

## *Glycoproteins and Proteoglycans*

A glycoprotein is defined as a protein or polypeptide to which a carbohydrate is attached by a covalent bond. These conjugated proteins are of major biological importance, comprising enzymes, hormones, antibodies, membranes, and the ground substance of every cell. They include not only most of the soluble globulins of the plasma, but insoluble proteins of connective tissue and the lubricant secretions of the various organs of the bodies, the mucoproteins.

Some proteins of the body, such as albumin, retinol-binding protein, thyroxine-binding prealbumin, and hemoglobin contain no carbohydrate. Others, such as the blood group substances, may contain as much as 80% carbohydrate. The proteins of the complement system and hemostasis are glycoproteins. Haptoglobin and ceruloplasmin contain about 20 and 10% carbohydrate, respectively.

Study of the composition of the plasma glycoproteins, especially those which occur in low concentrations, was hampered by the fact that they were heat labile, turning black on heating because of the browning reaction. When heated, the aldehyde group of the carbohydrate portion of the molecule reacts with an amino group of the protein to form a Schiff base, which then polymerizes, turning brown or black. Furthermore, microtechniques were needed for investigating the composition of proteins which occurred in minute amounts in the serum. In the last 25 years, the aminoacid and carbohydrate sequences have been elucidated for many of the glycoproteins.

### *5.1 ISOLATION OF THE PLASMA GLYCOPROTEINS*

When 95% ethanol is added to plasma or serum in a ratio of 100:1, some of the proteins of the serum will precipitate. On examina-

TABLE 5.1  
*Normal Distribution Obtained for the Glycoprotein Fractions  
of Serum by Electrophoresis<sup>a</sup>*

Prealbumin and albumin, %	Globulins, %			
	$\alpha_1$	$\alpha_2$	$\beta$	$\gamma$
2-9	18-22	25-35	22-27	13-18

<sup>a</sup> Results are in percentage of total carbohydrate.

tion, these are found to contain substantial amounts of carbohydrate. These alcohol precipitable, carbohydrate-carrying proteins are the major glycoproteins.<sup>(1,2)</sup>

If 0.75 N perchloric acid is added to serum in the ratio of 8:1, then most of the proteins in the serum will be precipitated. A more soluble carbohydrate-containing fraction remains in solution and may be precipitated with phosphotungstic acid. This latter mixture of proteins is often referred to as the *mucoprotein fraction*. It comprises mainly the  $\alpha_1$ -acid *glycoprotein* which travels with the  $\alpha_1$  globulin fraction on electrophoresis.<sup>(3)</sup>

If serum is spotted on paper, cellulose acetate or some other medium and subjected to electrophoresis at pH 8.6, one may stain for the protein fractions, namely albumin,  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ , and the  $\gamma$  globulins. If one stains instead with the *periodic acid-Schiff reagent* (PAS), the carbohydrates are revealed<sup>(4,5)</sup>. Carbohydrate occurs in all the protein fractions but mainly in the  $\alpha$  and  $\beta$  globulin regions. The distribution of these carbohydrate-containing fractions may be seen in Table 5.1.

Six monosaccharides comprise the main components of the polysaccharide linked with plasma proteins. These are the hexoses, galactose and mannose, the hexosamines, glucosamine and galactosamine, and fucose and the sialic acids. Xylose is also found occasionally.<sup>(6)</sup> The concentration of these components of serum proteins are listed in Table 5.2. Adding the mean values of Table 5.2, one obtains

TABLE 5.2  
*Concentration of Monosaccharides in Plasma Proteins,  
mg/100 ml of Serum*

Hexoses	Hexosamines	Sialic acid	Fucose
121 $\pm$ 2.1	83 $\pm$ 4.9	60 $\pm$ 3.7	8.9 $\pm$ 0.6

**TABLE 5.3**  
*Distribution of Monosaccharides Among the Various  
 Electrophoretic Fractions of Human Serum, g monosaccharide/100 g  
 Protein*

Protein	Hexoses	Hexosamine	Sialic acid	Fucose
Albumin	0.20	0.06	0.10	0.01
$\alpha_1$ Globulin	7.5	6.3	4.1	0.55
$\alpha_2$ Globulin	5.9	4.2	3.0	0.40
$\beta$ Globulin	2.9	1.9	1.5	0.20
$\gamma$ Globulin	1.9	1.5	1.2	0.30
Total	1.6	1.1	0.87	0.11

a total of 273 mg/100 ml of protein-bound carbohydrate. This value increases by a factor of two or three in the inflammatory and neoplastic diseases, the increase being in all of the components.<sup>(7)</sup> The distribution of these monosaccharides among the various protein fractions of the serum is given in Table 5.3.

### *Separation of the Carbohydrate Moiety*

The sugars found in the glycoproteins are galactose, *N*-acetylgalactosamine, *N*-acetylglucosamine, mannose, *N*-acetylmannosamine, *N*-acetylneuraminic acid, fucose, xylose, and in the cell wall of bacteria, *N*-acetylmuramic acid. The structures of these sugars are given in Figures 5.1 and 5.2. None of the plasma glycoproteins have been shown to contain glucose, except for Clq, a component of the complement system. However, the tissue (e.g., collagen) and membrane glycoproteins do contain glucose.<sup>(8)</sup> The carbohydrate units of glycoproteins are first isolated as glycopeptides with a minimum number of amino acids attached after extensive enzymic proteolytic digestion.<sup>(9)</sup> From a study of these glycopeptides, the size, number, and composition of these carbohydrate units can be estimated. The point of attachment to the polypeptide then can be determined.

The carbohydrate units range in size from a molecular weight of approximately 3500 (as in fetuin) to single monosaccharide residues of molecular weight 162 (as in the collagens). There may be only a single carbohydrate unit per molecule as in ribonuclease, or there may be as many as 800, as in the ovine submaxillary glycoprotein. Since

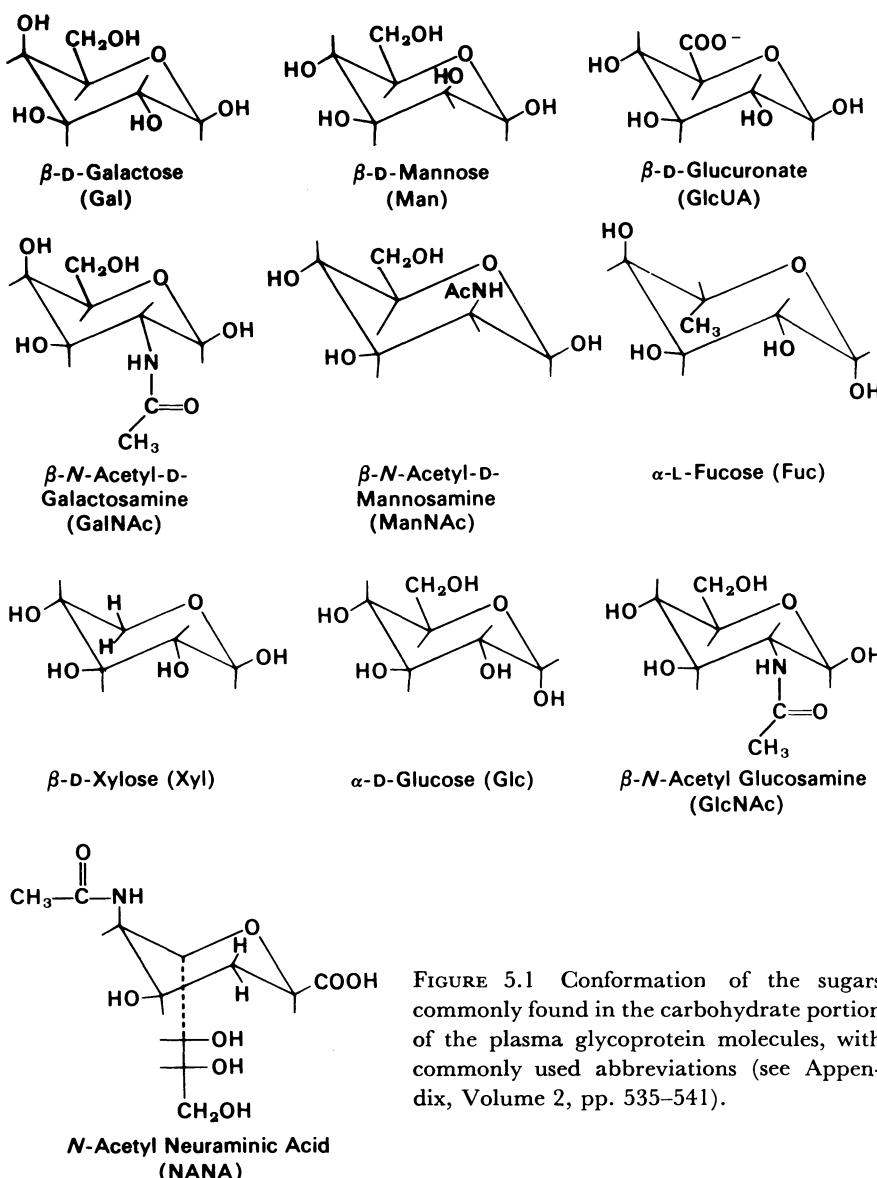


FIGURE 5.1 Conformation of the sugars commonly found in the carbohydrate portion of the plasma glycoprotein molecules, with commonly used abbreviations (see Appendix, Volume 2, pp. 535–541).

glycoproteins vary so much in molecular weight, from 14,500 for ribonuclease to  $1 \times 10^6$  for the ovine submaxillary glycoprotein, the average spacing of the carbohydrate units along the peptide chain (that is, the number of amino acids per carbohydrate unit) is a better

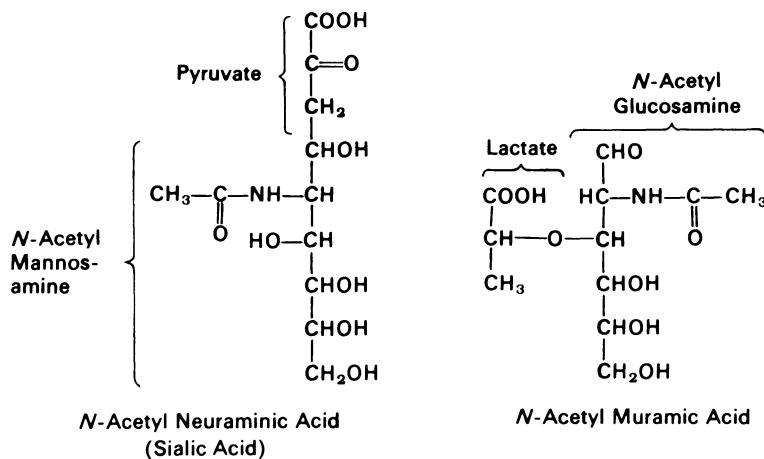


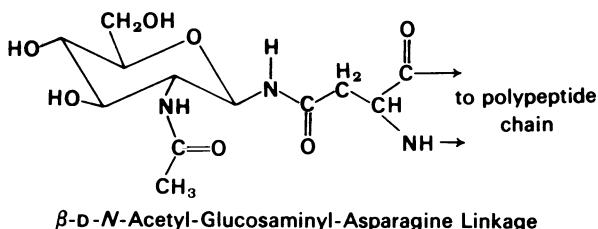
FIGURE 5.2 Comparison of the structure of *N*-acetyl neuraminic acid and *N*-acetyl muramic acid. Neuraminic acid is derived from *D*-mannosamine and pyruvic acid; *N*-acetyl muramic acid is *D*-glucosamine connected by ether linkage with lactic acid. In sialic acid the acetyl group may be replaced with the glycyl residue.

index of the extent of carbohydation of the protein than the number of units per molecule. This spacing of carbohydrate units may be seen to vary considerably, with as few as six amino acid residues per unit in the ovine submaxillary glycoprotein, to as many as 779 amino acid residues per carbohydrate unit in the IgG immunoglobulins.

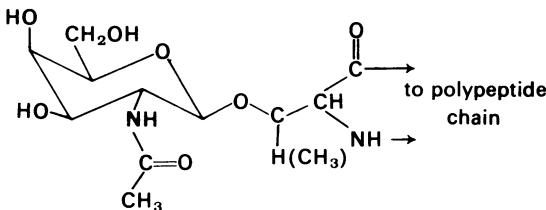
It may be noted, that most glycoproteins contain only one type of carbohydrate unit. A few, however, like thyroglobulin and the glomerular basement membrane, contain more than one distinct type.

## 5.2 THE NATURE OF THE CARBOHYDRATE PROTEIN LINKAGE

The covalent attachment of the carbohydrate units of glycoproteins has been uniformly shown to involve carbon 1 of the most internal sugar residue, and a functional group on an amino acid in the peptide chain. The three distinct types of glycopeptide bonds have been shown to occur in protein from animal tissues (see Figure 5.3). *The first glycopeptide bond to be ascertained was between carbon 1 of *N*-acetyl-glucosamine and the amide nitrogen of asparagine. This represents a glyco-*



$\beta$ -D-N-Acetyl-Glucosaminyl-Asparagine Linkage



$\beta$ -D-N-Acetyl-Galactosaminyl-Serine Linkage  
(threonine linkage, CH<sub>3</sub> for H)

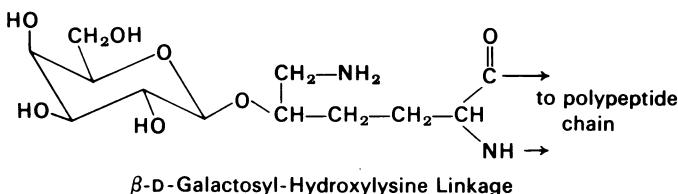


FIGURE 5.3 Linkages of the carbohydrate portion of the glycoprotein molecules in the polypeptide chain.

*sialamine type linkage.*<sup>(10)</sup> It was originally described in ovalbumin,<sup>(11)</sup> and has subsequently been found in a large variety of glycoproteins from diverse sources, including IgG immunoglobulin,<sup>(12)</sup> ribonuclease,<sup>(13)</sup> deoxyribonuclease,<sup>(14)</sup>  $\alpha_1$ -acid glycoprotein,<sup>(11)</sup> fetuin,<sup>(13)</sup> thyroglobulin,<sup>(14)</sup> and ovomucoid.<sup>(15)</sup> It is the most common type of linkage of carbohydrate to protein in the plasma proteins.

*A second glycopeptide linkage involves an O-glycosidic bond to serine or threonine.* It is easily split by very mild alkali. The sugar component of this type of linkage is often N-acetylgalactosamine. The N-acetylgalactosamine is involved in the linkage of various mucins.<sup>(16)</sup> The molecular weight of these proteins is generally on the order of several millions. In these glycoproteins, the number of oligosaccharide residues per total amino acid residue is very high and solutions of these proteins

are very viscous. In the mucins, serine and threonine account for up to 40–50% of amino acid residues present, and a large proportion of these are substituted by carbohydrate groups. The sugar involved in this linkage is galactose or xylose, or more often *N*-acetylgalactosamine, usually in a  $\beta$  linkage<sup>(17)</sup> (see Figure 5.3). This type of linkage occurs in the immunoglobulins.

*A third type of glycoprotein linkage occurs in basement membranes<sup>(18)</sup> and collagens.<sup>(19)</sup> It involves an O-glycosidic linkage between a galactose residue and the hydroxyl group of hydroxy lysine. This linkage is an unusual one, for it involves a glycosidic substitution on to a hydroxyl group which at physiological pH values is positively charged. Contrary to the O-glycosides of serine and threonine, this glycopeptide bond is very stable to alkaline hydrolysis permitting the isolation of the carbohydrate unit attached to hydroxylysine in very high yield. This linkage occurs in the Clq component of the complement system. It occurs commonly in collagen.*

Studies of a number of glycoproteins such as ovalbumin, various ribonucleases, the immunoglobulins, and others, have revealed that where an oligosaccharide is attached to the amide of aspartate through *N*-acetylglucosamine, the sequence is always Asn-X-Thr- (or Ser-), where X is any amino acid with the exception of proline and the aromatic amino acids.<sup>(19–26)</sup> For example, in IgM, X can be alanine,

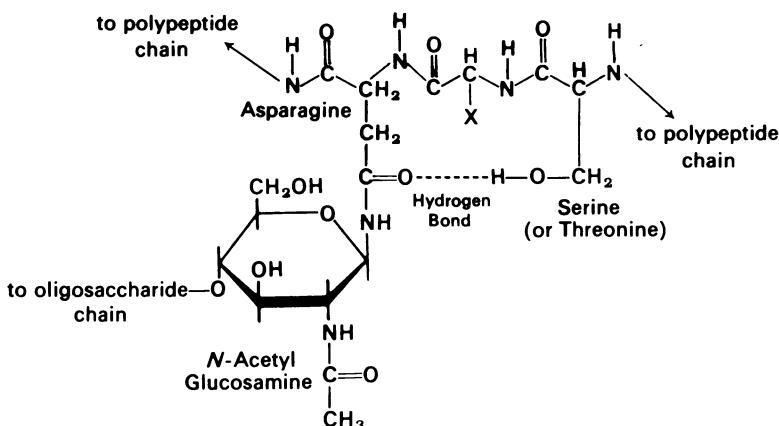


FIGURE 5.4 Proposed reason for occurrence of glycoside attachment at sequence —Asn-X-Ser(Thr)-. Hydrogen bond loosens hydrogen on amide, permitting substitution of glycoside residue.

asparagine, glycine, isoleucine, or leucine. For  $\alpha_1$  acid glycoprotein, X occurs as lysine, threonine, or alanine. In ceruloplasmin, X can be valine or leucine. In transferrin, X occurs as valine and lysine. These few examples suggest that this is more than a coincidence. It has been suggested that, in order for the N-acetylglucosamine to be attached, the hydroxyl group of the serine or threonine residue needs to form a hydrogen bond with the carboxyl group of the asparagine (Figure 5.4). This serves to activate the hydrogen on the amide and permit substitution of the glycosidic linkage.<sup>(27)</sup>

### 5.3 STUDIES OF THE STRUCTURE OF THE HETEROSACCHARIDES

In order to comprehend the function of the oligosaccharides attached to the polypeptides in the glycoproteins, certain facts need to be explored. These may be listed as follows:

1. The monosaccharide units which make up the oligosaccharides need to be identified.
2. The nature of the carbohydrate-peptide linkage needs to be ascertained.
3. The monosaccharide sequences and branching need to be unraveled.
4. The sites on the polypeptide chain where the oligosaccharides are attached need to be located.

Elucidation of the structure of the heterosaccharides attached to the plasma proteins is a complex problem. Examination of Figure 5.1 reveals that, after attachment of the monosaccharide to the polypeptide chain, there remain four or five hydroxyl groups to which a second monosaccharide may be attached. Thus, the sequence of a simple disaccharide, GlcNAc—Gal, may signify at least four isomers. If another monosaccharide is added, it can then be bound to GlcNAc or Gal, in any of seven different locations. The linkage of the hydroxyl groups in the 1 position can also be  $\alpha$  or  $\beta$ . Thus an oligosaccharide comprising only a few monosaccharides can give rise to a huge number of possible structures.

Another problem which presents itself is the fact that synthesis of

the heterosaccharide side chains attached to polypeptides is relatively inaccurate, when compared to the precision with which the polypeptides are synthesized. The polypeptides are synthesized on a template which guides their formation. No such template exists for the addition of the monosaccharides. These sugars are added one by one by a mechanism which is presented in Section 5.4. If a monosaccharide is in short supply, it may be omitted. This gives rise to glycoproteins prepared in the same individual which may vary slightly. This phenomenon is called *microheterogeneity*, and accounts for some of the heterogeneity observed when isolating glycoproteins such as ceruloplasmin, the haptoglobins, and others.

In spite of the complexity of this field of study, substantial progress has been made in elucidating the structure of the polysaccharide side chains of a number of proteins. The term used by the experimenters in this field for these side chains is *heterosaccharide* to indicate a structure made up of different sugars. *Oligosaccharide* derives from the Greek *oligos*, meaning little or a few, and *saccharide*, meaning sugar. Thus the term oligosaccharide denotes a lesser number of monosaccharides forming a chain or matrix. *Polysaccharide* is reserved for the large complex carbohydrate structures, usually made up of a single monosaccharide such as glycogen and cellulose (glucose), inulin (fructose), mannan (mannose), galactan (agar, galactose), and others.

A major means for unraveling the sequence and linkages of the glycoproteins is the use of specific carbohydrases, all of which occur in the lysosomes of cells. These include neuramidinases, the  $\alpha$  and  $\beta$  galactosidases, fucosidases, glucosidases, hexoseaminidases, and xylosidases and finally  $\beta$ -aspartylglucosylamine: amidohydrolase to release the sugar from the aspartylamide of the polypeptide chain. This is outlined schematically in Figure 5.5.<sup>(26)</sup>

In the normal individual, ingested glycoproteins are dismantled with the use of the enzymes shown in Figure 5.5. Genetic deficiency of any of these enzymes results in the accumulation of glycoproteins, glycolipids, and polysaccharides, resulting in a number of diseases, including the lipidoses, Hurler's disease, and related syndromes. In addition, where sulfate is attached to the carbohydrate, as in collagen, sulfatases are required for digestion. These sulfatases also serve in the elucidation of the complex carbohydrate structures.

Digestion of certain proteins is incomplete, unless the carbohydrate is first removed. Thus, where carbohydrate forms a high percentage of

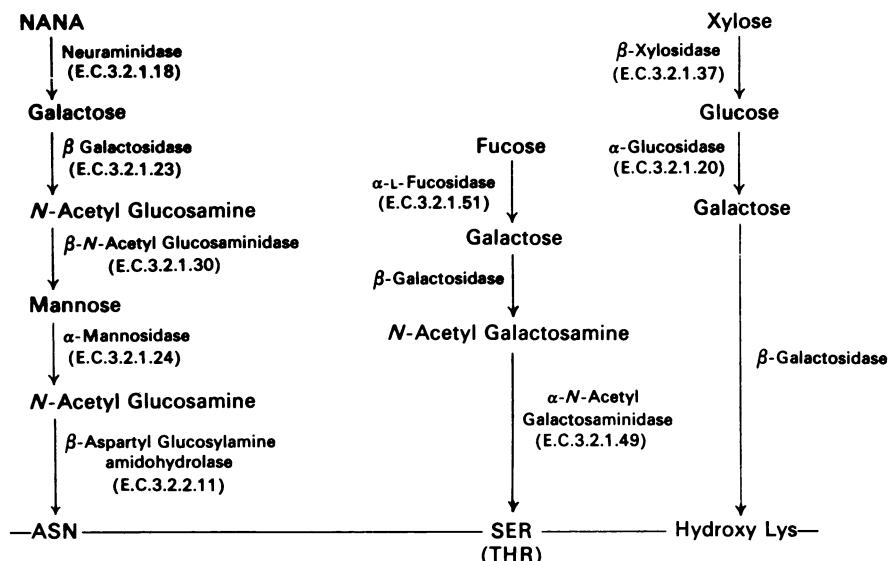


FIGURE 5.5 Representation of the lysosomal enzymes employed in studies of heterosaccharide structure and in digestion of glycoproteins.

the molecule, it serves to protect the polypeptide chain from proteolytic attack.

#### 5.4 HETEROSACCHARIDE SYNTHESIS

Under the electron microscope, the endoplasmic reticulum of the cell appears as an extensive network of tubules, vesicles, and lamellae (variant of *laminae*) which in Latin means thin flat layers or leaves. It comprises a complex of membranes of extensive surface, which are composed of 60–70% protein and 30–40% phospholipid by weight. During homogenization of cells the endoplasmic reticulum breaks up, forming closed vesicles called *microsomes*. Many of these vesicles carry ribosomes on their outer surface. The cytochromes  $b_5$  and P450-NADPH enzyme systems are located in the endoplasmic reticulum as well as a substantial number of other enzymes, especially those involved in the synthesis of the carbohydrate moiety of the glycoproteins.

Some of the endoplasmic reticulum of the cell appears “rough” because of the accumulation of ribosomes on its surface. It is here that

the polypeptides are assembled.<sup>(27-29)</sup> A message derived from RNA translation (mRNA) results in the synthesis of the polypeptides on the ribosomes bound to the endoplasmic reticulum (see Figure 5.6). It will be noted that protein synthesis also takes place on ribosomes bound to the nuclear membrane, which is, in effect, part of the endoplasmic reticulum.

The purpose of the embedding of the ribosomes in the endoplasmic reticulum is so that the formed peptide chain can pass into the cisternal space beneath. This isolates the newly formed molecule for further processing. In the cisternal compartment, some modification takes place, such as disulfide bond formation and hydroxylation of lysine and proline by the  $b_5$  and P-450 systems. This is required for collagen synthesis and the glycosylation steps, which are the topic of discussion in this section.

The polypeptide moves along the smooth surface of the cisterna with energy supplied from ATP and encounters areas where specific transferases are located for sequentially glycosylating the particular glycoprotein being synthesized by the cell. This is shown schematically in Figures 5.6 and 5.7.

Most commonly, as far as the plasma glycoproteins are concerned, *N*-acetylglucosamine is the first monosaccharide to be attached to the amide nitrogen of an asparagine of the polypeptide chain (Figure 5.4).<sup>(30-32)</sup> In some cases, as indicated in Figure 5.5, attachment is to the hydroxyl appendage of serine or threonine. In this case, it is not

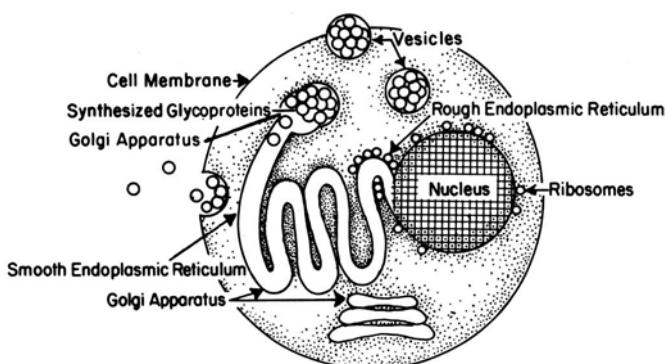


FIGURE 5.6 Schematic representation of the function of the endoplasmic reticulum and Golgi apparatus in the synthesis of glycoproteins, formation of vesicles, and discharge of the glycoproteins into the blood stream.

