INTRODUCTION

CHAPTER-I

INTRODUCTION

Glycoproteins have a wide distribution in nature and serve a vast number of functions. Recently there has been an increased interest in the structure, biosynthesis, catabolism and functions of glycoproteins, which has led to a commensurate increase in our knowledge of all these aspects of glycoproteins.

Gottschalk A.(1966); Spiro R.G.(1970); Marshall and Neuberger A. (1972); Hubbard S.C. and Ivatt R.J. (1981) and Kornfeld R. and Kornfeld S. (1985) have studied in detail about the chemistry and metabolism of glycoproteins.

Almost all the plasma proteins of humans, except albumin, are glycoproteins. Many proteins of cellular and extracellular membranes contain substantial amounts of carbohydrates. Proteins of mucous secretions, hormones, enzymes and both soluble and insoluble components of connective tissue also contain carbohydrates (Spiro R.G., 1969).

Glycoproteins are proteins that have oligosaccharide chains covalently attached to their polypeptide back bones. They are distinguished from other carbohydrate polymers, namely proteoglycans by the absence of the repeating units and hexuronic acids.

Glycoproteins have characteristic sugar components which includes D-galactose, D-mannose, D-glucose, L-fucose, N-acetyl-D-glucosamine, N-acetyl-D-galactoseamine, N-acetyl neuraminic acid, D-xylose and L-arabinose. The carbohydrate portion of a glycoprotein consists of one to seven of these sugar types. Glycoproteins are not characterised by a unique amino-acid composition.

The carbohydrate contents of glycoproteins are termed as their prosthetic moieties. The term protein-bound carbohydrate and serum glycoproteins are equivalent, protein-bound hexose (PBH), protein-bound hexosamine (PBHA), protein-bound sialic acid (PBSA) and protein-bound fucose (PBF) means that hexose, hexosamine, sialic acid and fucose respectively are found combined with proteins. Hence the amount of protein-bound carbohydrate in serum is a direct measure of the serum glycoproteins.

Various methods like automated ion-exchange separation (Kesler R.B.; 1967; Lee Y.C. et al, 1969) and gas liquid chromatography (Kim et al, 1969 and Lehnhardt W.F. and Winzler R.J., 1968) have been applied for the analysis of the sugar components of glycoproteins. The sequential application of the Smith periodic acid degradation has been used in the study of several glycoproteins (Spiro R.G., 1964; Makino M, Yamashina I, 1966; Yamaguchi H. et al, 1969). A number of purified glycosidases have been described which have been useful in the study of sugar sequences in glycoproteins (Spiro R.G., 1962).

The carbohydrate units of glycoproteins range in size from simple monosaccharide or dissacharide as in mucins (Graham E.R.B. and Gottschalk A., 1960; Carlson D.M., 1968) and collagens (Spiro R.G., 1969) to complex heteropolysaccharide units as in plasma glycoproteins (Sprio R.G. 1968). Some glycoproteins such as ovalbumin, ribonuclease B, deoxyribonuclease or Taka amylase have only a single carbohydrate unit per molecule, while submaxillary glycoproteins have multiple units reaching up as high as 800 (Graham E.R.B., Gottschalk A., 1960). Table No.1 shows the number of carbohydrate units of various glycoproteins. Because of the wide variations in the molecular weight of glycoproteins, the extent of carbohydration of these molecules can be judged in terms of the number of amino-acids per carbohydrate unit as seen in Table No.1.

A detailed study of the structure of carbohydrate units of a large number of glycoproteins has revealed a relatively conservative structural patterns with certain preferred sequences recurring in many proteins. Figure No.1 shows some of these structural patterns.

Figure 1A represents a frequently occuring structural pattern. A heteropolysaccharide unit consisting of oligosaccharide chains with the sequence sialic acid (or fucose) → galactose →N-acetylglucosamine → linked to a core of mannose and additional N-acetylglucosamine residue, occurs in fetuin (Spiro R.G., 1962 and 1964), the alpha-1-acid glycoprotein (Huges C.R. and Jeanlox R.W., 1966; Sato T. et al, 1967; Wagh P.V., 1969), the alpha-2-macroglobulin (Dunn J.T. and Spiro R.G., 1967), thyroglobulin (Spiro R.G. and Spiro M.J., 1965; and Spiro R.G., 1965), the

