. (2005) *Nat. Cell Biol.*, **7**, 847-849.
64. Saftig, P., and Klumperman, J. (2009) *Nat. Rev. Mol. Cell Biol.*, **10**, 623-635.
65. Figura, K. V., and Hasilik, A. (1986) *Annu. Rev. Biochem.*, **55**, 167-193.
66. Kornfeld, S., and Mellman, I. (1989) *Ann. Rev. Cell Biol.*, **5**, 483-526.
67. Ghosh, P., Dahms, N. M., and Kornfeld, S. (2003) *Nat. Rev. Mol. Cell Biol.*, **4**, 202-212.
68. Braulke, T., and Bonifacino, J. S. (2009) *Biochim. Biophys. Acta*, **1793**, 605-614.
69. McMahon, H. T., and Boucrot, E. (2011) *Nat. Rev. Mol. Cell Biol.*, **12**, 517-533.
70. Fontes, C. M. G. A., and Gilbert, H. J. (2010) *Annu. Rev. Biochem.*, **79**, 655-681.
71. Hiraiwa, M., Saitoh, M., Arai, N., Shiraishi, T., Odani, S., Uda, Y., Ono, T., and O'Brien, J. S. (1997) *Biochim. Biophys. Acta*, **1341**, 189-199.
72. Kolter, T., and Zandhoff, K. (2005) in *Neuroglycobiology* (*Molecular and Cellular Neurobiology*) (Fukuda, M., Rutishauser, U., and Schnaar, R. L., eds.) Oxford University Press, pp. 199-223.

73. Aureli, M., Bassi, R., Loberto, N., Regis, S., Prinetti, A., Chigorno, V., Aerts, J. M., Boot, R. G., Filocamo, M., and Sonino, S. (2012) *J. Inherit. Metab. Dis.*, **35**, 1081-1091.
74. Brown, J., Novak, E. K., Takeuchi, K., Moore, K., Medda, S., and Swank, R. T. (1987) *J. Cell Biol.*, **105**, 1571-1578.
75. Wiederschain, G. Ya., and Rosenfeld, E. L. (1971) *Biochem. Biophys. Res. Commun.*, **44**, 1008-1014.
76. Wiederschain, G. Ya., and Prokopenkov, A. S. (1973) *Arch. Biochem. Biophys.*, **158**, 539-543.
77. Wiederschain, G. Ya., Rosenfeld, E. L., and Kolibaba, L. G. (1973) *Clin. Chim. Acta*, **46**, 305-310.
78. Wiederschain, G. Ya., Beyer, E. M., Klyashchitsky, B. A., and Shashkov, A. S. (1981) *Biochim. Biophys. Acta*, **659**, 434-444.
79. Wiederschain, G. Ya. (1982) *Adv. Clin. Enzymol.*, **2**, 150-157.
80. Beyer, E. M., and Wiederschain, G. Ya. (1982) *Clin. Chim. Acta*, **123**, 251-259.
81. Beyer, E. M., and Wiederschain, G. Ya. (1982) *Usp. Biol. Khim.*, **23**, 103-122.
82. Pearl, L. H. (2000) *Mutation Research/DNA Repair*, **460**, 165-181.
83. Zharkov, D. O., Mechetin, G. V., and Nevinsky, G. A. (2010) *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **685**, 11-20.
84. Wiederschain, G. Ya., Koul, O., Bovin, N. V., Nifantiev, N. E., and McKluer, R. Kh. (2000) *Bioorg. Khim.*, **26**, 448-451.
85. Wiederschain, G. Ya., Koul, O., Aucoin, J., Smith, F. I., and McCluer, R. H. (1998) *Glycoconj. J.*, **15**, 379-388.
86. Wiederschain, G. Ya. (1979) *Usp. Sovr. Biol.*, **20**, 46-70.
87. Bakker, H., Friedmann, I., Oka, S., Kawasaki, T., Nifant'ev, N., Schachner, M., and Mantei, N. (1997) *J. Biol. Chem.*, **272**, 29942-29946.
88. Jungalvala, F. B. (2005) in *Neuroglycobiology* (*Molecular and Cellular Neurobiology*) (Fukuda, M., Rutishauser, U., and Schnaar, R., eds.) Oxford University Press, pp. 59-94.