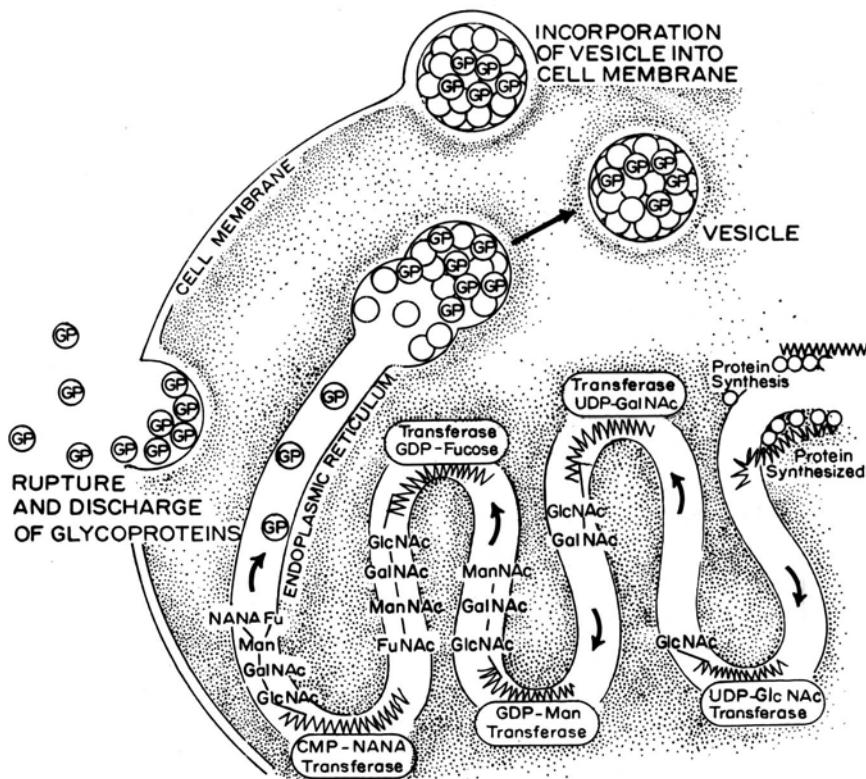
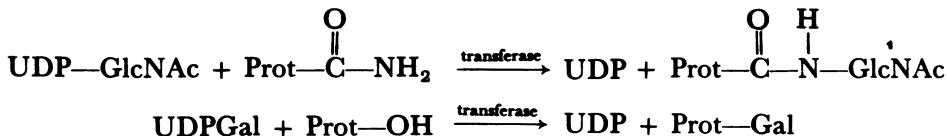


FIGURE 5.7 The role of the various transferases and activated monosaccharides in glycoprotein synthesis. In this hypothetical case, the sequence should be, NANA (Fu)-Man-Ga1NAc-G1cNAc-.

unusual for the *N*-acetylgalactosamine to be the first sugar attached. In special proteins, such as collagen, the attachment to hydroxylysine also occurs. The innermost monosaccharide is then often galactose.

The transferase always acts by exchange of the monosaccharide bound to a nucleotide, such as in uridine diphosphoglucosamine (UDP-GlcNAc), for a hydrogen on the —CO:NH<sub>2</sub> or —OH group of the polypeptide, for example:



The monosaccharide bound to the nucleotide acts as a substrate for a particular transferase and, in this form, is said to be *activated*. The nucleotide to which the monosaccharide is attached is not the same in every case. For example, for glucose, galactose, xylose, arabinose, and their *N*-acetyl amino derivatives, uridine diphosphate is bound to the sugar. For mannose and fucose, guanosine diphosphate is the nucleotide portion of the activated complex. For the sialic acids (*N*-acetyl-, glycyll-, or glycolylneuraminic acids), cytidine monophosphate forms the activating complex. The structures of some of these activated substrates are shown in Figure 5.8.

All monosaccharides found in the glycoproteins are derived from glucose. Figure 5.9 shows this relationship. In this figure, the activated forms of the monosaccharides used in the synthesis of the plasma glycoproteins are placed in a box. A total of ten are shown; however, the list is incomplete. For example, iduronate occurs as a component of some heterosaccharides.

Referring to Figure 5.7, after the first monosaccharide is added, the polypeptide moves along to the next station at which a second sugar is added. Branching may occur, as indicated. After NANA is added, no other sugar is added except that a second or third molecule of NANA may be attached to NANA. After fucose is added to a chain, it indicates the end of that chain or branch. It is not unusual for the same monosaccharide to be repeated in a chain. For example, as many as six mannose residues commonly occur in sequence in some glycoproteins.

After the glycoprotein is synthesized, it may be sulfonated by a sulfotransferase using 3'-phosphoadenylylsulfate (PAPS) as the sulfate donor (see Volume 1, pp. 179-183).

When the glycoprotein is finally formed, it is pushed along the cisternae to a dead end, where the protein is concentrated in an enlarged balloon-like space. An approximately spherical portion, engorged with glycoprotein, breaks off to form a vesicle loaded with glycoprotein. This moves to the surface of the cell and fuses with the cell membrane. The vesicle then breaks open, expelling the contents of the vesicle into the blood stream.

The endoplasmic reticulum and bulbar end resemble in appearance a deflated toy balloon. This shape permits the structures to be stacked one on top of each other. The complex so formed was first noted by Golgi and is therefore referred to as the *Golgi apparatus*.<sup>(33-37)</sup>

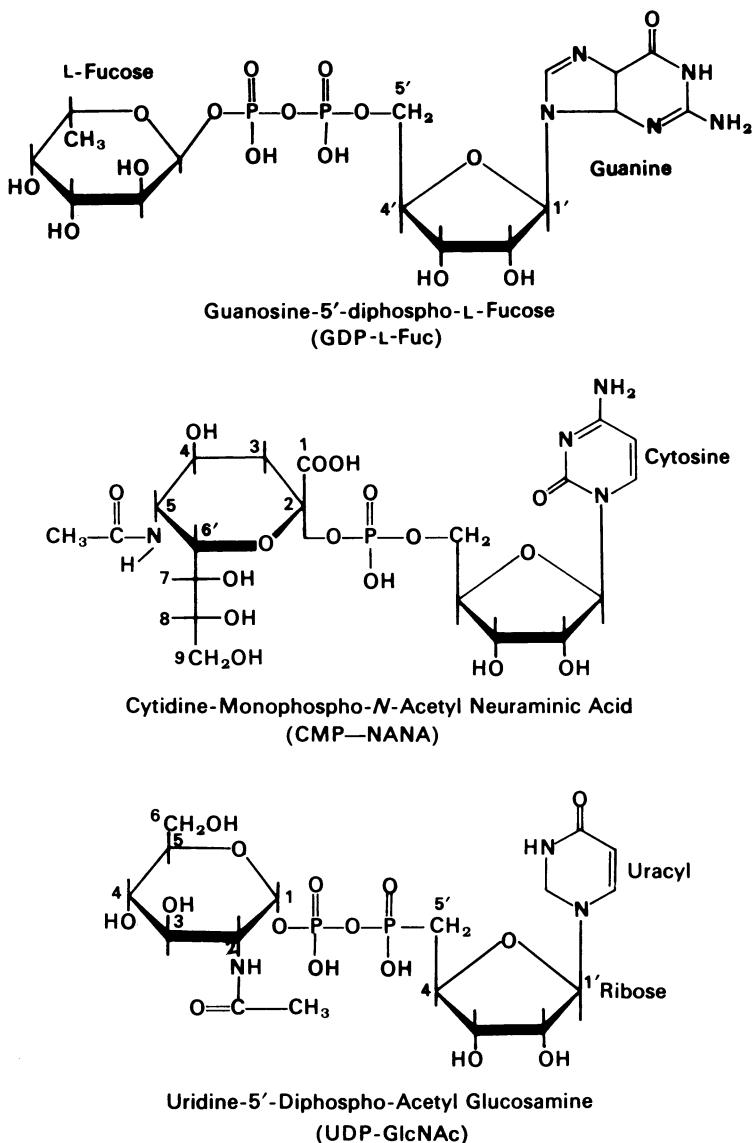
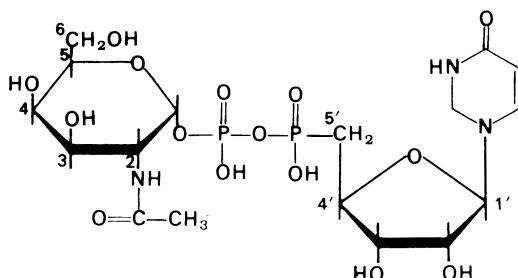
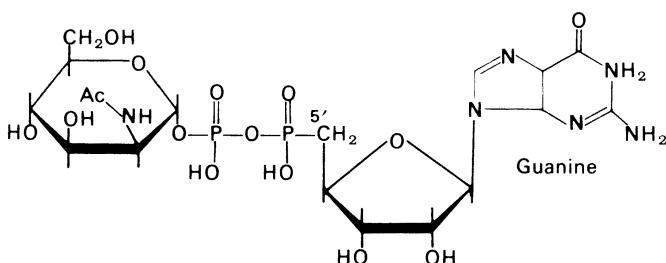


FIGURE 5.8 Activated forms of the various monosaccharides employed in the synthesis of the heterosaccharides. UDP-Gal and UDP-Glc are not shown. They have structures similar to that of UDP-Glc-NAc and UDP-Gal-NAc, respectively, with the *N*-acetyl group replaced by an —OH group. (See Volume 2, p. 120.)



Uridine-5'-Diphospho-Acetyl Galactosamine  
(UDP-GalNAc)



Guanosine-5'-Diphospho-Acetyl Mannosamine  
(GDP-ManNAc)

FIGURE 5.8 (continued)

Figure 5.10 is a reproduction of an electron micrograph showing the structure and stacking of the Golgi complex.

### Dolichol and Retinol in Glycoprotein Synthesis

From various sources, such as milk, hen oviduct, mammary gland, submaxillary gland, and other tissues rich in glycoprotein, oligosaccharides were isolated attached to a nucleotide. Typical examples are UDP ← GlcNAc ← Gal; UDP ← GlcNAc ← Gal ← NANA; UDP ← GlcNAc ← Gal ← Fuc; UDP ← GlcNAc ← sulfate; and others. From this the concept derived that an oligosaccharide attached to a single nucleotide could be transferred as a whole. This would be a more efficient system, since it could take place at one location on the endoplasmic reticulum.

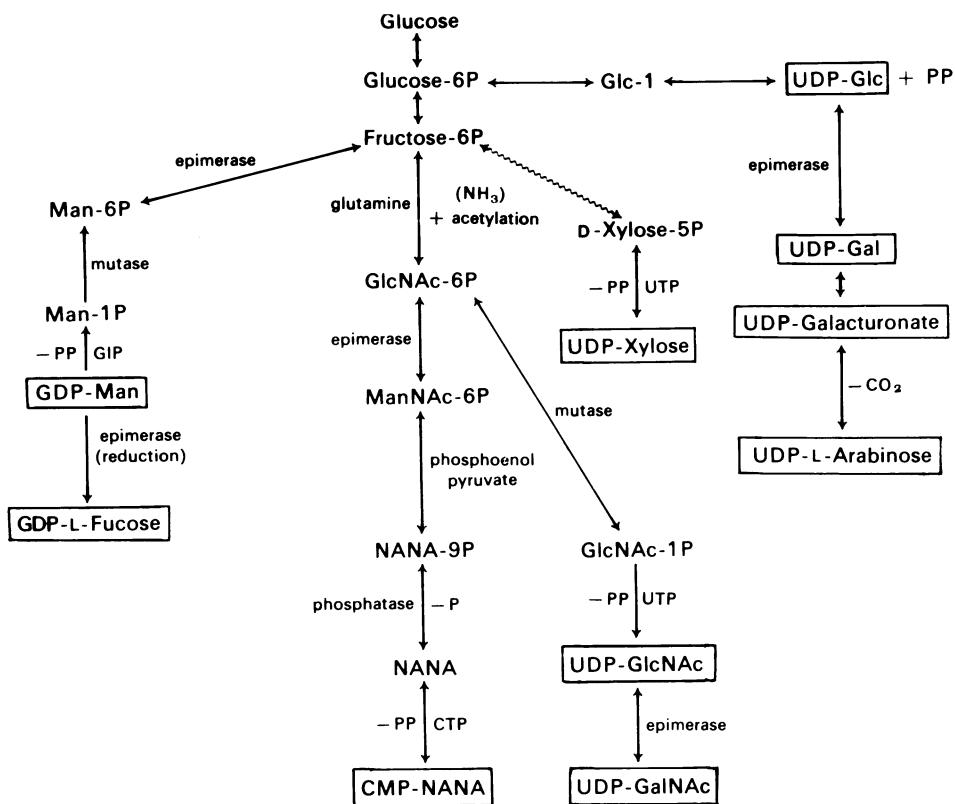


FIGURE 5.9 Formation of the activated monosaccharides from glucose. The activated sugars which take part in glycoprotein synthesis are underlined. See Volume 2, pp. 114–125 and 157 for the relationship between glucose, fructose, and UDP-glucose. All sugars are in the D-form except where indicated as L. A wavy arrow indicates a complex pathway.

Earlier it was pointed out that the endoplasmic reticulum was 30–40% phospholipid. Thus it would be reasonable to assume that a lipid anchored at a site could serve to collect the monosaccharides before transferring them to the newly synthesized protein. This seems to be the case for many of the glycoproteins synthesized. The lipids involved in the transport have been identified as *dolichol* and *retinol* (*vitamin A*).<sup>(38–46)</sup> See Figure 5.11.

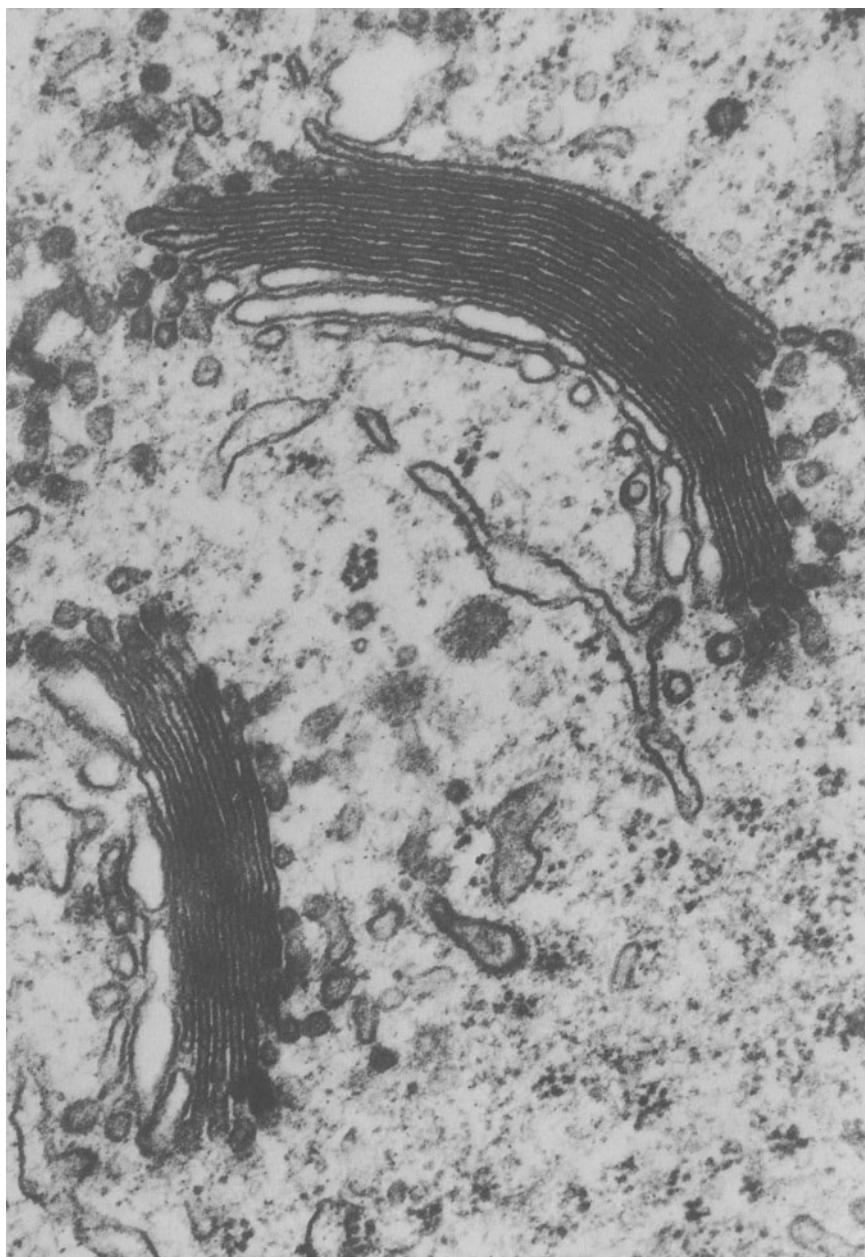


FIGURE 5.10 Electron micrograph showing the stacking of the Golgi apparatus. Scale: 1 in. = 0.37  $\mu$ m. Courtesy of M. Dauwalder, Cell Research Institute, University of Texas at Austin.

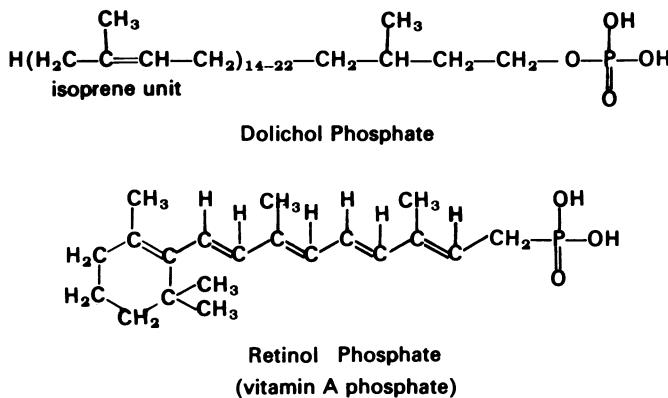
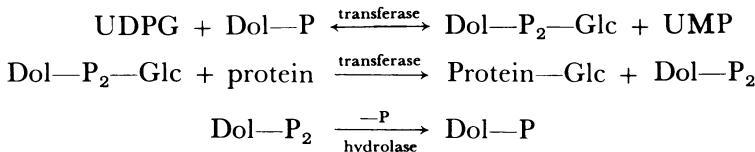


FIGURE 5.11 Structures of dolichol and retinol monophosphate. Both are polymers of isoprene. Dolichol is of variable composition, comprising 15–22 isoprene units. Retinol (vitamin A) contains only 4 isoprene units.

Each of the monosaccharide donors (Figure 5.8) donates its sugar to the dolichol by means of its transferase. These transferases are highly specific for nucleotide and sugar structure and are tightly bound to the “rough” and smooth endoplasmic reticulum. Retinol can substitute for dolichol.<sup>(47a,b)</sup> Thus, the glycosides are formed in a hydrophobic environment. This system is used to attach the first carbohydrate core chain to the newly synthesized protein. The system outlined in Figure 5.7 seems to add the side chains. The reaction taking place may be typified as follows, using UDPG (uridine diphosphoglucose) as an example:



A typical buildup of a core oligosaccharide is shown in Figure 5.12. This structure, transferred to the newly formed protein, serves as a base for addition of other sugars and possible sulfation before being released into the blood stream or some other secretion, such as mucus.

It has been known for some time that vitamin A was involved in some way with the formation of connective tissue. For example, a fetus could not retain its implantation in the uterus without vitamin A. The

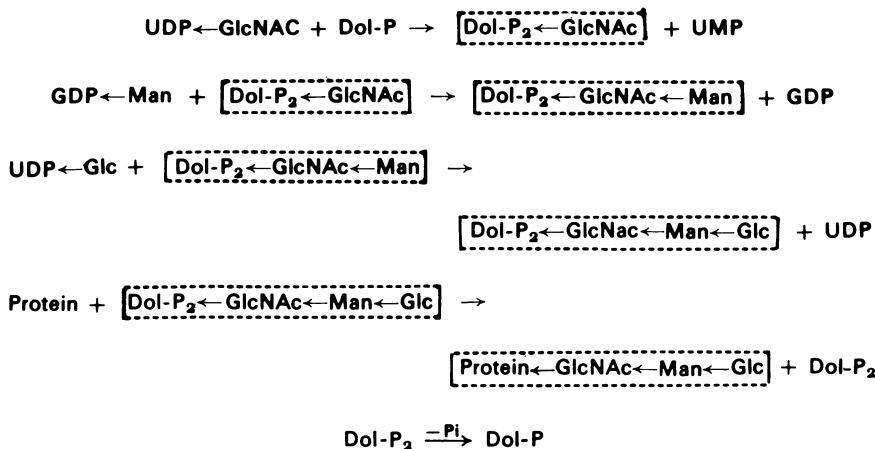


FIGURE 5.12 Representation of the role of dolichol monophosphate in core glycosyl synthesis and transfer to form a glycoprotein. Each of the steps can be repeated; thus the final product may contain repeating units of GlcNAc and especially mannose.

mechanism discussed gives a role to vitamin A in glycoprotein synthesis, and thus the synthesis of ground substance and connective tissue. Its relationship to dolichol is not clear at present. Retinoic acid (oxidized retinol) relieves most symptoms of vitamin A deficiency. Reduction of retinoic acid to retinol has not been demonstrated in animal tissue. These problems need clarification.

Dolichol is synthesized from farnesyl pyrophosphate (Figure 5.13 and Appendix). Thus deficiencies in mevalonate synthesis or any

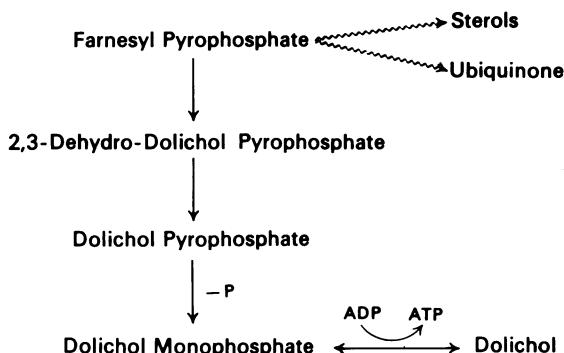


FIGURE 5.13 Synthesis of dolichol monophosphate in human tissue.

of the intermediate steps leading to dolichol synthesis may account for symptoms noted in certain diseases.

## 5.5 GLYCOPROTEIN HETEROGLYCOSACCHARIDE STRUCTURE

The mechanism of heterosaccharide synthesis proposes that an oligosaccharide is built up on dolichol, which is then transferred to the newly synthesized protein. This is then added to as indicated in Figures 5.6 and 5.7. For this reason, it would be expected that the core structure of the oligosaccharides of the glycoproteins would resemble each other, and this is actually the case. A structure proposed for an oligosaccharide unit attached to dolichol is shown in Figure 5.14.<sup>(45,47)</sup>

One of the first glycoprotein oligosaccharide sequences determined was that for transferrin. Transferrin has two oligosaccharides which are identical. Their approximate structure is shown in Figure 5.15. In spite of extensive work on this problem, unresolved discrepancies still exist as to the structural detail.<sup>(48-50)</sup>

Another group of plasma proteins, where substantial progress has been made, is in the oligosaccharide structure of the immunoglobulins. This problem is complicated by the fact that all of the oligosaccharide chains are not identical. For example, IgE has four oligosaccharides per heavy chain, and IgM has five per heavy chain.<sup>(51-53)</sup>

An oligosaccharide, common to the immunoglobulins IgG, IgA, and IgE, is shown in Figure 5.16. This resembles that for transferrin, except for the attachment of the fucose residue. In these immunoglobulins, a high-mannose-containing oligosaccharide is also found.

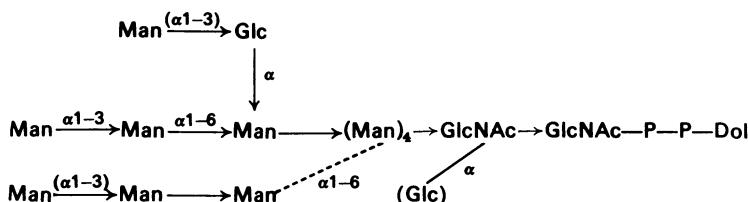


FIGURE 5.14 Provisional structure proposed for a dolichol-P-P-oligosaccharide.

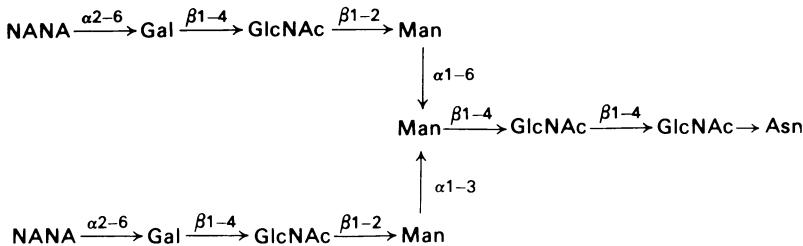


FIGURE 5.15 Structure of the heterosaccharides of transferrin. The transferrin molecule has two such identical structures.

Two of these structures are shown in Figure 5.16. A disaccharide isolated from human A erythrocytes is also shown in this figure.

These high-mannose-containing oligosaccharides resemble the single oligosaccharide attached to hen ovalbumin (Figure 5.17).<sup>(54)</sup>

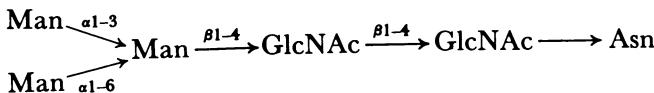
Human albumin does not contain a carbohydrate residue. One similar to that of the hen albumin oligosaccharide has been found in thyroglobulin.<sup>(55)</sup> Thyroglobulin also contains a unique oligosaccharide comprising a repeating glucuronate-galactosamine disaccharide attached to the peptide chain through a galactosyl xylosyl-serine linkage.<sup>(56)</sup> This structure also occurs in the proteoglycans (see Figure 5.42).