TABLE 1 CARBOHYDRATE UNITS OF REPRESENTATIVE GLYCOPROTEINS: SPACING AND NUMBER

Protein	Amino acids per carbohydrate unit[a]	Number of units per molecule
Asn-linked:		
α_1-Acid glycoprotein	41	5
Ribonuclease (porcine)	42	3
Ovomucoid (hen)	56	3
Haptoglobin (human)	113	13
Fetuin	120	3
Ribonuclease B (bovine)	124	1
α_2-Macroglobulin (human)	209	31
Deoxyribonuclease	270	1
Thyroglobulin (calf)	296	19
Transferrin (human)	375	2
Taka-amylase (Asp. oryzae)	433	1
Ovalbumin (hen)	507	1
IgG immunoglobulin (human)	776	2
Hemagglutinin (soy bean)	860	1
Ser(thr)-linked:		
Submaxillary glycoprotein (ovine)	6	800
Submaxillary glycoprotein, A^+ (porcine)	8	496[b]
Hyl-linked:		
Lens capsule (cow)	27	
Glomerular basement membrane (cow)	58	
Corneal collagen (rabbit)	173	19
Skin collagen (calf)	435	8
Tendon collagen (bovine)	556	6
Swim bladder collagen (carp)	716	5
Skin collagen (rat)	770	4
Scleral collagen (rabbit)	1,000	3

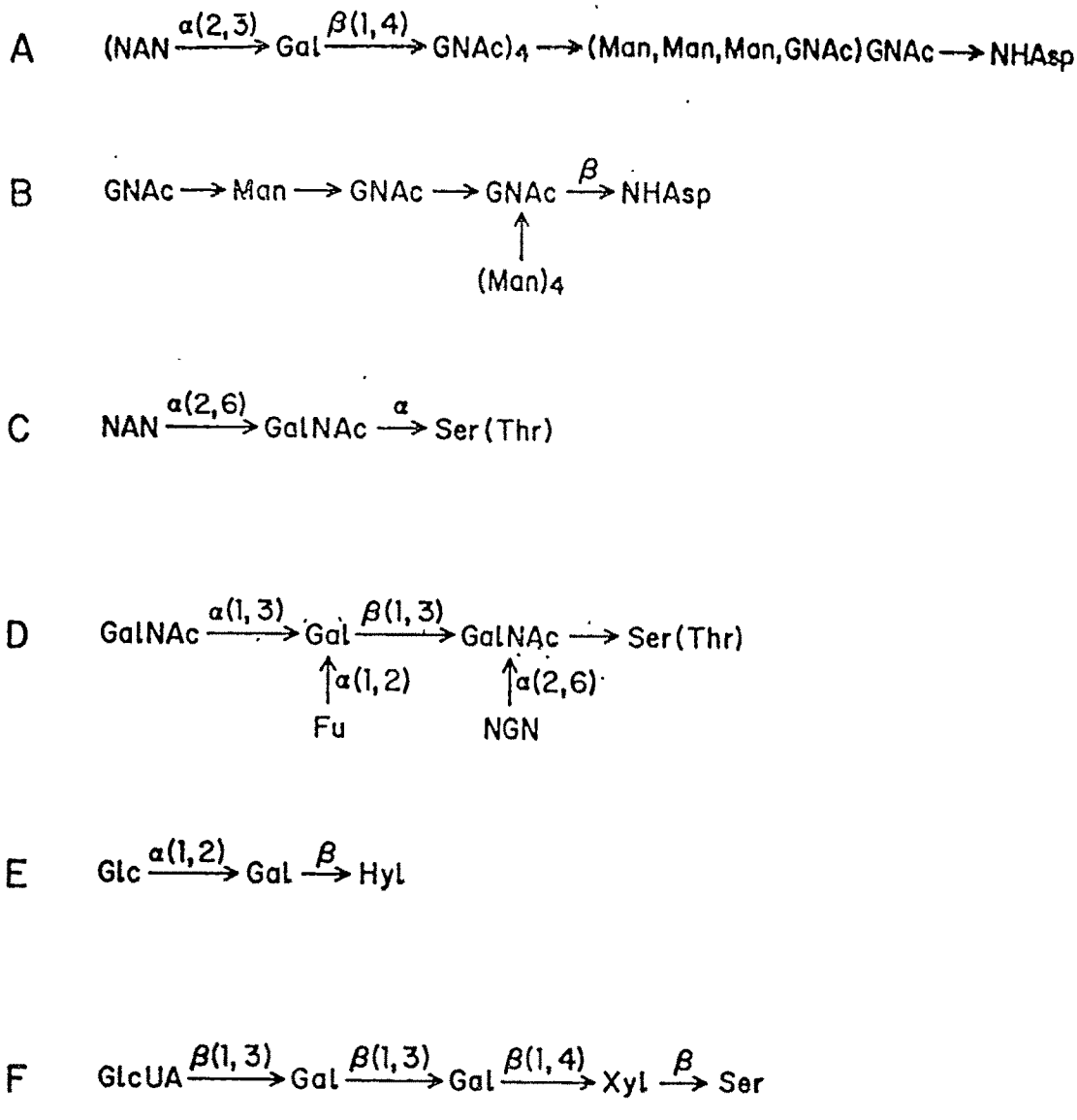
[a] Total number of amino acids in glycoprotein divided by the number of carbohydrate units shown in the third column.