89. Eylar, E. (1965) *J. Theor. Biol.*, **10**, 89-113.
90. Ashwell, G., and Harford, J. (1982) *Ann. Rev. Biochem.*, **51**, 531-554.
91. Stockert, R. J. (1995) *Physiol. Rev.*, **75**, 591-609.
92. Veer, C., and van der Poll, T. (2008) *Nat. Med.*, **14**, 606-608.
93. Crewal, P. K., Uchiyama, S., Ditto, D., Varki, N., Le, D. T., Nizet, V., and Marth, J. D. (2008) *Nat. Med.*, **14**, 648-655.
94. Haltiwanger, R. S., Lehrman, M. A., Eckhardt, A. E., and Hill, R. L. (1986) *J. Biol. Chem.*, **261**, 7433-7439.
95. Varki, A. (1993) *Glycobiology*, **3**, 97-130.
96. Moran, A. P., Holst, O., Brennan, P. J., and von Itzstein, M. (eds.) (2009) *Microbial Glycobiology. Structures, Relevance and Applications*, Elsevier, Academic Press, Amsterdam-Boston-Heidelberg-London-New York-Oxford-Tokyo.
97. Cummings, R. D., and Piers, J. M. (eds.) (2009) *Handbook of Glycomics*, Elsevier, Academic Press, Amsterdam-Boston-Heidelberg-London-New York-Oxford-Tokyo.
98. Fukuda, M. (ed.) (2010) *Methods in Enzymology*, **478** (*Glycomics*); **479** (*Functional Glycomics*); **480** (*Glycobiology*), Elsevier Inc., Academic Press.
99. Issitt, P. D., and Anstee, D. J. (1998) *Applied Blood Group Serology*, 4th Edn., Montgomery Scientific Publications, Durcham, North Carolina, USA.
100. Derevitskaya, V. A. (1988) *Bioorg. Khim.*, **14**, 1605-1625.
101. Hakomori, S.-I. (2008) *Biochim. Biophys. Acta*, **1780**, 325-346.
102. Wiederschain, G. Ya., and Rosenfeld, E. L. (1969) *Bull. Soc. Chim. Biol.*, **51**, 1075-1084.
103. Daniels, G., and Withers, S. G. (2007) *Nat. Biotechnol.*, **25**, 427-428.
104. Liu, Q. P., Sulzenbacher, G., Yuan, H., Bennet, E. P., Pietz, G., Saunders, K., Spence, J., Nudelman, E., Levery, S. B., White, T., Neveu, J. M., Lane, W. S., Bourne, Y., Olsson, M. L., Henrissat, B., and Clausen, H. (2007) *Nat. Biotechnol.*, **25**, 454-464.
105. Brondz, B. D. (1987) *T-Lymphocytes and Their Receptors in Immunological Recognition* [in Russian], Nauka, Moscow.
106. Dyatlovitskaya, E. V., and Bergelson, L. D. (1987) *Biochim. Biophys. Acta*, **907**, 125-143.
107. Feizi, T., and Childs, R. A. (1987) *Biochem. J.*, **245**, 1-11.
108. Caraway, K. L., and Spielman, J. (1986) *Mol. Cell. Biochem.*, **72**, 109-120.
109. Varki, A., and Gagneux, P. (2012) *Ann. N. Y. Acad. Sci.*, **1253**, 16-36.
110. Benjamini, E., Coico, R., and Sunshine, G. (2000) *Immunology. A Short Course*, 4th Edn., Wiley-Liss, New York-Toronto.
111. Liu, D. Y., Petschek, K. D., Remold, H. G., and David, J. R. (1982) *J. Biol. Chem.*, **257**, 159-162.
112. Stolman, L. M. (1989) *Cell*, **56**, 907-910.
113. Wiederschain, G. Ya. (1986) *Usp. Biol. Khim.*, **27**, 117-135.
114. Callachan, J. W., and Lowden, J. A. (eds.) (1981) *Lysosomes and Lysosomal Storage Disease*, Raven Press, New York.
115. Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D. (eds.) (2001) in *The Metabolic and Molecular Bases of Inherited Disease*, 8th Edn., McGraw-Hill, Vol. III, pp. 3371-3894.
116. Durand, P., and O'Brien, J. S. (eds.) (1982) *Genetic Errors of Glycoprotein Metabolism*, Edi-Ermes, Milano, Springer-Verlag, Berlin, Heidelberg, New York.
117. Barranger, J. A., and Brady, R. O. (eds.) (1984) *Molecular Basis of Lysosomal Storage Disorders*, Academic Press, Orlando-San Diego-New York-London.