A glycopeptide has been isolated from human urine comprising glucose, fucose, and threonine. This is unusual in the fucose-threonine linkage, as follows<sup>(57)</sup>:



This is probably a fragment of an unidentified glycoprotein and suggests a normal type of linkage to the polypeptide chain.

In almost all of the glycoproteins found in substantial quantities in the plasma where attachment to asparagine occurs it is now apparent that an oligosaccharide core structure seems to be present. This may be represented by



Another example of such a structural type is that found in  $\alpha$  protease inhibitor (antitrypsin). This is seen in Figure 5.18. This

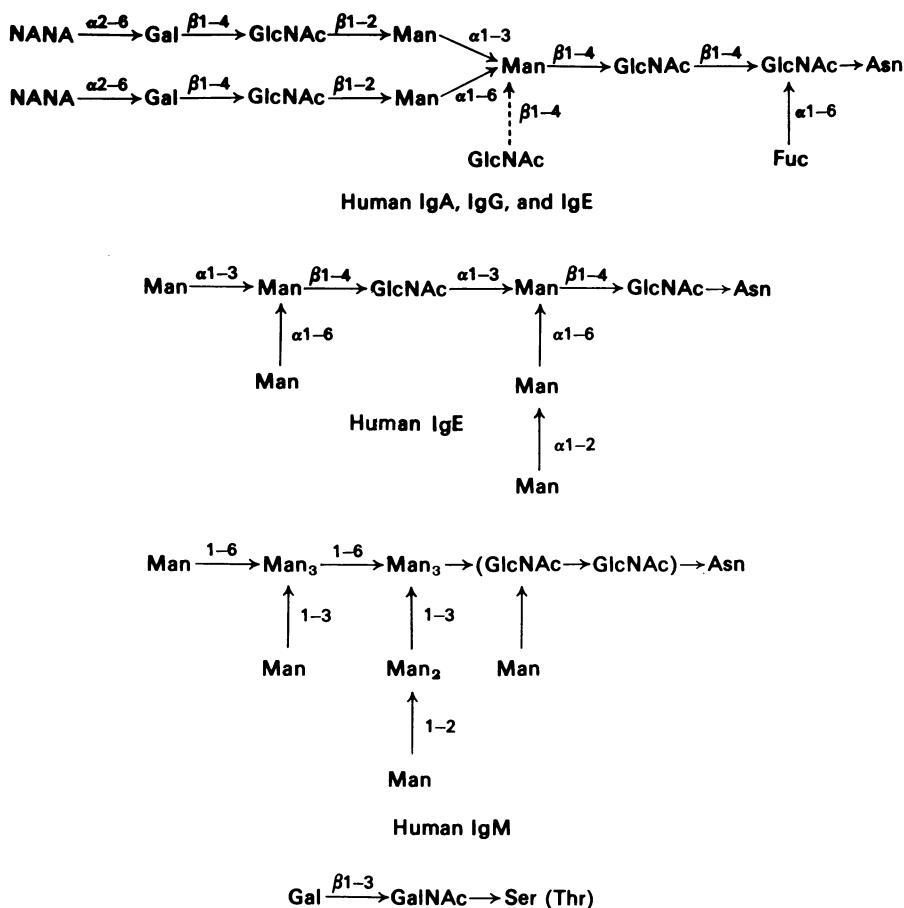


FIGURE 5.16 Some of the oligosaccharides of the immunoglobulins. The structure with the broken line attached to GlcNAc is found in IgE.

molecule has four oligosaccharides, two of which have a trisaccharide (NANA-Gal-GlcNAc) attached and two without.<sup>(58)</sup>

### 5.5.1 Heterosaccharide Structure of the Blood Group Glycoproteins

The major carrier of the oligosaccharides on the erythrocyte responsible for the immunological characteristics of blood groups is glycophorin (Volume 2, pp. 37–39, 51–55). This molecule is 60%

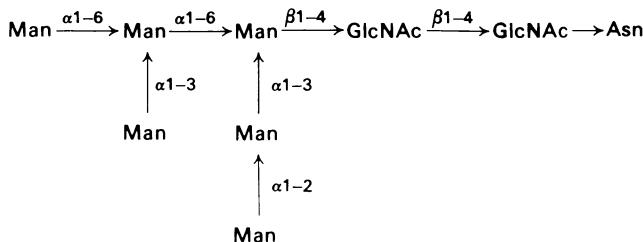
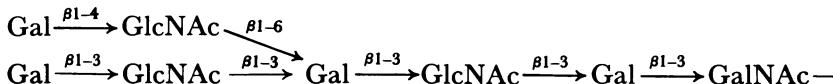


FIGURE 5.17 The glycopeptide of hen ovalbumin. Ovalbumin (mol. wt. 45,000) has only one oligosaccharide per molecule.

carbohydrate, of which sialic acid (NANA) represents more than 40%. The polypeptide chain traverses the cell wall. The carbohydrate-containing portion extends outside the cell (see Figure 5.19).<sup>(59)</sup> Variations in the structures of the oligosaccharides of glycophorin are responsible for the determination of some of the blood groups.

Glycoproteins having the blood group specificities are present in the secretions of the body such as saliva gastric juice and ovarian cyst fluid. These are attached to the protein at the hydroxyl group of a serine or threonine residue in the polypeptide chain. The blood group specificity is determined by the nature and linkage of the monosaccharides at the nonreducing end of the heterosaccharide matrix. The core, not involved in determining specificity, seems to be the same in all blood groups. It probably has the following structure:



To this oligosaccharide are attached fucose, galactose, and *N*-acetyl-galactosamine, to form the determinants of the ABH and Lewis specificities as shown in Figure 5.20.<sup>(60-66)</sup>

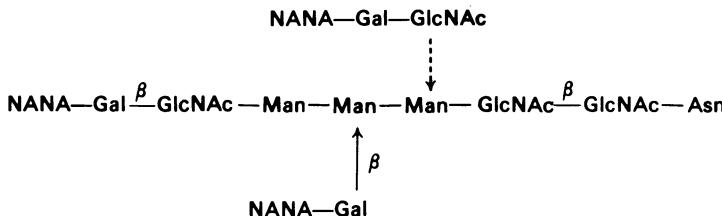


FIGURE 5.18 Oligosaccharides found in  $\alpha$ -protease inhibitor (antitrypsin). There are four oligosaccharides per molecule, two with the NANA  $\rightarrow$  Gal  $\rightarrow$  GlcNAc, and two without.

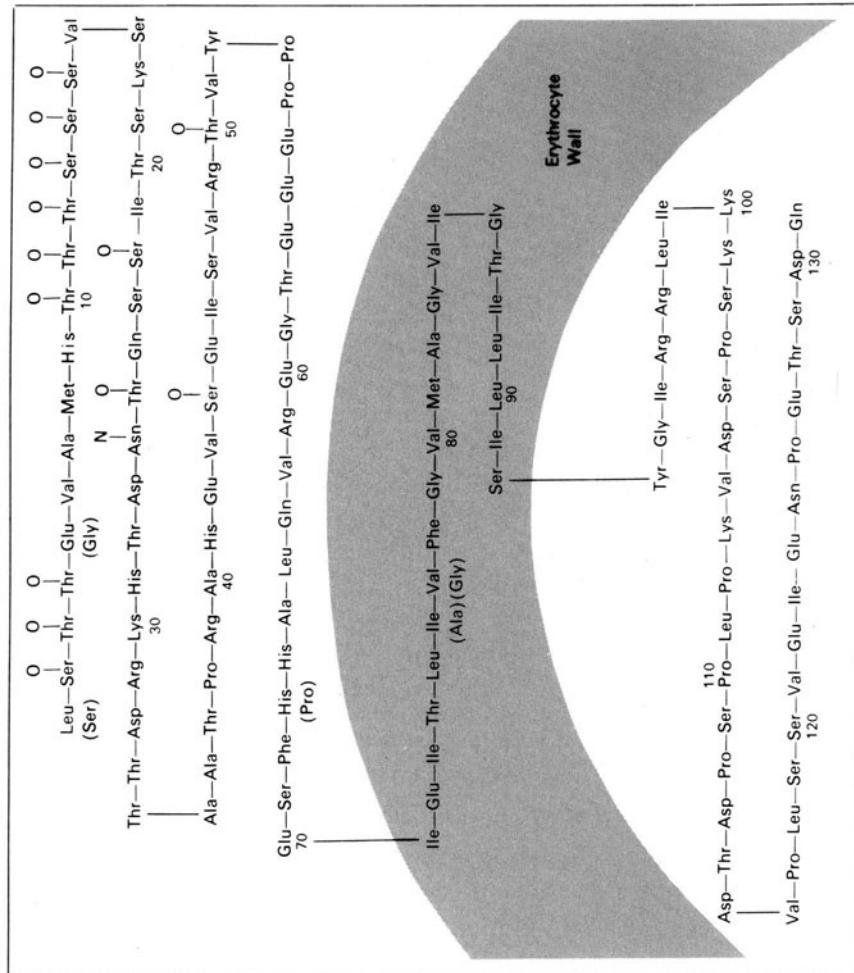


FIGURE 5.19 The arrangement of glycoporphin on the erythrocyte wall. See Volume 1, p. 39. The symbol “O” represents the ether linkage of the oligosaccharide to serine or threonine, and the “N” represents the linkage to asparagine.

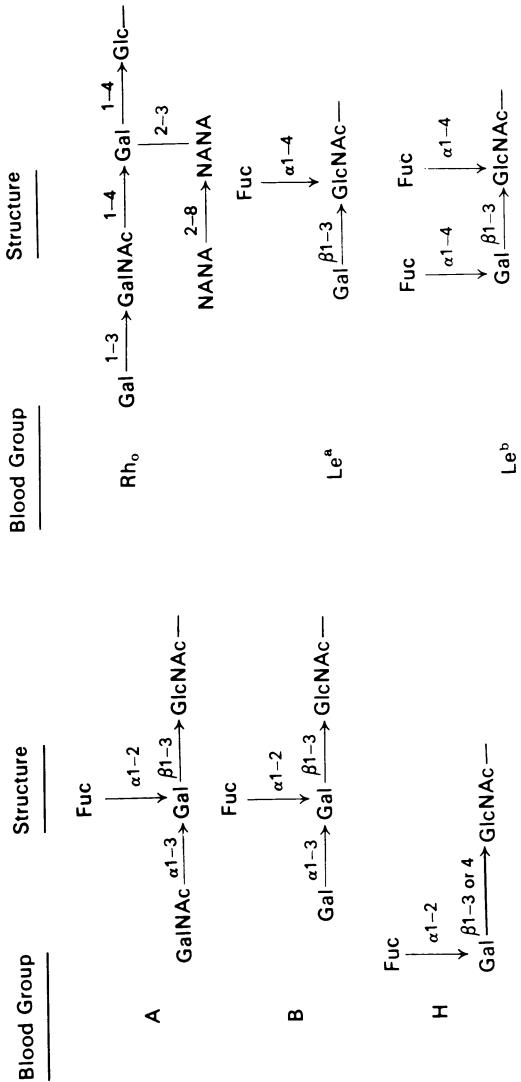


FIGURE 5.20 Structures determining A, B, H, Rh<sub>o</sub>, and Lewis specificities of the glycoproteins.

The Lewis blood group system is primarily one of saliva and serum antigens rather than red cell antigens.<sup>(67)</sup> The red cells acquire their Lewis phenotype simply by adsorbing Lewis substances from the serum.<sup>(68)</sup> Since some individuals are nonsecretors (about 20%) they will not possess these antigens unless obtained by transfusion from some donor who is a secretor. Although rare, severe hemolysis during transfusion has been reported with Lewis incompatibility between donor and recipient.

The immunodominant sugar for each specificity is fucose for H, GalNAc for A, Gal for B, Fuc for Le<sup>a</sup>, and two fucoses for Le<sup>b</sup>. The ability to form the structures shown in Figure 5.20 is controlled by the action of genes at four independent loci, ABO, Lele, Hh, and Sese. *These genes are responsible for the synthesis of the specific transferases for the formation of the structures shown in the figure.* Thus the *A* gene produces a GalNAc-transferase, the *B* gene a Gal-transferase and the *H* and *Le* genes produce two different Fuc transferases. These enzymes have been identified in the milk, submaxillary glands, and gastric mucosa of mothers carrying the particular blood type.

The reactions mediated by the specific transferases during the formation of the erythrocyte are shown in Figure 5.21. Thus, the

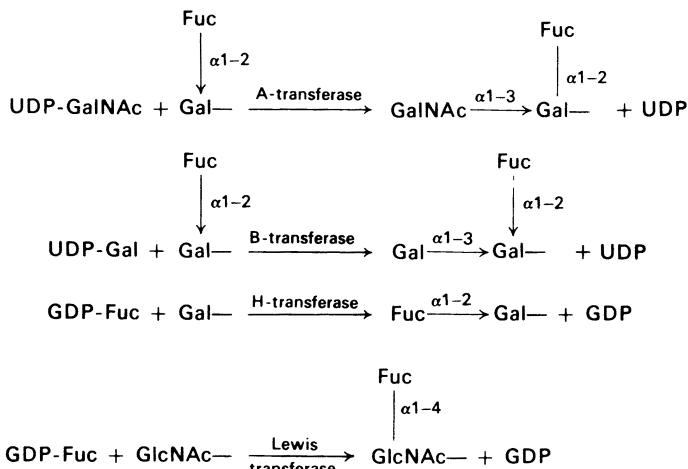


FIGURE 5.21 The mechanisms for the synthesis of the various blood group structures.

*inheritance of blood groups, is the inheritance of the ability to synthesize certain glycosyl transferases and the relative activity of these transferases.* The study of the structure of the numerous blood groups has developed into a study of the heterosaccharide structures and the enzymes responsible for their synthesis.

By the action of a suitable specific carbohydrate, types A and B can be converted to type H.<sup>(62)</sup> If erythrocyte type A is acted upon by an  $\alpha$ -N-acetylgalactose-aminidase, and type B by an  $\alpha$ -galactosidase, then both types of erythrocytes can be converted to O(H) by the use of suitable enzymes. This suggests the practicability of creating a large central erythrocyte bank of all type O blood, obtained naturally or by the action of enzymes on types A and B. Alternatively, by the use of a suitable transferase, a sugar can be added to an H antigen to make the A or B antigen.<sup>(64)</sup>

It is of interest that patients with type A blood lack the specific  $\alpha$ -hexosaminidase and patients with B blood lack the  $\alpha$  galactosidase. *Thus, patients with types A and B blood may be considered as patients with genetic deficiencies.*

A similar situation, as with the ABH system, exists for the MN system. For example, M specificity is readily transformed to N by incubation at pH 2 and 56°C.<sup>(65,66)</sup> This treatment removes one sialic acid, exposing the N-active terminal structure. This has another terminal sialic residue resistant to hydrolysis (Figure 5.22).

A composite structure indicating the location of the specific blood group antigens on the oligosaccharide is shown in Figure 5.23.<sup>(62)</sup> It can be readily seen from the figure, that it is possible to have one GalNAc on one leg of the structure and Gal on the other to make the AB blood type of the erythrocyte.

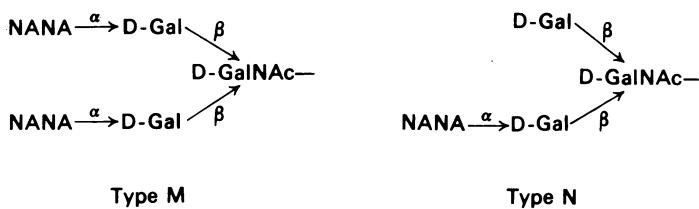


FIGURE 5.22 Proposed terminal immunoreactive structure for the MN system. Removal of one sialic residue yields the N terminal group.  $\beta$ -Galactosidase inactivates both types.

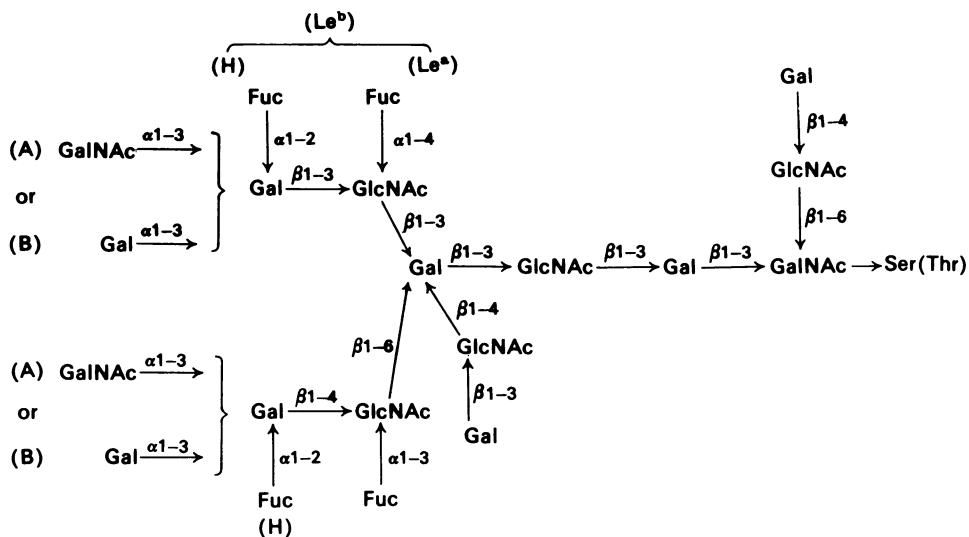


FIGURE 5.23 Composite structure of the blood-group-specific antigens on the erythrocyte.

### 5.5.2 Mucin Heterosaccharides

The mucins function as lubricants and thickening agents. In this group of glycoproteins the oligosaccharide chains are linked mainly to the hydroxyl group of serine and/or threonine of the peptide chain. The simplest structure found in most submaxillary mucins is one in which sialic acid is attached to the hydroxyl radical at C-6 of Gal-NAc. Typical oligosaccharides structures found in submaxillary mucin are shown in Figure 5.24.<sup>(69-72)</sup>

Four major groups of glycoproteins are identified by histochemical means: neutral, sialylated glycoproteins sensitive to neuraminidase, sialylated glycoproteins resistant to hydrolysis, acid glycoproteins (sulfated), and proteoglycans. These can all be located histochemically in the secretory granular cells.<sup>(73)</sup> In inflammation, the secretory cells of the airway increase in number, swell the tissue, and may block the airway. In chronic bronchitis, a higher percentage of sulfated glycoprotein is found and there is an increase in the neuraminidase-resistant glycoprotein fraction.

The blood group oligosaccharide structures are found in the mucus of the individual provided he is a secretor. For unexplained reasons,

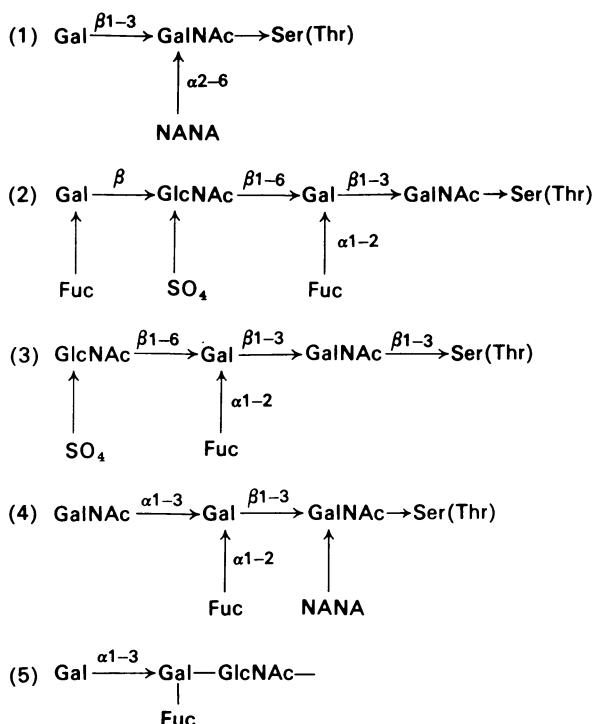


FIGURE 5.24 Some oligosaccharides found in submaxillary mucin. Number 4 has blood group A immunoactivity and 5 has B immunoactivity.

some humans do not secrete their blood type oligosaccharide structures and are referred to as *nonsecretors*. It is from these structures and from oligosaccharides of human milk that much of the structure of blood group oligosaccharides has been determined.<sup>(74,75)</sup>

### 5.5.3 Collagen Heterosaccharides

Substantial amounts of the heterosaccharides of collagen and the basement membranes are attached to the protein at the hydroxyl group of hydroxy lysine (Hyl). An oligosaccharide core common to these glycoproteins is  $\text{Glc} \xrightarrow{\beta 1-2} \text{Gal} \rightarrow \text{Hyl}$  (see Figure 5.25).<sup>(76)</sup> A characteristic sequence occurs around the point of attachment to the polypeptide. This is —Gly—X—Hyl—Gly—Y—Arg—, where X is alanine, serine, leucine, or phenylalanine and Y is glutamate,

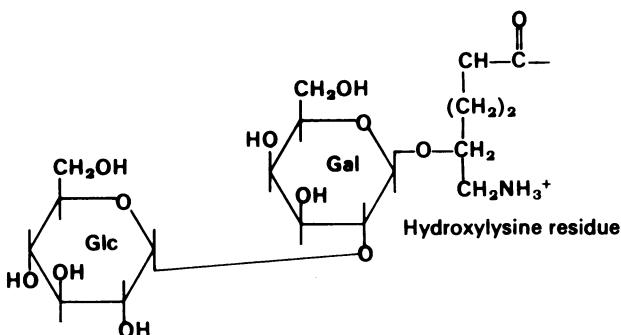


FIGURE 5.25 Core structures of the oligosaccharides of collagen and basement membranes.

histidine, or isoleucine.<sup>(77)</sup> Associated with collagen are the *proteoglycans*. These carry the acidic glycosaminoglycans as their carbohydrate moiety. They are discussed in Section 5.11.

Certain microorganisms carry the sequence, Glc  $\xrightarrow{\beta 1-2}$  Gal  $\xrightarrow{\beta}$ , in their carbohydrate capsule. This is also a terminal sequence in the heterosaccharide of the basement membrane. High titer antibodies, produced in response to infection with these organisms, are antibodies against the glomerulus basement membrane. This plays a significant role in the pathogenesis of some forms of glomerulonephritis.<sup>(78)</sup> *These are autoimmune diseases, where circulating antibodies to the basement membrane serve to destroy it.*

## 5.6 FUNCTION OF THE OLIGOSACCHARIDES OF GLYCOPROTEINS

Most proteins have some oligosaccharide attached to the polypeptide chain. This includes most of the plasma proteins, enzymes, structural proteins, membrane proteins, hormones, antibodies, and others. A notable exception is human albumin. However, there are certain characteristics unique to certain glycoproteins, where the functional property is in the heterosaccharide structure. In these cases, the protein may be considered the carrier of a group of heterosaccharides. This is the case in the proteoglycans.

For the structural proteins, such as collagen and the basement membranes, *the function is that of a base for the construction of a form*. In this

regard, cellulose (as in wood) may be considered a typical example. The basement and other membranes and ground substances may be compared to cellophane. The use of sulfate for crosslinking and binding provides for a rigid structure, as in collagen, or a flexible container, such as the aorta or skin.

A second function is the property of the oligosaccharides *to increase viscosity or as "thickening" agents.*<sup>(74,75)</sup> Commercial methylcellulose serves such a purpose. In the human, the mucus is used as a lubricant and to retain moisture in the airways in the face of exchange of large volumes of air. This property is impaired in cystic fibrosis. In the plasma, the oligosaccharides maintain blood viscosity so as to retain the cells in suspension. In addition, the sialic acids are the major source of the charge on the cells.<sup>(79)</sup> This prevents pooling of the cells and the plugging of the fine capillaries. The sedimentation rate is a measure of the viscosity of the plasma.

*A major function of the oligosaccharides is to provide a "fingerprint" so that the immunochemical system can distinguish those cells or particles which are foreign and those which are native to the particular human.* If the sialic acid end groups of the plasma proteins are removed by neuraminidase exposing a galactose residue they are phagocytized in the liver.<sup>(79)</sup> The insulin receptor in the fat cells is a glycoprotein. If treated with neuraminidase and then galactosidase, it loses its ability to bind insulin.<sup>(80)</sup>

*A most important set of oligosaccharides are those controlled by the histocompatibility gene complex which determines the carbohydrate configuration on the surface of cells.* Rejection or acceptance of organ transplants depends upon whether the lymphocytes recognize the cells as foreign or native. A structure has been proposed for the nature of the type of heterosaccharide as would be found in cells, such as the leukocytes used for histocompatibility matching of patient and donor for transplant (see Figure 5.26).<sup>(81)</sup>

The implications of these studies are of major importance. *Development of techniques for determining oligosaccharide structure from a tissue sample (e.g., leukocytes) would permit matching of donor to recipient on a rational chemical basis.*

Another function attributed to the oligosaccharide portion of a plasma protein is that of *increasing solubility.* The sialic acid provides a charge distribution to maintain the colloidal state of the protein. In the case of a membrane, the charge on the sialic acid can be used to create a barrier and selectively permit only certain ions to pass the

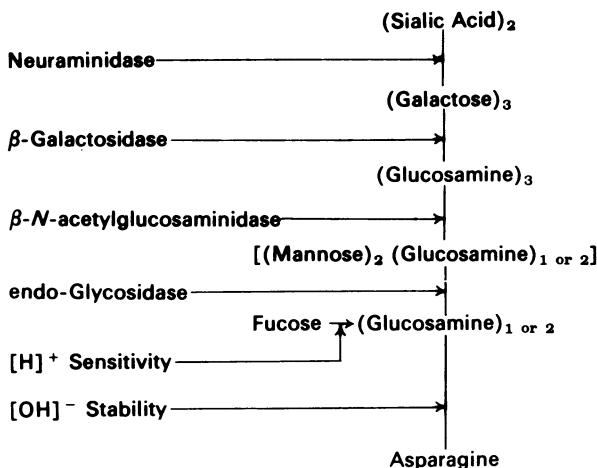


FIGURE 5.26 Proposed model of the structure of the H-2 oligosaccharides of the mouse. On the left are the enzymatic or chemical treatment which has been used in determining the structure.

membrane.<sup>(79)</sup> This is the chemical basis for the *blood brain* barrier which protects the nervous system against toxic substances.