[b] Number of units calculated from galactose content of porcine submaxillary glycoprotein; since incomplete units without galactose also occur, this is a minimal value.

Adopted from Spiro R.G. (1970)

FIGURE-I

SUGAR SEQUENCES AND THE GLYCOPEPTIDE LINKAGES OF GLYCOPROTEINS



ADOPTED FROM SPIRO R.G. (1970)

IgG (Rothfus J.A. and Smith E.L., 1963) and IgA immunoglobulin (Dawson G. and Clamp J.R., 1968), fibrinogen (Bray B.A. and Laki K., 1968), and glomerular basement membrane.(Spiro R.G.,1967a)

Figure 1B shows units of simpler patten consisting only mannose and N-acetylglucosamine residues present in ovalbumin (Johansen P.G. et al, 1961).

Figure 1C shows mannose N-acetylglucosamine carbohydrate units. They have a significantly greater number of mannose residue as seen in thyroglobulin (Spiro R.G., 1968), Ribonuclease B (Plummer T.H.,Jr. and Hirs C.H.W., 1964), Taka-amylase (Yamaguchi H., et al, 1969) and deoxyribonuclease (Catley B.J. et al, 1969).

The carbohydrate units of mucins, many having blood group activity, are made up of galactose, N-acetylgalactosamine, N-acetylglucosamine, N-acetylneuraminic acid and fucose. They do not contain mannose residues.

Figure 1D exibits the structure of the sialyl-N-acetylgalactosamine disaccharide and pentasaccharide from the ovine submaxillary gland (Graham E.R.B. and Gottschalk A., 1960) and procine submaxillary gland (Carlson D.M., 1968) respectively, having blood group activities.

Figure 1E shows the alpha (1 $\rightarrow$ 2) glucosylgalactose disaccharide present in collagens and basement membranes (Spiro R.G., 1967; Spiro R.G., 1969 and Spiro R.G. and Fukushi, 1969).

While most proteins contain carbohydrate units of only one structural pattern, the occurrance of more than one type of carbohydrate unit has been reported in several glycoproteins. All of these proteins contain the complex heteropolysaccharide unit (Fig 1.A) but in addition, an IgA immunoglobulin contains both a mannose-N-acetylglucosamine unit and sialic acid-galactose-N-acetylgalactosamine units (Dawson G., Clamp J.R., 1968) and the glomerular basement membrane contains glycosylgalactose disaccharides (Spiro R.G., 1967). The fetal plasma glycoprotein, fetuin, in addition to its complex heteropolysaccharide units, contains sialic acid, galactose and N-acetylgalactosamine which are linked by the galactosamine to serine residues in the peptide chain (Spiro R.G. - unpublished data).

Spiro R.G. (1970) suggested the occurrance of microheterogeneity in glycoproteins due to the presence of their carbohydrate units in varying stages of completion or minor modifications. Investigations on the alpha-2-macroglobulin of human plasma, exhibit microheterogeneity (Dunn J.T., Spiro R.G., 1967), containing 31 heteropolysaccharide units showed marked differences in size and composition. It is also observed that the heterogeneity may also be due to positional isomerism as seen in the alpha-1-acid glycoprotein. The sialic acid in this glycoprotein can be linked to carbon 3,4 or 6 of the galactose (Jeanlox R.W., 1966).

Carbohydrate-Protein Linkage :

The attachment of the carbohydrate to the peptide portion represents the most unique structural feature of glycoprotein. This linkage involves the carbon-1 of the most internal sugar and a functional group of an amino acid

within the peptide chain. Several distinct types of glycopeptide bonds have been described as follows :

1. The first is a glycosylamine bond, in which N-acetylglucosamine is linked by a β-glycosidic bond to the amide group of asparagine (Marshall R.D. and Neuberger A., 1964). This glycopeptide bond occurs in large number of glycoproteins including plasma proteins, enzymes, hormones, immunoglobulins etc. as shown in Table No.2.

2. Another major type of carbohydrate - peptide linkage involves an O-glycosidic bond to serine and threonine (Anderson B. et al, 1964). The sugar component of this linkage can be either N-acetylgalactosamine as in mucins (Tanaka K. et al, 1964; Carbelli R., et al, 1965) and cartilage keratan sulfate (Bray B.A., 1967) or galactose as in the earthwarms (Muir L., Lee Y.C., 1969).