118. Hers, H. G., and van Hoof, F. (eds.) (1973) *Lysosomes and Storage Diseases*, Academic Press, New York-London.
119. Rosenfeld, E. L., and Popova, I. A. (1989) *Inborn Disorders in Glycogen Metabolism* [in Russian], Meditsina, Moscow.
120. Platt, F. M., and Walkley, S. U. (eds.) (2004) *Lysosomal Disorders of the Brain. Recent Advances in Molecular and Cellular Treatment*, Oxford University Press.
121. Futerman, A. H., and Zimran, A. (eds.) (2007) *Gaucher Disease*, CRC, Taylor and Francis Group, Boca Raton, FL, USA.
122. Freeze, H. (2009) in *Essentials of Glycobiology* (Varki, A., Cummings, R. D., Esko, J. D., Freeze, H., Stanley, P., Bertozzi, C. R., Hart, G. W., and Etzler, M. E., eds.) 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 567-583.
123. Mehta, A., and Winchester, B. (eds.) (2012) *Lysosomal Storage Disorders: a Practical Guide*, Wiley-Blackwell.
124. Kleta, R., Morse, R. P., Orvisky, E., Krasnewich, D., Alroy, J., Ucci, A. A., Bernardini, I., Wenger, D. A., and Gahl, W. A. (2004) *Mol. Genet. Metab.*, **82**, 137-143.

125. Aula, N., and Aula, P. (2006) *Prenat. Diagn.*, **26**, 655-658.
126. Wiederschain, G. Ya. (1977) *Usp. Biol. Khim.*, **18**, 185-210.
127. Beyer, E. M., Ivleva, T. S., Artykova, G. T., and Wiederschain, G. Ya. (1995) *Biochim. Biophys. Acta*, **1270**, 7-11.
128. Ivleva, T. S., and Wiederschain, G. Ya. (1994) *Cell Biol. Int.*, **18**, 647-653.
129. Beyer, E. M., Ivleva, T. S., Artykova, G. T., and Wiederschain, G. Ya. (1993) *Biochem. Mol. Biol. Int.*, **30**, 367-375.
130. Futerman, A. H., and van Meer, G. (2004) *Nat. Rev. Mol. Cell Biol.*, **5**, 554-565.
131. Hopwood, J., Bate, G., and Kirkpatrick, P. (2006) *Nat. Rev. Drug Disc.*, **5**, 101-102.
132. Freeze, H. (2013) *J. Biol. Chem.*, **288**, 6936-6945.
133. Varki, A., and Freeze, H. (2009) in *Essentials of Glycobiology* (Varki, A., Cummings, R. D., Esko, J. D., Freeze, H., Stanley, P., Bertozzi, C. R., Hart, G. W., and Etzler, M. E., eds.) 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 601-615.
134. Varki, A., Kannagi, R., and Toole, B. (2009) in *Essentials of Glycobiology* (Varki, A., Cummings, R. D., Esko, J. D., Freeze, H., Stanley, P., Bertozzi, C. R., Hart, G. W., and Etzler, M. E., eds.) 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 617-632.
135. Nizet, V., and Esko, J. D. (2009) in *Essentials of Glycobiology* (Varki, A., Cummings, R. D., Esko, J. D., Freeze, H., Stanley, P., Bertozzi, C. R., Hart, G. W., and Etzler, M. E., eds.) 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 537-551.
136. Litwack, G. (2008) *Human Biochemistry and Disease*, Elsevier, Amsterdam-Boston-New York-London.
137. McGrath, B. M., and Walsh, G. (eds.) (2006) *Directory of Therapeutic Enzymes*, CRC Press, Taylor and Francis Group, Boca Raton-London-New York-Washington.
138. Townsend, R. R., and Hotchkiss, A. T., Jr. (eds.) (1997) *Techniques in Glycobiology*, Marcel Dekker, New York.
139. Hounsell, E. (ed.) (1998) *Glycoanalysis Protocols*, 2nd Edn., Humana Press, Totowa, NJ, USA.
140. Brockhausen, I. (ed.) (2006) *Glycobiology Protocols*, Humana Press, Totowa, NJ, USA.
141. Packer, N. H., and Karlsson, N. G. (eds.) (2009) *Glycomics. Methods and Protocols*, Humana Press, Springer Science, New York.
142. Yuriev, E., and Ramsland, P. A. (eds.) (2013) *Structural Glycobiology*, CRC Press, Taylor and Frances Group, Boca Raton, FL, USA.