On the other hand, for certain functions, the oligosaccharide portion of the protein seems to be superfluous and serves no definite function. For example, pancreatic ribonuclease exists in two forms, one with carbohydrate and one without.<sup>(82)</sup> Both have the same activity and amino acid sequence. *Homozygotes of the rare En(a)-erythrocyte type lack glycophorin in their erythrocytes.*<sup>(83)</sup> As a result, they develop antibodies to all blood types. The En blood group was discovered in England in 1965 in a patient whose serum agglutinated all erythrocytes tested. This patient had been transfused before, and had developed antibodies to A, B, and H cells. The name En was chosen to indicate the word *envelope* signifying the abnormal cell wall. En(a) was used for the second patient discovered in Finland with this abnormality.<sup>(84)</sup> None of these patients show any abnormalities except when sensitized by blood transfusion.

Although glycosyl transferases are found mainly on the endoplasmic reticulum, they do occur on the outer surface of cells. When this is the case, *the glycosyl transferase will bind its specific sugar located on the outer wall of a second cell or structure, thus binding to the second cell. Transfer of the sugar can initiate a biologically significant reaction.* It is this mechanism

which has been proposed for the adhesion of platelets to collagen fibrils, adhesion between gametes, glycosylation of adjacent cells in tissue culture (a signal for stopping cell growth), and other processes.<sup>(85,86)</sup>

A large glycoprotein, probably related to glycophorin, exists on the surface of cells such as fibroblasts and myoblasts. This has been named *large external transformation sensitive glycoprotein*, or simply LETS protein or LETS glycoprotein. *LETS glycoprotein serves to stimulate the cells to adhere to tissue and other cells.* Removal of LETS protein has a mitogenic effect. This results from the action of proteases and mitogens, like cytochalasin B. This has aroused substantial interest because of its significance in the proliferation and metastasis of tumors.<sup>(86a)</sup>

Regardless of the mechanism, polysaccharides on the surface of cells are of major importance in the recognition and interaction between circulating cells, such as between lymphocytes and the macrophage.

## 5.7 ACUTE-PHASE REACTANTS

The term *acute-phase reactants* (AP-reactants) refers to protein components of the plasma whose concentration is increased significantly in the acute phase of inflammatory processes.<sup>(87-89)</sup> These proteins are all glycoproteins whose carbohydrate moiety is in relatively high concentration and they are synthesized in the liver parenchymal cells. They are thus *trauma-inducible liver-produced plasma glycoproteins*. Table 5.4 lists the proteins commonly considered as the AP-reactants. The immunoglobulins which also contain carbohydrate are normally not considered acute phase reactants.

In addition to the proteins listed in Table 5.4, there are a substantial number of glycoproteins which satisfy the definition of an acute-phase reactant but are present only in small amounts in normal serum. From Table 5.4 it can be seen that a group of proteins is listed with diverse functions and with little relationship to each other. The reason for this is the fact that these proteins were found in high concentration with inflammatory disease, and it was assumed that they were part of this process.

With recent developments, it is possible to explain the reason

TABLE 5.4  
*Typical Acute-Phase Proteins*

Protein	Concentration in normal plasma, mg/100 ml	Carbohydrates, %	Molecular weight	Isoelectric point, pI
$\alpha_1$ Acid glycoprotein	75–100	41.4	44,000	2.7
$\alpha_1$ Antiprotease (antitrypsin)	210–287	12.4	45,000	~3
Ceruloplasmin	27–63	8.0	160,000	4.4
C-reactive protein	Trace	—	138,000	$\beta$ mobility
Fibrinogen	200–600	2.5	341,000	5.8
Haptoglobin (1-1)	30–190	19.3	85,000	4.1
$\alpha_2$ Macroglobulin (plasmin and trypsin inhibitor)	110–310	8.0	725,000	5.4

for the increase of the concentration of these proteins. For example, in severe infection there is substantial hemolysis and a need for increased haptoglobin synthesis to dispose of the released hemoglobin (Section 9.11). For the same reason, ceruloplasmin concentration is increased in inflammatory processes (Section 8.7). Ceruloplasmin is also a glycoprotein containing 8% carbohydrate, the structure of whose oligosaccharide portion has been explored.<sup>(88)</sup> This would be expected as a defense mechanism against excessive bleeding. The clotting and inflammation reactions are part of the same system in defense against invasion by foreign bodies. The  $\alpha_1$  protease inhibitor ( $\alpha_1$  antitrypsin) and  $\alpha_2$  macroglobulin are also part of the clotting and inflammation mechanism and will be considered in Volume 4 of this series. Serum concentrations of  $\alpha_1$  antiprotease and  $\alpha_2$  macroglobulin are also increased significantly with inflammation. C-reactive protein and  $\alpha_1$  acid glycoprotein will now be discussed.

### 5.7.1 *C-Reactive Protein*

The cell walls of pneumococci are protected by an outer coat of polysaccharide. In pneumonia, antibodies to this polysaccharide are prepared in the human in defense against the invading organism. In

1930 it was noted that "acute phase" serum from a patient who had pneumonia formed a copious precipitate when a solution of an anti-serum to the polysaccharide, prepared from pneumococci, was added.<sup>(90)</sup> The polysaccharide preparation is referred to as *C polysaccharide*. For this reason, the component of serum reacting with this polysaccharide, is called *C-reactive protein (CRP)*.

Since the 1930s, CRP has been estimated in the serum of patients with a wide variety of inflammatory diseases, such as tumors, abscesses, peritonitis, acute infectious hepatitis, viral infections, autoimmune diseases, and especially in rheumatic fever and the acute phase of rheumatoid arthritis.<sup>(91-97)</sup>

CRP acts as an activator of the complement system.<sup>(98)</sup> In this regard, it resembles the immunoglobulins. CRP also affects the response of the platelets to inflammation.<sup>(99)</sup> It would therefore be expected that CRP is a member of the family of immunoglobulins. At this writing, no substantial evidence has been adduced that this is the case. The reason for this is the extremely low concentration normally present in serum. It is only recently that CRP has been detected in normal serum,<sup>(100)</sup> even though sensitive immunochemical techniques have been available for several decades. Electrophoretically, it travels with the  $\beta$  globulins and has a molecular weight of about 138,000.

When the erythrocyte sedimentation rate (ESR) is increased, it is recommended that the CRP test be done, particularly in exploring cases of rheumatoid arthritis.<sup>(101)</sup> In cardiovascular disease, elevated CRP levels are often found in myocardial infarction but not in angina pectoris or arteriosclerotic heart disease.

The test for CRP is easy to perform. The serum from the patient and the antiserum (Burroughs Wellcome Co., Research Triangle Park, North Carolina) are taken up in a capillary and the height of the precipitate is noted.<sup>(102)</sup>

### 5.7.2 $\alpha_1$ Acid Glycoprotein

In 1948 it was shown that a 7% solution of perchloric acid did not precipitate all of the proteins. A highly soluble group of proteins remained. These could be precipitated by phosphotungstic acid.<sup>(103)</sup> The soluble fraction contained about 10% of the protein-bound carbohydrate of the serum. It was then variously referred to as *seromucoid*,

*orosomucoid*, or *mucoprotein-1 (MP-1)*. Most of this fraction was shown to consist of a single glycoprotein, moving with the  $\alpha_1$  plasma protein fraction. If borate is added to starch gel, this glycoprotein shows the highest electrophoretic mobility since it has a high binding capacity for borate. Because of its high negative charge, even at pH 4.5, it was considered an acid and labeled  $\alpha_1$  acid glycoprotein. It can be prepared in large quantities from commercially available Cohn fraction V supernatant, from the process for preparing human albumin, and globulins.<sup>(104)</sup>

$\alpha_1$  Acid glycoprotein is about 45% carbohydrate. Its molecular weight is 40,000 and it contains 14 residues of sialic acid, 34 residues of neutral hexoses, 31 residues of *N*-acetylglucosamine, and two residues of fucose. The hexoses are galactose and mannose.<sup>(105)</sup>

The sialic acid residues are located terminally and linked to the heterosaccharide groups. Six of them are readily cleaved by neuraminidase. However, the others are buried in the molecule and are somewhat resistant to hydrolysis.<sup>(106,107)</sup> A structure for a typical heterosaccharide attached to the polypeptide chain is shown in Figure 5.27.<sup>(108)</sup> This structure is similar to that proposed for the glycoprotein found in fetal calves blood (fetuin).<sup>(109)</sup> There are five such carbohydrate units attached to the polypeptide chain.<sup>(110)</sup>

The structure of the polypeptide chain of  $\alpha_1$  acid glycoprotein is shown in Figure 5.28. There is substantial heterogeneity in the composition of this protein from different individuals and some of the substitutions are shown in the figure.<sup>(111,112)</sup>

There is considerable homology between the sequences of  $\alpha_1$  acid glycoprotein, haptoglobin, and the immunoglobulins. It is logical to assume that these proteins evolved from a single precursor in the

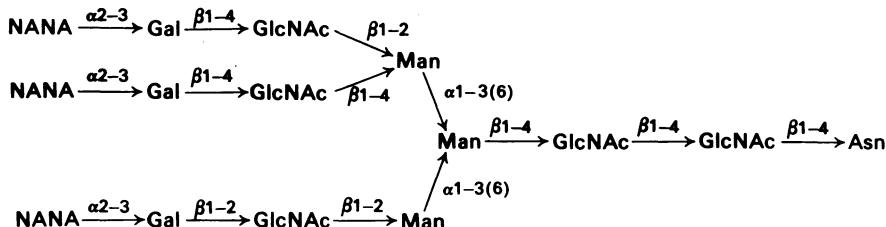


FIGURE 5.27 Proposed structure for a typical heterosaccharide unit of  $\alpha_1$  acid glycoprotein.

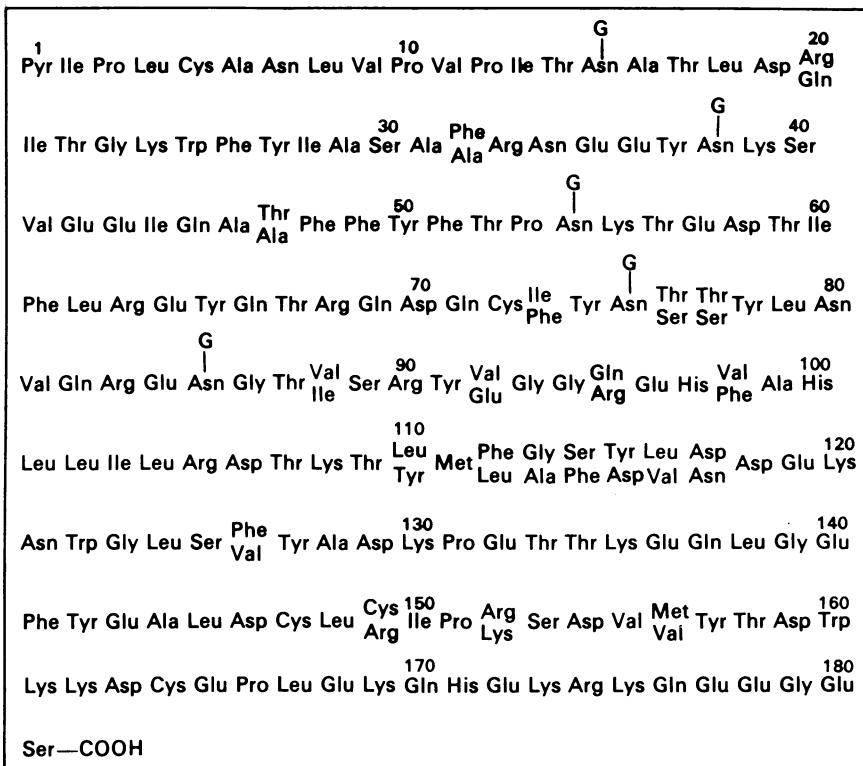


FIGURE 5.28 The amino acid sequence of  $\alpha_1$  acid glycoprotein showing some of the substitutions. Pyr stands for pyrrolidone carboxylic acid which is the anhydride of glutamic acid. G represents carbohydrate.

distant past. However neither haptoglobin nor  $\alpha_1$  acid glycoprotein has any of the properties of the immunoglobulins in neutralizing invading organisms.

#### *Biological Role of $\alpha_1$ Acid Glycoprotein*

Increased concentration of  $\alpha_1$  acid glycoproteins is the major factor in the increased concentration of glycoproteins in serum with inflammation.<sup>(113)</sup> The concentration of this glycoprotein is also increased in various unrelated conditions and diseases, such as during wound healing, pregnancy, rheumatoid arthritis, cancer, pneumonia, and numerous others.<sup>(113-115)</sup> The common denominator in all of these conditions is cell proliferation. *Tissues with a high cell proliferation*

rate release a factor which stimulates  $\alpha_1$  acid glycoprotein synthesis.<sup>(116)</sup> The significance of these observations is still not clear.

Normal human platelets carry substantial amounts of  $\alpha_1$  acid glycoprotein tightly bound to their membrane.<sup>(117)</sup> Platelets adhere to collagen. Dipyridamol inhibits binding of platelets to collagen. This property can be restored if  $\alpha_1$  acid glycoprotein is added to the system.<sup>(118)</sup> Since the clotting and inflammation are part of one overall mechanism, it would be logical for  $\alpha_1$  acid glycoprotein to be secreted for the purpose of increasing platelet adhesion to collagen when faced with an inflammatory reaction.

When  $\alpha_1$  acid glycoprotein is added to a solution of collagen, striated fibers are formed containing about 50%  $\alpha_1$  acid glycoprotein and 50% collagen. Under the microscope, the fibers show regularly spaced dense repeating zones 1200 Å apart. These fibers resemble, in appearance, the more closely spaced fibers (640 Å) seen in bone cartilage sections or produced when chondroitin sulfate is added to soluble collagen. The more widely spaced fibers contain 6 to 8 molecules of  $\alpha_1$  acid glycoprotein for every collagen molecule.<sup>(119)</sup>

The more widely spaced fibers of the type formed from  $\alpha_1$  acid glycoprotein and collagen are seen in normal Descemet's membrane.<sup>(120)</sup> They are also seen in certain tumors of the nervous system.<sup>(121,122)</sup> They are known to contain collagen and a glycoprotein.  $\alpha_1$  Acid glycoprotein is therefore implicated in the formation of certain membranes and fibers in combination with soluble collagen. *The crosslinking of collagen with  $\alpha_1$  acid glycoprotein seems to serve the formation of a mesh for the purpose of preparing a membrane or semirigid structure.*

### 5.7.3 Low-Concentration Acute-Phase Glycoproteins

From time to time, reports have appeared on the recognition of new glycoproteins, present in increased quantities in inflammatory conditions or disease. A number of these, like  $\alpha_1$  fetoglobulin and CEA have become of diagnostic importance. Some of these will be presented here with whatever data are available at present.

#### 5.7.3.1. Pregnancy-Associated Glycoprotein

Although all of the acute-phase reactants are elevated in pregnancy, certain glycoproteins are associated only with pregnancy. They are generally referred to as *pregnancy-associated glycoproteins, or acute-phase*

*proteins of pregnancy.*<sup>(123,124)</sup> There are at least four such proteins. These include the pregnancy-specific  $\beta_1$  glycoprotein,<sup>(123)</sup> pregnancy-associated plasma protein A (PAPP-A), placental lactogen, and chorionic gonadotrophin. Others which have been found to be elevated in pregnancy, such as steroid-binding  $\beta$  globulin (Section 14.8) and pregnancy-associated  $\alpha_2$  glycoprotein ( $\alpha_2$  PAG) are normal components of serum.

*Pregnancy-specific  $\beta_1$ -glycoprotein* was prepared from placenta.<sup>(126)</sup> An antiserum was prepared from this material and various sera tested. Only sera from pregnant women were positive in this test. The plasma protein travels with the  $\beta_1$  globulin fraction on electrophoresis.

*Pregnancy-associated  $\alpha_2$ -glycoprotein* is normally present in all sera at a concentration of about 1–2 mg/100 ml. This protein was noted first in 1959 and has been reported repeatedly as a new protein by numerous investigators. It has been called, Xh protein, Xm factor, pregnancy zone protein,  $\alpha_2$  pregnoglobulin, new  $\alpha_2$  macroglobulin, and others. The term pregnancy-associated  $\alpha_2$  glycoprotein ( $\alpha_2$  PAG) seems to be generally accepted at present. Some still call this protein *pregnancy-associated  $\alpha_2$  macroglobulin*.<sup>(127)</sup>

Human  $\alpha_2$ PAG contains 10–11% carbohydrate and has a molecular weight of about 359,000.<sup>(128)</sup> It is not produced by the fetus since it cannot be detected in cord blood. The serum concentration of this protein rises sharply on estrogen administration. It has no significant binding properties for any of the steroid hormones. However,  $\alpha_2$  PAG is also elevated in cancer, rheumatoid diseases, and other inflammatory conditions.<sup>(125–128)</sup>

Although the concentration of  $\alpha_2$ PAG is about 1–2 mg/100 ml in normal serum, it rises to 50–200 mg/100 ml in normal pregnancies.<sup>(128)</sup> *If  $\alpha_2$ PAG levels do not rise significantly in the 9th to 12th week of the pregnancy, then it is claimed that there is 16 times the chance for spontaneous abortion.*

One function of  $\alpha_2$ PAG seems to be as an immunosuppressive agent, to prevent the rejection of the fetoplacental unit.  $\alpha_2$ PAG suppresses phytohemagglutinin-induced lymphocyte transformation and also the mixed leukocyte reaction.<sup>(129,130)</sup> It is synthesized by human leukocytes.<sup>(129)</sup>

### 5.7.3.2 Zinc- $\alpha_2$ Glycoprotein

A glycoprotein found in normal serum in 1961, at a concentration of 20–200 mg/liter, binds zinc.<sup>(131,132)</sup> It is found in the  $\alpha_2$  region on

electrophoresis, and for this reason is called *Zn- $\alpha_2$ -glycoprotein* (*Zn- $\alpha_2$ -GP*). Its molecular weight is 41,000 and its sedimentation constant is 3.2. Its electrophoretic mobility is 4.2 and the isoelectric point is at pH 3.8.

In the urine, the concentration of *Zn- $\alpha_2$ -glycoprotein* is about 1 mg/liter.<sup>(134)</sup> The mean concentration found in amniotic fluid is about 9.8 mg/liter and is proportional to the protein content of the amniotic fluid.<sup>(133)</sup> *Zn- $\alpha_2$ -GP* is found in almost all the fluids of the body including sweat and saliva. With renal pathology, *Zn- $\alpha_2$ -GP* is lost in the urine because of its relatively low molecular weight.<sup>(132-134)</sup> In newborns, *Zn- $\alpha_2$ -GP* concentration in serum is lower than in adults by a factor of about one-half.

The function of *Zn- $\alpha_2$ -GP* is unknown. Zinc binds also to albumin and this is probably its major means of transport. Zinc is a required coenzyme for carbonic anhydrase and a number of enzymes in normal metabolism.<sup>(135-137)</sup> It is essential for the formation of normal mucin.<sup>(138)</sup> However, no relationship between zinc metabolism and *Zn- $\alpha_2$ -GP* has been established.

#### 5.7.3.3 Seromucoid Proteins

In studies with perchloric acid precipitation of serum proteins it has been pointed out that a substantial amount of glycoprotein remains in solution. These proteins with high carbohydrate content have been referred to as *seromucoids* or *mucoproteins*.  $\alpha_1$  Acid glycoprotein is a major component of this fraction. These proteins are of relatively low molecular weight, and appear in Cohn fraction VI. *Zn- $\alpha_2$*  Glycoprotein is another member of this group. A number of others have also been identified.

Numerous proteins which have been identified, such as members of the clotting and complement systems, enzymes such as cholinesterase and lysozyme have, what might be called, seromucoid or mucoprotein properties since they have low sedimentation rates and high carbohydrate content. These will be considered elsewhere. Here are presented those seromucoids whose function has not been identified at the present writing.

A protein, with a sedimentation coefficient of 2.9, is known as  $\beta_2$  *glycoprotein I*. This protein has been crystallized.<sup>(139)</sup> It has the electrophoretic mobility of  $\alpha_1$  globulin and its molecular weight is about 48,000.<sup>(140)</sup> The protein occurs in serum at a concentration of

15–30 mg/100 ml.<sup>(141)</sup> There are two other proteins associated with  $\beta_2$  glycoprotein I, and these are designated  $\beta_2$  glycoprotein II and III.  $\beta_2$  Glycoprotein II has been identified as C3 activator in the complement system.  $\beta_2$  Glycoprotein III has a molecular weight of about 35,000 and occurs in plasma at a concentration of 5–15 mg/100 ml. No function has been ascribed to  $\beta_2$  glycoproteins I or III.

If serum is diluted 1:2 with a pH 6.0 buffer and shaken with carboxymethylcellulose (CM cellulose), four proteins will be found to adhere. These include C1q of the complement system, lysozyme, and two glycoproteins designated *3.8S- $\alpha_2$  globulin* and *9.5S- $\alpha_1$  globulin*.<sup>(142)</sup> The *9.5S- $\alpha_1$  globulin* crystallizes readily. The designations *3.8S- $\alpha_2$*  and *9.5S- $\alpha_1$*  signify the sedimentation coefficient and electrophoretic mobility of these proteins. Because of their affinity for carboxymethylcellulose, these two proteins have also been designated CM-I and CM-III. C1q is referred to as CM-II, and lysozyme as CM-IV. The function of these proteins is unknown. They bind CM cellulose, heparin, and bivalent ions such as  $\text{Ca}^{2+}$ . They may be of significance in the clotting mechanism.

The *3.8S- $\alpha_2$  globulin* has been found to be rich in histidine, with a molecular weight of about 58,500. The molecule is made up of two identical subunits, held together by ion binding. It is present in serum of adults at  $9.2 \pm 4.5$  mg/100 ml and in newborns at  $3.5 \pm 0.9$  mg/100 ml. The protein binds heparin and can be displaced by protamine. It is referred to as the *histidine-rich glycoprotein*.<sup>(143)</sup>

Another mucoprotein with high carbohydrate content (30%) is *8S- $\alpha_3$  glycoprotein*. This protein contains about 9% of sialic acid. Its sedimentation coefficient is 7.87 and its molecular weight is about 220,000. It is present in serum at a concentration of 3.1–4.5 mg/100 ml.<sup>(144)</sup> It has a high solubility in perchloric acid and trichloroacetic acid because of its high carbohydrate content. The protein can be dissociated to lower-molecular-weight polypeptides by disulfide reducing agents.

Recently, a leucine-rich seromucoid of sedimentation constant 3.1 has been isolated. It has  $\alpha_2$  mobility and a molecular weight of about 49,000. It carries about 23% carbohydrate. It is present in normal serum at a concentration of about 2.1 mg/100 ml. Its leucine content is almost 17%. One out of every five amino acids is leucine. It is designated as *leucine-rich, 3.1S- $\alpha_2$  glycoprotein*.<sup>(145)</sup>

Related to Zn- $\alpha_2$  glycoprotein, a protein which precipitates with  $\text{Zn}^{2+}$ , is a protein which is readily precipitable by  $\text{Ba}^{2+}$ . This is an

$\alpha_2$  protein with a sedimentation rate of 3.3, a molecular weight of about 49,000, and an electrophoretic mobility of 4.2. It is present in normal serum in a concentration of 40–85 mg/100 ml. The function of this protein is unknown. It is usually referred to as  $\alpha_2$  HS-glycoprotein or Ba- $\alpha_2$  glycoprotein.<sup>(146)</sup>

Another seromucoid with a sedimentation rate of 3.3 occurs in the  $\alpha_1$  region on electrophoresis and is distinguished by the fact that it contains very little tryptophan. It is present in serum at a concentration of 5–12 mg/100 ml. It is designated  $\alpha_1$  T-glycoprotein.<sup>(147)</sup>

A mucoprotein with a sedimentation rate of 3.8, which is readily precipitated with protein precipitants, is designated  $\alpha_1$  B-glycoprotein. Its molecular weight is about 50,000 and it is found in the  $\alpha_1$  region on electrophoresis. Its concentration in normal serum is 15–30 mg/100 ml.<sup>(148)</sup>

Two seromucoids present in very low concentration in serum have been used in the diagnosis of certain types of tumors. One is  $\alpha$  fetoglobulin<sup>(149)</sup> and the other is carcinoembryonic antigen (CEA).<sup>(150)</sup>

$\alpha$  Fetoglobulin, which is present in fetal serum, also appears in the serum of patients with hepatomas. For this reason, it is of great interest in the diagnosis of these types of tumors. Its characteristics are listed in Table 5.5.<sup>(149)</sup>

Carcinoembryonic antigen has a molecular weight of 180,000 and is 49.9% carbohydrate. Thus it is a seromucoid. It moves with the  $\alpha$  globulins on electrophoresis and has a sedimentation constant of 7. Sequential measurement of CEA provides an early warning of the recurrence and metastasis of large bowel cancer. The upper limit of normal for CEA is usually taken as 15  $\mu\text{g}/\text{liter}$ . Values above 30  $\mu\text{g}/\text{liter}$  suggest a high probability of an underlying cancer.<sup>(151)</sup>

The properties of the seromucoids present in serum in low concentration are listed in Table 5.5. All of these are most probably of biological significance. This area of knowledge needs to be investigated more thoroughly.