3. The third type of glycopeptide-bond involves the amino acid hydroxylysine. This bond involves a β-glycosidic linkage between the galactose residue and the hydroxyl group of hydroxylysine (Spiro R.G., 1967). Contrary to the O-glycosidic linkages to serine and threonine, the hydroxyl groups involved in the linkage to hydroxylysine is on the delta-carbon and therefore is stable to strong alkali treatment. This type of bond is found in glomerular basement membrane (Spiro R.G., 1967) and has been found in other collagens (Spiro R.G., 1969) and basement membranes (Spiro R.G.; Fukushi S., 1969).

TABLE 2: CLASSIFICATION OF CARBOHYDRATE UNITS OF GLYCOPROTEINS[a]

	Glycopeptide bond				Carbohydrate unit		Glycoproteins[c]
	Amino acid	Sugar	Type	Stability to alkali	Composition[b]	Mol wt range	
1.	Asn	GlcNAc	N-glycoside	Stable to weak alkali; split by moderate alkali	NAN, NGN, Fu, Gal, GlcNAc, GalNAc, Man	2000–3500	Plasma proteins, fetuin hormones, immunoglobulins, thyroglobulin, porcine ribonuclease, basement membranes
	Asn	GlcNAc	N-glycoside	Stable to weak alkali; split by moderate alkali	Man, GlcNAc	1200–4900	Enzymes, egg white proteins, thyroglobulin, IgA immunoglobulin, soybean hemagglutinin
	Asn	GlcNAc	N-glycoside	Stable to weak alkali; split by moderate alkali	NAN, Fu, Gal, GlcNAc, Man, sulfate	<10,000[d]	Corneal keratan sulfate-protein[g]
2.	Ser, Thr	GalNAc[e]	O glycoside	Split by mild alkali through β-elimination	NGN, NAN, Fu, GalNAc, GlcNAc	203-1225[f]	Mucins, cell membranes, immunoglobulins, fetuin
	Ser, Thr	GalNAc	O-glycoside	Split by mild alkali through β-elimination	NAN, Fu, Gal, GlcNAc, GalNAc, sulfate	3500–10,000[d]	Cartilage keratan sulsulfate -protein[g]
	Ser, Thr	Gal	O-glycoside	Split by mild alkali through β-elimination	Gal	162–486[h]	Annelid cuticle
3.	Ser	Xyl	O-glycoside	Split by mild alkali through β-elimination	GlcUA, IdUA, GlcN (Ac, SO_3H) GalNAc, Gal, Xyl, O-sulfate	13,000-29,000[d]	Proteoglycans (protein complexes of chondroitin sulfates, dermatan sulfate, heparin, heparan sulfate)
4.	Hyl	Gal	O-glycoside	Stable to strong alkali	Glc, Gal	162–324	Basement membranes and collagens
5.	Hyp	Ara	O-glycoside	Stable to strong alkali	Ara[i]	i	Plant cell walls

Adopted from Spiro R.G. (1970)

4. Another type of glycopeptide linkage involving hydroxyproline is seen to occur in the cell wall of higher plants (Lamport D.T.A., 1969). In this bond L-arabinose is believed to be attached by an alkali-stable O-glycosidic bond to the hydroxyproline.

Classification of Glycoproteins

Table No.2 shows the classification of glycoproteins by Spiro R.G. (1970). Despite the diversity of the proteins listed in Table No.2, they are considered togather as a group because of the presence of covalently linked carbohydrate which necessitates the occurrance of certain special characteristics in their structure and requires the involvement of extra enzymatic steps in their assembly.

As shown in Table No.2 glycoproteins are chemically classified into four classes on the basis of the structural features of the carbohydrate units and the type of glycopeptide linkages. They are :

a) Asparagine-linked carbohydrate units.
b) Serine-threonine-linked carbohydrate units.
c) Hydroxylysine-linked carbohydrate units, and
d) Hydroxyproline-linked carbohydrate units.

a) Asparagine-linked carbohydrate units are linked to the peptide chain by an N-glycosidic bond. On the basis of their carbohydrate composition they

are further sub-divided into three major forms (Kornfeld K. and Kornfeld S., 1985) like :