143. Shibaev, V. N. (1985) in *Progress in Chemistry of Carbohydrates* [in Russian], Nauka, Moscow, pp. 149-173.
144. Withczak, Z., and Nieforth, K. A. (eds.) (1997) *Carbohydrates in Drug Design*, Marcel Dekker, New York.
145. Hanessian, S. (ed.) (1997) *Preparative Carbohydrate Chemistry*, Marcel Dekker, New York.
146. Wang, P. G., and Bertozzi, C. R. (eds.) (2001) *Glycochemistry*, *Principles, Synthesis, and Applications*, Marcel Dekker, New York.
147. Yarema, K. (ed.) (2005) *Handbook of Carbohydrate Engineering*, CRC Press, Taylor and Frances Group, Boca Raton, FL, USA.
148. Garg, H., Linchardt, R., and Hales, C. A. (eds.) (2005) *Chemistry and Biology of Heparin and Heparan Sulfate*, Elsevier, Amsterdam-Boston-New York-London.
149. Iozzo, R. (ed.) (2001) *Proteoglycan Protocols*, Humana Press, Totowa, NJ, USA.
150. Plou, F. J., de Segura, A. G., and Ballesteros, A. (2007) in *Industrial Enzymes Structure, Function and Applications* (Polaina, J., and MacCabe, A. P., eds.) pp. 141-160.
151. Li, J. (ed.) (2010) *Functional Glycomics. Methods and Protocols*, Humana Press, Springer Science, New York.
152. Redini, F. (ed.) (2012) *Proteoglycans. Methods and Protocols*, Humana Press, Springer Science, New York.
153. Taniguchi, N., Suzuki, A., Ito, Y., Narimatsu, H., Kawasaki, T., and Hase, S. (eds.) (2008) *Experimental Glycoscience. Glycochemistry*, Springer, Tokyo-Berlin-Heidelberg-New York.
154. Large, D. G., and Warren, C. D. (eds.) (1997) *Glycopeptides and Related Compounds. Synthesis, Analysis, and Applications*, Marcel Dekker, New York.
155. Thibault, P., Honda, S. (eds.) (2002) *Capillary Electrophoresis of Carbohydrates*, Humana Press, Totowa, NJ, USA.
156. Volpe, N. (ed.) (2011) *Capillary Electrophoresis of Carbohydrates. From Monosaccharides to Complex Carbohydrates*, Humana Press, Springer Science, New York.
157. Liu, Y., Palma, A. S., and Feizi, T. (2009) *Biol. Chem.*, **390**, 647-656.
158. Musto, C. J., and Suslick, K. (2010) *Curr. Opin. Chem. Biol.*, **14**, 758-766.
159. Kiessling, L. L., and Splain, R. A. (2010) *Annu. Rev. Biochem.*, **79**, 619-653.
160. Croci, D. O., Cumashi, A., Ushakova, N. A., Preobrazhenskaya, M. E., Piccoli, A., Totani, L., Ustyuzhanina, N. E., Bilan, M. I., Usov, A. I., Grachev, A. A., Morozevich, G. E., Berman, A. E., Sanderson, C. J., Kelly, M., Gregorio, P., Rossi, Di C., Tinari, N., Iacobelli, S., Rabinovich, G. A., and Nifantiev, N. E. (2011) *PLoS ONE*, **6**(2), e17283, doi:10.1371/journal.pone.0017283.
161. Usov, A. (2011) *Adv. Carbohydr. Chem. Biochem.*, **65**, 115-217.
162. Cumashi, A., Ushakova, N. A., Preobrazhenskaya, M. E., D'Incecco, A., Piccoli, A., Totani, L., Tinari, N., Morozevich, G. E., Berman, A. E., Bilan, M. I., Usov, A. I., Ustyuzhanina, N. E., Grachev, A. A., Sanderson, C. J., Kelly, M., Rabinovich, G. A., Iacobelli, S., and Nifantiev, N. E. (2007) *Glycobiology*, **17**, 541-552.
163. Pomin, V. H., and Mourao, P. A. S. (2008) *Glycobiology*, **18**, 1016-1027.
164. Ghosh, T., Chattopadhyay, K., Marshall, M., Karmakar, P., Mandal, P., and Ray, B. (2009) *Glycobiology*, **19**, 2-15.
165. Sola, R. J., and Griebenow, K. (2010) *BioDrugs*, **24**, 9-20.
166. Beck, A. (ed.) (2013) *Glycosylation Engineering of Biopharmaceuticals. Methods and Protocols*, Humana Press, Springer Science, New York.