## 5.8 GLYCOPROTEIN CATABOLISM

*In vitro*, glycoproteins can be degraded by hydrolyzing the polypeptide chains with proteases, and then attacking the polysaccharide structure, as indicated in Figure 5.26. However, *in vivo*, the problem is

TABLE 5.5  
*Seromucoids Present in Small Amounts in Serum and of Unknown Function*

Designation <sup>a</sup>	Alternative designation	Molecular weight	Sedimentation rate	Electrophoretic mobility	Concentration, mg/liter	Carbohydrate, %
$\alpha_1$ B-GP	Easily precipitable	50,000	3.8	$\alpha_1$	15-30	13.3
$\alpha_1$ T-GP	Tryptophan poor	60,000	3.3	$\alpha_1$	5-12	13.7
$\alpha_1$ F	Fetoglobulin	64,000	4.5	$\alpha_1$	<0.01	7.4
9.5S $\alpha_1$	$\alpha_1$ Macroglobulin	308,000	9.5	$\alpha_1$	3-8	—
C.E.A.	Carcinoembryonic antigen	180,000	7.0	$\alpha_1$	<0.015	49.9
3.1S $\alpha_2$	Leucine rich	49,600	3.1	$\alpha_2$	~2.1	23
3.8S $\alpha_2$	Histidine rich	58,500	3.8	$\alpha_2$	5-15	14
Zn- $\alpha_2$	Zn- $\alpha_2$ GP	41,000	3.2	$\alpha_2$	2-15	18.2
$\alpha_2$ HS-GP	Ba- $\alpha_2$ GP	49,000	3.3	$\alpha_2$	40-85	13.4
$\alpha_2$ PAG	Pregnancy-associated macroglobulin	359,000	—	$\alpha_2$	1-2	12
8S $\alpha_3$	8-S- $\alpha_3$ GP	220,000	7.87	$\alpha_3$	3.1-4.5	30
PS $\beta_1$ -GP	Pregnancy-specific GP	120,000	4.6	$\beta_1$	—	—
$\beta_2$ I	$\beta_2$ Mucoid	48,000	2.9	$\gamma$ (1.6)	15-30	17.1
$\beta_2$ III	$\beta_3$ Mucoid	35,000	—	—	5-15	—

<sup>a</sup> GP stands for glycoprotein.

more complicated. Some sialic acid and fucose residues usually surround the glycoprotein with a protective coat which resists the action of proteolytic enzymes. These need to be removed before catabolism can proceed. Others are removed subsequently.

An *endo- $\beta$ -N-glycosaminidase* then cleaves the polysaccharide from the polypeptide between the two *N*-acetylglucosamines (GlcNAc) which are attached to the asparagine chain. This yields a polysaccharide and a disaccharide (Fuc—GlcNAc—) still attached to the polypeptide. This is shown in Figure 5.29.<sup>(152)</sup> This enzyme is an “endo-” enzyme since it attacks the molecule from *within* the chain.

If fucose is attached to the *N*-acetylglucosamine, as it is in some glycoproteins, the peptide is then cleaved by a fucosidase. The GlcNAc is finally cleaved from the polypeptide by an *N-aspartylglucosaminidase*.

From Figure 5.29, it can be seen that hydrolysis proceeds sequentially removing each monosaccharide by an enzyme specific for that linkage. The sugars are then reutilized for glycoprotein synthesis or metabolized to yield energy for metabolism. Intermediates of varying carbohydrate composition also appear normally in the urine.

From Figure 5.29 it can be seen that, other than the proteolytic enzymes, at least seven enzymes are involved in the process of dismantling the heterosaccharides. These enzymes are located in the lysosomes. However, they can be released within and outside the cell for appropriate use. For example, when culturing fibroblasts from patients, these enzymes are released from the cells into the culture medium.

Any one of these enzymes can be deficient in a particular patient, giving rise to a variety of genetic disorders. The sphingolipidoses with carbohydrase deficiencies have been discussed in Volume 2, pp. 75–109. In addition, deficiency of mannosidase and fucosidase have been referred to in Volume 2, pp. 51–52. See also pp. 119 and 121 of this volume.

### 5.8.1 *Abnormal Glycoprotein Catabolism*

Deficiencies of almost every enzyme listed in Figure 5.29 have been reported in humans. The nature of the disease is usually discovered by finding substantial amounts of heterosaccharides in the urine. The composition of the heterosaccharide usually indicates which enzyme system is defective. From these data and direct enzymic analysis of

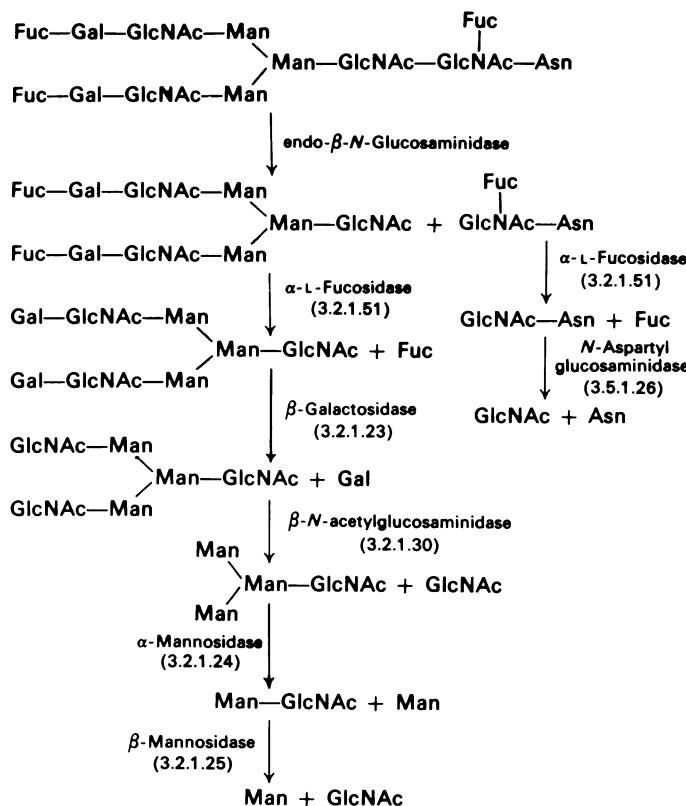


FIGURE 5.29 Steps in the catabolism of a typical heterosaccharide structure. The number in parentheses under each enzyme is its E.C. designation.

biopsy tissue the diagnosis can be made. In some cases, fibroblast culture permits the determination of the nature of the disease. This can be ascertained by adding the enzyme to the culture medium. If a specific abnormality exists, this will correct the deficiency.<sup>(152,153)</sup>

### 5.8.2 Mannosidosis

A patient was studied in 1967 with symptoms which resembled, to some extent, those of Hurler's syndrome. These symptoms included psychomotor retardation, slight gargoylelike facies, vacuolized lymphocytes, and recurrent infections.<sup>(154)</sup> This condition was identified as an  $\alpha$  mannosidase deficiency. In this condition, tissue  $\alpha$  mannosidase is reduced substantially. The disease, now called *mannosidosis*, had also been

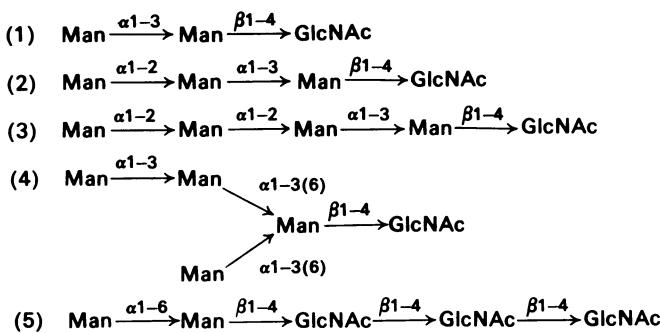


FIGURE 5.30 Polysaccharides found in human (Nos. 1-4) and bovine (No. 5) urine with mannosidosis.

observed in Angus cattle since at least the 1950s, but its etiology was not ascertained until 1972.<sup>(155)</sup> Polysaccharides found in human brain and other tissue and in urine from patients with mannosidosis are shown in Figure 5.30.<sup>(156)</sup> A sequence of three *N*-acetylglucosamines in succession, as observed in cattle, has not been identified in human glycoproteins.

Three different mannosidases have been isolated from human liver; A and B have their pH optima at acid pH (about 4.5) and C has its optimum pH at about 7.0. Forms A and B differ only in sialic acid content.<sup>(157)</sup> These enzymes are  $\alpha$  mannosidases and do not hydrolyze the inner mannose linkages which are  $\beta$  linkages (Figure 5.30). *The deficiency in humans and cattle is an  $\alpha$  mannosidase deficiency of all three mannosidases.*

The diagnosis of mannosidosis can be made by assay for the enzyme from serum, leukocytes, or biopsy material or by determination of the trisaccharide,  $\text{Man} \xrightarrow{\alpha} \text{Man} \xrightarrow{\beta} \text{GlcNAc}$ , in the urine. For this purpose the trisaccharide is isolated by gel filtration on a column. The aldehyde group is reduced with sodium borohydride and the resultant alditol is methylated. The methylated trisaccharide can be determined by gas chromatography.<sup>(158)</sup> Normally, this trisaccharide is not detected in urine. With mannosidosis values of 200–800 mg/liter are found.

The enzymatic method is most practical for routine use. The reaction can be carried out with leucocytes or serum and heterozygotes can be detected by determining their ratio to *N*-acetyl- $\beta$ -glucosaminidase normally present.<sup>(159)</sup>

### 5.8.3 Fucosidosis

Fucosidosis, as a deficiency of  $\alpha$ -L-fucosidase, was first discovered in 1966.<sup>(160-162)</sup> The clinical manifestations include progressive motor and mental deterioration, coarseness of facial features, cardiomegaly, hepatomegaly, and skeletal abnormalities including short stature. The tissues show vacuolated cytoplasm of epithelial and stromal cells suggestive of a lysosomal storage disease. The homozygote is mentally retarded and may develop grand mal seizures. The course is progressive so that by about 10 years of age the patients require complete nursing care being severely mentally retarded. The disease is transmitted as an autosomal recessive.

On electrophoresis in gels, leucocyte  $\alpha$ -L-fucosidase shows an isoenzyme pattern resembling that for lactate dehydrogenase, with five or six major bands.<sup>(163)</sup> Fractionation on DEAE cellulose of kidney fucosidase yields two enzymes, one with an optimum pH at 6.5 and the other at pH 4.4. The pH 6.5 enzyme has a high molecular weight and is heat labile, whereas the enzyme with pH maximum at 4.4 is heat stable. When serum is incubated at 70°C for 20 min, normal serum retains 65% of its  $\alpha$  fucosidase activity. No measurable activity is observed in the heat-treated serum of patients with fucosidosis.<sup>(164,165)</sup> Thus the deficiency is a deficiency in a specific enzyme.

With fucosidosis, fucose-containing polysaccharides are found

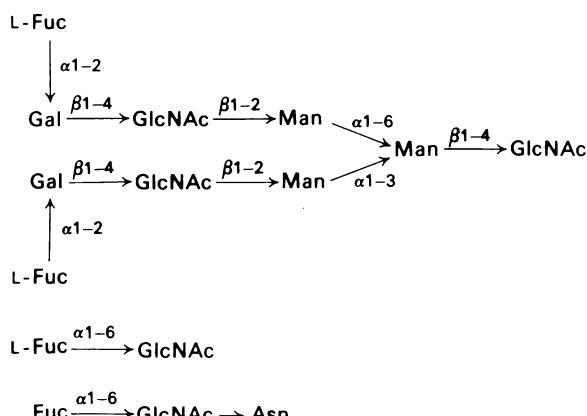
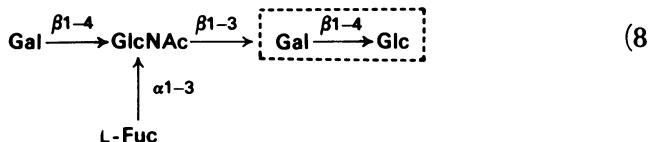
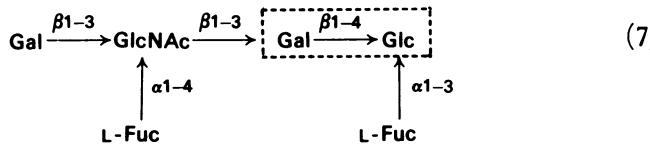
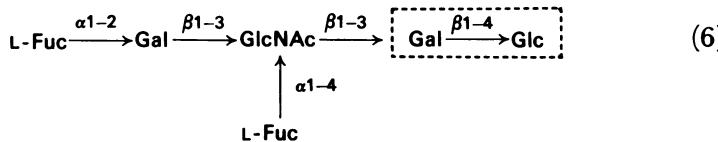
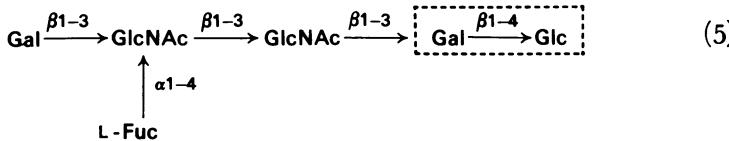
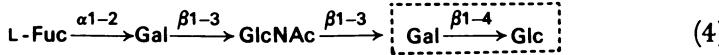
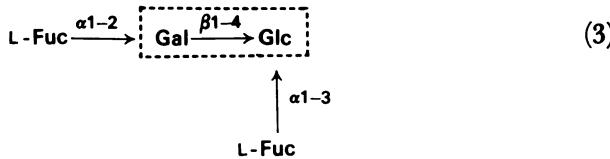


FIGURE 5.31 Polysaccharide fragments found in urine and tissues of patients with fucosidosis.



**FIGURE 5.32** Some heterosaccharides containing fucose which are found in human milk, probably as lactose precursors. The fucoses are all of the L type, with the  $\alpha$  linkage. The lactose portion of each molecule is enclosed in the dotted lines.

deposited in various tissues in the body especially in the lysosomes. This accounts for the cardiomegaly and hepatomegaly.

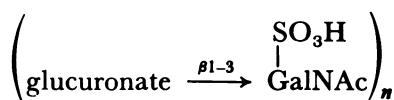
The disaccharide fragment, L-Fuc  $\xrightarrow{\alpha 1-6}$  GlcNAc, is found in brain and urine, along with other polysaccharide fragments containing fucose,<sup>(160)</sup> see Figure 5.31.

The disease is variable, some of the patients living to adulthood with less severe symptoms. The reason for this is partially the variability of the polysaccharide chains. For example, a patient secreting Le<sup>b</sup>-type blood group antigen will secrete more fucose-containing polysaccharides than one with the Le<sup>a</sup> antigen (Figure 5.23). On the other hand the nonsecretor is at an advantage with this disease, since he does not release these fucose-containing heterosaccharides into the body fluids and secretions. The diet is important. Milk contains substantial amounts of fucose-containing polysaccharides. Other foods contain less. These polysaccharides cannot be metabolized by the infant with mannosidosis and some deposit in the lysosomes (Figure 5.32).

From Figure 5.32 it can be seen that all these polysaccharides contain lactose (Gal  $\xrightarrow{\beta 1-4}$  Glc). *This seems to indicate that lactose, a predominant component of human milk (5%) derives from a higher polysaccharide containing fucose.*

## 5.9 GLYCOSAMINOGLYCANs: THE MUCOPOLYSACCHARIDES

The *mucopolysaccharides* (*glycosaminoglycans*) comprise a family of polymeric disaccharides with *N*-acetylchondrosine as a repeating unit and about one sulfate group per repeating disaccharide. *Chondrosine* is a disaccharide made up of a uronic acid, such as glucuronic or iduronic, linked to *N*-acetylgalactosamine. The chondroitin sulfates (see Figure 5.33) have the general formula



When glucuronic acid (GlcUA) is replaced by its epimer at the 5' position, it becomes iduronic acid (IdUA). In this case the polymer is

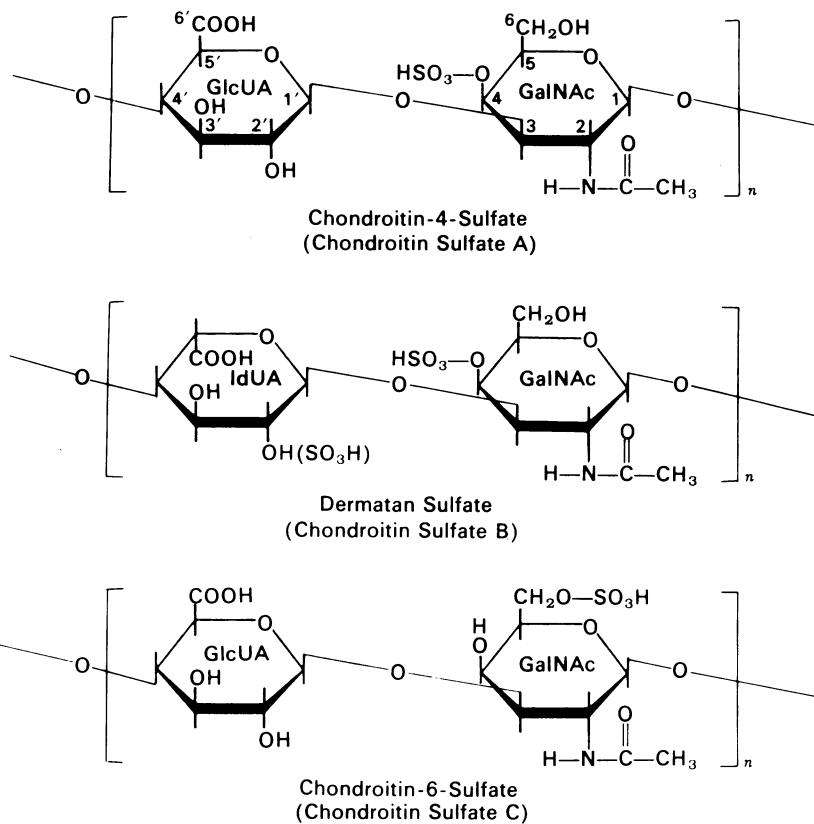
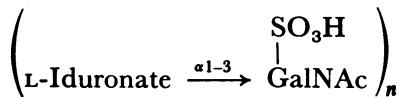


FIGURE 5.33 Structure of the repeating units of the various chondroitin sulfates. In dermatan sulfate, iduronic acid (IdUA) replaces glucuronic acid (GlcUA).

referred to as *dermatan sulfate*. The structure then becomes:



Chondroitin sulfate acts to cross-link collagen, creating a flexible linkage between the tough protein filaments of cartilage. This serves as a resilient matrix for calcification to form bones and teeth and for the formation of structures such as the nasal septum. The sulfate is on the 4 or 6 position of the galactosamine. When in the 4 position, we have

chondroitin sulfate A. When in the 6 position, we have chondroitin sulfate C.

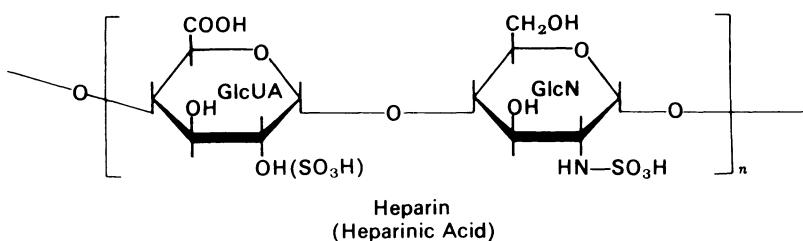
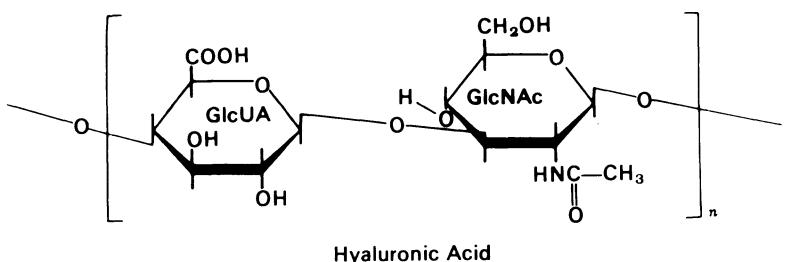
Dermatan sulfate, also called chondroitin sulfate B, is present in soft connective tissue and is the main cross-linking substance for collagen in skin, arterial walls, and cartilage. The sulfate is in the 4 position on the GalNAc portion of the disaccharide repeating unit. *Cross-linking of collagen with dermatan sulfate produces a softer and more flexible structure suitable for construction of tissues where more flexibility is required.*

In addition to the structural mucopolysaccharides, a number of others serve varied functions. *Hyaluronic acid*, a sulfate-free polysaccharide, related to dermatan sulfate in structure, is made up of repeating units of a disaccharide, GlcUA  $\xrightarrow{\beta 1-3}$  GlcNAc. Its molecular weight ranges from 50,000 to as high as 8 million.<sup>(168-170)</sup> Hyaluronic acid is a thickening agent and lubricant. It is present in serum, synovial fluid, vitreous humor, umbilical cord (Wharton's jelly), and sperm fluid, and coats the walls of the gastrointestinal tract, where it protects against bacterial invasion and digestion by the gastrointestinal enzymes.

Pathogenic bacteria secrete enzymes called *hyaluronidases* (E.C. 3.2.1.35 and 3.2.1.36), which hydrolyze hyaluronic acid and thus gain access to the tissues. Sperm can release hyaluronidases, which along with acid phosphatase of semen dissolve the wall of the ovum permitting one of the sperm to penetrate the egg. The structure of hyaluronic acid is shown in Figure 5.34. Hyaluridase is also known as the "spreading factor." It can be assayed by its effect in lowering the viscosity of a solution of hyaluronic acid. Its activity accounts for the increased sedimentation rate of erythrocytes in rheumatoid disease.

*Heparin*, originally isolated from liver as its name indicates, is a major anticoagulant in the human protecting against thrombosis. It is present in liver, lung, and mast cells in relatively high concentration. It is composed of repeating units of a disaccharide having the structure GlcUA  $\xrightarrow{\alpha 1-4}$  GlcN (see Figure 5.34).<sup>(171,172)</sup> Its molecular weight, after removing the sulfate esters, ranges from 600 to 20,000, depending upon the source and method of isolation. It occurs sulfonated on the nitrogen in the 2 position of glucosamine. Some sulfate ester also occurs at position 6.

Related to heparin is *heparitin sulfate (heparan sulfate)*.<sup>(173)</sup> It is a variable structure (microheterogeneity), composed mainly of repeating units of a disaccharide similar to that of heparin, but where D-glucuronic acid (GlcUA) is replaced by its epimer, L-iduronic acid (IdUA)



**FIGURE 5.34** Comparison of the structures of heparin and hyaluronic acid. Heparin carries sulfate on the nitrogen, and often on the 2' position of the glucuronate and 3 position of the glucosamine moiety.

(IdUA  $\xrightarrow{\alpha 1-4}$  GlcNAc). In addition, disaccharides with D-glucuronic acid, also occur in the polymer, resulting in heterogeneity of the structure. As distinct from heparin, some of the glucosamine is acetylated. A typical sequence would be:

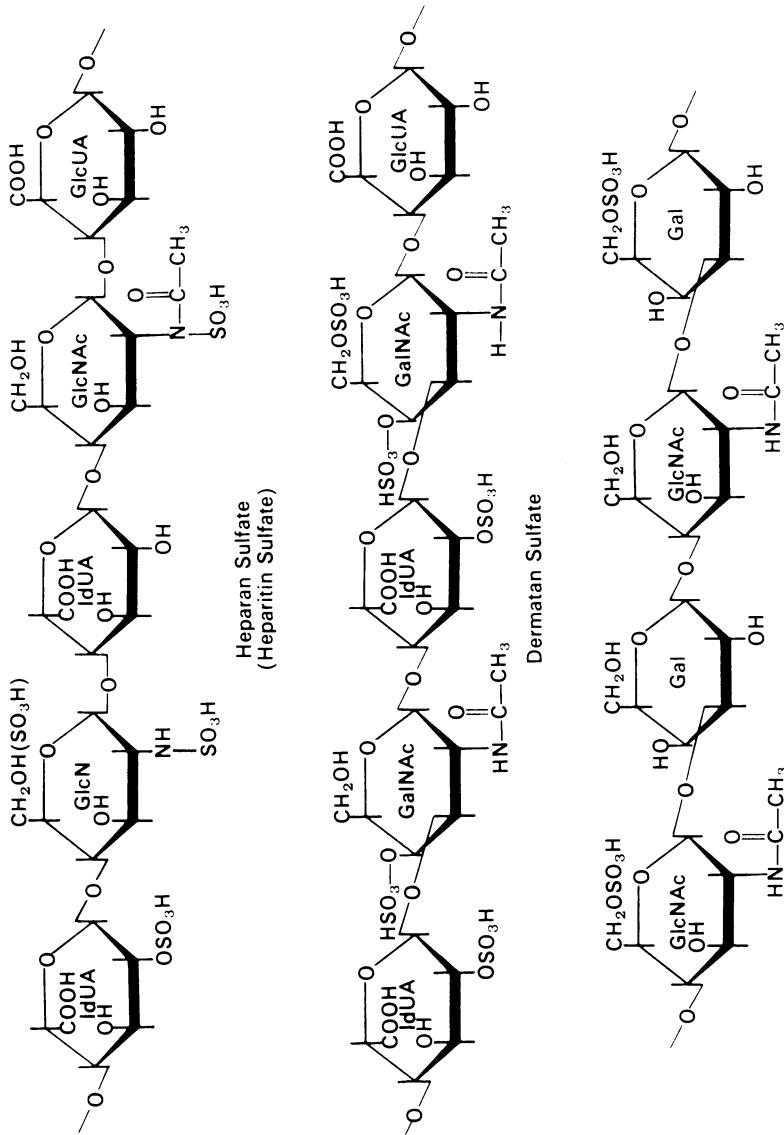


This structure is shown in Figure 5.35.

Heparitin sulfate occurs normally in liver, lung, spleen, and other organs, especially of the reticuloendothelial system, and its formation is related to heparin synthesis. It has no anticoagulant activity, however.

The structure of *dermatan sulfate* as indicated in Figure 5.33 is a simplification. When isolated, it contains a variable amount of glucuronic acid, which is integrated into the polymer chain. A typical structure is shown in Figure 5.35 and in simplified form can be represented as,

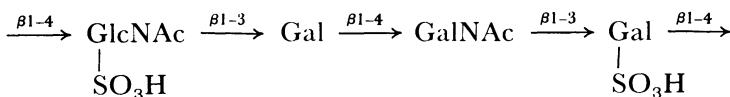




**Keratan Sulfate**

FIGURE 5.35 Structures of heparan sulfate, dermatan sulfate, and keratan sulfate.