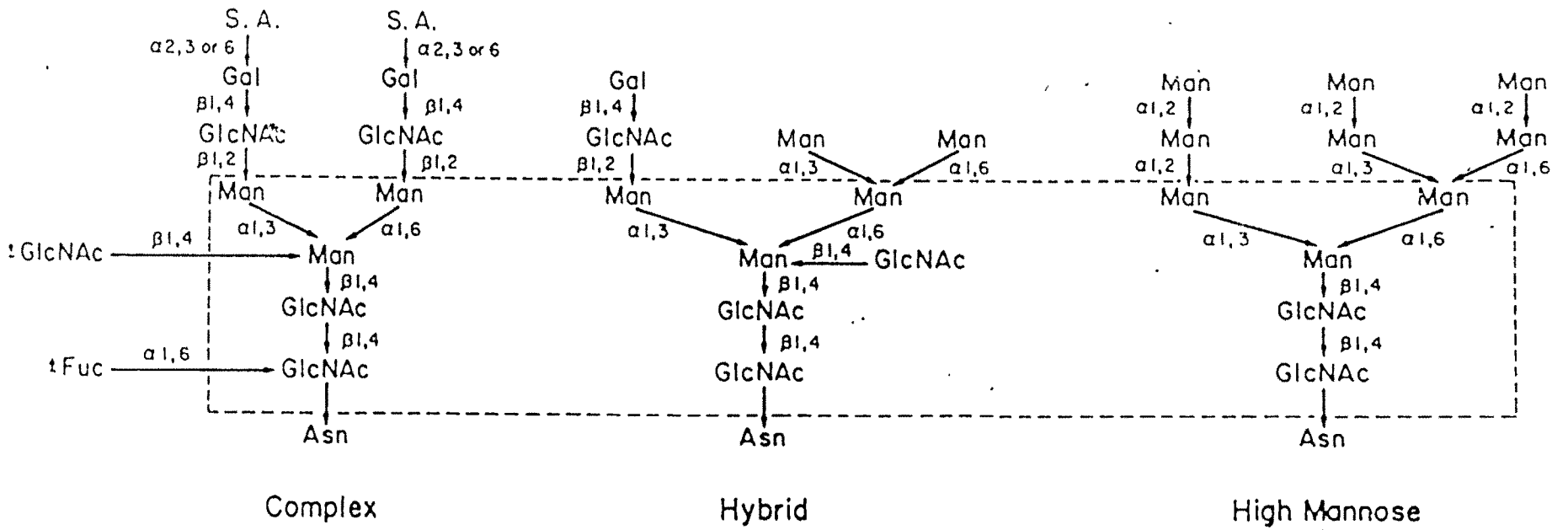
I) High mannose
ii) Hybride, and
iii) Complex form.

All these three forms share the common pentasaccharide core structure Man-α 1→3 (Man α 1→6) Man β-1→4 Glu NAc-β 1→4-Glu NAc-Asn, but differ in their outer branches. As shown in Figure 2, the high mannose-type oligosaccharides typically have two to six additional mannose residues linked to the pentasaccharide core.

The hybrid molecules as the name suggests, have features of both high mannose and complex type oilgosaccharides. Most hybrid molecules contain a 'bisecting' N-acetylglucosamine-linked β 1→4 to the β-linked mannose residue, although some exceptions exist (Varki and Kornfeld, 1983 and Yamashita K. et al, 1983a).

The complex type structure shown in figure 2 contains two outer branches with the typical sialyl lactosamine sequence and shows two other commonly found substituents, namely, a fucose in alpha 1,6 linkage to the inner most N-acetyl glucosamine residue and a 'bisecting' N-acetylglucosamine-linked beta 1,4 to the beta-linked mannose residue. This complex type structure may be modified both by the addition of extra branches on the mannose residue or by the addition of extra sugar residues that elongate the outer

FIGURE-II : STRUCTURES OF THE MAJOR TYPES OF ASPARAGINE-LINKED OLIGOSACCHARIDES. THE BOXED AREA ENCLOSES THE PENTASACCHARIDE CORE COMMON TO ALL N-LINKED STRUCTURES.



ADOPTED FROM KORNFELD, K. AND KORNFELD, S. (1985)

chains. Majority of this complex type oligosaccharides contain 2,3 or 4 outer branches, but units with 5 outer branches have been found in avian ovomucoids (Paz-Parente et al, 1982 and Yamashita K. et al, 1983b). Krusius T., et al (1978) and Jarnefelt J. et al, (1978) discovered another outer branch structure consisting of repeating lactosamine disaccharide on erythrocyte membrane glycoprotein and called erythroglycans. These polylactosamine oligosaccharide may be substituted in various ways and have been shown to carry A B H and I blood group antigens (Childs R.A. et al 1978 and Krusius T., 1978).

All of the asparagine-linked oligosaccharide structures have the common pentasaccharide core structure as they all arise from the same biosynthetic precursor, lipid-linked oligosaccharide which is transferred to nascent peptide chains and then processed to form these various structures (Kornfeld and Kornfeld S., 1985).

b) **Serine-Threonine-linked carbohydrate units** :

The serine and threonine-linked units are alkali labile O-glycosides. They are also sub-divided into 3 types depending on the sugar involved in the glycopeptide bond and the nature of the carbohydrate unit (Spiro R.G., 1970) as shown in Table No.2.