A mucopolysaccharide occurs in the costal cartilage and cornea of the eye and other tissues, which is referred to as *keratosulfate or keratan sulfate*. It is composed of equimolar amounts of sulfate, *N*-acetylglucosamine, and galactose. The structure of keratan sulfate is variable and may contain mannose, L-fucose, and sialic acid, as isolated from various sources.<sup>(174-176)</sup> A representative sequence is elaborated in Figure 5.35. This may be represented as follows:



### *Catabolism of the Mucopolysaccharides (Glycosaminoglycans)*

Most likely, the glycosaminoglycans are synthesized initially as *glycoproteins* as described in Section 5.4. This is supported by the finding of fragments of heparan sulfate in the urine which contain serine and the monosaccharides normally associated with a glycoside linkage in the glycoproteins, GlcNAc, Gal, and xylose.<sup>(175,177)</sup> They are then cleaved from the protein by various endoglycosidases.<sup>(178)</sup> The catabolism of the major heparan, dermatan, or keratan sulfate moiety then proceeds sequentially from the nonreducing end.<sup>(179)</sup>

It should be pointed out that glycosaminoglycans form complexes with polypeptides and it is possibly that they are transported in this form to sites of cartilage and ground substance synthesis, where they form complexes with collagen to build a structure such as cartilage, a blood vessel or a membrane.

Those studying glycosaminoglycan catabolism have assigned sequential numbers to the various enzymes, approximately in the order in which they act on heparan, dermatan, and keratan sulfates. These are listed in Table 5.6 in the order in which they act on the glycosaminoglycans.

Figures 5.36 and 5.37 indicate the steps in the degradation of typical mucopolysaccharides.

In the *mucopolysaccharide (glycosaminoglycan) storage diseases* one or more of these steps cannot take place at the normal rate. This results in accumulation of the glycosaminoglycans in the lysosomes. The lysosomes become engorged and swell, resulting in the swelling of the liver,

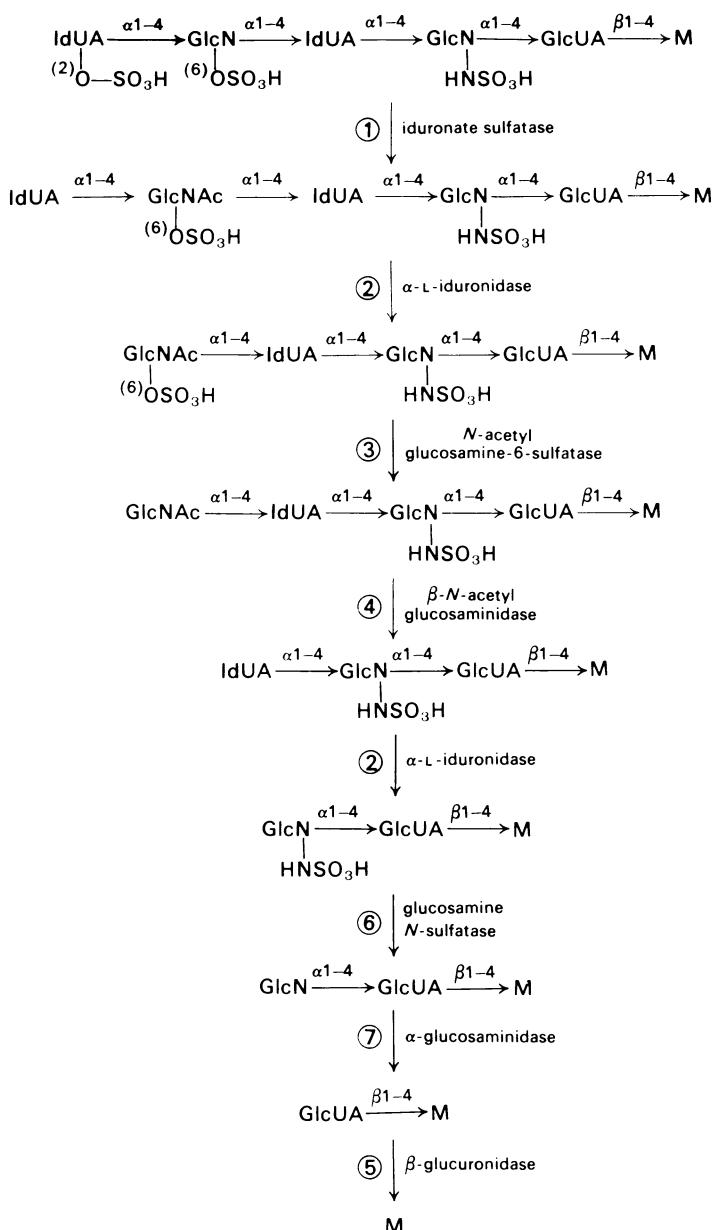


FIGURE 5.36 Steps in the catabolism of heparan sulfate. Heparan and dermatan sulfates have the same uronic acid components, but differ in that the amino sugar is galactosamine in dermatan sulfate, with  $\beta$  linkages. M represents the repeating units of the mucopolysaccharide. The circled numbers refer to the enzyme numbers in Table 5.6.

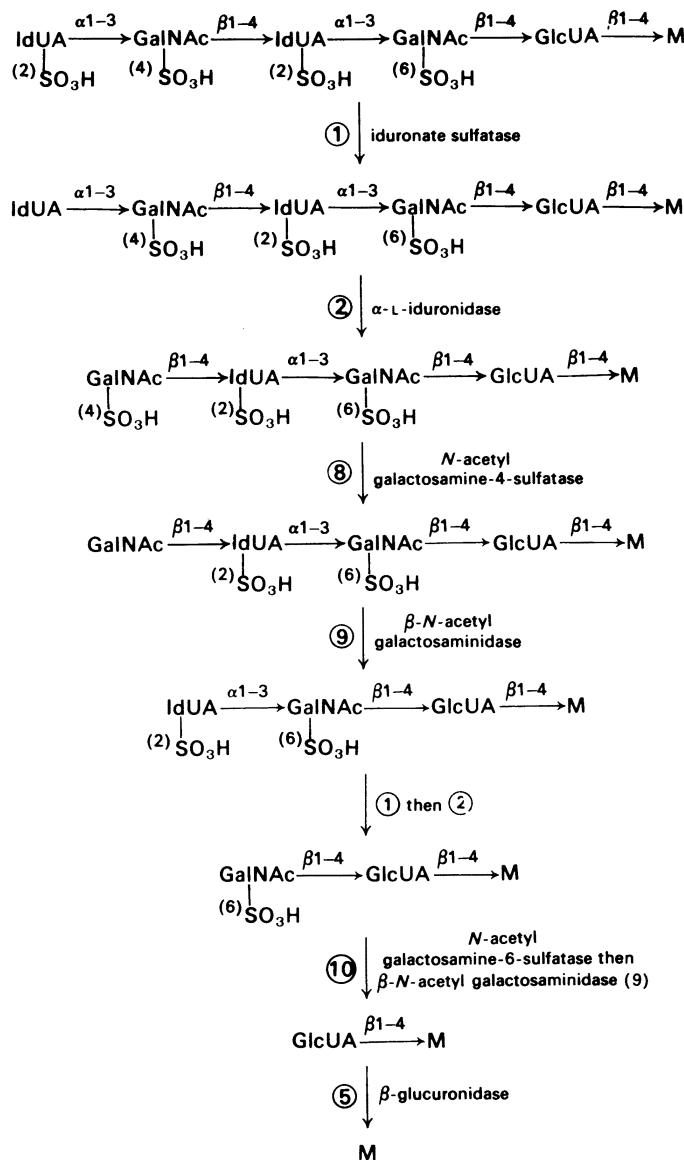


FIGURE 5.37 Steps in the catabolism of dermatan sulfate. M represents the continuation of the mucopolysaccharide chain. The circled numbers refer to the enzyme numbers in Table 5.6. The numbers in parentheses refer to the position of attachment on the sugar.

TABLE 5.6

*Enzymes Responsible for the Catabolism of the Glycosaminoglycans in Approximate Order of Their Action on the Substrate*

Sequence number <sup>a</sup>	Alternative designation	E.C. designation	Substrate
1	L-Iduronate sulfatase	—	L-Iduronate-2-sulfate (heparan and keratan sulfates)
2	$\alpha$ -L-Iduronidase	3.2.1.76	Iduronate $\alpha$ 1-4 linkage (heparan and keratan sulfates)
3	<i>N</i> -acetylglucosamine-6-sulfate sulfatase (aryl-sulfatase B)	3.1.6.1 <sup>b</sup>	<i>N</i> -Acetylglucosamine-6-sulfate
4	$\beta$ -N-acetyl- $\alpha$ -glucosaminidase	3.2.1.50	$\alpha$ 1 Linkage of <i>N</i> -acetylglucosamine
5	$\beta$ -Glucuronidase	3.2.1.31	$\beta$ -Glucuronides
6	(Heparan <i>N</i> -sulfatase) Sulfamidase ( <i>N</i> -sulfatase)	—	$\alpha$ -Glucosamine- <i>N</i> -sulfate
7	$\alpha$ -Glucosaminidase	—	$\alpha$ -Glucosamine, 1-4 linkage
8	<i>N</i> -acetylgalactosamine-4-sulfatase	3.1.6.1 <sup>b</sup>	<i>N</i> -Acetylgalactosamine-4-sulfate
9	$\beta$ -N-Acetyl-galactosaminidase	—	<i>N</i> -Acetylgalactosamine- $\beta$ 1-4 linkage
10	<i>N</i> -Acetylgalactosamine-6-SO <sub>4</sub> -sulfatase	3.1.6.1 <sup>b</sup>	<i>N</i> -Acetylgalactosamine-6-sulfate

<sup>a</sup> Sequence numbers of McKusick *et al.*<sup>(180)</sup>

<sup>b</sup> The same number is assigned to a group of aryl sulfatases.

heart, spleen, and other tissues. In addition, the mechanism for laying the foundation for the mineralization of bones and teeth is compromised, and a general dysostosis (defect in the ossification of cartilage) develops in severe cases called *dysostosis multiplex*. Figure 5.38 is an electron micrograph of the appearance of the lysosomes in a lysosomal storage disease.



**FIGURE 5.38** Electron micrograph showing enlarged lysosomes (arrows) due to storage of huge amounts of glycolipids in a granulocyte. Courtesy of Joseph Dardano, NASA Research Laboratories, Houston ( $\times 13000$ ).

## 5.10 GENETIC GLYCOSAMINOGLYCAN STORAGE DISEASES

The first clear descriptions of patients with genetic glycosaminoglycan storage diseases were those of Hunter in 1917 and Hurler in 1919.<sup>(181)</sup> Subsequently, these syndromes were referred to as *gargoylism*, from the facial appearance of the patients, *dysostosis multiplex*, from the skeletal deformities, and *lipochondrodystrophy*, on the assumption that the storage material was lipid in nature. In 1952, it was demonstrated by metachromatic staining of tissue, that the stored material was composed of mucopolysaccharides. The name *mucopolysaccharidosis* was then used<sup>(182)</sup> for Hurler's, Hunter's, and related syndromes.

In 1957, large quantities of heparan and dermatan sulfate were isolated from the urine of a patient with Hurler's syndrome.<sup>(179)</sup> Subsequently, this finding was confirmed by others. It was soon found that patients with related symptoms also excreted mucopolysaccharides, but of different composition. It then became possible to classify the diseases biochemically, first by the nature of the polysaccharides accumulating, and subsequently by the enzymatic defect which caused certain glycosoaminoglycans to deposit in the tissues and be excreted in the urine.<sup>(180)</sup> The classification is based on the enzymes listed in Table 5.6 and referred to in Figures 5.36 and 5.37. Table 5.7 classifies the mucopolysaccharidoses and identifies the genetic defect.

### 5.10.1 Hurler's Syndrome (Type I H)

Hurler's syndrome is designated MPS-1, the MPS standing for mucopolysaccharidosis. The MPS distinguishes these type numbers from those used for the glycogenoses, lipidoses, and others. We will dispense with this designation except where its deletion would result in confusion.

Hurler's syndrome (Type I H) is a progressive disease resulting in death at about age 10. The infants appear to be normal at birth. Subsequently they progressively deteriorate mentally and physically. Dwarfing becomes evident at about 2–3 years of age, at which point growth ceases, the cornea becomes cloudy, and the facies become coarse taking on the appearance of the gargoyle on medieval castles. Joints become stiff and claw hands develop. These patients also become

TABLE 5.7  
*Classification of the Mucopolysaccharidoses*

Type	Syndrome	Genetic deficiency	Major urinary mucopolysaccharide	Symptoms
I H	Hurler's	$\alpha$ -L-Iduronidase	Dermatan sulfate, heparan sulfate	Mental retardation, skeletal deformities with gargoyleism, corneal clouding, death before age 10
I S	Scheie's <sup>a</sup>	$\alpha$ -L-Iduronidase	Dermatan sulfate, heparan sulfate	Similar to Hurler's but much milder; normal intelligence, probably normal life span, mild skeletal changes, especially at the joints, corneal opacity
I H/S	Hurler/Scheie	$\alpha$ -L-Iduronidase	Dermatan sulfate, heparan sulfate	Symptoms intermediate that of Hurler's and Scheie's
II	Hunter's (severe or mild)	Iduronate sulfatase	Dermatan sulfate, heparan sulfate	Similar to Hurlers I, but less severe, moderate mental retardation, no corneal opacity, inherited as an X-linked recessive, death usually at about 15 years; in mild form patients survive to 30–60 years and are of fair intelligence

III A	Sanfilipo A	Heparan N-sulfatase	Heparan sulfate	Severe mental retardation in homozygote, very little to no corneal opacity
III B	Sanfilipo B	N-acetyl- $\alpha$ -D-glucosaminidase	Heparan sulfate	Symptoms similar to that of Sanfilipo A, but different etiology
IV	Morquio's	N-Acetylhexosamine-6-sulfatase	Keratan sulfate	Dwarfism, skeletal deformity, osteoporosis, defect in epiphyses of vertebral bodies resulting in shortening of the neck, no mental retardation, occasional corneal opacity
V	Scheie's	Now classified as type I S	Dermatan sulfate	Severe skeletal deformity and corneal opacity. No mental retardation, valvular heart disease. White cell inclusions.
VI	Maroteaux-Lamy	N-acetylgalactosamine-4-sulfatase		
VII	$\beta$ -Glucuronidase deficiency	$\beta$ Glucuronidase	Dermatan sulfate, heparan sulfate	Normal intelligence. Survives to age 20. Variants can be mild or intermediate in symptoms Hepatosplenomegaly, white cell inclusions, dysostosis multiplex, mental retardation

<sup>a</sup> A variant of Hurler's syndrome; formerly Type V.

deaf. Involvement of the heart valves and lung occur with stertorous breathing. Skeletal abnormalities develop with widening of the medial end of the clavicle. The sella turcica is enlarged, the optic chiasm being elongated, creating the J-shaped or  $\omega$  sella.<sup>(179-182)</sup>

The disease is inherited as an autosomal recessive. It is estimated that there is an incidence of 1 out of 100,000 births. About 1 in 150 are carriers for the disease. Death usually results from pneumonia or heart disease.<sup>(183)</sup>

The basic defect, as indicated in Table 5.7, is a deficiency of  $\alpha$ -L-iduronidase, enzyme No. 2 in Table 5.6, and Figures 5.36 and 5.37. It is therefore apparent that except for some removal of sulfate, *dermatan sulfate and heparan sulfate cannot be metabolized*. These patients possess adequate amounts of glucuronidase. For this reason, chondroitin sulfates A and C and keratan sulfate can be metabolized (see Figures 5.33 and 5.35).

Diagnosis can be first made by metachromatic staining by the urine of a paper impregnated with alcian or toluidine blue. Cetyl pyridinium salts will precipitate the mucopolysaccharides from urine. Albumin acidified with dilute acid will also precipitate the mucopolysaccharides.<sup>(184-186)</sup>

A most practical method for identifying some of the glycosaminoglycans is by agarose and acrylamide gel electrophoresis. Since the sulfated mucopolysaccharides are highly charged, they move fairly rapidly at pH 9.0 and separate. Chondroitin sulfates A, B, and C are available commercially. Heparan sulfate is readily prepared from beef lung. Electrophoresis can be carried out before and after the action of chondroitinases, which are available commercially. By these techniques, it is practicable to distinguish Hurler's from Hunter's syndrome or Sanfilippo A and B.<sup>(187)</sup>

Although it is practicable to demonstrate the accumulation of [<sup>35</sup>S]-mucopolysaccharides by cultured fibroblasts and the correction of this defect by adding  $\alpha$ -L-iduronidase,<sup>(188)</sup> a much simpler approach is to determine  $\alpha$ -L-iduronidase in the leukocytes of the patient.<sup>(189-192)</sup> The use of  $\alpha$ -L-iduronidase in cultured fibroblasts of amniotic fluid is useful in detecting Hurler's syndrome prenatally.<sup>(188)</sup> The heterozygote can be detected since the  $\alpha$ -L-iduronidase concentration of leukocytes is about half that of the normal.

There is no treatment for Hurler's syndrome which will affect the course of the disease process. Infusions of  $\alpha$ -L-iduronidase have been

tried in several patients. The urinary excretion of mucopolysaccharides decreased but the effect was transient and did not influence the progressive course of the disease significantly.<sup>(194)</sup> This needs to be explored further.

### 5.10.2 Scheie's Syndrome (*Type IS*)

A syndrome was discovered in 1962 which resembled Hurler's disease, except that it was observed in adults.<sup>(195)</sup> Enzyme studies revealed a deficiency of  $\alpha$ -L-iduronidase.<sup>(196)</sup> Since the disease is a milder form of Hurler's disease, it was expected that some  $\alpha$ -L-iduronidase activity was present. This could not be confirmed with cultured fibroblasts.<sup>(197)</sup> In tissue culture studies, Scheie cells will not correct the Hurler cell deficiency.

The patient with Scheie's syndrome shows severe clouding of the cornea, deformity of the bone structure, and involvement of the aortic valve. Intelligence is normal. Patients with Scheie's syndrome include college graduates and professionals. Deafness is common among these patients.

The disease is usually associated with corneal clouding and pigmentary degeneration of the retina and glaucoma leading to visual disability. Facies are coarse, with the broad mouth characteristic of Hurler's disease. Stiff joints develop as the patient matures with claw hand and other skeletal deformities and the development of carpal tunnel syndrome. Patients complain of stiff, painful legs which interferes with walking. Feet are misshapen. Stature can be normal, although short stature is usually reported in these patients.

Scheie's disease is inherited as an autosomal recessive. A woman with Scheie's disease is reported to have given birth to a normal son.<sup>(195)</sup> The frequency of Scheie's syndrome is estimated at 1 in 500,000 births.<sup>(198)</sup>

It is postulated that Hurler's and Scheie's syndrome represent homozygosity for two different alleles at the structural locus for  $\alpha$ -L-iduronidase synthesis. This concept is supported by a phenotype with  $\alpha$  iduronidase deficiency intermediate Hurler's and Scheie's syndrome. This is designated *Type I H/S mucopolysaccharidosis*.

Symptoms in patients with the hybrid Hurler-Scheie's syndrome, are intermediate in severity to the two primary syndromes. These

patients are short with dysostosis multiplex, mental retardation, and severe involvement of the heart and liver. The cornea is cloudy. Stiff joints and claw hand occur in these patients. Valvular heart lesions are a constant finding. There is a tendency to a receding chin. There is marked destruction in the sella turcica region and cribiform plate, with the development of an arachnoid cyst. Patients tend to be blind with degenerative optic nerve changes. Spinal fluid block at the level of C2 and C5 occurs.

Patients with the Hurler–Scheie's syndrome have been known to survive to their 20s and some females have become pregnant. On autopsy, mucopolysaccharide storage in connective tissue is observed throughout the body. The dura is thickened and contains infiltrates of foamy macrophages loaded with mucopolysaccharides.

The mucopolysaccharides found in the urine in Scheie's disease are the same as in Hurler's syndrome. In the less severe Scheie's disease with normal intelligence, eye surgery for glaucoma and corneal transplantation have been successful.<sup>(199)</sup>

The laboratory tests for Hurler's and Scheie's syndrome do not distinguish between these conditions. This can only be done clinically. However, the absence of  $\alpha$ -L-iduronidase in the leucocytes of a patient who has gone beyond puberty is diagnostic of Scheie's or the compound disease, depending upon the clinical behavior of the patient.<sup>(200)</sup>

### 5.10.3 *Hunter's Syndrome (Type II)*

Hunter's syndrome is a disease of glycosaminoglycan accumulation in the lysosomes, as is Hurler's, but differs in its cause. It is a much milder disease and is distinct from Hurler's since there is no clouding of the cornea. The prominent features of this condition are stiff joints, dwarfing, coarse facial features, progressive deafness, mental deterioration, and progressive loss of vision due to papilledema and retinitis pigmentosa. In the severe form of the disease, patients live to about 15 years of age. In these cases hydrocephalus develops. The autonomic nervous control system is defective and diarrhea is a common problem in these patients.<sup>(201)</sup>

In the mild form of Hunter's syndrome the patients show rosy cheeks (plethoric appearance), the voice is hoarse, and heart disease is chronic caused by valvular, myocardial, and ischemic factors. Death

usually results from a heart attack. Degenerative arthritis and lung disease with airway obstruction is seen in the adults. With the mild form of Hunter's disease, patients have survived to beyond 60 years of age. Some show normal intelligence and minimum skeletal symptoms.<sup>(202)</sup>

*The defect in the Hunter's syndrome is a deficiency of the enzyme L-iduronate sulfatase* (see Figures 5.36 and 5.37 and Table 5.6). The first step in the catabolism of dermatan sulfate and heparan sulfate is blocked. As a result, these glycosaminoglycans accumulate in all tissues of the body, and are excreted in huge quantities in the urine. Keratan sulfate and chondroitins A and C are metabolized normally (Figures 5.33 and 5.35) since they do not contain the iduronate-2-sulfate residue. Since sulfates have a tendency to hydrolyze nonenzymatically to some extent, some metabolism of dermatan and heparan sulfate is possible. This may be a factor in reducing the severity of the disease in Hunter's as compared to Hurler's syndrome. An ameliorating factor is that with the sulfate attached, the glycosaminoglycans are more soluble. Thus, in Hurler's syndrome the material deposited in the lysosomes is more insoluble and accumulates more rapidly, resulting in more severe symptoms.

Hunter's syndrome is readily noted as a suspected mucopolysaccharidosis with the screening tests for mucopolysaccharides in the urine, as described under Hurler's disease.<sup>(184-186)</sup> Electrophoresis is useful, since the more highly sulfated dermatan and heparan sulfates have an increased mobility over the lesser sulfated mucopolysaccharides.<sup>(187)</sup> If Hunter's disease is suspected, a direct test for iduronate sulfatase is practicable in serum and leukocytes.<sup>(199,203,204)</sup>

Confirmation of Hunter's disease can be made by adding the normal cells to cultured fibroblasts from the patient to see whether this corrects the accumulation of [<sup>35</sup>S]-mucopolysaccharides. Cloning can reveal the Hunter cells in heterozygotes. Occasionally the heterozygote cells become spontaneously enriched with Hunter cells during cell culture.<sup>(205)</sup> The use of cell cultures is still the preferred technique for prenatal determination of Hunter's syndrome in the homo- and heterozygote.<sup>(193)</sup>

In contrast to Hurler's disease, which is inherited as an autosomal recessive, Hunter's syndrome is inherited as an X-linked recessive. Mutations at the same X-chromosome locus probably account for the variability of the severity of this disease.

The Lyon hypothesis (see Volume 2, pp. 217, 218, 473) designed to explain mosaicism in erythrocytes, proposes that only one X chromo-

some is functional in each cell. There would therefore be two populations of cells in the Hunter heterozygote (trait) whether the maternal (Hunter) or paternal (normal) X-chromosome is active. This hypothesis is used to explain the mosaicism seen in cell cultures when the cells obtained from heterozygotes are stained with metachromatic stains to reveal the deposited mucopolysaccharides.

Attempts have been made to treat the Hunter's syndrome with plasma and lymphocyte transfusions to supply iduronate sulfatase.<sup>(194,206,207)</sup> Results have been only minimally effective. *An interesting approach is the use of a skin graft from a histocompatible sibling.*<sup>(208)</sup> This was effective in significantly decreasing heparan and dermatan sulfate excretion in the urine.

#### 5.10.4 Sanfilippo Syndromes A and B (Type III)

Sanfilippo syndrome was first reported (1961) as a variant of Hurler's disease.<sup>(209)</sup> This case was subsequently shown to have the Type B form of the disease. Two years later the condition was described more fully and recognized as a disease different from Hurler's or Hunter's.<sup>(210)</sup>

The clinical course is severe. Progressive mental retardation is usually not marked until about 5–6 years of age. Subsequently, the patients lose the power of speech and survive only to about age 20. There is no clouding of the cornea, hepatosplenomegaly is slight, and the bone changes are less severe than in Hurler's or Hunter's disease. Dwarfing is moderate. The calvaria tends to be unusually dense and dorsolumbar vertebral bodies show ovoid dysplasia. Hirsutism is common. Mucopolysaccharide concentration in urine is not very high and may be negative. Cardiac valvular defects develop and in one case the mitral valve was replaced in a child 3 years old.<sup>(211)</sup>

Both types of Sanfillipo syndrome are inherited as autosomal recessives. The A type of the disease is more common by at least a factor of two.<sup>(212)</sup>

*The defect in Sanfillipo A is the absence of the sulfatase which hydrolyzes the sulfate from the nitrogen in heparan.*<sup>(213)</sup> This is listed as enzyme No. 3 in Figure 5.36 and Table 5.6. This enzyme is not required for the hydrolysis of dermatan sulfate (Figure 5.37) and dermatan sulfate is not found in the urine of these patients in increased amounts. *This*

distinguishes Sanfillipo from Hurler's and Hunter's disease. This enzyme is sometimes referred to as *heparan-N-sulfatase* and *heparin sulfamidase* since it acts on these substrates.