All the mucins contain carbohydrate units linked to serine and threonine by N-acetylgalactosamine residues. The sugar components present in these units includes sialic acid, fucose, galactose,

N-acetylgalactosamine and N-acetylglucosamine (Carlson D.M., 1968). Some cell membrane glycoproteins are also linked to the peptide chain by N-acetylgalactosaminyl bonds to serine and threonine (Thomas D.B., Winzler R.J., 1969; Winzler R.J. et al, 1967).

All the immunoglobulins contain most of the carbohydrate in the form of asparagine-linked units, a serine-linked unit consisting of sialic acid, galactose and N-acetylgalactosamine has been observed in the IgA myelomaprotein (Dawson G., Clamp J.R., 1968).

A group of sulphated glycoproteins is found to occur in gastric mucosa (Pamer T. et al., 1968) and colonic mucosa (Inone S., Yosizawa Z., 1966) as well as in the submaxillary gland (Bignardi C., et al, 1964). These glycoproteins contain galactose, N-acetylglucosamine, N-acetylgalactosamine, sialic acid, fucose and sulfate esters. The very high content of threonine and serine residues in these glycoproteins suggest that the carbohydrate is linked to the peptide chain through these amino acids.

c) **Hydroxylysine-linked carbohydrate units**

These are simple units consisting of 2-0-alpha-D-glucosylgalactose disaccharides linked to the hydroxyl group of hydroxyllysine by an alkali stable O-glycosidic bond. This type of carbohydrate units is observed in a variety of collagens obtained from tendon, skin, cornea, sclera (Spiro R.G., 1969), the renal glomerular basement membrane (Spiro R.G., 1967) and the lens capsule of the eye (Spiro R.G. and Fukushi S., 1969).

d) **Hydroxyproline-linked carbohydrate units**

The hydroxyproline-linked carbohydrates isolated from the cell walls of plants are monosaccharide and oligosaccharide units (upto four residues in length) made up of arabinose residues (Lamport D.T.A., 1969). It is not established how the other sugar components of plant cell relate to this linkage region.

The classification of carbohydrate units in Table No.2 indicates that there is a strong tendency for certain biological groups of glycoproteins to have similar carbohydrate units, as in case of the plasma proteins with asparagine-linked complex heteropolysaccharide units and the mucins having the serine-threonine-N-acetylgalactosamine linkage. However, a number of glycoproteins, like thyroglobulin (Spiro R.G. 1965), IgA immunoglobulin (Dawson G., 1968), fetuin (Spiro R.G. - unpublished data) glomerular basement membrane (Spiro R.G., 1967), lens capsule (Spiro R.G. and Fukushi S., 1969) contain more than one distinct type of unit and even in some cases, more than one type of glycopeptide linkage.

Functions of Glycoproteins

Glycoproteins participate in large number of normal and disease-related functions of clinical relevance (Marshall R.D., 1979). For example, many of the proteins in the outer cellular membrane are glycoproteins. Such proteins within the cell surface are antigens, which determine the blood antigen system (A,B,O) and the histocompatibility and transplantation determinants

of an individual. Immunological antigenic sites and viral and hormone receptor sites in cellular membranes are often glycoproteins. In addition, the carbohydrate portions of glycoproteins in membrane provide a surface, code for cellular identification by other cells and for contact inhibition in the regulation of cell growth. As such, changes in the membrane glycoproteins can be correlated with tumorigenesis and malignant transformation in cancer (Schachter H., 1986).

Most of the important plasma proteins, except albumin, are glycoproteins. These plasma proteins include the blood clotting proteins, the immunoglobulins and many of the complement proteins.

Some of the protein hormones, such as follicle stimulating hormone and thyroid stimulating hormone, are glycoproteins. The important structural protein, collagen contains carbohydrate . The proteins found in the mucus secretions are carbohydrate, containing proteins, where they perform a role in lubrication and in the protection of epithelial tissue. The antiviral protein, interferon, is a glycoprotein.

Biosynthesis of Glycoproteins

Liver is the major site for the synthesis of carbohydrate and protein moieties of plasma glycoproteins (Robinson et al, 1964; Macbeth et al, 1965). Similar observations have also been reported by Chandler and Neuhans (1964) and Weimer et al (1965).

Biosynthesis of glycoproteins take place besides liver in kidney, spleen, lung, muscle and intestine (Shetlar, 1961; Robinson et al, 1964), the retina (Berman, 1964); submaxillary gland (Elchberg and Karnovsky, 1963); gastric mucosa (Kornfeld et al, 1965); lymphoid cells (Smiley and Jasin, 1965) and thyroid (Seed and Goldberg, 1965).

Synthesis of O-linked Glycoproteins

The polypeptide chains of these and other glycoproteins are encoded by m-RNA, because most glycoproteins, membrane-bound or secreted, are generally translated on membrane-bound polyribosomes.