The enzyme deficiency of the B form of Sanfillipo disease is unrelated to that of the A form. In this case there is a deficiency of  $\beta$ -N-acetyl- $\alpha$ -glucosaminidase. This is listed as enzyme No. 4 in Table 5.6 and Figure 5.36. This enzyme is required to hydrolyze heparan sulfate (Figure 5.36) but not dermatan sulfate (Figure 5.37). Thus in both Sanfillipo A and B only heparan sulfate is found in increased amounts in the urine. Addition of N-acetyl- $\alpha$ -D-glucosaminidase to a culture medium of fibroblasts from patients with Sanfillipo B disease corrects the defect.<sup>(214)</sup>

In a case of Sanfillipo B disease, the presence of an inactive form of the N-acetyl- $\alpha$ -glucosaminidase was shown by immunochemical techniques.<sup>(215)</sup>

Urinary excretion of heparan sulfate with the clinical symptoms described, is evidence for pursuing the concept that the patient has the Sanfillipo syndrome. Serum or leukocyte assay for N-acetyl- $\alpha$ -glucosaminidase is effective in the identification of the Sanfillipo homo- and heterozygote.<sup>(216)</sup> The differences in structure of the heparan in the A and B syndrome has been used to differentiate these two conditions.<sup>(217,218)</sup> Prenatal diagnosis using cultured fibroblasts is also a practical procedure.<sup>(219)</sup>

### 5.10.5 Morquio's Syndrome (Type IV)

This syndrome is not uncommon among the French Canadians of Eastern Quebec Province.<sup>(220)</sup> It bears the name of Morquio who first described the syndrome in 1929. The disease is characterized by short limbs, out of proportion to the size of the torso. The neck is contracted so the head rests almost directly on the shoulders. As our understanding of the mucopolysaccharidoses developed, it became apparent that Morquio's syndrome was a mucopolysaccharidosis. Inclusions in leukocytes and brain cells, excessive secretion of keratan sulfate but not heparan or dermatan sulfate, coarse facies, cardiorespiratory insufficiency, prominence of the lower ribs noticed at 12–18 months, all pointed to a glycosaminoglycan storage disease.<sup>(221–224)</sup>

With Morquio syndrome, growth stops after age 6–7. In severe cases, dental enamel is abnormally thin.<sup>(223)</sup> Clouding of the cornea is

mild and deafness is an invariable finding. The joints tend to be excessively loose and instability at the wrist tends to incapacitate the patient. A constant feature of the syndrome is absence or severe hypoplasia of the odontoid process of the second cervical vertebra. Atlantoaxial subluxation is a major problem. Aortic regurgitation develops in some patients.

By the time these patients reach their teens, keratan sulfate excretion in the urine is reduced so that it is almost undetectable. Although knock-knees is a serious problem in the severe form of the disease, some of these patients with mild symptoms have straight legs.<sup>(222)</sup>

Patients with the severe form of Morquio's syndrome do not survive much beyond 20–30 years of age. Patients with the milder forms have lived beyond 60 years of age.

Figure 5.33 illustrates the fact that chondroitin sulfate contains sulfate ester on *N*-acetylgalactose at both the 4 and 6 position. Dermatan sulfate contains sulfate only in the 4 position. The sulfate in keratan sulfate is in the 6 position on *N*-acetylglucosamine and galactose (Figure 5.35). When fibroblasts cultured from a Morquio patient were incubated with chondroitin sulfate preparations, only sulfate at the 4 position could be hydrolyzed. Thus, the defect in Morquio's syndrome seems to be in deficiency of *N*-acetylgalactosyl-6-sulfate sulfatase.<sup>(225)</sup> Keratan sulfate, however, has the sulfate on galactose and *N*-acetylglucosamine and not on *N*-acetylgalactosamine (Figure 5.35). It is probable that the enzyme specificity is for sulfate on the 6 position and the *N*-acetyl group and the configuration of the monosaccharide does not interfere in its action as long as it is in the D-form.<sup>(226)</sup>

At present, the diagnosis of Morquio's syndrome depends upon the clinical appearance and behavior of the patient and the finding of keratan sulfate in the urine.

No effective treatment for Morquio's syndrome has been developed.

A syndrome which combines the features of Morquio's and Sanfillippo syndromes has been reported in a patient. *This 5-year-old male had an N-acetylglucosamine-6-sulfate sulfatase deficiency.*<sup>(227a)</sup> This is enzyme No. 3 in Table 5.6 and Figure 5.36. The patient was of short stature, with excessive coarse hair, hepatomegaly, mild dysostosis multiplex, and hypoplasia of the odontoid. The cornea was clear. Both keratan and heparan sulfate were found in the urine. Circulating

lymphocytes, stained with toluidine blue, showed ring-shaped deposits under the cell membrane.

Unlike Morquio's syndrome, cultured fibroblasts accumulated [<sup>35</sup>S]-mucopolysaccharides. Both heparan and keratan sulfates contain sulfated *N*-acetylglucosamine, which is the substrate for the missing enzyme. Both parents showed partial deficiency of the enzyme. The syndrome is therefore inherited as an autosomal recessive.

#### 5.10.6 Maroteaux-Lamy Syndrome (Type VI)

In 1963, a report appeared describing a patient with dysostosis multiplex, but differing from Hurler's syndrome in that the urine contained chondroitin sulfate (Figure 5.33). The syndrome is named after the authors.<sup>(227)</sup> In Type VI mucopolysaccharidosis the bone abnormalities become obvious at about age 2 with genu valgum (knees twisted outward), lumbar kyphosis (hunchback), contracture of the fingers, and anterior sternal protrusion.<sup>(228)</sup> There is severe restriction of movement of the joints. The corneas are cloudy and cardiac abnormalities, seen in Hurler's syndrome, are present. Hydrocephalus, requiring a shunt, is common, and there are neurological complications resulting from atlantoaxial subluxation (partial dislocation of the first vertebra).<sup>(229)</sup> The joints, especially the head of the femurs, are severely affected. Patients with the severe form of the disease rarely survive past their 20's.

Milder cases occur where the stiffness at the joints are minimal, stature is short, and corneas are cloudy. However, these patients are able to work at various occupations.<sup>(230)</sup> Cases intermediate the mild and severe form also occur. These patients resemble Scheie's syndrome patients but are shorter in stature. They may be of superior intelligence. However, there are substantial changes at their joints and hips.<sup>(231)</sup>

Even in the mild form of Maroteaux-Lamy syndrome, there is myelopathy because of compression of the cervical region of the spinal cord by thickened dura. Neurological deterioration proceeds slowly but progressively. Aortic stenosis also occurs.<sup>(229,231)</sup>

Some of the patients with a mild form of Maroteaux-Lamy syndrome have become pregnant. Severe neurological deterioration then occurs, especially in the last trimester.<sup>(229)</sup>

*The defect in the Maroteaux-Lamy syndrome is the inability to hydrolyze the sulfate which occurs at the 4 position of N-acetylgalactosamine of dermatan*

sulfate (Figure 5.35). N-Acetylgalactosamine-4-sulfatase and arylsulfatase B have been shown to be the same enzyme.<sup>(232)</sup> This distinguishes this disease from metachromatic leukodystrophy, where the defect is in arylsulfatase A (see Volume 2, p. 95).<sup>(233)</sup> The defect is in enzyme No. 8 of Table 5.6 and Figure 5.37.<sup>(236)</sup>

Lysosomal inclusions in the Kupffer cells and abnormal metachromatic staining in the leukocytes is severe in Type VI disease.<sup>(234,235)</sup> Inclusions are also seen in the cornea, conjunctiva, and skin.

Urinary excretion of dermatan sulfate exclusively suggests the Maroteaux-Lamy syndrome. This can be detected by acrylamide gel electrophoresis before and after treatment of the urine with arylsulfatase B. The determination of arylsulfatase B activity in the leukocytes is the simplest specific test for Type VI mucopolysaccharidosis.<sup>(233-235)</sup> Prenatal diagnosis is also practicable with the use of synthetic substrates.<sup>(234)</sup>

#### 5.10.7 Sly Syndrome: $\beta$ -Glucuronidase Deficiency (Type VII)

In 1973, the first case of  $\beta$ -glucuronidase deficiency was reported.<sup>(237)</sup> This is listed as enzyme No. 5 in Figures 5.36 and 5.37 and Table 5.6. This patient was a black male, 7 weeks of age when first examined. He showed umbilical hernia, hepatosplenomegaly, metatarsus adductus, and the facies of the mucopolysaccharidoses patients. Circulating and bone marrow granulocytes showed metachromatic staining granules. Subsequently, he developed anterior chest deformity and bilateral inguinal hernias (common in the mucopolysaccharidoses) which were repaired. Between 2 and 3 years of age mental deterioration became apparent. Mucopolysaccharide levels in urine were only slightly elevated.

Several other patients have been reported subsequently. There are, however, substantial differences in the clinical course and biochemical findings. In the first patient, the cornea was clear and the patient was mentally retarded. Others showed severe corneal clouding and some showed normal intelligence.<sup>(238,239)</sup> All the patients which have been reported (about ten) showed the coarse facies and cardiac involvement. Several have shown the lung anomalies with repeated episodes of pneumonia common to the mucopolysaccharidoses.

$\beta$ -Glucuronidase deficiency is inherited as an autosomal recessive, and the locus for the gene for  $\beta$ -glucuronidase synthesis has been found on chromosome No. 7.<sup>(240)</sup>

The deficiency of  $\beta$ -glucuronidase can be shown in the fibroblasts and leukocytes.<sup>(238,239)</sup> From Figures 5.36 and 5.37 it can be seen that the block occurs in enzyme No. 5. The disease is of varied expression. In some patients, only dermatan sulfate is found in the urine. In others, heparan sulfate is found. In some, both heparan and dermatan sulfate are found. In still others, only lower-molecular-weight polysaccharide fragments appear in the urine.<sup>(238)</sup>

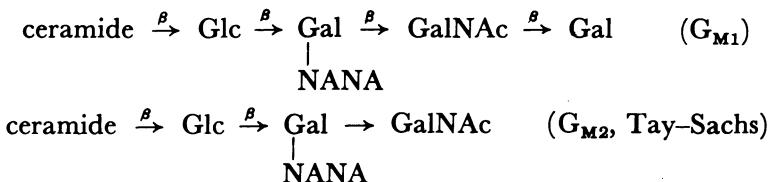
Total deficiency of  $\beta$ -glucuronidase would be incompatible with life because  $\beta$ -glucuronidase is utilized for many purposes in the human. The fact that these patients have survived to term, indicates that they have varying amounts of glucuronidase activity. On immunoassay of tissue extracts and serum from these patients, cross-reacting material with antibodies for  $\beta$ -glucuronidase has been demonstrated.<sup>(241)</sup> Thus variable mutations in a structural gene for the enzyme is the probable defect in these cases.

The diagnosis of  $\beta$ -glucuronidase deficiency is readily made by measuring the  $\beta$ -glucuronidase concentration in fibroblasts, leukocytes, or serum using synthetic substrates available commercially.<sup>(242)</sup> Technology for demonstrating this condition prenatally is available.

### 5.10.8 Glycosaminoglycan Lysosomal Storage Diseases

The mucopolysaccharidoses are only one group of conditions where deposits of glycosaminoglycans are found in the lysosomes. The sphingolipidoses (discussed in detail in Volume 2, Chapter 3), are another example of this condition. They are grouped together for convenience since they concern the metabolism of globoside and related glycolipids. These are also deficiencies of some lysosomal carbohydrases.

In generalized gangliosidoses and Tay-Sachs disease, the gangliosides  $G_{M1}$  and  $G_{M2}$  accumulate because of a  $\beta$ -galactosidase and hexosaminidase deficiency, respectively, as follows:



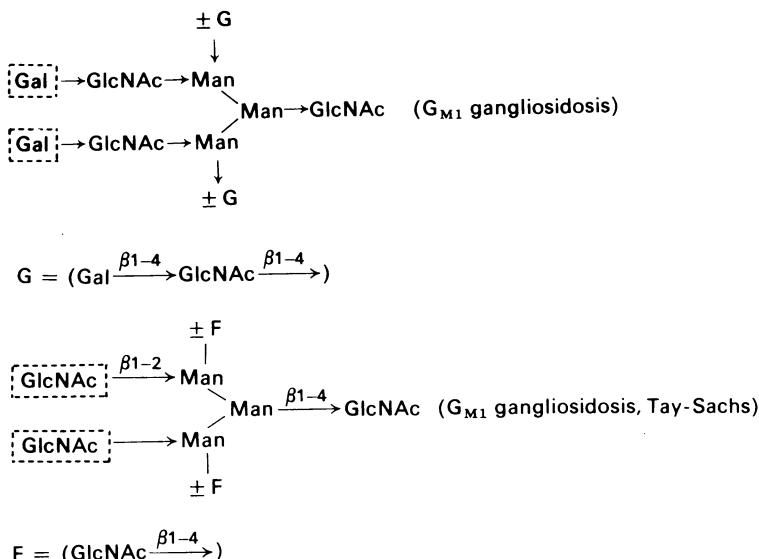


FIGURE 5.39 Polysaccharides found stored in the liver lysosomes of patients with generalized gangliosidosis and Tay-Sachs disease. The enzyme defect is emphasized by placing the monosaccharide which cannot be removed in a dotted box.

However, in addition to being unable to degrade the glycolipids, these patients also accumulate polysaccharides; their structure is shown in Figure 5.39.<sup>(242-244)</sup>

From Figure 5.39 it can be readily seen that these carbohydrates derive from a glycoprotein by endoglucosaminidase action (see Figure 5.29). *Thus the gangliosidoses and mucopolysaccharidoses are closely related to fucosidosis and mannosidosis.* They are all manifestations of defects in glycoprotein metabolism which results in storage of heterosaccharide complexes in the lysosomes. Thus they are all *lysosomal storage diseases*. Another example of this generalization is the condition which results from the inability to hydrolyze the last *N*-acetylglucosamine from the asparagine residue attached to a glycoprotein polypeptide chain (see Figure 5.29). This results in a severe lysosomal storage disease, resembling the most severe cases of Hurler's syndrome. *This disease is called aspartylglucosaminuria.*

#### 5.10.8.1 Aspartylglucosaminuria

In Figure 5.29 it is pointed out that an endo- $\beta$ -*N*-glucosaminidase cleaves most of the heterosaccharide chain from its attachment to the

polypeptide. This usually leaves one or two monosaccharides still attached to the polypeptide chain. In Figure 5.29, fucose is then removed leaving only *N*-acetylglucosamine attached to an asparagine residue. To cleave this last sugar from the polypeptide requires *N*-aspartylglucosaminidase (E.C. 3.5.1.26). A deficiency of this enzyme was first demonstrated in two mentally retarded siblings who had excreted large quantities of *N*-acetylglucosaminylasparagine (GlcNAC-Asn) in the urine.<sup>(245)</sup> Subsequently, this disorder was diagnosed in Finland<sup>(246,247)</sup> and the United States.<sup>(248)</sup> It is referred to as *aspartyl-glucosaminuria* or AGU. AGU, in Finland, occurs in 1 of 26,000 live births.

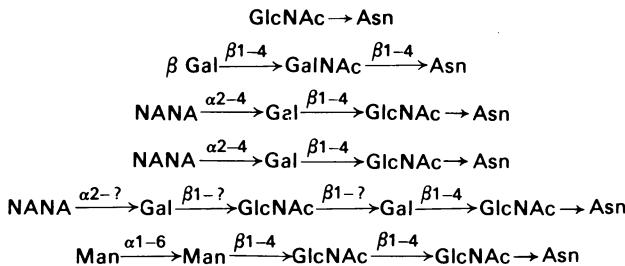
Symptoms of AGU resemble those of Hurler's disease. Features are coarse, and there is the typical visceromegaly and swollen lysosomes, vacuolated lymphocytes, impaired speech and motor clumsiness. Connective tissue lesions include dysostosis multiplex, umbilical hernia, and hypermobile joints. The cardiac symptoms of Hurler's and Hunter's disease are present with mitral insufficiency.

The major tissue change, as observed with the electron microscope, is enlargement of the lysosomes. Kupffer cell and kidney lysosomes are also involved.<sup>(245-249)</sup> Fibroblasts from skin biopsy also show deposits resembling the mucopolysaccharidoses. The cytoplasm of the neuronal cells from the cerebral cortex, cerebellum, and thalamic regions are filled with membrane bound vacuoles of electron-lucent granular material and electron-dense membranous or granular material with occasional lipid droplets.<sup>(248)</sup> The variety of lysosomal changes seen in aspartylglucosaminuria exceeds that seen in the mucopolysaccharidoses discussed above.

Reduced amounts of the enzyme *N*-acetylglucosaminyl-aspart-amidohydrolase [2-acetamido-1-( $\beta$ -L-aspartamido)-1,2-dideoxy- $\beta$ -D-glucose aspartamidohydrolase, E.C. 3.5.1.26] have been demonstrated in leukocytes, seminal fluid, brain, liver, and spleen.<sup>(249-251)</sup> Measurement of the enzyme in cultured fibroblasts permits detection of heterozygotes and the condition can be detected prenatally.<sup>(252)</sup> The urine contains substantial amounts of glycoasparagines other than the simple GlcNAc-Asn compound. These are shown in Figure 5.40.<sup>(253)</sup>

#### 5.10.8.2 *The Mucolipidoses (ML): Neuraminidase Deficiency*

Certain lysosomal storage diseases which result in the deposition of mucopolysaccharides in the lysosomes have been designated *muco-*



**FIGURE 5.40** Glycosparagine containing heterosaccharides from the urine of patients with aspartylglucosaminuria.

*lipidoses*. These conditions are distinct from the sphingolipidoses and related diseases in that the urine contains glycosaminoglycans but not lipid-carbohydrate complexes. In this group of diseases, there are at least four syndromes which resemble each other. These are labeled ML-I, ML-II, ML-III, and ML-IV. All are characterized by increased excretion of heterosaccharides containing high concentrations of sialic acids. Lysosomal deposits are also rich in sialic acid. *All show deficiency of  $\alpha$ -N-neuraminidase, at least in some tissue.* Clinically, all these diseases resemble Hurler's syndrome of various degree of severity.<sup>(254,255a)</sup>

In ML-I, the condition is generalized. Abnormal storage of glycoprotein and/or glycolipids occurs in neuronal, mesenchymal, and visceral tissue.<sup>(256,257)</sup> The only enzyme defect is in lysosomal  $\alpha$ -N-acetyl-neuraminidase, determined in cultured fibroblasts. Clinically, the disease resembles a severe type of Hurler's syndrome, with skeletal dysplasia, neurodegeneration with progressive ataxia, impaired speech and mental retardation. Death usually occurs before the end of childhood. These patients show the cherry red spot seen in Tay-Sach's disease. The urine contains numerous different heterosaccharide fragments containing sialic acid. Typical structures are shown in Figure 5.41.

In contrast to ML-I, ML-II and ML-III show enzyme defects not only in  $\alpha$ -N-neuroaminidase but in other hydrolases as well. In addition, the disease is limited to certain tissues and lysosomal deposits are found only in the fibroblast cultures.

Clinically, mucolipidoses II and III resemble severe and milder forms of Hurler's disease. Mucolipidoses II, also called inclusion cell (I-cell) disease, is the more severe condition with severe mental retardation, hepatomegaly, cardiomegaly, recurrent upper respiratory

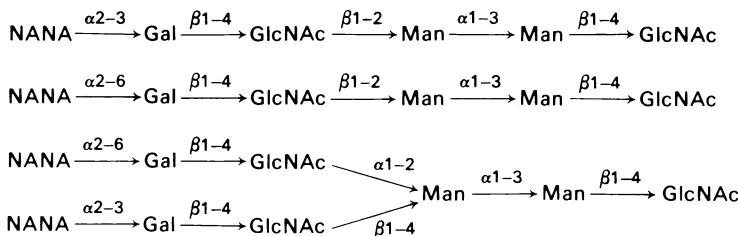


FIGURE 5.41 Typical sequences of heterosaccharides found in the urine of patients with the mucolipidoses. NANA represents  $\alpha$ -N-acetylneuraminic acid.

infections, and umbilical hernia. All these conditions are progressive. Corneas remain clear. Most of these patients die before 6 years of age.<sup>(256,257)</sup> *Mucolipidosis IV* is distinguished from the others by the fact that these patients develop cloudy corneas.<sup>(255b)</sup> In other respects it resembles ML-I.

*Mucolipidosis III* is the most common of the conditions. Patients with *mucolipidosis III* can survive to adulthood. They develop the coarse facies and skeletal changes typical of Hurler's syndrome, with stiff joints, claw hands, severe changes in the lung structure, and the murmur of aortic regurgitation.<sup>(258)</sup> They are mentally retarded, and are usually assigned to the ungraded class in school. Cases have been reported where patients with *mucolipidosis III* have raised large families.<sup>(258,259)</sup>

Both conditions are inherited as autosomal recessives. The genetic relationship between *mucolipidoses II* and *III* is unknown, but it is apparent that they are variants of the same disease.<sup>(258-260)</sup>

*A remarkable characteristic of the mucolipidoses is the marked elevation of lysosomal hydrolases in the serum.* This includes almost all the hydrolases involved in carbohydrate metabolism including,  $\alpha$ -L-iduronase, iduronate sulfatase,  $\beta$  glucuronidase, *N*-acetyl- $\beta$ -hexosaminidase, aryl-sulfatase A,  $\beta$  galactosidase,  $\alpha$ mannidase, and  $\alpha$ -L-fucosidase. It is as though the lysosomes were disintegrating and dumping their contents into the body fluids. For this reason some have proposed a defect in the cell wall of the lysosomes as the cause of the disease. This has not been confirmed.<sup>(258,260)</sup>

In contrast to their high concentration in body fluids, the concentration of the hydrolases in connective tissue and fibroblasts is very low. As a result, mucopolysaccharides and glycolipids accumulate in fibro-

blast cultures. This can be demonstrated in tissue culture.<sup>(261)</sup> The fibroblasts of these patients tend to crack up on freezing because of an apparent defect in the cell membrane.

Sialyl polysaccharide derivatives are present in high concentration in the urine of patients with the mucolipidoses (seven to eight times the normal).<sup>(262)</sup> In Hurler's syndrome, sialoglucides are not elevated to more than two to three times the normal. In addition, deficiency in neuraminidase in fibroblasts and leukocytes has been reported in the mucolipidoses.<sup>(263,264)</sup> Sialic acid levels are also increased three- to fourfold in cultured fibroblasts from patients with ML-II syndrome.<sup>(263)</sup> Furthermore, the lysosomal hydrolases excreted by ML-II fibroblasts have an abnormal electrophoretic mobility as compared to the normal which is corrected with neuraminidase. *This has suggested that the mucolipidoses are primarily a neuraminidase deficiency in connective tissue.* The hydrolases elaborated contain excessive amounts of sialic acid, which normally would be removed. This increases their solubility and tendency to move out of the cells into the surrounding circulating fluids. The low concentration of hydrolases remaining in the lysosomes then causes polysaccharides to accumulate in the lysosomes of the connective tissue since they cannot be metabolized.

Diagnosis of the mucolipidoses is made by clinical findings of symptoms resembling Hurler's disease without excessive amounts of the mucopolysaccharides in the urine. The disease is confirmed by measuring *N*-acetyl- $\beta$ -hexosaminidase and arylsulfatase A levels in serum. These should be elevated to about three times the normal. Prenatal diagnosis has been made by measuring the elevated enzyme levels in the amniotic fluid. The serum electrophoretic isoenzyme patterns of  $\beta$  hexosaminidase are abnormal.<sup>(265)</sup> Sialic acid levels measured in urine are helpful.

## 5.11 PROTEOGLYCANS

For centuries, bone and cartilage extracts were made for nutritional purposes (jellies) and glues. Interest was shown only in the collagen component of cartilage. Toward the end of the 19th century, attention was directed to acid-insoluble organic substances and it was discovered that these could be extracted from cartilage with dilute alkali. They

were, therefore, acids. In addition, they contained a high percentage of carbohydrate. Since they contained ester sulfates these compounds were labeled *chondroitin sulfates*, from the Greek *chondros* meaning cartilage.<sup>(266-268)</sup>

For the next 50 years, intensive studies were made on the structure of the chondroitin sulfates (now also called mucopolysaccharides, since related substances were found in mucins). During all this time, very little attention was paid to the polypeptides to which the mucopolysaccharides were attached. It was tacitly assumed that the mucopolysaccharides were synthesized and attached to a polypeptide to form a proteoglycan. The polysaccharide portion of the proteoglycan was then sulfated and subsequently split from the proteoglycan. The sulfated polysaccharide then reacted with collagen to form a polar bond.<sup>(269)</sup>

This assumption was supported by the observation that chondroitin sulfates coprecipitated with collagen to form insoluble striated fibers seen in cartilage, with the striated fibers 640 Å apart. For this reason, chondroitin sulfates were always purified by treatment with proteolytic enzymes to remove any adhering polypeptides (impurities). In spite of this treatment, it was always difficult to remove the serine residue clinging to the chondroitin sulfate.<sup>(270,271)</sup>

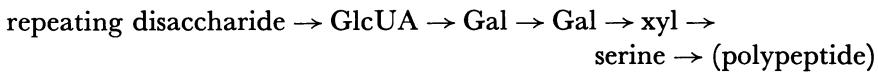
A new approach was taken in the 1950s when attempts were made to isolate the chondroitin sulfate-polypeptide complex intact by extracting with water or dilute saline.<sup>(270)</sup> These studies culminated in 1968 with the isolation of the chondroitin sulfate polypeptide. This was done by extraction with 4 N guanidine sulfate which serves to break the hydrogen bonds binding protein-chondroitin complexes to each other.<sup>(272-274)</sup>

The compounds isolated then came to be called *protein polysaccharides*, to distinguish them from the glycoproteins. Unlike the glycoproteins, they were sulfated and had been oxidized to form uronic acids. It was established soon that these mucopolysaccharides existed in covalent linkage with the polypeptides.<sup>(273,274)</sup>

In 1970, a review of this field appeared entitled "Protein Polysaccharides of Cartilage."<sup>(266)</sup> At that time, the generic name glycosaminoglycans was introduced and used internationally for what had been called the *chondroitins* or *mucopolysaccharides*. Only 3 years later, the term *protein polysaccharides*, which emphasizes the carbohydrate moiety, was replaced by the term *proteoglycans*, which stresses their existence as proteins.<sup>(267)</sup>

The average proteoglycan molecule, also called proteoglycan subunit (PGS), has a protein core of a molecular weight of about 200,000 and length of about 340 nm. To this core are attached about 100 chains of chondroitin and 50 chains of keratan sulfate; 90% of the proteoglycan subunit is carbohydrate and only 10% polypeptide. The molecular weight of the keratan sulfate is about 4000–8000 and of the chondroitins about 20,000. The total molecular weight is about 2.5 million.<sup>(275–277)</sup>

The repeating dipeptides of the glycosaminoglycan in cartilage are attached by a series of monosaccharides to serine of the polypeptide chain.<sup>(275–279)</sup> This series comprises xylose attached to serine, followed by galactose, another galactose, galacturonic acid, and then the repeating unit, glucuronate → *N*-acetylgalactosamine, of chondroitin sulfate. The sulfate esters are at the 4 or 6 position. For dermatan sulfate, the repeating unit is iduronate—*N*-acetylgalactosamine (Figure 5.33). Thus, the sequence for the core polysaccharide structure is



This structure is elaborated in Figure 5.42. In cartilage, keratan sulfate is linked to the serine or threonine residues of the core protein by *N*-acetylgalactosamine.<sup>(277)</sup>

### 5.11.1 *Proteoglycan Aggregates*

It had been known for some time that articular cartilage contained substantial amounts of chondroitin sulfates and hyaluronic acid and lesser amounts of keratan sulfate. Studies with the electron microscope coupled with biochemical studies, showed how these three substances were bound together to form the structure of cartilage (Figure 5.43).<sup>(280,281)</sup>

The proteoglycan aggregates consist of proteoglycan subunits arising laterally at fairly regular intervals (20–30 nm) from the opposite sides of an elongated filamentous structure made up of hyaluronic acid, which ranges in length from 400 to 4000 nm.