The sugars of the oligosaccharide chains of the O-glycosidic type of glycoproteins are built up by the step-wise donation of sugars from nucleotide sugars, such as UDP Gal NAc, UDP Gal and CMP-Neu Ac (Carlson D.M. et al, 1964 and McGuire E.J. and Roseman S., 1967). The enzymes catalyzing this type of reaction are membrane-bound glycoprotein-glycosyltransferases. Several glycoprotein glycosyltransferases involved in the synthesis of oligosaccharides with A B H and Lewis blood group have been found in the particle fraction from gastric mucosa (Ziderman D. et al, 1967). Two separate fucosyl-transferases (Shen L., et al, 1968; Grollman E.F., et al, 1969), two distinct galactosyltransferases (Zinderman D., 1967; Race C., et al, 1968: Kobata A., et al 1968), one N-acetylgalactosaminyl transferase (Hearn V.M. et al, 1968) and one N-acetylglucosaminyl transferase (Zinderman D., 1967) have been observed.

The enzymes catalyzing addition of the inner sugar residues are located in the endoplasmic reticulum. The addition of the first sugar occurs during translation (i.e. cotranslational modification of the proteins occurs). The enzymes adding the terminal sugars, like acetyl-neuraminic acid, are located in the golgi apparatus.

On the basis of electron microscopic studies conducted on cells engaged in the export of proteins, it is believed that the peptide synthesized on the polysomes by usual methods of protein synthesis move through the channels of the endoplasmic reticulum (Warshawsky H., et al, 1963; Nadler N.J., et al, 1964). As it progresses through these channels, membrane-bound transferases (Horowitz A.L., Dorfman A., 1968), probably attach one sugar at a time to the growing carbohydrate unit. After the passage through the endoplasmic reticulum, the glycoprotein is believed to reach the golgi region where it is concentrated and packed for secretion. In cells active in the synthesis of glycoproteins, the golgi complex is quite prominent and reacts intensely with glycoprotein stains (Rambourg A., et al, 1965).

Synthesis of N-linked Glycoproteins

Hubbard S.C., et al (1981) and Snider M.D. (1984) reviewed the steps in the synthesis of the lipid-linked oligosaccharide precursor. Leloir (1951) and his colleagues described the occurrence of a lipid carrier, dolichol-phosphate (oligosaccharide-pyrophosphoryl dolichol), which plays a key role in the biosynthesis of N-linked glycoproteins.The N-linked glycoproteins is the major class of glycoproteins and has been studied in detail, since the most

readily accessible glycoproteins like plasma proteins, belong to this group. It includes both membrane bound and circulating glycoproteins. The biosynthesis of N-linked type is quite different from that of the O-linked glycoproteins.

The oligosaccharide chain of this group generally has the structure $(Glc)_3$ $(Man)_9$ $(Glu\ NAc)_2$-R, (R = P-P-Dol). The sugars of this compound are first assembled on the pyrophosphoryl-dolichol backbone and the oligosaccharide chain is then transferred enbloc to suitable asparagine residue of acceptor apoglycoproteins during their synthesis on membrane-bound polyribosomes. The process of synthesis occurs in two stages :

i) Assembly and transfer of oligosaccharide-pyrophosphoryl dolichol, and
ii) Processing of the oligosaccharide chain.

The efficient glycosylation of proteins is dependent on a sufficient pool of completely assembled and glycosylated lipid-linked oligosaccharide donor, an adequate activity of oligosaccharide transferase and a properly oriented and accessible asparagine - x - serine (threonine) sequence in the acceptor (x = any amino acid except proline and aspartic acid).

Asparagine-linked oligosaccharide may undergo further post-translational modification, including phosphorylation of mannose residues, sulfation of mannose and N-acetylhexosamine residues, and O-acetylation of sialic acid residues.

Regulation of the Glycosylation of Glycoproteins

It is evident that glycosylation of glycoproteins is complex process, involving a large number of enzymes. One index of its complexity is that seven distinct Glu NAc-transferases involved in glycoprotein biosynthesis have been reported, and a number of others are theoretically possible. Difference in the relative activity of these enzymes among species and tissues can account for many of the variations in oligosaccharide structures that occur.

Second determinant of oligosaccharide processing is the location of the protein in the cell. Thus, resident endoplasmic reticulum glycoproteins, not having been exposed to the golgi processing enzymes, would be expected to have only high mannose units (Liscum, et al, 1985).

Oligosaccharide processing of proteins that exit the endoplasmic reticulum is controlled by a number of factors, including the conformation of the polypeptide backbone (Hunt L.A., et al, 1983). Differences in the conformation of closely related proteins influence the extent of oligosaccharide processing. Even protein structure influence the oligosaccharide processing (Green M., 1982).