The fragment of the proteoglycan adjacent to the hyaluronic acid binding site contains the keratan sulfate. The chondroitin sulfate chains

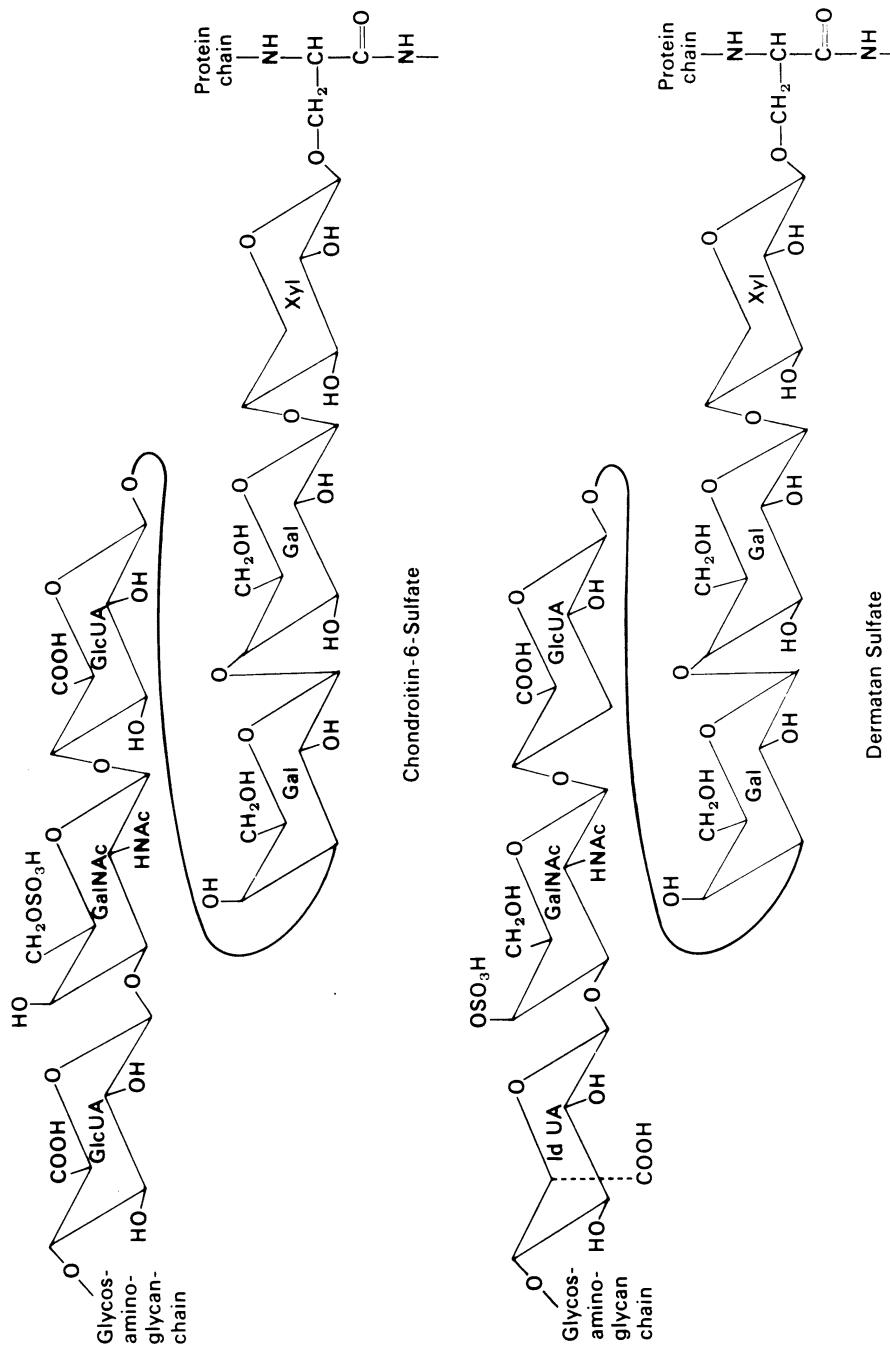


FIGURE 5.42 Linkage of repeating disaccharide units of chondroitin and dermatan sulfates to the polypeptide to form the proteoglycans.

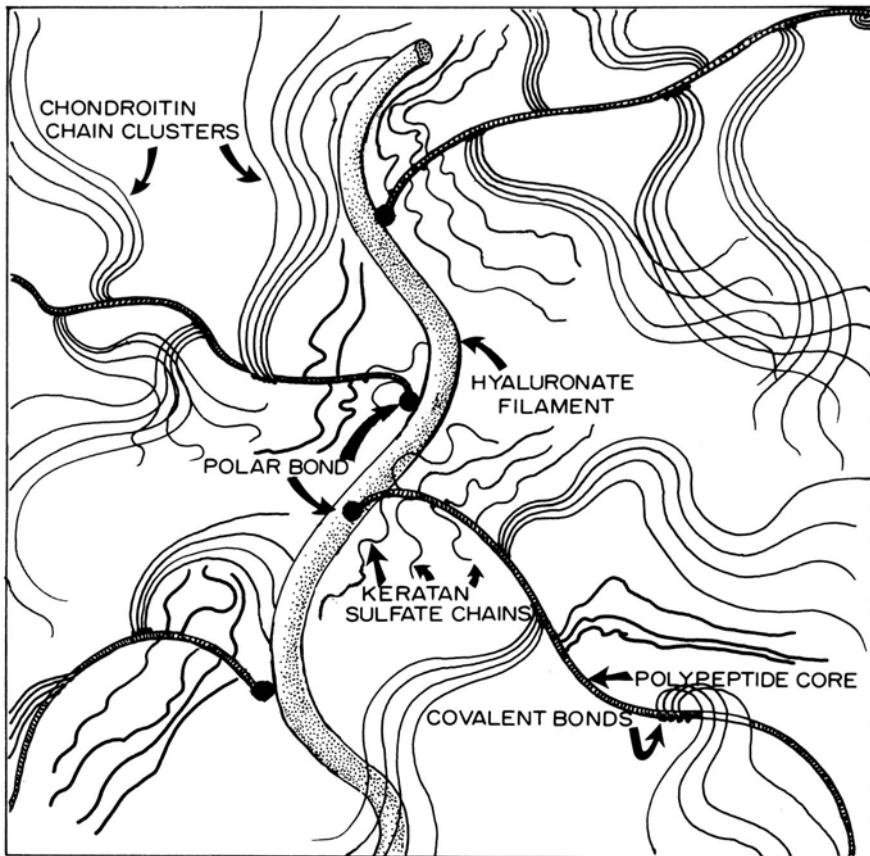


FIGURE 5.43 Schematic illustration of the structure of a proteoglycan aggregate. The polypeptide core is attached to a central hyaluronate filament. Attached to the polypeptide core are clusters of chondroitin sulfate chains. As the filament is approached, single short strands of keratan sulfate extend from the polypeptide chains. See Figure 5.44.

are further out, on the polypeptide chain. A schematic representation of this arrangement is shown in Figure 5.43. Figure 5.44 is an electron micrograph of a proteoglycan aggregate.

In cartilage, the core polypeptide and chondroitin chains of the monomer structure shown in Figure 5.43 tend to polymerize, the suspended chains entangling each other into a semi-rigid structure. This can be disentangled with 4 N guanidine salt solution at pH 7.4, which breaks the hydrogen bonding and solubilizes the monomers.

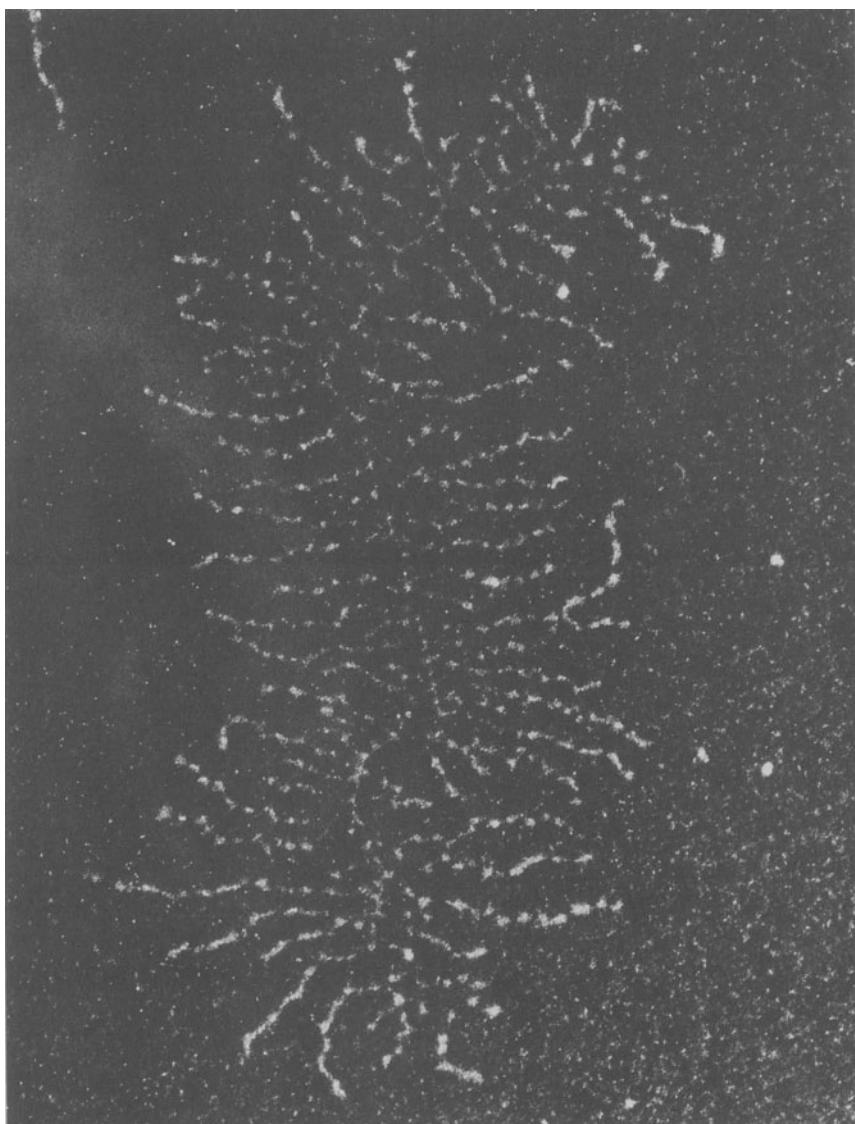


FIGURE 5.44 Proteoglycan–proteohyaluronate aggregate from bovine articular cartilage with 77 proteoglycan subunits arising from a filamentous backbone (hyaluronate), 1700 nm in length. Electron, dark-field, micrograph of proteoglycan–cytochrome-*c* complex. (Courtesy of Lawrence Rosenberg, Montefiore Hospital and Medical Center, Bronx, New York.)

*In the cornea, the keratan sulfate is bound to the core polypeptide, not to serine, but through N-acetylglucosamine to asparagine, as in the glycoproteins of plasma (see Figures 5.3 and 5.4).<sup>(282-284)</sup> Cartilage keratan sulfate is bound to serine or threonine of the core polypeptide through N-acetylgalactosamine, as mentioned above.*

*Heparan sulfate, which occurs in many tissues of the body, is also a macromolecular complex attached to a common protein core, as determined in brain proteoheparan sulfate.<sup>(285)</sup> The heparan sulfate in the brain is a polymer of a disaccharide of glucuronate and glucosamine sulfates, sulfated at the nitrogen and at the 6 position of the glucosamine. Thus, it is also a proteoglycan. The polypeptide core contains serine and glycine in equimolar proportions.<sup>(285)</sup>*

The proteoglycan structure of the neutral hyaluronate filament, to which the hyaluronic acid proteoglycans are attached, is still uncertain in 1979. The hyaluronate filament seems to be a single unbranched polymer attached to a protein differing from the other branched proteoglycans attached to it (Figure 5.43).<sup>(286,287)</sup>

### 5.11.2 Proteoglycan Synthesis

Almost every cell of the body has the capacity to synthesize proteoglycans. The mechanism of synthesis is similar to that described for the glycoproteins (see Section 5.4). The core protein is synthesized, and carbohydrates are then attached.<sup>(278,279,288)</sup> Retinol and dolichol are involved, and vitamin A deficiency results in impaired synthesis of the proteoglycans and disintegration of the cartilage.

The monosaccharides are transferred to the growing polysaccharide chain by transferases, as for the glycoproteins. Xylosyl transferase, galactosyl transferase I, galactosyl transferase II, and glucuronosyl transferase I serve to build the core polysaccharide chain. For chondroitin sulfate, this is followed by the action of N-acetylgalactosaminyl transferase I and glucuronosyl transferase II alternating to build the repeating units of chondroitin sulfate.

As the polysaccharide units are added to the growing chain, the molecules are sulfonated by "active sulfate." For this, 3'-phosphoadenylylsulfate (PAPS), and PAPS-transferase, are utilized (see Volume 1, pp. 180-182). In addition, *sulfation factor (somatomedin)* is required. Somatomedin is derived from the liver by action on the growth hormone. *If viable costal cartilage is added to a tissue culture medium containing sulfate,*

*the sulfate will not be incorporated unless somatomedin is present.* Growth hormone deficiency, as in the pituitary dwarf, results in defective sulfation of chondroitin and other aminoglycosoglycans, retarding the development of cartilage and thus the skeleton. A similar effect results from severe cirrhosis of the liver, under which condition somatomedin cannot be formed. It has been proposed that degenerative arthritis is partly a somatomedin deficiency resulting from a liver degenerative process or decrease in secretion of growth hormone.<sup>(289)</sup>

In the embryo, along with the synthesis of proteokeratans and proteochondroitins, there is simultaneous synthesis of hyaluronic acid and collagen so as to form the cartilage structure. Hyaluronidases appear at certain stages of embryo development. This permits break down of hyaluronate and remolding of the cartilage as the embryo develops. Circulatory hyaluronate removal is also necessary for cell differentiation and tissue construction.<sup>(290)</sup>

Proteoglycans synthesized by chondrocytes can be extruded so as to form cartilage (see Figures 5.6 and 5.7). On the other hand, proteoglycans and the glycosaminoglycans can be incorporated into cells such as fibroblasts by pinocytosis.<sup>(291)</sup>

The structure of the glycosaminoglycans and proteoglycan molecules vary, even when being synthesized by a single cell, because of the lack of precision in building the carbohydrate chains. This results in the heterogeneity of the synthesized proteoglycans and glycosaminoglycans as has been observed with the glycoproteins.<sup>(292-294)</sup>

Proteoglycan fragments which are formed with tissue destruction, as in injury or disease, need to be metabolized. For this purpose, carbohydrases are present, which serve to disintegrate and solubilize the carbohydrate fragments. Hyaluronidase is one such example. Various cathepsins present in liver and other tissues serve to hydrolyze the polypeptide core.<sup>(295)</sup> Healthy viable cartilage is protected from the cathepsins, since the intricate entanglement of chondroitin sulfate chains acts as a barrier to its action.

### 5.11.3 *Function of Proteoglycans in Health and Disease*

In different tissues, the type of glycosaminoglycan being synthesized varies also and the proportion in which their proteoglycans form a structure by complexing with collagen and elastin.

There are three major types of cartilage: fibrous, elastic, and hyaline. Fibrous cartilage is made up of parallel bundles of collagen fibers. Elastic cartilages are networks of fibers containing collagen and elastin. Hyaline cartilage is made up mostly of proteoglycans. In hyaline cartilage, only after removal of the proteoglycans by trypsin and alkali can the collagen fibrils be seen.

Cartilage may be easily deformable as skin or tendon, or it may have the hardness of bone cartilage. With advancing age, costal cartilage increases in stiffness. Cartilage, such as that of the vertebral disks, becomes dehydrated as the patient ages. This does not reflect changes in the osmotic properties of the proteoglycans but changes in their state of cross-linking and polymerization.<sup>(296)</sup>

Recent developments in our understanding of the structure of collagen has stimulated greater interest in the study of the biochemical changes which take place in cartilage in disease processes such as osteoarthritis. Although the subject is too novel for its applications to be apparent, questions are raised here which can be studied in the light of our recently acquired knowledge of the chemistry of the proteoglycans.

Joint cartilage can be damaged by trauma, inflammation, and mechanical or biochemical factors. A similar sequence of events can be produced by any of these factors. Osteoarthritis which results is therefore a heterogeneous disease. The earliest lesions seen in arthritis are on the surface of the cartilage. Alterations in the deeper zones with the disintegration of cartilage-forming clefts is progressive. The final phase is a failure of the reparative process, resulting in disintegration of the matrix, cell death, and total loss of cartilage integrity.<sup>(297)</sup> As the disease progresses, the rate of polysaccharide and DNA synthesis decreases. The cartilage which is synthesized shows formation of immature proteoglycan monomers, as indicated by alterations in the percentages of keratan and chondroitins being synthesized.<sup>(298,299)</sup> Cathepsins and other hydrolases are then released from the lysosomes which attack the partially degraded proteoglycans.

Where the disease is complicated by bacterial infection and inflammation, hyaluronidases are secreted by the bacteria which serve to disintegrate the filaments on which the proteoglycans are attached leading to disintegration of the tissue.

In rheumatic fever and rheumatoid arthritis, the inflammation affects not only the joints but also the proteoglycans of which the heart valves are formed, resulting in the typical heart defects observed with

these diseases. This has been repeatedly referred to in discussing the various mucopolysaccharidoses.

In periodontal disease, the cartilage supporting the teeth depolymerizes and disintegrates causing loosening and loss of the teeth. This occurs from trauma, as with malocclusion where excessive pressure is exerted at a particular point. More commonly it occurs with aging. The cause of this could possibly be connected with the decrease in liver function with age and decreased conversion of growth hormone (somatotropic hormone, STH) to the sulfation factor, somatomedin.

Major interest has been shown over the past 60 years in the role of cartilage in promoting mineralization of tissue and bone and teeth formation. If fibers are made by mixing collagen and chondroitin sulfate, both prepared from nasal septum, calcification will take place if placed in a calcifying solution (i.e., a solution containing  $\text{Ca}^{2+}$  and phosphate at the concentrations of normal serum). However, calcification does not take place in nasal septum *in vivo*. What factors guide the mineralization process?

Proteoglycans bind cations such as  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , and others. These in turn cause adherence of anions such as phosphate, chloride, citrate, and sulfate. As a result, 90% of the NaCl in the body is found in the cartilage and connective tissue. This serves as a huge reservoir for NaCl, and is of major importance for animals who can only make periodic trips to salt licks since it is distant from their food supply. It is also probable that mineralization of bones and teeth starts with  $\text{Ca}^{2+}$  binding which then binds phosphate to form the hydroxyapatite structure.

Not only in the rheumatic diseases, but in other conditions including multiple epiphelial dysplasia<sup>(300)</sup> and cancer, such as in malignant glial cells,<sup>(301)</sup> abnormal proteoglycans are synthesized.

Another disease which deserves further study in connection with the proteoglycans is *cystic fibrosis*. The end group, on the nonreducing end of keratan sulfate is fucose. Thus in fucosidosis, keratan sulfate appears in the urine.<sup>(302)</sup> In cystic fibrosis, increased amounts of fucose have been reported in the urine.<sup>(303)</sup> This relationship needs to be explored.

The significance of the problems raised above are now being explored in the light of our recent understanding of the biochemistry of the proteoglycans.<sup>(304)</sup>

## 5.12 RECAPITULATION

*Glycoproteins* are proteins in which carbohydrate is attached by a covalent bond. *Proteoglycans* are acidic glycoproteins where some of the monosaccharides have been oxidized to uronic acids; they occur often as sulfate derivatives giving them a strongly acidic character.

Almost all the plasma proteins are glycoproteins. A notable exception is albumin. However, albumin of the hen's egg is a glycoprotein.

Glycoproteins are synthesized on the endoplasmic reticulum. A sequence of polysaccharides first accumulates on dolichol or retinol (Vitamin A). This core is transferred to a newly synthesized polypeptide in the Golgi apparatus and additional monosaccharides are added sequentially. Finally, sialic acid and fucose are added. In the case of the proteoglycans, sulfation proceeds as the carbohydrate chain is being constructed. The monosaccharides and sulfate are all added to the growing polysaccharide by transferases, specific for each monosaccharide or sulfate added.

The attachment of the heterosaccharide to the polypeptide chain is to the nitrogen of asparagine or to the hydroxyl group of serine, threonine, or hydroxylysine. The core monosaccharides attached to each type of linkage are characteristic. To asparagine, *N*-acetylglucosamine residues followed by mannose are attached most often. The mannose is then attached to two or three chains to form branches. *N*-acetylgalactosamine is usually the monosaccharide attached to serine or threonine. In the proteoglycans, xylose is attached to the hydroxyl group, followed by two galactoses and then a glucuronate residue. To this is attached repeating units of disaccharides. The repeating units for chondroitin sulfate are glucuronate-*N*-acetylgalactosamine sulfate. For dermatan sulfate, the repeating unit is sulfonated L-iduronate-GalNAc. For keratan, the repeating unit is sulfonated *N*-acetylglucosaminegalactose.

Heparin occurs in various tissues bound to a protein and is derived from a proteoglycan called heparan sulfate. The repeating unit in heparan sulfate is glucuronate-glucosamine. Hyaluronic acid has a similar sequence with the glucosamine acetylated.

In inflammation, the glycoproteins of the plasma such as  $\alpha_1$ -acid glycoprotein, C-reactive protein, haptoglobin, ceruloplasmin, and those of the complement and clotting system are elevated. These are

called the acute-phase reactants or acute-phase proteins. They are all involved with different functions in the mechanism of defense against invading foreign bodies. Certain glycoproteins are associated with pregnancy. These are also acute-phase reactants.

There are at least 30 glycoproteins in plasma occurring in low concentration associated mainly with the  $\alpha$  electrophoretic fractions of the serum. They tend to have a high percentage of carbohydrate and do not precipitate with dilute perchloric acid. They are referred to as seromucoids. The term *mucoprotein* is usually used to indicate a glycoprotein of high polysaccharide content, like those found in the mucus.

Glycoproteins and proteoglycans are degraded by lysosomal hydrolases, specific for each linkage in the heterosaccharide chain. Absence of any one of these results in accumulation of polysaccharides in the lysosomes. This results in swelling of the lysosomes and thus of the tissue. Accumulation of carbohydrate occurs in brain interfering with its function. This results in mental retardation. This occurs also with the sphingolipidoses and related diseases discussed in Volume 2 of this series.

When mannose or fucose cannot be hydrolyzed from the heterosaccharide, mannose- or fucose-containing polysaccharides appear in the urine. These diseases are called *mannosidosis* and *fucosidosis*, respectively. If the *N*-acetylglucosamine cannot be hydrolyzed from the aspartyl linkage of the polypeptide chain, the disease is called *aspartyl-glucosaminuria*.

A group of diseases occurs where the heterosaccharide chains, attached to the polypeptide of the proteoglycans, cannot be catabolized because of a lysosomal hydrolase defect. Hurler's and Hunter's syndrome are the prototypes for these diseases. They are referred to as the *mucopolysaccharidoses* after the nature of the polysaccharide found in the urine and deposited in the lysosomes. The mucopolysaccharides found in the urine and tissues in these diseases are dermatan and keratan sulfates. They are characterized by skeletal changes and characteristics of the lysosomal storage diseases.

Another group of lysosomal storage diseases are the *mucolipidoses*. In these conditions, the enzymes of the lysosomes are released into the plasma and are therefore in low concentration in the cells. The disease is limited to the cells producing cartilage, connective tissue, and skin. Some evidence has been adduced to suggest a neuraminidase deficiency in these patients.

In inflammatory disease, the proteoglycan aggregates which make up collagen are depolymerized. This exposes them to the action of cathepsins and hyaluronidase which serves to cause the cartilage to disintegrate.

A major function of the glycoproteins is for the purpose of distinguishing, by the defense mechanisms of the body, which cells are native and which are the invaders. For this purpose, heterosaccharide complexes are attached to polypeptides on the cell surface, unique for the individual. These are the histocompatibility antigens. Similarly, the erythrocytes have glycoproteins bound to the cell wall which are characteristic of the various blood groups.

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