There is evidence that host-dependent factors influence the extent of oligosaccharide processing. These difference in processing among cell types could be due to intrinsic difference in the cellular processing enzymes, differences in duration of intracellular transit of glycoproteins, or difference

in the physical accessibility of oligosaccharides during processing (Hsieh P., et al, 1983).

Williams and Lennarz (1984) have obtained evidence which indicates that the substrate specificities of the processing enzymes can differ between tissues and/or species.

Degradation of the Oligosaccharide Moieties of Glycoproteins

The lysosomes are subcellular organ cells which contain enzymes for the degradation of a large number of macromolecules (de Duve C; 1965). It has been shown that the glycosidases degrade the carbohydrate units of glycoproteins (Aronson N.N.; de Duve, 1968 and Mahadevan S., et al, 1969). Aronson N.N. et al (1968) and Mahadevan S. et al (1969) pointed that the lysosomes from kidney and liver can break down in a step-wise manner the sialic acid-galactose-N-acetylglucosamine chains of the fetuin carbohydrate units. The exoglycosidases remove sugars sequentially from the non-reducing end, exposing the substrate for the subsequent glycosidase. The absence of a particular glycosidase prevents the action of the next enzyme resulting in cessation of catabolism and accumulation of the product, as observed in aspartylglycosylaminuria, due to the absence of β-aspartyl-N-acetylglucosamine aminohydrolase (Mahadevan S.,Tappel A.L, 1967; Ohgushi T., Yamashina I., 1968), and α-N-acetylgalactosaminidase (Weissmann B., Hinrichsen D.F., 1969). There is also evidence for the presence of endoglycosidases with broader specificity. However, the sequence of action of endo- and exoglycosidases in the catabolism of

glycoproteins is not well understood. It is shown that liver lysosomes can degrade 80% of the disaccharide units of ovine submaxillary mucin, with the release of free N-acetylneuraminic acid and N-acetylgalactosamine (Aronson N.N., de Duve, 1968).

The primary degradation process occurs in lysosomes but there are also specific microsomal glycosidases involved in the processing of glycoproteins during their synthesis.

Disorders of Degradation

Various pathological conditions arise due to the disturbances in the degradation of glycoproteins. To ennumerate :

1. Fucosidosis - Fucose accumulates in tissues due to the absence of α-fucosidase (Van Hoof and Hers, 1968)

2. Gangliosidosis - Glycoprotein and glycolipid accumulates due to decrease in the lysosomal β-galactosidase in the liver and brain (Van Hoof and Hers, 1968).

3. Aspartylglycosaminuria - Large amounts of N-acetylglucosaminyl-asparagine are excreted in urine (Pollitt et al, 1968). There is defect in the degradation of glycoproteins due to the absence of the enzyme β-aspartyl-glucosylamine-amidohydrolase.

Glycoproteins in Various Pathological Conditions

Serum contains over 30 distinct glycoproteins. Almost all of the plasma proteins of humans, except albumin, are glycoproteins. Many proteins of cellular membrane contain substantial amounts of carbohydrates. Many of these glycoproteins have been identified, purified and analysed to know the nature of their carbohydrate units, sequence of monosaccharide residues, carbohydrate-peptide linkages and amino acid sequences.

The level of these glycoproteins is maintained within a narrow range in health, but is elevated in many pathological conditions (Winzler, 1955). In a disease, the overall rise seen in the level of glycoproteins is due to increase in the concentration of a few components (Winzler, 1965). Experiments performed by Ashwell (1974) suggested that an individual sugar could play an important role in governing at one of the biologic properties (i.e. time of residence in the circulation) of certain glycoproteins.

Since the glycoproteins vary in their relative content of the four monosaccharides, the net increase obtained in the whole level would also vary, depending upon the monosaccharide used for determination. For example, it is reported that in advanced cancer, an increase of 250% in the level of serum glycoproteins is obtained when estimated by hexosamine content, but the increase is only 50% when protein-bound hexose is used for determination (Winzler, 1955). In rheumatic fever, on the other hand, the rise indicated by hexosamine is negligible but that shown by hexose is about

100% (Kelly, 1952). Therefore, if any correlation exists between the serum glycoprotein levels and diseases, it is mosts likely to be brought out by study of all four monosaccharides.

In diabetes mellitus, the defect is an absolute or relative lack of insulin or decreased response of tissues to it. This leads to abnormalities of metabolism, not only of carbohydrate but also of protein and fat. Because of the central role which glucose plays in the formation of the monosaccharide components of glycoproteins, the biosynthesis of these compounds is of particular interest in diabetes, since it would seen reasonable that significant alterations in glucose metabolism could be related in the synthesis of carbohydrate containing proteins. Hence, the present study is undertaken, with the main emphasis on diabetes mellitus patients and in those patients who have developed renal, vascular and cardiac, neurological or neuro-muscular complications.